LARVAL COMPETITION AND ADULT SUSCEPTIBILITY TO ARBOVIRUS INFECTION IN CONTAINER MOSQUTIOES

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

This dissertation is dedicated to my mother, Barbara A. Larson, for her undying support

ACKNOWLEDGMENTS

I am thankful for valuable advice and reviews from my dissertation committee, L. P. Lounibos, S. Juliano, C. Lord, J. Maruniak, C. Mores, C. Osenberg, and W. Tabachnick. I am especially grateful to my advisor L. P. Lounibos for years of guidance, support, useful discussions, and reviews. I am grateful to J. Butler, B. Coon, and K. McKenzie for assistance with methods and equipment necessary to construct the silicon membrane system; C. Jennings for supplying me with citrated blood; G. O'Meara for providing me with cages for bloodfeeding trials and data on mosquito larval densities from field collections; P. Grimstad and R. Nasci for providing me with *Aedes* eggs; N. Nishimura, R. Escher, I. Tobar, and B. Wagner for daily maintenance of the competition studies and for measuring wing lengths; J. Maruniak, S. Higgs, and D. Bowers for advice with cell culture and Sindbis virus assays; S. Fernandez at Walter Reed Army Institute of Research for generously providing me with the plaque assay protocol and the dengue-2 virus (16803) used in the mosquito infection study; M. Reiskind for dengue virus RNA extraction and quantitative RT-PCR research, as well as valuable discussions and reviews that improved the dissertation; D. Baptiste for providing Vero cells and aiding in dengue virus RNA extraction; D. Chisenhall, J. Dyer, K. Pesko, and S. Richards for assistance in bloodfeeding mosquitoes.

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August 2006

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Larval competition is well-documented among container mosquitoes and influences life history traits such as survivorship, development, and adult size. Few studies have attempted to address how biological interactions experienced by larvae may impact adult susceptibility to arboviral infection, subsequent viral spread to secondary tissues (i.e., disseminated infection), and viral body titer.

With Sindbis, an arbovirus frequently used in vector research, *Aedes albopictus* mosquitoes had higher infection rates but lower body titer and dissemination rates than *A. aegypti*. For both *A. albopictus* and *A. aegypti*, competition affected population growth measurements, with uncrowded larval conditions consistently resulting in shorter time to adult emergence, increased survivorship, adult size, and better population performance than crowded conditions. For *A. albopictus*, but not for *A. aegypti*, more intense intra- and interspecific competition resulted in higher Sindbis virus infection rates, body titers, and dissemination rates compared to low competition conditions. Whole body titers of

virus increased with mosquito size irrespective of competition. However, between competitive treatments, mosquitoes from low competition conditions had greater mean size, with lower infection and lower whole body titers than smaller mosquitoes from high competition conditions. The results of experiments on this model system indicate the importance of the larval environment, especially competitive conditions, on adult vector competence.

With dengue virus, the most important arbovirus afflicting humans, *A. aegypti* had lower dengue virus infection rates and body titers but higher dissemination rates than *A. albopictus.* Higher levels of intra- and interspecific competition enhanced *A. albopictus* infection and dissemination rates with dengue virus. Similar effects of competition on mosquito infection parameters with unrelated Sindbis and dengue viruses suggest a generalizable mechanism of environmental influences on infection parameters. The experimental results indicate that larval conditions are an important aspect of vector competence and should be included in future epidemiological considerations and modeling of arbovirus transmission.

CHAPTER 1
INTRODUCTION AND REVEW OF THE LITERATURE

Introductory Statement

Larval competition is well-documented among container mosquitoes but its effects on adult susceptibility to arboviral infection remain unclear. This research addresses the question whether larval competition among and between mosquitoes *Aedes aegypti* and *A. albopictus* influences adult susceptibility to Sindbis and dengue virus infection. A silicon membrane bloodfeeding system was evaluated as a method to administer infectious bloodmeals for subsequent competition and infection studies. The purpose of the following literature review is to provide a working knowledge of container habitats and basic biology of the two mosquito species used in the current experiments. Also, I summarize the vector biology research of dengue and Sindbis viruses as well as place the current question concerning competition and susceptibility to arboviral infection in context to studies of similar nature.

Water-filled Containers

Phytotelmata are parts of terrestrial plants such as leaf axils of tank bromeliads, bamboo internodes, pitchers of carnivorous plants, *Heliconia* bracts, fallen leaves or fruit husks, and treeholes which hold bodies of water (Frank and Lounibos 1983). Artificial containers serve as analogs of phytotelmata and come in a variety of forms such as discarded tires, cans, vases, jars, cisterns, and plastic debris. Both natural and artificial containers are habitats for a variety of arthropods having aquatic life stages. Containers may be favorable model systems for investigating entomological and ecological

processes because they harbor small, discrete aquatic communities. Diptera are the most taxonomically diverse group among insect orders inhabiting phytotelmata (Fish 1983). In particular, mosquitoes are the most extensively studied dipterous insects within container communities, in part, because they often are the most abundant macroinvertebrates (Fish 1983) and may be vectors of arthropod-borne (arbo) viruses and other vertebrate pathogens.

Nutrient resources in these systems come in the form of inputs of allochthonous plant detritus (fallen leaves, flower parts) (Lounibos et al. 1993, 1992, Kitching 1971), macroinvertebrate carcasses (Daugherty et al. 2000, Sota et al. 1998, Heard 1994, Naeem 1988, Bradshaw and Holzapfel 1986), throughfall, and stem flow (Kaufman et al. 1999, Walker et al. 1991, Walker and Merritt 1988, Carpenter 1982a). The latter two resource inputs refer to precipitation that has fallen through the canopy or down the branches and trunk of trees, respectively. Decomposing plant detritus is recognized as the predominant nutrient base for treeholes, and, perhaps, artificial container communities (Maciá and Bradshaw 2000, Lounibos et al. 1992, Carpenter 1983, Fish and Carpenter 1982, Kitching 1971). Leaf litter is likely a lower quality nutrient resource compared to macroinvertebrate carcasses due to intrinsic differences in carbon:nitrogen ratios among these resources (Cloe and Garman 1996). Some macroinvertebrates may directly consume plant detritus (e.g., *Helodes* and *Prionocyphon* beetles, Paradise and Kuhn 1999, Barrera 1996a, Carpenter 1982b), however most container mosquitoes consume microorganisms associated with the detritus and water column (e.g., Walker and Merritt 1988, Fish and Carpenter 1982). Spatial and temporal differences in the quality and quantity of nutrient resources have important consequences for container communities.

For example, mosquito larval stages are confined to containers, and so habitat characteristics (e.g., nutrients, competition, predation, temperature) largely determine mosquito population growth measurements. Rate of leaf decomposition, and associated bacteria and algae, is often positively correlated with nutritional value and mosquito productivity (e.g., Dieng et al. 2002, Yanoviak 1999, Fish and Carpenter 1982, Swift et al. 1979), and differences in degradation among leaf types are due to the environmental conditions and properties of the leaf species (Yanoviak 1999, Léonard and Juliano 1995, Fish and Carpenter 1982).

Mosquitoes

Aedes albopictus

The Asian tiger mosquito *Aedes albopictus* (Skuse), native to Southeast (SE) Asia and the Pacific and Indian Ocean regions, invaded container habitats in the U.S., Europe, West Africa and South America during the last 30 years (reviewed in Eritja et al. 2005, Juliano and Lounibos 2005, Lounibos 2002). *Aedes albopictus* is second only to *A. aegypti* in terms of importance as a vector of dengue virus (DENV). Although small introductions of *A. albopictus* in the continental U.S. were found previously in used tires shipped to a port in Oakland, CA (Eads 1972) and a cemetery in Memphis, TN (Reiter and Darsie 1984), none became established. It is believed that *A. albopictus* first became established in the continental U.S. in Houston, Texas in 1985 (Sprenger and Wuithiranyagool 1986). Populations of *A. albopictus* in the U.S. are believed to be derived from temperate Japan (Hawley et al. 1987), whereas Brazilian *A. albopictus* are of tropical origin (Birungi and Munstermann 2002). Successful spread of *A. albopictus* in the eastern U.S. was facilitated by immature stages using artificial container habitats, in particular used or discarded tires (Moore 1999, Reiter 1998). *Aedes albopictus* is

adapted to both tropical and temperate climatic regions and capable of using a wide range of suitable container habitats such as man-made containers (e.g., discarded tires, cemetery vases, cans), natural tree holes, bamboo internodes, and other phytotelmata (Hawley 1988).

Adult females deposit desiccation resistant eggs on walls of containers, and these eggs hatch when flooded by water (Hawley 1988). Embryonated eggs are able to survive several months at 20-25°C and at moderate to high humidity (44-90%) (Sota and Mogi 1992, Hawley 1988). The embryonation period is temperature-dependent but usually can be completed within two days to just over a week. *Aedes albopictus* from temperate Japan have photoperiodically inducible egg diapause, whereas *A. albopictus* of tropical origins ordinarily do not (Lounibos et al. 2003a, Pumpuni et al. 1992, Hawley et al. 1987). In continental Asia and the U.S., 0ºC and -5ºC are the approximate northern maximum isotherms for overwintering range and northward expansion, respectively (Nawrocki and Hawley 1987).

Larvae in water-filled containers filter-feed and browse on decomposing plant detritus and microorganisms. Studies quantifying development time of larval stages have usually been performed at ~ 25°C and under optimal nutrition. These conditions allow for complete larval development in 5-10 days (Hawley 1988). Low temperature, crowded larval conditions, and nutrient deprivation greatly increase development time (e.g., Lounibos et al. 2002, Alto and Juliano 2001ab, Briegel and Timmermann 2001, Teng and Apperson 2000). The non-feeding pupal stage lasts ~ 1-3 days. Pupal size is determined to a large extent by larval density and food supply, however other factors (e.g., temperature) may also be important (Hawley 1988).

Studies quantifying adult longevity have largely been conducted in the laboratory and are likely to overestimate longevity in the field. Under laboratory conditions, females can live for several weeks and perhaps as long as a few months. High temperature coupled with low humidity decreases adult longevity (Alto and Juliano 2001b, Mogi et al. 1996). Female *A. albopictus* blood feed diurnally and are able to blood feed within 2-3 days of emergence (Hawley 1988). *Aedes albopictus* is an opportunistic biter taking blood meals from a variety of hosts, including humans (Ponlawat and Harrington 2005, Niebylski et al. 1994, Savage et al. 1993). It is capable of dispersing > 800 m in suburban settings (Honório et al. 2003). Female fecundity is positively correlated with size but other factors may also influence fecundity (e.g., temperature, size of blood meal) (e.g., Armbruster and Hutchinson 2002, Lounibos et al 2002, Briegel and Timmermann 2001, Blackmore and Lord 2000, Briegel 1990, 1985).

Aedes aegypti

The yellow fever mosquito *Aedes aegypti* (L.) has its origins in Africa. Water vessels aboard slave ships are thought to have transported immature stages of *A. aegypti* from West Africa to the Western Hemisphere during the 15th to 17th centuries (Christophers 1960). However, *A. aegypti* may have established in Portugal and Spain prior to its arrival in the Western Hemisphere (Tabachnick 1991). It is likely that *A. aegypti* spread subsequently to the Mediterranean, tropical Asian, and Pacific Islands during the 18th, 19th, and 20th centuries, respectively (Tabachnick 1991). In Sub-Saharan Africa, at least 2 forms of *A. aegypti* exist, differing genetically, morphologically, and behaviorally (Tabachnick et al. 1979, Mattingly 1957). The sylvan form, *A. aegypti formosus* (Walker), is darkly colored and found in natural phytotelmata (treeholes) and confined to East Africa (Christophers 1960). *Aedes aegypti formosus* feed on a variety of

vertebrates, including primates, but primarily feed on reptiles and small mammals (McClelland and Weitz 1963). The domestic form, *A. aegypti aegypti* (L.), is lighter in color and highly anthropophilic, blood feeding predominantly on humans and occupying artificial containers in its immature stages. The domestic form is referred to as *A. aegypti* and the sylvan form as *A. aegypti formosus*. Adults of *A. aegypti* commonly oviposit and blood feed in human dwellings. The adaptation to artificial container habitats and blood feeding on humans has made *A. aegypti* highly successfully in spreading throughout much of tropical to mild temperate regions (Lounibos 2002, Christophers 1960). *Aedes aegypti* is considered the primary vector of DENV.

Female *A. aegypti* adults deposit desiccation resistant eggs within a wide range of artificial containers, both outdoors and indoors, in urban environments. Eggs of *A. aegypti* are more resistant to mortality induced by high temperatures and desiccation as compared to *A. albopictus* (Juliano et al. 2002, Sota and Mogi 1992). The embryonation period is similar to that of *A. albopictus* and there is no evidence that eggs, or any developmental stage of *A. aegypti*, are capable of diapause.

A. aegypti larvae develop more rapidly than *A. albopictus* on artificial nutrient resources (e.g., yeast, albumin) but develop more slowly compared to *A. albopictus* on leaf litter (B.W. Alto, personal observation). This is consistent with the observation that larval resistance to starvation was maximized with leaf litter for *A. albopictus* and with liver powder (non-natural) food for *A. aegypti* (Barrera 1996b). Pupal developmental period is similar to *A. albopictus* and highly dependent on temperature (~2 – 3 d at 23-27ºC) (Christophers 1960).

In extreme cases, maximum survival of adult females in laboratory settings may be >100 days, however, longevity is highly dependent on abiotic conditions (temperature, humidity) (Mogi et al. 1996) as well as adult size and nutrient availability (water, carbohydrates, blood) (Christophers 1960). Regular access to carbohydrates and blood, coupled with high humidity and ~28ºC is optimal for *A. aegypti* adult longevity (Christophers 1960). A mark-release-recapture field study in Kenya determined longevity of adult *A. aegypti* during the rainy season (April-May 1972, environmental conditions unspecified) (Trpis and Hausermann 1986). Mean-maximum adult female and male longevity was 10.7-42 and 5.8-8 d, respectively. *Aedes aegypti* often prefer human hosts for blood meals and may imbibe multiple blood meals in a single gonotrophic cycle (Scott et al. 2000ab, 1993ab). Large *A. aegypti* consume more than twice as much blood as small individuals, and subsequent efficiency of yolk synthesis derived from the blood meal is positively related to female size (Briegel 1990). Adult dispersal may be hundreds of meters (e.g., 100 to >800 m) in rural and urban dengue endemic regions (Harrington et al. 2005, Honório et al. 2003).

Dengue Virus

Introduction

Dengue virus (DENV) consists of the dengue serotypes 1-4. These are the etiological agents of human disease that range in severity from undifferentiated dengue infection (asymptomatic or mildly symptomatic), classical dengue fever (DF), dengue hemorrhagic fever (DHF), to dengue shock syndrome (DSS) (Gubler 1997, Gubler et al. 1981). DF is characterized by an abrupt febrile illness with associated malaise, headache, retro-orbital pain, rash, and extreme muscle and joint pain. DF is not known to be associated with mortality. Initial symptoms of DHF resemble those of DF followed by

thrombocytopenia, hemorrhagic manifestations, and plasma leakage due to increased vascular permeability from the release of circulating factors in infected white blood cells (e.g., monocytes, T cells). DSS is characterized by severe DHF followed by shock where patients experience restlessness, rapid and weak pulse, subnormal temperature, and low blood pressure. DENV is considered among the most important vector-transmitted arboviruses and its geographic range places 2.5 billion humans at risk (review in Gubler 2002). Annually 50-100 million cases of DF occur in tropical cities with hundreds of thousands of cases of DHF (<1-15% DHF mortality) (Gubler 2002).

An *A. aegypti* eradication program initiated by the Pan American Health Organization in the 1940s and 1950s was successful at limiting *Aedes aegypti* distribution in the Americas (Gubler 1997). However, the program was disbanded in the 1970s followed by *A. aegypti* reinfestation in most areas which the program had targeted. Dengue activity has increased in recent decades and poses a major global public health problem (Gubler 1997). Although the reasons are complex and not fully understood, factors that may contribute to increased dengue activity include reinvasion of tropical America by the primary vector of DENV, *A. aegypti*, ineffective mosquito control in areas associated with dengue, unplanned urbanization, abundant man-made larval habitats, and increased and rapid human travel.

Human Infection

A study on naturally acquired DENV of serotypes 1, 3, and 4 in Central Java, Indonesia, showed patients with viremia ranging from $10^{3.8}$ to > $10^{8.0}$ MID_{50}/ml (Mosquito Intrathoracic Inoculation Dose for 50% infection) that lasted for 5-6 days in some cases (Gubler et al. 1979). Similarly in Jakarta, Indonesian patients with dengue fever showed a viremia range of 2-12 days with an average of 4-5 days (Gubler et al.

1981). Viral titer in these patients ranged from $10^{3.8}$ to $10^{7.2}$ MID_{50}/ml, with DENV serotype 4 infected patients showing ~ 10^2 times lower titer (Gubler et al. 1981). Gubler et al. (1981) did not find that disease severity was significantly affected by duration or magnitude of viremia. However, fatal DHF cases had large amounts of circulating DENV (Gubler et al. 1981). DENV pathogenesis is difficult to study because there are no *in vivo* or *in vitro* models manifesting pathology similar to humans (Leitmeyer et al. 1999). Despite unique human pathology, nonhuman primates and mice have served traditionally as human surrogates in dengue laboratory models (Gubler 1997).

Infection Cycle in the Mosquito Vector

The DENV transmission cycle includes a human reservoir and a mosquito vector, although sylvatic cycles occur between monkeys and mosquitoes in tropical Africa and Asia. After imbibing an infectious bloodmeal, arboviruses (e.g., DENV) are deposited in the mosquito midgut and an infection may initiate. Biological transmission of arboviruses includes acquisition from an infectious bloodmeal, replication in the mosquito, dissemination of virus throughout the body of a mosquito resulting in a generalized infection, movement of virus into salivary glands via hemolymph or neural pathways, and transmission to a host by subsequent bloodfeeding (Hardy et al. 1983). Additional biological routes include transovarial and venereal transmission.

Successful biological transmission of an arbovirus requires that several internal physical barriers must be overcome in the mosquito. The ingested infectious blood is deposited into the posterior midgut. Within a matter of hours viruses migrate toward the microvillar margins of the mesenteronal epithelial cells (midgut cells) (Hardy et al. 1983). Mechanisms by which viruses enter the midgut cells are not well known, but

attachment with receptor-mediated-entry is thought to be a common mechanism. Once in a midgut cell, the virus releases its nucleic acid and replicates. The midgut infection barrier is the first barrier that the arbovirus must overcome in the infection process (Gomez-Machorro et al. 2004, Bosio et al. 2000, Woodring et al. 1996, Hardy et al. 1983). Crossing of this barrier is thought to be dose-dependent such that the likelihood of infection increases with increased viral titer in the blood meal (Lord et al. 2006, Hardy et al. 1983). At this stage, arboviral infection is limited to the mesenteronal epithelial cells. The next barrier to overcome in the arboviral infection cycle is the midgut escape barrier (Bennett et al. 2005ab, Bennett et al. 2002, Myles et al. 2004, Woodring et al. 1996). Crossing of this barrier, which consists of multilayer basal laminae, is also thought to be dose-dependent (DeFoliart et al. 1987). If arboviruses fail to overcome the midgut escape barrier, then infections are limited to the mesenteronal epithelial cells. If the midgut escape barrier is overcome, the arbovirus enters the hemocoel from where it can disseminate, via hemolymph, to other tissues and organs (e.g., fat body, foregut, hindgut, ovarioles, salivary glands). These midgut barriers have important epidemiological significance because they, in part, determine whether mosquitoes become potential arboviral transmitters. Intrathoracic inoculation of arboviruses (ie., bypass midgut barriers) is highly efficient at infecting mosquitoes, and may effectively eliminate interspecific differences in vector competence, even species that may otherwise show refractoriness to arboviral infection or transmission, perhaps due to barriers, (i.e., genetic refractoriness) (Woodring et al. 1996, Hardy et al. 1978).

The final two barriers to transmission by mosquitoes are the salivary gland infection barrier and the salivary gland escape barrier (Grimstad 1985 et al., Hardy et al.

1983). Crossing of the salivary gland infection barrier is dose-dependent and time-dependent. The reason for the time-dependency is that, in many instances, the longevity of the female adult mosquito and the extrinsic incubation period are similar. The time from initial ingestion of the infectious blood meal until the time the mosquito can transmit the arbovirus is the extrinsic incubation period. Thus, for successful transmission of the arbovirus to another host, it must pass all the barriers and infect the salivary glands before the mosquito dies. The paired salivary glands consist of a single layer of cuboidal epithelial cells surrounded by a basal lamina. If the salivary gland infection barrier is overcome, the arbovirus may replicate in the cuboidal cells. Finally, if the salivary gland escape barrier is overcome, virus is incorporated into the saliva and is potentially transmitted to a vertebrate host during the next blood feeding. These barriers determine the intrinsic ability of a mosquito to become infected and subsequently transmit a pathogen (i.e., vector competence). Mosquitoes with disseminated DENV infection are capable of transmitting virus for the remainder of their life (Rodhain and Rosen 1997).

Sylvatic Dengue Cycles

Sylvatic dengue cycles are known to occur in West Africa and Malaysia involving *Aedes* species mosquitoes and monkeys (Diallo et al. 2003, Wang et al. 2000, De Silva et al. 1999, Gubler 1997, Rodhain 1991, Rudnick 1978, 1965). Sylvatic dengue cycles are not known in the Western Hemisphere (Rodhain and Rosen 1997). However, antibodies to DENV have been recovered from bats and other mammals in Costa Rica, Ecuador, and French Guiana (de Thoisy et al. 2004, Platt et al. 2000). Further research on sylvatic cycles in the Western Hemisphere is needed (e.g., viral isolation) since neutralizing antibodies may cross-react with other related viruses in those regions (Scott 2001, Innis

1997). Monkeys infected with DENV are not known to exhibit human-like DF or DHF symptoms. In West Africa, DENV-2 has been isolated from *A. africanus* (Theobald), *A. luteocephalus* (Newstead), *A. opok* (Corbet and Van Someren), *A. furcifer* (Edwards), and *A. taylori* (Edwards) (Diallo 2003, Wang et al. 2000). In Malaysia, it is believed that the canopy dwelling mosquito *A. niveus* (Ludlow) serves as the vector for all the DENV serotypes to monkeys (Wang et al. 2000). DENV isolates from nonprimate reservoirs are not known to exist, although neutralizing antibodies have been found from mammals in the Eastern Hemisphere (Rudnick 1965).

Viral Isolates and Experimental Infection/Transmission

DENV is one of > 70 arboviruses within the genus *Flavivirus* (family Flaviviridae) (White and Fenner 1994). Flaviviruses are enveloped, spherical virions with a diameter of 40-50 nm. They have a linear plus sense single stranded RNA genome of 10.5-11 kb and are capped at the 5′ terminus but not polyadenylated at the 3′ terminus. Endemic/epidemic dengue cycles between humans and the primary and secondary vectors, *A. aegypti* and *A. albopictus,* as well as *A. polynesiensis* (Marks). Field collections of naturally infected mosquitoes suggest that other vectors may include *A. mediovittatus* (Coquillett), *A. scutellaris* (Walker), *A. cooki* (Belkin), and *A. hebrideus* (Edwards) (Rodhain and Rosen 1997, Freier and Rosen 1988). It is thought that the DENV serotypes that now cause epidemics independently evolved from sylvatic progenitors ~ 100 to 1,500 years ago, presumably when DENV adapted to peridomestic *A. albopictus* and later to *A. aegypti* (Moncayo et al. 2004, Wang et al. 2000). Thus, sylvatic DENV serotypes, typically cycling between sylvatic mosquitoes and monkeys, differ from endemic/epidemic DENV serotypes transmitted by *A. aegypti, A. albopictus*,

and other anthropophilic *Aedes* mosquitoes (Moncayo et al. 2004). All sylvatic DENV serotypes are found in Malaysia whereas only sylvatic DENV-2 occurs in Africa, suggesting that DENV may have originated in the Asian-Oceanic region (Wang et al. 2000). If this hypothesis is true, then the peridomestic transmission of DENV was most likely initially vectored by *A. albopictus* since *A. aegypti* did not establish in Asia until the later half of the 19th century (Tabachnick 1991, Smith 1956). Both *A. albopictus* and *A. aegypti* are more susceptible to infection with endemic/epidemic DENV-2 than to sylvatic DENV-2, supporting the hypothesis of emergence of endemic/epidemic dengue by viral adaptation to peridomestic *Aedes* spp. (Moncayo et al. 2004).

Endemic/epidemic DENV serotypes differ in their ability to infect *A. albopictus* and *A. aegypti* (e.g., Moncayo et al. 2004, Armstrong and Rico-Hesse 2003, 2001, Rosen et al. 1985). Previous literature suggested that all DENV serotypes infected and disseminated in *A. aegypti* more poorly than in other *Aedes* species, including *A. albopictus* (Rodhain and Rosen 1997, Rosen et al. 1985, Gubler et al. 1979). However, these studies used highly adapted laboratory colonies of these *Aedes* species, which may have altered vector competence due to founder effects, genetic drift, and unintentional artificial selection imposed on laboratory colonies (Armstrong and Rico-Hesse 2001, Lorenz et al. 1984). A laboratory study on the F_1 - F_2 progeny of field collected mosquitoes in Vietnam and Thailand showed that *A. aegypti* were more readily orally infected than *A. albopictus* (mosquito head assays) with SE Asian DENV-2 (Vazeille et al. 2003). Similarly, laboratory colonies of *Aedes* collected in Taiwan ($\geq F_5$) showed *A. aegypti* had significantly higher salivary gland infection and transmission rates for DENV-1 compared to *A. albopictus* (Chen et al. 1993). The conflicting observations in

the cited studies demonstrate that it is unclear whether *A. albopictus* or *A. aegypti* is the more competent DENV vector. It appears that vector competence of these two species critically depends on underlying genetic differences in the strains of mosquitoes, the strains of DENV, and the environmental conditions under which the laboratory analyses are conducted. The complexity of investigating vector competence mechanisms and variation has been discussed elsewhere (Tabachnick 1994).

Vertical transmission of of arboviruses (e.g., transovarial) may serve as a mechanism to survive inhospitable environmental conditions (e.g., cold temperatures, drought). For arboviruses that cycle between mosquito and humans (e.g., DENV in the Western Hemisphere), vertical transmission may facilitate endemic maintenance, especially when human cases are not occurring. Experimental studies have shown that both *A. albopictus* and *A. aegypti* are capable of vertical transmission of DENV as determined by detection of DENV antigen or viral isolation among immature stages (e.g., Joshi et al. 2006, 2002 1996, Rodhain and Rosen 1997, Bosio et al. 1992, Rosen et al. 1983). For example, field collections of immature stages of *A. aegypti* in India and Burma showed definitive evidence of DENV-2 and DENV-3 vertical transmission because infected field mosquito supernatant fluid was inoculated in *Toxorhynchites splendens* (Weidemann) mosquitoes, allowed to replicate, and DENV antigen was positively recovered from head tissues (Thenmozhi et al. 2000, Khin and Than 1983).

Sindbis Virus

Introduction

Sindbis virus (SINV) was first isolated from mosquitoes *Culex univittatus* (Theobald), *Culex pipiens* (L.), and a juvenile hooded crow *Corvus corone sardonius* in 1952 in Sindbis Egypt, 30 km north of Cairo (Taylor et al. 1955). SINV has a wide

geographic distribution in Australia, Scandinavia, South Africa, Middle East, and Asia (Laine et al. 2004, Dohm et al. 1995, Niklasson 1989, Tesh 1982). SINV has been given distinct names based on the geographic region of isolation i.e., Ockelbo (Sweden), Pogosta (Finland), Karelian (Russia), and SINV (other regions) (Laine et al. 2004). In all instances the viruses are similar and represent geographically distinct genotypes (Kurkela et al. 2004, Laine et al. 2004, Sammels et al. 1999, Lundström 1999, Norder et al. 1996, Shirako et al. 1991, Lundström et al. 1993a, Olson and Trent 1985). SINV is not known to occur in the Americas. The wide distribution is partially attributable to migratory birds which serve as reservoirs and transport SINV over large distances (e.g., Buckley et al. 2003, Brummer-Korvenkontio et al. 2002, Lundström et al. 2001, Lundström et al. 1993b).

Human and Reservoir Infection

Clinical symptoms of SINV infection were described from Uganda in 1961 (Woodall et al. 1962). The most complete record of human cases include SINV epidemics in Sweden, Finland, and Russia during late summer and fall (August - October) (Laine et al. 2004, Lundström et al. 1991). Human SINV infections produce a self-limited febrile disease characterized by arthralgia, rash, headache, fatigue, and fever (Laine et al. 2004, 2000, Tesh 1982). It is not uncommon for chronic arthralgia to last for several years after full recovery from other symptoms (e.g., Kurkela et al. 2005, Laine et al. 2000, Turunen et al. 1998, Niklasson et al. 1988, Niklasson and Espmark 1986). SINV is not known to cause human mortality, although morbidity is common.

Although SINV has a wide geographic distribution, human disease with clinical symptoms has been limited to Northern Europe and South Africa (Lundström 1994, Niklasson et al. 1988). Nucleotide sequences of genes encoding capsid (C) and envelope

protein (E2) showed that SINV strains from Northern Europe were most closely related to those strains from South Africa compared to strains from other geographic regions (Norder et al. 1996, Shirako et al. 1991). Intercontinental exchange of SINV strains may be facilitated by migratory birds because 25% of the 252 bird species that breed in Scandinavia overwinter in Africa (Norder et al. 1996, Lundström et al. 1993a, Shirako et al. 1991).

Initially SINV infection was regarded as a minor human disease because there were few human cases. However, several outbreaks occurred in South Africa in 1974 involving *C. univittatus* and *Culex theileri* (Theobald) (Jupp et al. 1986a, McIntosh et al. 1976, 1967, 1964), and Northern Europe in the 1980's including Sweden (Lundström et al. 1991, Espmark and Niklasson 1984, Niklasson et al. 1984, Skogh and Espark 1982), Finland (Kurkela et al. 2004, Brummer-Korvenkontio et al. 2002, Brummer-Korvenkontio and Kuusisto 1981), and Russia (Lvov et al. 1984, 1982). Mosquito vectors of SINV in Northern Europe and Russia include *Culex* spp., *Culiseta* spp., *A. cinereus* (Meigen), and *Aedes communis* (DeGeer) (Lundström 1999, 1994). SINV is the most common virus isolated from mosquitoes in Australia where the principal vectors include *Culex annulirostris* (Skuse) and *Aedes normanensis* (Taylor) (Niklasson 1989). However, clinical symptoms in Australia have only been reported on a few occasions (Sammels et al. 1999).

Viral Isolates and Experimental Infection/Transmission

SINV is a prototype *Alphavirus* in the family Togaviridae. Alphaviruses are enveloped, spherical virions with icosahedral capsids and a diameter of 70 nm (White and Fenner 1994). They have a linear plus sense single stranded RNA genome 11-12 kb, are capped at the 5′ terminus, and are polyadenylated at the 3′ terminus. The most complete

record of SINV isolates from field-collected mosquitoes and experimental infection / transmission studies are from South Africa and Sweden. Typically, zoonotic circulation of SINV occurs between ornithophilic *Culex* and *Culiseta* spp. and passerine birds, although other vector species and avian orders may also be involved in transmission cycles (Lundström 1999, 1994, Lundström et al. 1993b, Francy et al. 1989). The single SINV isolate from an arthropod, not a mosquito, was from a *Hyalomma marginatum* tick (Koch) in Italy (Sicily) in 1975 (Gresikova et al. 1978). Viral isolates from field-collected birds during SINV epidemics in South Africa (1960s-1970s) showed high SINV immune rates for several bird species. Viral isolates from field-collected ornithophilic mosquitoes *C. univittatus* and *C. theileri* showed infection rates of 0.65% and 0.083%, respectively. *Culex univittatus* was observed to readily feed on humans and was regarded as the main epidemic vector for SINV in the region (McIntosh et al. 1978, 1976). However, *C. neavei* (Theobald) may be an important vector in costal South Africa, although it may not acquire a disseminated infection as readily as *C. univittatus* (Jupp et al. 1986b). Laboratory experiments were used to determine the vector potential of *Culex* spp. derived from South Africa (Jupp et al. 1972, Jupp and McIntosh 1970ab). *Culex univittatus* ($F_{6\text{-}15}$) was readily infected (50-83%) after SINV infectious bloodmeals (separate feeding trials with a range of viral titers $10^{3.6-5.6}$) and 57-66% transmitted SINV to avian hosts after taking a subsequent bloodmeal (Jupp and McIntosh 1970a). A similar experiment showed higher (74-100%) *C. theileri* ($F_{3\text{-}11}$) infection with similar SINV titers, but transmission was much lower (9%) (Jupp et al. 1972). Both infection (0-16%) and transmission (0-50%) in *C. pipiens* ($F_{4\text{-}10}$) were lower than for the other *Culex* spp. (Jupp and McIntosh 1970b).

Sweden is dominated by mammalophilic *Aedes* species (Francy et al. 1989, Jaenson and Niklasson 1986). Field collections made in central Sweden showed that 60% of all *Aedes* spp. collected were *A. cinereus*, whereas only 3.4 and 3.7% (of total mosquitoes collected) were *Culex* and *Culiseta* spp., respectively. Despite their infrequency in collections, *C. pipiens*, *C. torrentium* (Martini), and *C. morsitans* (Theobald) accounted for 80% of all SINV isolates (Francy et al. 1989). Minimum infection rates were ~7-14% for *C. pipiens* and *C. torrentium*, and ~2-5% for *C. morsitans*. Minimum infection rates for *A. cinereus* were 0.5%. Experimental inoculations in indigenous bird species showed that Passeriforms ($10^{5.8}$ to $10^{7.5}$ Plaque forming units/ml) (PFU/ml) had significantly higher and longer viremia compared to Anseriforms ($10^{3.7}$ to $10^{4.5}$ PFU/ml) (Lundström et al. 1993b). Passeriform thrushes (*Turdus* spp.) and finches (*Fringilla* spp.) are the major SINV reservoirs in Sweden (Lundström et al. 2001, Lundström 1994). The presence of neutralizing antibodies to SINV in passerine reservoirs was detected in summer but not spring bird migrants (Francy et al. 1989). It is likely that *Culex* and *Culiseta* spp. are important vectors in the enzootic cycle involving passerine birds, whereas *A. cinereus* and *A. communis* are probable bridge vectors to humans (Francy et al. 1989, Jaenson and Niklasson 1986, Lundström 1999, 1994). This epidemiological hypothesis is supported by SINV isolates from a suspected bridge vector *A. communis* of SINV human infections in Russia (Lvov et al. 1984).

Experimental infection and transmission studies were used to determine the vector potential of *Culex* spp. from central Sweden (Lundström et al. 1990ab). *C. torrentium* (F_{3-6}) infected and transmitted SINV to avian hosts more efficiently than *C. pipiens* (F_{4-10}). Even at low infectious bloodmeal titers (<2.0 PFU/ml), 50% of *C. torrentium* were

infected with SINV. Blood meal titers of >3.0 PFU/ml resulted in 90-100% infection and 100% transmission. For *C. pipiens*, 3.0-3.0 PFU/ml resulted in 4% infection, whereas higher titers (6.0-8.9 PFU/ml) resulted in 42 to 55% infection and 14 to 37% transmission (Lundström et al. 1990a).

A laboratory experiment determined the effect of natural temperature regimes (10, 17, 24, cyclic 10-24°C) during the transmission season in Sweden on *Culex* spp. vector competence (Lundström et al. 1990b). Low temperature significantly reduced transmission potential of *C. pipiens*, as measured by SINV dissemination, compared to higher temperatures. In contrast, dissemination in *C. torrentium* was rapid and unaffected by temperature regimes. This result was unexpected and contrary to the established thought that extrinsic incubation period is inversely related to temperature (e.g., Reisen et al. 2006). Thus, although both *Culex* spp. may serve as enzootic vectors in Sweden, *C. pipiens* transmission potential may be broken under cooler conditions, whereas *C. torrentium* is likely to persist as an efficient SINV vector in cool weather. An identical experiment using *Aedes* spp. showed similar SINV infection between temperature regimes, however, transmission was lower and occurred later at low temperature compared to high temperature (Turell and Lundström 1990). Low temperatures were associated with longer extrinsic incubation periods in *A. taeniorhynchus* and *A. aegypti,* which are not known as natural vectors of SINV (Lundström et al. 1990b).

Adult *A. communis*, *A. cinereus*, and *A. excrucians* were collected from Sweden and allowed to blood feed on SINV infected chickens ($10^{4.2}$ PFU/ml) (Turell et al. 1990). All three *Aedes* spp. were highly susceptible to SINV infection (96-100%) and had

dissemination rates ranging from 51-100%. Although *A. communis* failed to refeed, the other *Aedes* spp. had a 50% transmission rate. These *Aedes* spp. are competent SINV vectors and should be regarded as potential links between the enzootic SINV cycle and human infections in Scandinavia. SINV has been repeatedly isolated from both *A. communis* and *A. cinereus* during episodes of human infection. Further, these *Aedes* spp. are active day biters on mammals, including humans, but will also bite birds (Turell et al. 1990).

Laboratory experiments with SINV using easily colonized mosquito species (e.g., *A. aegypti*, *A. albopictus*) have proven useful to address questions about experimental infection and transmission, genetically modified arboviruses (e.g., SINV gene expression vectors), and the dynamics of arboviral tissue tropism and pathology in mosquito vectors (e.g., Bowers et al. 2003, Bowers et al. 1995, Jackson et al. 1993, Xiong et al. 1989). High ($10^{8.4}$ PFU/ml) SINV titers resulted in greater infection compared to moderate ($10^{5.3}$ PFU/ml) SINV titers (Percent infected for high-moderate titers; *A. albopictus*, 90-49%; *C. pipiens*, 48-0%, *A. aegypti*, untested-18%). *Aedes albopictus* had greater dissemination (66%) and transmission rates (53%) compared to *A. aegypti* (9 and 7%) at the lower titer (Dohm et al. 1995).

A study on SINV replication and tissue tropism following intrathoracic inoculation in *A. albopictus* showed temporal and organ-specific distribution of the virus during the extrinsic incubation period (Bowers et al. 1995). Many organs had maximal infection within 3-4 days after infection because the gut barriers were bypassed by intrathoracic inoculation. Some organs were refractory to infection (e.g., ovarioles, malpighian tubules), whereas others had transient or persistent infections, perhaps indicating viral

modulation by the mosquito vector or SINV (e.g., Bowers et al. 1995, Luo and Brown 1993, Murphy et al. 1975). SINV-associated pathology of the salivary glands and midgut muscle tissue of *A. albopictus* has been observed (Bowers et al. 2003). Typically, arboviruses have few cytopathic affects on mosquito cells (*in vitro* and *in vivo*) (Hardy et al. 1983). Similarly, a study on *A. aegypti* following oral infection showed rapid infection of many organs within several days after feeding with the salivary glands being infected by day 5 (Jackson et al. 1993). As was the case for *A. albopictus*, some organs of *A. aegypti* were refractory to infection (e.g., ovarioles, malpighian tubules). Unlike *A. albopictus*, there was no indication that the distribution of SINV in organs changed from days 6-14 (Jackson et al. 1993).

Competition and Vector Competence

Classic laboratory and field research established evidence for the importance of interspecific competition for a variety of systems (e.g., Connell 1961, Birch 1953, Hairston 1951, Crombie 1947, Park 1948). Despite numerous studies, establishing the existence and importance of competition in nature may be difficult and has been historically a topic of debate (e.g., Hairston et al. 1960). Reviews on this topic have provided concise evidence that interspecific competition is widespread in natural systems for a variety of organisms (e.g., Reitz and Trumble 2002, Connell 1983, Schoener 1983, Crombie 1947). More recently, interspecific competition has been invoked as a mechanism by which competitively superior invasive plant and animal species alter the distribution and abundance of established species (e.g., Juliano and Lounibos 2005, Levine et al. 2002, Reitz and Trumble 2002, Byers and Goldwasser 2001, Mack et al. 2000, Holaway 1999, Petren and Case 1996, D'Antonio and Vitousek 1992).

Intra- and interspecitic competition between larval mosquitoes is common and plays an important role in dermining population growth measurements. Competition has been demonstrated in laboratory and field experiments for several mosquito vector species that occupy a variety of aquatic habitats (e.g., Costanzo et al. 2005ab, Peck and Walton 2005, Juliano and Lounibos 2005, Braks et al. 2004, Juliano et al. 2004, Ye-Ebiyo et al. 2003, Gimnig et al. 2002, Gleiser et al. 2000ab, Schneider et al. 2000, Juliano 1998, Barrera 1996b, Léonard and Juliano 1995, Broadie and Bradshaw 1991). Mechanisms involved in mosquito competition have largely been attributable to limiting resources (e.g., food) (e.g., Juliano 1998, Barrera 1996b), although interference competition, mediated by direct physical contact or chemical excretions, may also be important. However, studies with mosquitoes have yielded mixed results and additional studies are needed to evaluate the role of interference competition in natural systems (Broberg and Bradshaw 1995, Broadie and Bradshaw 1991, Dye 1984, 1982, Moore and Whitacre 1972, Moore and Fisher 1969). Resource type (e.g., leaves) and abundance and larval density affect mosquito fitness such that high intra- and interspecific larval density and low resources result in increased larval development time and mortality and decreased adult size, fecundity, longevity, and per capita rate of growth (e.g., Alto et al. 2005, Costanzo et al. 2005ab, Peck and Walton 2005, Juliano et al. 2004, Lounibos et al. 2003b, 1993, Gimnig et al. 2002, Daugherty et al. 2000, Schneider et al. 2000, Teng and Apperson 2000, Yanoviak 1999, Juliano 1998, Léonard and Juliano 1995, Hawley 1985).

Competition is well documented among container mosquitoes and may be important to some mosquitoes in other aquatic habitats. Arbovirus-mosquito research has mainly focused on intrinsic (e.g., genetic) and extrinsic (e.g., temperature, blood meal

viral titer) factors of adult biology that determine vector competence (Tabachnick 1994). Few studies have attempted to address how biological conditions experienced by larvae may determine subsequent adult vector competence.

It is likely that effects of larval competition have an impact on the adult stage and influence adult vector competence of arboviruses. The most extensive research has been conducted on the effect of nutrient deprivation on mosquito vector competence. Low food availability among *Ochlerotatus triseriatus* (Say) produced smaller adults. These small adults transmitted La Crosse virus (LACV) at higher rates than did larger adults that resulted from well-fed larvae. However, the infection rates in these mosquitoes were independent of adult body size (Grimstad and Haramis 1984, Grimstad and Walker 1991). Enhanced transmission efficiency of small *O. triseriatus* adults was associated with higher virus titers and dissemination rates compared to larger adults. Additional support for size-dependent transmission comes from field-collected pupae that were orally infected as adults with LACV and had dissemination and transmission rates that were inversely correlated with adult size (Paulson and Hawley 1991).

The effect of larval nutrition and adult size on infection parameters has been investigated in other mosquito species. Large *A. aegypti* adults produced under varying conditions of larval crowding and food availability had a greater proportion of DENV-2 disseminated infection (New Guinea C strain) than did smaller females (Sumanochitrapon et al. 1998). Similar results were found for *A. aegypti* susceptibility to infection with Ross River virus (RRV) over a range of blood meal titers. Differences between infection of small and large adults became less distinct at greater blood meal titers (Nasci and Mitchell 1994), perhaps suggesting that high titers simply overwhelm

the differences seen at lower titers. Conversely, titer (midgut and head) as well as DENV-2 (Puerto Rico and Ibo strains) dissemination were independent of *A. aegypti* body size (Bosio et al. 1998). Low food availability among larvae of *C. tritaeniorhynchus* (Giles) produced smaller adults that had shorter periods between initial infection of Japanese encephalitis virus (JEV) and subsequent virus secretion in the saliva than did larger adults from well-fed larvae. Additionally, small *C. tritaeniorhynchus* adults had greater JEV transmission than did large adults (Takahashi 1976). Baqar et al. (1980) showed a trend, although not significant, that increased larval densities and decreased larval nutrition resulted in small adults with increased infection susceptibility of *C. tritaeniorhynchus* to West Nile virus (WNV). Infection and transmission rates of Murray Valley encephalitis virus (MVEV) were unaltered between two larval nutritional regimes producing different sized adult *C. annulirostris*. Additionally, neither body nor salivary gland viral titers were altered by larval nutrition (Kay et al. 1989). Similar nonsignificant effects of the larval environment and adult size were found for *C. tarsalis* (Coquillett) infection and transmission of St. Louis encephalitis virus (SLEV) and Western Equine encephalomyelitis virus (WEEV) (Reisen et al. 1997).

Size-dependent differences in mesenteronal tissues may, in part, explain differences in dissemination and transmission of arboviruses by adults of different sizes (Grimstad and Walker 1991). Fewer basal lamina layers were present in the mesenteron of small adult *O. triseriatus* (4-6 layers) as compared to large adults (10-16 layers) which weakened the midgut escape barrier (MEB) and thus enhanced dissemination and transmission rates (Grimstad and Walker 1991, Paulson and Hawley 1991). An alternative hypothesis explaining observed negative relationships between size (wing

length) and vector competence may be related to the number of viral particles imbibed relative to mosquito body size. Large mosquitoes imbibe greater volume of blood, and thus virus, than smaller mosquitoes. Additional support comes from greater viral titers found in freshly bloodfed large adults than in small adults (Nasci and Mitchell 1994). However, when the amount of virus imbibed was corrected for mosquito body weight, small adults imbibe proportionally more virus than large adults in proportion to their body weight (Nasci and Mitchell 1994, Grimstad and Haramis 1984). This explanation may hold true for a number of mosquito species since blood meal titer is positively related to infection, dissemination, and transmission (e.g., Turell et al. 2001, Dohm et al. 1995, Grimstad and Haramis 1984, Kramer et al. 1981).

The previous examples illustrate that larval nutrition affects adult mosquito infection and transmission of mosquito arboviruses. However, the mechanism and details are dependent on the particular mosquito-virus system. Some of the studies support the hypothesis that larval resource competition enhances vector competence. Resource competition alters numerous mosquito life history traits, however, these studies have limited the focus to a single life history trait, adult size. Further, they did not address whether the effect of resource competition on vector competence was causally related to adult size, or alternatively (additionally) related to other physiological conditions correlated with adult size (Grimstad and Walker 1991, Paulson and Hawley 1991). Further, drawing conclusions about common themes from a limited number of studies would be premature and perhaps misleading. Thus, controlled experiments are required to determine quantitatively the effects of larval competition on vector competence for multiple mosquito-virus systems, as well as to disentangle which mosquito life history

traits (e.g., size, development time) are most important in determining vector competence parameters (infection, body viral titer, disseminated infection). Results from such experiments may support with greater quantitative detail the hypothesis that resource competition affects mosquito vectoring ability, or may offer alternative explanations for the larval competition-adult vector competence relationship. The chapters that follow describe the development of an artificial bloodfeeding system used in delivering arbovirus infectious blood meals as well as experiments that evaluate the effects of competition on *A. aegypti* and *A. albopictus* population growth measurements and SINV and DENV infection parameters. The use of these *Aedes* species to investigate the relationship between competition and arboviral infection is important because; competition is well-documented between these *Aedes* species, competition has important ecological effects on their distribution and abundance, and these *Aedes* are the most important vectors of human arboviruses.

CHAPTER 2
AGE-DEPENDENT BLOODFEEDING OF *Aedes aegypti* AND *Aedes albopictus* ON ARTIFICIAL AND LIVING HOSTS

Introduction

Since its introduction to the Americas in the mid 1980s (Hawley et al. 1987, Sprenger and Wuithiranyagool 1986), *Aedes albopictus* has spread rapidly and colonized much of the southeastern U.S. and Brazil. In parts of the eastern U.S., the invasion of *A. albopictus* is associated with declines in the abundance, and in some instances displacement, of *Aedes aegypti* in rural and suburban areas (Mekuria and Hyatt 1995, O'Meara et al. 1995, Hornby et al. 1994, Hobbs et al. 1991). However, these *Aedes* coexist in urban areas of south Florida. Recent comparative studies attempting to explain the observed distributions of these *Aedes* have investigated egg desiccation (Juliano et al. 2002, Sota and Mogi 1992), larval competition (Lounibos et al. 2002, Daugherty et al. 2000, Juliano 1998, Barrera 1996a), adult desiccation (Mogi et al. 1996), and reproductive and metabolic differences (Klowden and Chambers 1992).

One major concern about the *A. albopictus* invasion in the Americas has been its potential as an arboviral disease vector (e.g., DENV). In recent decades, the range of *A. aegypti*, the primary vector of DENV in the Americas, has increased, and dengue activity has surged (Gubler 1997). The range of *A. albopictus* in the U.S. is more extensive than that of *A. aegypti*, and its range in the U.S. is likely to continue to expand (e.g., Madon et al. 2002). *Aedes albopictus* is a competent laboratory vector of numerous arboviruses

(Mitchell 1991, Shroyer 1986) including DENV in Asia and Hawaii; however, the degree to which *A. albopictus* is involved in arbovirus transmission in the Americas is unclear.

With exceptions of transovarial and venereal transmission, successful biological transmission of arboviruses requires acquisition of an infectious bloodmeal, or at least probing behavior. For *A. aegypti*, research investigating factors that influence the normal sequence of events in successful acquisition of a bloodmeal (e.g., host-seeking, probing, bloodfeeding) have mainly focused on measurements of host-seeking behavior (Klowden and Fernandez 1996, Klowden and Briegel 1994, Bowen 1991, Klowden et al. 1988, Klowden and Lea 1984, 1979ab, 1978). Davis (1984) showed a linear increase in host-seeking behavior of *A. aegypti* from 1 to 5 days post-emergence followed by a constant high response until the end of observations at 15 days. A study measuring probing behavior in *A. aegypti* over a 21-day period showed a rhythmic pattern in probing activity in response to a convection current of constant heat and moisture, but no probing pattern was observed in response to a human host (Burgess 1959). However, the design of this latter experiment was weak (e.g., experimental units were not replicated), and there was little statistical support for the conclusion of rhythmic behavior. Few studies have measured age-related acquisition of the initial bloodmeal, an important factor in determining vector potential. Those that have done so have focused on bloodfeeding over a short interval. Seaton and Lumsden (1941) showed a general increase in bloodfeeding associated with age for 1-5-day-old starved virgin *A. aegypti* followed by decreased bloodfeeding on day 6. They suggested that the decreased response on day 6 was attributable to female exhaustion. A similar increase in bloodfeeding with increasing age was found for 3 strains of 1-4-day-old starved *A. aegypti* fed on chickens and

membrane systems (Bishop and Gilchrist 1946). In order to quantify age-dependent acquisition of a bloodmeal, the present study compares bloodfeeding patterns of *A. albopictus* and *A. aegypti* starting from the time of first responsiveness to a bloodmeal (Hawley 1988, Christophers 1960) up to 15 days post-emergence.

Materials and Methods

Experimental Protocol

Aedes eggs used to initiate the experiments were derived from laboratory colonies at the Florida Medical Entomology Laboratory in Vero Beach, FL. Both *Aedes* spp. originated from Fall, 2000, field collections of larvae from water-filled cemetery vases in Hillsborough County, FL, near Tampa. Colonies were housed in 0.03 m^3 cages at (mean ± SD) 24.6 ± 0.4°C, 76.6 ± 6.7% RH, and a 14:10 (L:D) h photoperiod regime including a 1 h dawn and dusk. Colonies had access to ≈ 20% sucrose solution *ad libitum* and weekly bloodmeals from domestic chickens (handled in accordance with the National Institutes of Health guidelines for the use of laboratory animals). Females were provided with water-containing cups lined with paper towel as oviposition substrates. Eggs were hatched, by species, in metal pans with 1.0 liter tap water and 0.30 g of a 1:1 lactalbumin and brewers yeast mixture. Following hatching, approximately 300-500 larvae were reared in pans, with water and food substrate changed every 2 days.

As soon as pupation occurred, inspections of the rearing pans were made daily, and pupae were transferred into 40-ml vials with water until emergence. Vials were checked daily between 1600 and 1800 h for newly emerged adults. These adults were transferred, by species, to cylindrical cages (11 x 9.5 cm, ht x diam) with nylon mesh tops and maintained under similar conditions as the parental generation except for

bloodfeeding. Female density per cage ranged from 5 to 47 with means ± SE of 14.8 ± 9.9 and 14.2 ± 8.2 for *A. aegypti* and *A. albopictus*, respectively. At least one male was present in each cage for every 3-4 females, although many cages had equal numbers of males and females. Examination of scatter plots of residuals versus predicted values (Draper and Smith 1966) showed no evidence that the number of males per cage was in any way related to proportions of females that bloodfed.

In Experiment 1, cages with *Aedes* females were haphazardly assigned to an age treatment (e.g., 3, 4, …, 15 days old). Each cage containing same-age adults ranging from 3 through 15 days old was offered a bloodmeal from a silicon membrane feeding system (Butler et al. 1984). Thus, same-age females were tested on many different days. Females were deprived of sucrose, but not water, 24 h prior to bloodfeeding trials. Before the start of a feeding trial, citrated bovine blood was heated to (mean ± SD) 37.8 ± 1.1°C in 1.5 ml circular wells and covered with a silicon membrane. Next, the membrane feeding system was positioned over the mesh top of the cage for two 15-min periods separated by a 15-min interval. Feeding trials were performed at (mean ± SD) 23.2 ± 0.5°C and 48.1 ± 4.2% RH. After a feeding trial, the number of females that successfully acquired a bloodmeal was recorded. If blood was visually detected in the female gut, it was scored as having a bloodmeal. Thus no attempt was made to distinguish between meals of different volumes. All feeding trials were performed in the late afternoon, within 2-3 h of each other.

For Experiment 2, 3-15 day old *Aedes* were allowed to bloodfeed from a restrained domestic chicken. The methods for mosquito husbandry and adult exposure during feeding trials were the same as those used in Experiment 1. For all feeding trials,

uniformly sized and aged (6-8 wks old) chickens were restrained inside 0.03 m^3 cages into which adult *Aedes* were released and allowed to feed for 30 min. Female density per cage ranged from 5 - 51 with means ± SE of 28.1 ± 9.9 and 24.1 ± 9.7, for *A. aegypti* and *A. albopictus*, respectively. Larger cages were used for bloodfeeding in Experiment 2 to provide greater space for the normal sequence of events involved in bloodmeal acquisition (Clements 1999). Immediately following feeding trials, chickens were removed from the cages, and then *Aedes* were removed from the cage using an electric aspirator and killed by placing them at -20°C for < 1h. The number of female *Aedes* that had successfully bloodfed was recorded as in Experiment 1.

Data Analyses

For Experiments 1 and 2, proportions bloodfed for each species were calculated as the numbers of females that acquired a bloodmeal during a trial divided by the total numbers of females offered the bloodmeal. Experimental units were defined as the cage of adult *Aedes* offered blood. Difficulties in predicting the number of females that would emerge and survive to the day of feeding precluded equal sample sizes for each unique species-by-age treatment. For Experiment 1, the numbers of replicates for each *A. aegypti* and *A. albopictus* by age treatment were (mean ± SD) 5 ± 1 and 5 ± 2, respectively (127 total cages). In Experiment 2, each unique species-by-age treatment was restricted to 3 replicates, except for 15-day old *A. aegypti,* which had 4 replicates (79 total cages).

For both experiments, effects of female density per cage was tested as a continuous variable (PROC GLM, SAS Institute 1989, Sokal and Rohlf 1995). Raw data adequately met assumptions of normality and homogeneous variance except for membrane-fed *A.*

albopictus where the proportion bloodfed was transformed by $\log_{10}(x + 1)$ to meet the assumption of normality. Because effects of female density on proportion bloodfed were all non-significant ($P >> 0.10$ in all cases), analysiss of effects of female age were performed on proportion bloodfed, which was assessed by treating age as a continuous independent variable and comparing regression lines for each species by feeding protocol treatment. This tests for equal slopes among species-feeding protocol groups to determine whether the regression relationships were similar (SAS Institute 1989, Sokal and Rohlf 1995).

Graphical presentation of the data appeared to show age-dependent periodicity in feeding incidence. Therefore, separate Runs Up and Down Tests were performed for the proportion bloodfed for each species-feeding protocol combination (Sokal and Rohlf 1995, Zar 1996). A run was defined as a temporal sequence of increases or decreases in the proportion bloodfed. Difference between mean proportion bloodfed for consecutive age groups was determined and resulted in a sequence of positive and negative changes in proportion bloodfed across female ages (e.g., + + + - - = 2 runs). These tests determined whether the number of runs for proportion blood-fed among females of different ages was significantly different from random expectation.

As an additional test to address the apparent age-dependent periodic pattern of feeding, four regressions were run (one for each species-feeding protocol combination) of proportion blood-fed versus age, each with a sine function of age according to the model:

$$y = a + bA + c \sin(dA),$$

where *y* is the proportion bloodfed, *a* is the intercept, *b* is the slope, *A* is female age, *c* is a parameter affecting the amplitude of the sine function, and *d* is a parameter affecting the frequency of the sine function. Several different initial parameter estimates were used to determine whether the addition of a sine wave function improved the fit of the regression (SAS Institute 1989, PROC NLIN). If either *c* or *d* parameters were not significant, the slope (*b*) was removed and the reduced model tested. Subsequently, if either *c* or *d* were not significant, both *c* and *d* were removed from the model and a linear regression was performed, including the slope, to determine whether or not there was a trend in age-dependent blood-feeding.

Results

Regardless of age, a higher proportion of both *A. albopictus* and *A. aegypti* bloodfed on the restrained chicken (mean ± SE; 59.8 ± 2.4 and 81.3 ± 2.3%, respectively) compared to the membrane system (mean ± SE; 30.8 ± 2.7 and 55.6 ± 2.6%, respectively).

Treating age as a continuous independent variable, there were significant age, species, feeding protocol, and age x feeding protocol effects (Table 2-1). All other effects were not significant. Slopes of proportion bloodfed vs. age were significantly positive for both *Aedes* species feeding on the membrane system and were not significantly different from zero for both *Aedes* species feeding on the restrained chicken (Table 2-2). Although these slopes were significant, the low r^2 values suggest that the linear relationships were weak (Fig. 2-1, Table 2-2). In addition, the sine function contributed significantly to the regression for *A. aegypti* ($P < 0.0001$, $r^2 = 0.391$, proportion fed = 0.188 + 0.042*(age) + 0.11*sine (9.91*age)). For both *Aedes* species

fed on the restrained chicken, the sine function did not contribute significantly to the regression (Table 2-2). Averaged over both species, slopes for proportion bloodfed on the membrane system were significantly greater, as shown by the age x feeding protocol interaction, than those for *Aedes* fed on the restrained chicken (Tables 2-1, 2-2).

Runs Up and Down Tests for *A. albopictus* and *A. aegypti* fed on restrained chickens showed that the number of runs was significantly different from random (both $P < 0.0275$) with the number of runs being greater than expected compared to random (Fig. 2-2). Thus, proportion bloodfed on chickens showed a significant pattern of alternate increases and decreases on alternate days of female mosquito age. Runs Up and Down Tests were not significant (both $P > 0.05$) for either *Aedes* species fed on the membrane system (Fig. 2-1).

Discussion

In Experiment 1, using the membrane feeders, there was a significant increase in proportion bloodfed as age increased (Table 2-2, Fig. 2-1). In Experiment 2, with restrained chickens, there was no significant increase in bloodfeeding associated with increased age (Table 2-2, Fig. 2-2). Further, the membrane-fed and chicken-fed mosquitoes showed significantly different trends (Table 2-2). Thus, the temporal pattern of bloodfeeding is strongly affected by the blood source used in experiments. Host-related cues (e.g., CO_2, surface area) may be partially responsible for the observed differences in pattern of bloodfeeding and should be taken into consideration in bloodfeeding research using *Aedes* mosquitoes of different ages, especially for silicon membrane systems. The lack of significantly positive slopes for chicken-fed mosquitoes is likely due to a higher proportion of bloodfed younger *Aedes* as compared to the membrane-fed mosquitoes.

Results showed significant age effects on bloodfeeding for both *A. aegypti* and *A. albopictus.* Davis (1984), in a study with naïve *A. aegypti* females of uniform ages ranging from 1 through 15 day old, showed a linear increase in host-seeking behavior for 1-5-day-old females, whereas females > 5 days old showed a consistently high (e.g., ≈ 94%) response to a human hand. Results from the current study suggest that bloodfeeding for these *Aedes*, over a similar period of time, shows some similarities to host-seeking response observed by Davis. However, proportions bloodfeeding appear additionally to exhibit distinct periodic patterns on alternate days of female mosquito age. Significant but weak positive relationships were found for *A. albopictus* and *A. aegypti* feeding versus age on the membrane system (Fig. 2-1, Table 2-2), and no positive relationships for feeding versus age on the restrained chicken (Fig. 2-2, Table 2-2). Additionally, slopes for the two *Aedes* species, as a single group, fed on the membrane system were significantly different from those fed on the restrained chicken (Table 2-2). Also, there appears to be an age-dependent periodic pattern in bloodfeeding incidences. The periodic pattern is most obvious among 3-13-day-old adults of each species fed on the restrained chicken, where the number of runs was significantly greater than that expected for random daily variation. Likewise, for the proportion of bloodfed *A. aegypti* on the membrane system versus age, a sine function made a significant contribution to the fit, providing further evidence for periodicity This result is surprising, because this was a short time series and typically, time series analyses have the potential to provide good fits when there are > 50 observations (Chatfield 1989). Unlike some previous research, *Aedes* in the current study were experimentally naïve (i.e., never given a previous bloodmeal), thus any periodicity in the time series is likely attributable to

endogenous factors. Periodicity could be an artifact of unknown exogenous factors, although most obvious factors were controled (e.g., temperature, humidity, feeding times). These results lend support to previous reports of a possible periodic pattern in probing behavior of non-bloodfed *A. aegypti* (Burgess 1959) and host-seeking behavior in non-bloodfed *Anopheles gambiae* sensu stricto (Takken et al. 1998). Hormone levels (e.g., juvenile hormone, ecdysteroids) vary at different times throughout the duration of adult female life. Juvenile hormone has been shown to be involved in initiating bloodfeeding for *Culex pipiens* (L.) and *C. quinquefasciatus* (Say) (Meola and Petralia 1980), and *C. nigripalpus* (Theobald) (Hancock and Foster 2000). The processes by which synergistic and antagonistic effects of juvenile hormone and ecdysteriods, from day to day, influence consumption of the initial bloodmeal, especially long after emergence (e.g., 15 days), is unknown. Given the lack of data on endogenous hormone fluctuation during the life span of unfed females, it is speculative to suggest that these hormones may contribute to the apparent age-dependent differences observed in proportion bloodfed of *A. aegyp*ti and *A. albopictus*.

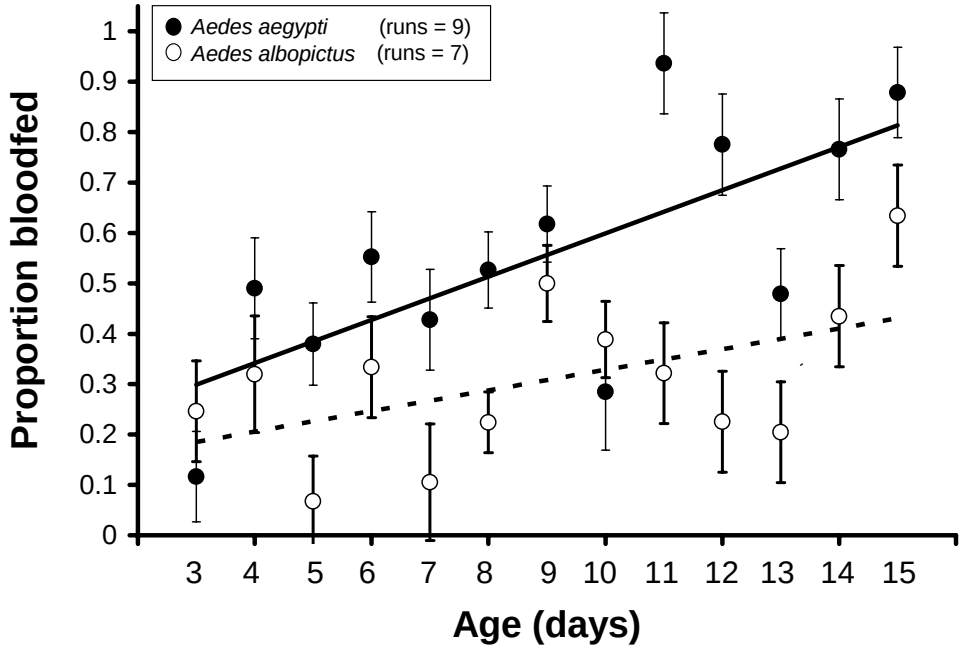

Figure 2-1. Least squares means (± SE) for proportion bloodfed females on the silicon-membrane system for 3-15 day old *Aedes albopictus* and *A. aegypti*. Line drawn through means shows the best-fit linear regression for *A. aegypti* (solid) and *A. albopictus* (broken).

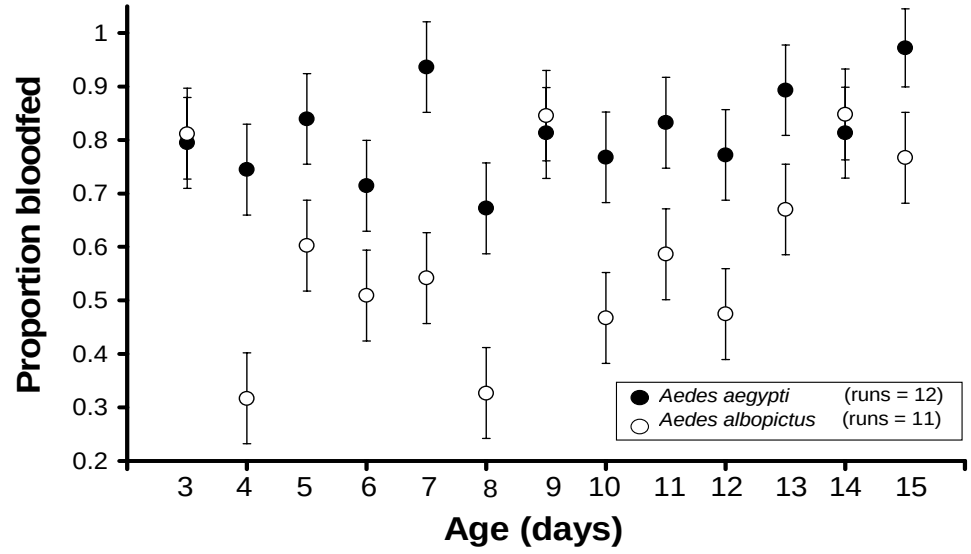

Figure 2-2. Least squares means (± SE) for proportion bloodfed females on restrained chickens for 3-15 day old *Aedes albopictus* and *A. aegypti*.

Table 2-1. Test for equal slopes among regressions of proportion bloodfed of *Aedes aegypti* and *A. albopictus* versus age

Source	df	Type III SS	MS	*F*	*P*
Age	1	1.3739	1.3739	30.69	< 0.0001
Feeding Protocol	1	1.4164	1.4164	31.64	< 0.0001
Species	1	0.1824	0.1824	4.07	0.0449
Feeding Protocol X Species	1	0.0697	0.0697	1.56	0.2136
Age X Feeding Protocol	1	0.2671	0.2671	5.97	0.0155
Age X Species	1	0.0412	0.0412	0.92	0.3384
Age X Feeding Protocol X Species	1	0.1094	0.1094	2.44	0.1197
Error df	198				

Table 2-2. Intercept and slope estimates for simple linear regressions of proportion bloodfed of *Aedes aegypti* and *A. albopictus* versus age. Slopes for groups followed by different letters are significantly different.

Source		Intercept ± SE	Slope ± SE		r^2	df	F	P
Membrane System	*A. aegypti*	0.1703 ± 0.0779	0.0434 ± 0.0082	a	0.3171	1, 61	28.33	< 0.0001
	A. albopictus	0.1084 ± 0.0778	0.0226 ± 0.0081		0.1110	1, 62	7.74	0.0071
Chicken Host	*A. aegypti*	0.7225 ± 0.0593	0.0103 ± 0.0059	b	0.0723	1, 38	2.96	0.0934
	A. albopictus	0.4600 ± 0.0879	0.0153 ± 0.0090		0.0720	1, 37	2.87	0.0987

CHAPTER 3
LARVAL COMPETITION DIFFERENTIALLY AFFECTS ARBOVIRUS INFECTION IN *Aedes* MOSQUITOES

Introduction

Biotic interactions among organisms play an important role in regulating population growth and in shaping communities. Among biotic interactions, competition has received a great deal of attention especially in the field of invasion biology where competitively superior invasive species displace or otherwise alter the distribution of established species (e.g., Juliano et al. 2004, Holway 1999, Petren et al. 1993). Although the most obvious effects of competition are reduced growth and survivorship, there are less obvious indirect effects mediated by competitively induced differences in life history traits (e.g., morphological or behavioral trait-mediated indirect effects; Abrams 1995).

Indirect effects describe interactions between two species mediated by a third species (e.g., exploitative competition, apparent competition, trophic cascades, indirect mutualism, interaction modifications) (Wootton 1994, Osenberg et al. 1992). Although different authors have applied multiple terms to similar types of indirect interactions (e.g., Morrison 1999), there is a consensus on classifying indirect effects as "density-mediated" or "trait-mediated" (Altwegg 2002). Density-mediated indirect effects occur when abundance of one species indirectly alter the abundance of another species through effects produced by altering the abundance of an intermediate species. Trait-mediated indirect effects occur when one species alters traits (e.g., behavioral, morphological) in a second species in ways that change the interaction between the second and third species.

The most frequently studied trait-mediated indirect effects involve predatory species that induce prey behavioral modification (e.g., reduced activity, increase use of refuges) that indirectly alter competitive interactions among those prey (Relyea 2000, Werner and Anholt 1996, Werner 1992, 1991).

Less attention has been given to indirect effects of competition among organisms with complex life cycles, where the impact of competition in one life stage has consequences for species interactions in subsequent stages (Altwegg 2002). Adult life-history traits of organisms with complex life cycles are, to a large extent, products of their larval environment. For example, effects of competition include reduced growth, development, and survivorship. Competitive-induced differences in adult life-history traits such as size may alter species interactions with enemies, including predators, pathogens, and parasites. Although nutrient limited conditions and physiological stress indirectly result in greater susceptibility to infection with pathogens or parasites in a single life stage (Kiesecker and Skelly 2001, Murray et al. 1998, Oppliger et al. 1998, Matson and Waring 1984), little is known about effects of competition in juvenile life history stages on susceptibility to infection in subsequent adult stages.

Water-filled containers are well suited to investigations of competitively induced indirect effects because they harbor simple communities subject to variable resource availability. Among the organisms occupying aquatic container communities, mosquitoes are the best studied because of the role of adults as vectors of pathogens. Resource availability and larval density in containers both influence mosquito survivorship, growth, and adult size (e.g., Juliano et al. 2004, Lounibos et al. 2002). Effects of competitive interactions among larval stages may carry over to the adult stage

and affect vector competence, which describes the ability to become infected and subsequently to transmit a pathogen after imbibing an infectious bloodmeal (Hardy 1988).

Biological transmission of arboviruses includes acquisition of the virus by the vector from an infectious bloodmeal, replication, dissemination of virus to the salivary glands, and transmission to a host by bite (Higgs 2004, Hardy 1988). Successful completion of this process requires that infection and dissemination barriers within the mosquito be overcome (Hardy 1988, Hardy et al. 1983). For example, if arboviruses fail to pass through the midgut, then infection is limited to the midgut cells and, although the mosquito is 'infected,' it cannot transmit virus (Hardy et al. 1983). Larval competition may have important consequences for adult arbovirus infection parameters. Typically, pupal and adult sizes of container breeding mosquitoes are positively related to the feeding rate experienced by larvae (e.g., Christophers 1960). Resource competition, and associated low food availability, among larvae of the treehole mosquito *Ochlerotatus triseriatus* produced smaller adults that transmitted La Crosse encephalitis virus (LACV) at higher rates than did larger adults from well-fed larvae. Infection rates were independent of adult body size (Grimstad and Walker 1991, Grimstad and Haramis 1984), although when *O. triseriatus* reared from field-collected pupae were orally infected with LACV, disseminated infection and transmission rates were negatively correlated with adult size (Paulson and Hawley 1991). In contrast, large *Aedes aegypti* adults produced under varying conditions of larval crowding and food availability disseminated dengue virus serotype 2 (DENV-2) more efficiently than did smaller females (Sumanochitrapon et al. 1998). Thus, it appears that ecological conditions

encountered by larvae can have variable effects on the interaction of mosquitoes with arboviruses. Investigations of competitive effects on pathogen transmission, other than size-related effects, remain rare.

The goal of our study was to determine the effects of larval competition on growth and survivorship of two well known container mosquito species, *A. albopictus* and *A. aegypti*, as well as their subsequent competence for arboviral infection and dissemination using Sindbis virus (SINV). SINV is a model *Alphavirus* that cycles between reservoir bird hosts and *Aedes* and *Culex* vector species (Seabaugh et al. 1998 and therein), and is widely used in experimental vector biology research (Olson et al. 1996, Dohm et al. 1995). *Aedes albopictus* is an invasive container breeding mosquito native to Asia which became established in large areas of the U.S., Europe, Africa, and South America during the last two decades (Lounibos 2002). In the southern U.S., the spread of *A. albopictus* coincided with reductions in range and abundance of the resident exotic *A. aegypti* in artificial containers (reviewed by Juliano et al. 2004). *Aedes albopictus* is an important vector of several arboviruses affecting humans and second only to *A. aegypti* in global importance as a vector of DENV (Lounibos 2002, Gubler and Kuno 1997). These species frequently encounter each other in artificial containers, in which interspecific competition has been well documented (Braks et al. 2004, Juliano et al. 2004, Barrera 1996b, Black et al. 1989, Ho et al. 1989), which probably explains displacements of *A. aegypti* by *A. albopictus* (Juliano 1998). This study tests whether variation in population growth parameters known to arise from intra- and interspecific competition (Juliano et al. 2004, Lounibos et al. 2002) have carryover effects in the adult stage, and are associated with variation in susceptibility to SINV infection dynamics.

Materials and Methods

Competition Study

Aedes albopictus Lake Charles strain (Nasci et al. 1989) and *A. aegypti* Rockefeller strain were used in the experiments. These mosquitoes were the progeny of genetically well-characterized strains. *Aedes albopictus* was obtained from a collection made at Lake Charles, Louisiana in 1987 and has been propagated under laboratory conditions since 1987. The *A. aegypti* Rockefeller strain was obtained from a long-standing colony at the University of Notre Dame. The competition experiment between *A. albopictus* and *A. aegypti* used 5-liter plastic containers filled with 4000 ml of tap water, 500 ml oak leaf infusion water (O'Meara et al. 1989), and 0.2 g of larval food (1:1, by weight, albumin: yeast). Three days after adding the initial contents to containers, a supplemental 500 ml oak infusion and 0.2 g larval food was added. Initial food resources were incubated for 5 d before the addition to each container of first instar (< 24 h old) mosquitoes. Ten days later, I removed 50% of the liquid contents, except larvae, and added 0.1 g larval food, 250 ml oak infusion water, and 2,250 ml tap water. Previous studies showed that this protocol provided sufficient resources for mosquitoes to complete development without negating the effects of larval competition (B.W. Alto, *unpublished data*). Competition treatments consisted of species/densities of *A. albopictus*: *A. aegypti* -- 160:0, 320:0, 160:160, 0:320, and 0:160. Ten replicates were used per treatment, for a total of 50 containers kept at 28 ± 1°C and 14:10 L:D regime. Containers were checked daily, and pupae transferred to sealed 20 ml vials with tap water until adult emergence. Emerged adults were kept, by species, in cylindrical cages (11 x 9.5 cm, ht. x diameter) and provided with 10% sucrose and an oviposition cup. The experiment was maintained until the last adult had emerged.

Measurements of population growth correlates were used to estimate the effect of competition on female *A. albopictus* and *A. aegypti* population growth. Mean female size (wing length) and mean time to emergence were calculated for each replicate. Female survivorship per replicate was calculated as (number of adult females) / (total number of original larvae) of a given species. An estimated finite rate of increase (λ') was also calculated for each replicate container.

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

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

A. aegypti (Briegel 1990)*:*

$f(w_x) = 2.50(w_x^3) - 8.616$

$r^2 = 0.875$, $N = 206$, and $P<0.001$

A. albopictus (Lounibos et al. 2002)*:*

$f(w_x) = 78.02\ (w_x) - 121.24$

$r^2 = 0.713$, $N = 91$, and $P<0.001$

In both cases w_x = wing length in mm. Effects of *A. albopictus* and *A. aegypti* competition were analyzed by individual Multivariate Analyses of Variance (MANOVA) to determine competitive treatment effects on the population growth correlates time to emergence, survivorship to emergence, and adult size. Raw data adequately met assumptions of univariate normality and homogeneous variances for all correlates used in the MANOVAs. For all analyses, significant effects were further analyzed by contrasts of pairs of main effect multivariate means with a sequential Bonferroni adjustment for experimentwise α=0.05. Standardized canonical coefficients (SCC) were used to determine the relative contribution of each of the response variables to significant multivariate effects as well as their relationship to each other (e.g., positive or negative) (Scheiner 2001). Competitive effects on *A. albopictus* and *A. aegypti* λ' were analyzed using one-way ANOVAs with treatment as a categorical variable (SAS Institute 1989). Significant effects were further analyzed by pairwise comparisons of main effect means (Ryan-Einot-Gabriel-Welsch test, SAS Institute 1989).

Infection Study

For each replicate from the competition study, newly emerged females and males were housed, by species, in cages (11 x 9.5 cm, ht. x diameter) and provided 10% sucrose and an oviposition cup. This arrangement facilitated mating and oviposition and enabled

the delivery of infectious bloodmeals to multiple females of approximately the same age. Because larval competition increases developmental time, adults from the competition containers emerged over several weeks. Therefore, multiple cages were used to house adults for each replicate to ensure that the females given an infectious blood meal were of similar ages (4-10 d old). SINV infection rates do not differ over the age range of 4-10 d for these *Aedes* species (Dohm et al. 1995). Thus, tests for the effects of larval competition on subsequent adult infection were performed on the same individual mosquitoes. Adults were housed in cages within an incubator at 26 ± 1°C and 14:10 L:D photoperiod. Adult females of each species were deprived of sucrose but not water for 24 h, then allowed to bloodfeed for 30 min. on a citrated bovine blood-SINV mixture maintained at 37 ± 1°C in a silicon membrane system (Butler et al. 1984). SINV (MRE-16 strain) titers used in bloodfeeding trials were $10^{5.3}$ tissue culture dose required to infect 50% of wells ($TCID_{50}$) (Reed and Muench 1938). $TCID_{50}$ is the quantity of virus that is required to infect 50% of the tissue cultures, so that viral titers (= number of virus particles / ml) can be determined. Viral titer refers to the amount of virus in solution. Virus titers were similar to those produced in wild bird reservoirs in nature (Ockelbo virus, a closely related strain of Sindbis virus (Lundstrom et al. 1993)). Titers were determined by 10-fold serial dilutions in 96-well plates seeded with 6.0 x 10^5 Vero cells / ml (10 wells per dilution). $TCID_{50}$ was determined by cytopathic effects after a 7 d incubation (Reed and Muench 1938). Vero cells infected with SINV virus exhibit stereotypical cytopathic effects, so infection was unambiguous. To avoid the possibility of reductions in titer with repeated thawing and freezing, all blood meals had virus derived from single stock placed in 1.5ml aliquots that were frozen (-80°C) and thawed

only once. The infection study was conducted in a biosafety level-2 facility appropriate for SINV at the Florida Medical Entomology Laboratory in Vero Beach, Florida.

Females that failed to take a blood meal during the first trial were given a second trial 18 h later. After the second feeding attempt, unfed females were removed from the cages, and bloodfed females were held for a 16 d extrinsic incubation period (EIP). The time from initial ingestion of the infectious blood meal until the time the mosquito can transmit the arbovirus is the EIP. Females surviving the EIP were killed and individually stored at -80°C and, subsequently, their wings were removed (to be measured as an indicator of female size). Bodies and legs were ground into a powder separately in 1 ml diluent (Leibovitz L-15 media, 5% fetal bovine serum, and gentamicin), centrifuged at 21000 m/s^2 for 12 min. at 4°C, and filtered (0.22μm). Proportion females infected, body titer (log_{10} $TCID_{50}$), and proportion of infected females with disseminated infection (i.e., with positive infected legs) were determined using 10-fold serial dilutions in triplicate wells of 96-well plates seeded with Vero cells by $TCID_{50}$.

Infection was determined using a 1/10 dilution of the body stock solution, and body titer was determined using a full range of dilutions. When describing infection of mosquitoes, "negative" describes the absence of a viral infection, and "positive" describes a mosquito with a viral infection in the midgut, and perhaps other organs. An infection limited to the midgut is called an "isolated infection," whereas an infection spread beyond the midgut, infecting secondary target organs (e.g., salivary glands, head, legs), is called a "disseminated infection." Disseminated infection is a recognized indicator of a mosquito's ability to transmit virus via biting (Gubler and Kuno 1997). So, dissemination of infection in positive females was determined by assaying undiluted leg

stock solution (Turell et al. 1984). In this study, isolated infections refer to mosquitoes with positively infected bodies, but absence of infection in legs, whereas disseminated infections refer to positively infected bodies and legs. Assaying salivary glands may be a more direct indicator of a mosquito's ability to transmit virus. However, extraction of the salivary glands may result in contamination with surrounding tissue. Thus, assays of mosquito legs were used in order to avoid this contamination problem and still obtain a good indication of ability to transmit (Turell et al. 1984).

Prior to analyzing effects of competitive treatment on arboviral infection, interspecific differences in susceptibility were analyzed using MANOVA and SCC on the response variables proportion infected, body titer, and proportion with disseminated infection. Next, individual MANOVAs for *A. albopictus* and *A. aegypti* were used to determine the effect of larval competition on response variables: proportion infected, body titer, and proportion with disseminated infection as described above. Multivariate contrasts with sequential Bonferroni adjustment for experimentwise α=0.05 (Rice 1989, Scheiner 2001) were used to compare high density treatments (320:0, 160:160) vs. the low density treatment (160:0), and then to compare the two high density treatments.

For *A. albopictus* and *A. aegypti*, effects of mean female size on body titer were tested by treating size as a covariate in an analysis of covariance (ANCOVA) with competitive treatment and competition x size interactions. Significant effects were further analyzed by all possible pairwise comparisons of treatment means (sequential Bonferroni adjustment; Rice 1989). Effects of mean female size on body titer were expected to be most pronounced in females with disseminated infections, and this analysis was the primary interest.

Product-moment correlation coefficients ($r_{1,2}$) were used to describe the relationship between population growth measurements (time to emergence, survivorship, size, λ′) and infection parameters: proportion infected, body titer of females with isolated infection, body titer of females with disseminated infection, and proportion with disseminated infection among competitive treatments of *A. albopictus* and *A. aegypti.* These analyses allowed for a test of the strength of positive or negative relationships among population growth measurements and infection.

Results

Competition Study

For both *A. albopictus* and *A. aegypti,* competitive treatments significantly affected population growth measurements (Table 3-1), with uncrowded larval conditions consistently resulting in shorter time to emergence, greater survivorship, and greater adult size compared to crowded conditions (Figs. 3-1, 3-2). For *A. albopictus*, SCC showed that differences in adult size followed by survivorship to emergence contributed the most to the significant competition effect as well as to subsequent treatment differences (Table 3-1). Although time to emergence was shorter at uncrowded larval conditions, it contributed less than the other population growth measurements (*A. albopictus* time to emergence ± SE d; 160:0, 13.58 ± 0.23, 320:0, 15.62 ± 0.23, 160:160, 15.93 ± 0.24) (Table 3-1). For *A. aegypti*, SCC showed that differences in survivorship to emergence followed by time to emergence contributed the most to the significant competition effect as well as pairwise-differences (Table 3-1). Size contributed far less to the significant competition effect (*A. aegypti* mean wing length ± SE mm; 0:160, 2.65 ± 0.05, 0:320, 2.39 ± 0.05, 160:160, 2.47 ± 0.07) (Table 3-1). For both species, competitive treatments significantly affected λ′ ($F_{2, 26} = 191.84$, $P < 0.0001$; $F_{2, 19} = 51.94$, $P < 0.0001$; *A.*

albopictus and *A. aegypti*, respectively) and λ′ was significantly greater in the pattern: 160 larvae > 320 larvae > 160+160 larvae (Fig. 3-3). Thus, inter- and intraspecific competition had major population-level effects.

Infection Study

Prior to analyzing effects of competition on arboviral infection, interspecific differences in susceptibility were first examined. Proportions infected, whole body titer, and proportions with disseminated infection were significantly different between *A. albopictus* and *A. aegypti* [Pillai's trace (3, 37) = 0.76, $P < 0.0001$]. Proportion infected (SCC = 1.23) was the most important variable in the overall interspecific difference, followed by proportion with disseminated infection (SCC = -0.66) and whole body titer (SCC = -0.57). The opposite signs of the SCC showed that there was a negative relationship between the variables across the species, so that *A. albopictus* had a greater proportion of infected individuals, a lower body titer, and a lower proportion disseminated infection compared to *A. aegypti* (LS means ± SE for *A. albopictus* and *A. aegypti* proportion infected, 0.94 ± 0.03 and 0.58 ± 0.04; body titer, 4.08 ± 0.14 and 5.58 ± 0.22 $TCID_{50}$; and proportion with disseminated infection, 0.67 ± 0.03 and 1.00 ± 0, respectively).

Interspecific competition had significant effects on proportion infected, whole body titer, and proportion of *A. albopictus* with disseminated infection [Pillai's trace (6,24) = 0.52, $P = 0.025$]. Proportion infected (SCC = 1.12) made the greatest contribution to the multivariate differences among treatments, and body titer (SCC = 0.23) and proportion with disseminated infection (SCC = - 0.06) contributed less. *Aedes albopictus* at low density alone (160/container) had a significantly lower proportion infected, lower

proportion with disseminated infection, and lower body titer compared to high density treatments [Pillai's trace (3,25) = 0.38, $P = 0.011$] (Fig. 3-4, A and B). Proportion infected was the major contributor to this effect (SCC = 1.14), whereas titer (SCC = 0.27) and proportion with disseminated infection (SCC = -0.08) contributed little. The two high density treatments did not differ significantly [Pillai's trace (3,24) = 0.14, $P = 0.319$] (Fig. 3-4, A and B). For the infection study, mortality during the extrinsic incubation period resulted in few *A. aegypti* females from the 160:160 treatment. Therefore, only means for intraspecific density treatments are reported for *A. aegypti*. There were no significant effects of competition on infection parameters for *A. aegypti* [Pillai's trace (2,11) = 0.23, $P = 0.301$]. Mean ± SE females assayed per treatment replicate were; 160:0 (10.0 ± 1.84), 320:0 (11.89 ± 1.15), 160:160 (albo) (6.44 ± 0.63), 0:320 (6.11 ± 1.12), and 0:160 (5.20 ± 0.92).

Females with disseminated infections are capable of transmitting virus and therefore are of epidemiologic significance. For these females, an analysis of covariance with mean female size as a covariate showed significant effects of size and competition on whole body viral titer for *A. albopictus* with disseminated infections, but no significant size x competition interaction (Table 3-2). Thus, effects of mean body size and of competition are independent. Estimated slopes were positive, indicating that within a competitive treatment body titer increased with size for mosquitoes with disseminated infection (Fig. 3-5). Pairwise comparisons of adjusted means among treatments showed that significant differences in body titer followed the pattern 160:160 > 320:0 > 160:0 (mean ± SE: 5.80 ± 0.23, 5.13 ± 0.22, 3.23 ± 0.32 $TCID_{50}$, respectively).

For *A. aegypti* with disseminated infections, there were no significant competitive treatment or covariate effects (Table 3-2).

Product-moment correlations showed significant relationships between infection and all correlates of population growth for *A. albopictus* (Table 3-3). In particular, increased time to emergence, a result of intra- and interspecific competition, was positively correlated with infection rate for *A. albopictus*, but survivorship, size, and λ' were negatively correlated with infection rate (Table 3-3). Also, survivorship and λ' were significantly negatively correlated with mean *A. albopictus* body titer for females with disseminated infections. All correlations between population growth parameters of *A. aegypti* and infection parameters were non-significant (Table 3-3).

Discussion

The two experiments in this study were designed to quantify the effects of intra- and interspecific larval competition, and then to determine whether competitive effects carried over into the adult stage and influenced competence for arbovirus infection. For both *Aedes* species in the competition experiment, all population growth measurements clearly showed that higher larval densities resulted in poorer performance (Figs. 3-1, 3-2, 3-3). Analyses of survivorship, time to emergence, and size at emergence suggested that the effects of intra- and interspecific competition were similar. However, for both *Aedes* species, a synthesis of multiple growth measurements (λ') showed that interspecific competition was more intense than intraspecific competition (Fig. 3-3).

A variety of model systems have shown that the outcome of interspecific competition depends on resource type (e.g., Sanders and Gordon 2003, Tilman 1982). Contrasting outcomes have been obtained with these two *Aedes* species, *A. aegypti*

having the competitive advantage over *A. albopictus* with nutritious larval food (e.g., liver powder, yeast), but not with low-nutrient, more natural resources (e.g., leaf litter) (Braks et al. 2004, Juliano 1998, Barrera 1996b, Black et al. 1989). The current experiment used a combination of natural (leaf infusion) and supplemental (albumin, yeast) resources and, for both *Aedes* species, interspecific competition was greater than intraspecific competition as measured by λ'. The intention in designing the competition experiment was not to mimic natural resources, rather to use a resource base known to maximize the production of *Aedes* females for the infection study, without negating the effects of competition. These objectives were met since competitive interactions were detected and sufficient numbers of adults were obtained for the infection study.

Although the experimental design was constrained to maximize adult production without negating competition, mosquito densities and sizes conformed to observations from field conditions. In the current experiment, densities were 0.032 and 0.064 larvae/ml for the 160 and 320 larvae treatments, respectively. Sampling of the entire contents of water-holding golf cart tires in Broward, Indian River, and Monroe counties in Florida (Dec. 1996 or Jan. 1997 – April 1998) showed that larval densities were within the range observed in tires occupied by *A. albopictus*, *A. aegypti*, or both species (N=790, mean ± SE, 0.17 ± 0.02, range 0.00083 - 3.08 larvae/ml) (G. F. O'Meara, unpublished). Also, *A. albopictus* adult female wing lengths (Fig. 3-1) were within the range of *A. albopictus* collected at tire sites in East St. Louis, USA (N = 180, mean ± SE, 2.43 ± 0.02 mm, range 1.84 - 2.95) (B.W. Alto and S.A. Juliano, unpublished). Similarly, both *A. albopictus* and *A. aegypti* female wing lengths in the current study were within the range of field-collected females of these species from tire sites in southwestern Louisiana

(N=150, mean ± SE 2.68 ± 0.02 mm, range 2.04 - 3.12; N=115, 2.64 ± 0.03, range 1.92 - 3.12, respectively) (Nasci 1990). Wing length, as a surrogate of adult size, is a good indicator of larval environmental conditions (e.g., food resources, larval density) (Juliano 1998, references therein). Thus, the experimental set-up produced adult females that parallel those sizes found in nature.

The infection component revealed that larval competition altered adult mosquito susceptibility to arboviral infection and potential for virus transmission. In particular, competitively stressed *A. albopictus* females were more likely to become infected and have higher SINV titers and dissemination than females reared with less competition. Results are consistent with other model systems where competition, in the form of nutrient-limitation or stressors, enhanced susceptibility to infection with pathogens or parasites (Kiesecker and Skelly 2001, Murray et al. 1998, Oppliger et al. 1998, Matson and Waring 1984). In the current study, infection rate was the variable most sensitive to the impact of larval competition. Intra- and interspecific competition altered subsequent *A. albopictus* interactions with SINV, suggesting that biotic interactions in early developmental stages may be important in determining adult arboviral infection parameters among mosquitoes. This type of indirect effect may be viewed as an interaction modification since "a change in density of one species alters the nature of a direct interaction between two other species" (Wootton 1993). On the other hand, effects of competition on *A. aegypti* infection parameters were not observed, and reasons for differences between the two *Aedes* species in responses to competitive treatments are unknown. Although there was less statistical power in the *A. aegypti* tests due to lower sample sizes, biological explanations could include species-specific qualitative

differences in the availability of midgut receptor sites used by SINV or escape barriers (e.g., midgut escape barrier) that may be differentially affected by competition. These results suggest species-specific differences in how larval competition affects adult competence for arboviral infection parameters. Similarly, in plant communities, studies have demonstrated species-specific responses to indirect effects (e.g., indirect facilitation), most likely attributable to differences among species in life history traits (e.g., Pages et al. 2003, Levine 1999).

The correlation coefficients demonstrating that infection rates were significantly associated with all correlates of population growth (Table 3-3) represent the first evidence that life history traits, in addition to adult size, change parameters associated with vector competence. Furthermore, correlations between life-history traits and infection parameters in *A. albopictus* showed that negative effects of competition on population growth are associated with enhanced vector potential (Table 3-3). The observation that larger *A. albopictus* females with disseminated infections had significantly greater body titer can be explained as simply a size phenomenon, i.e., more tissue is available for virus propagation. For body titer, there were independent effects of both mean adult size and competition. The lack of a significant size x competition interaction showed that the effect of size on body titer was similar among competitive treatments (Fig. 3-5). More importantly, density-dependent differences in body titer were found, with greater mean body titer among *A. albopictus* reared under competitive conditions. Significant density-dependent differences in body titer were identical to significant density-dependent differences in λ', except in the opposite direction (Figs. 3-3, 3-5). Specifically, more intense competition as measured by a lower λ' resulted in

greater body titer. These results demonstrate both size-dependent and size-independent effects of competition on infection dynamics that have opposite effects across competition treatments. Within a competitive treatment, larger mosquitoes have greater body titer, but between competitive treatments larger mosquitoes from low-competition larval rearing conditions have lower body titer and a lower proportion infected, compared to smaller mosquitoes emerging from high-competition conditions. Overall, the results demonstrate that competitive stress experienced by *A. albopictus* larval stages carried over to the adult stage and significantly influenced susceptibility to infection and dissemination.

Over and above the effects of competition, the two *Aedes* species differed in susceptibility to infection and dissemination. Other studies have shown interspecific differences for quantitative aspects of infection (Turell et al. 2001, Gubler et al. 1979). However, previous research using these *Aedes* species, as well as other mosquito species, did not quantify the variables most important for interspecific differences (e.g., infection, body titer, dissemination) or the positive and negative interrelationships among these variables across species (i.e., see SCC). *A. albopictus* was significantly more susceptible to infection than was *A. aegypti*, as seen in other studies (Turell et al. 2001, Gubler et al. 1979). Conversely, although *A. aegypti* had lower infection rates, those females that were infected had significantly higher body titer and dissemination rates compared to infected *A. albopictus*. Infection contributed approximately twice as much as body titer and dissemination to interspecific differences. Factors limiting body titer and dissemination (e.g., midgut escape barrier) were less efficient (or not expressed) in *A. aegypti* compared to *A. albopictus* under these conditions. These results suggest fundamental differences in

physiology between these *Aedes* species that alter their susceptibility to arboviral infection and dissemination, and these differences are likely to have important epidemiological consequences.

If effects of competition on vector infection with SINV apply to arboviruses such as DENV and West Nile virus, these results may have important implications for human health. The current study suggests that competition experienced by larval *A. albopictus* may enhance the threat posed by this species in pathogen transmission. Uncrowded larval rearing at low densities, used in most laboratory studies of vector competence, do not accurately reflect conditions in nature where competition is often strong and widespread (Juliano et al. 2004). The current study suggests that indirect effects are important in determining mosquito vector ability, and that the effect may be species specific. Failure to consider larval stresses may result in misleading estimates of relative susceptibility to infection for *A. albopictus* and *A. aegypti*, and by extension, other arboviral vectors. This report is the first to quantify how larval competition may affect arbovirus infection in adult mosquitoes, and demonstrates the species-specificity of the process from infection to dissemination. Future assessments of vector potential should consider the species-specific effects of larval conditions that reflect competitive conditions observed in nature.

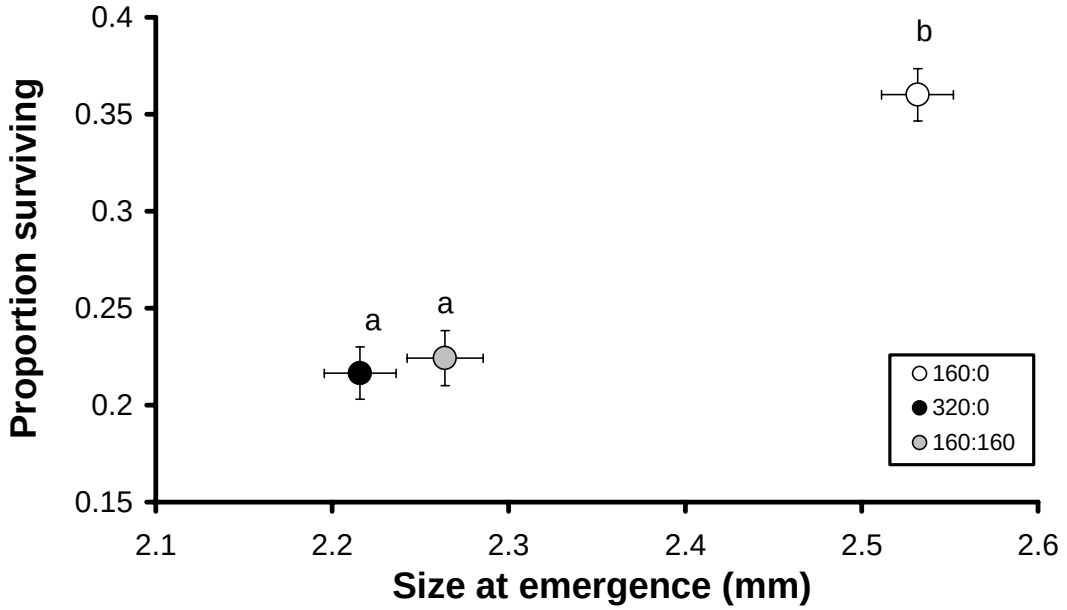

Figure 3-1. *Aedes albopictus* least squares means (±SE) for female survivorship and size at emergence. Different lowercase letters indicate significant differences between bivariate means. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti*—160:0, 320:0, and 160:160.

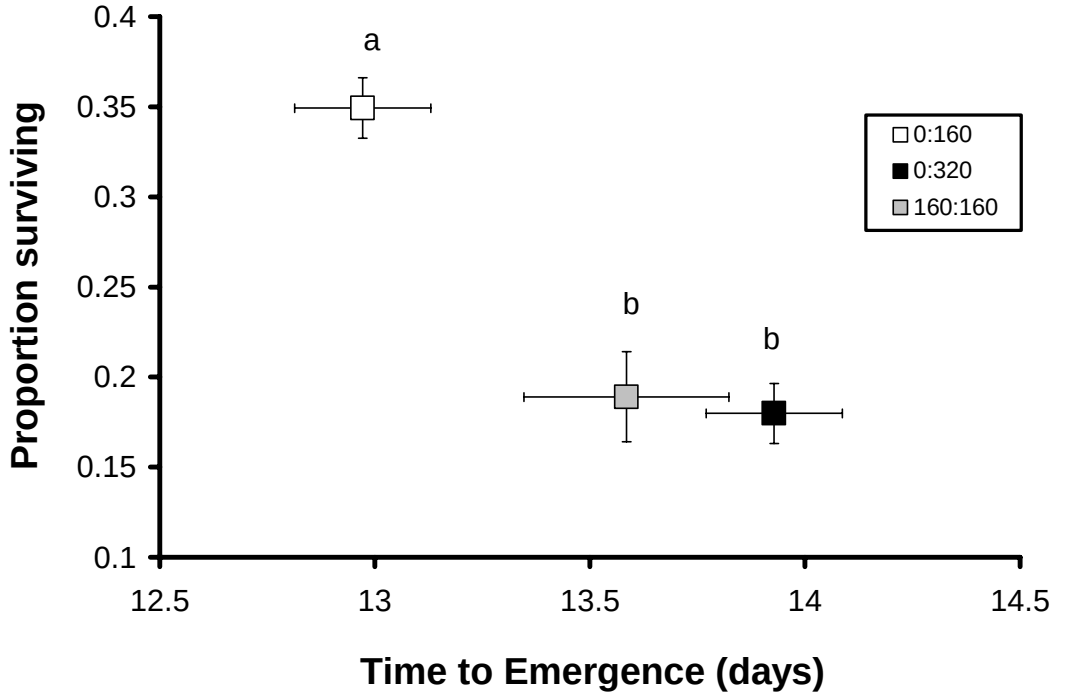

Figure 3-2. *Aedes aegypti* least squares means (±SE) for female survivorship and time to emergence. Different lowercase letters indicate significant differences between bivariate means. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti*—0:160, 0:320, and 160:160.

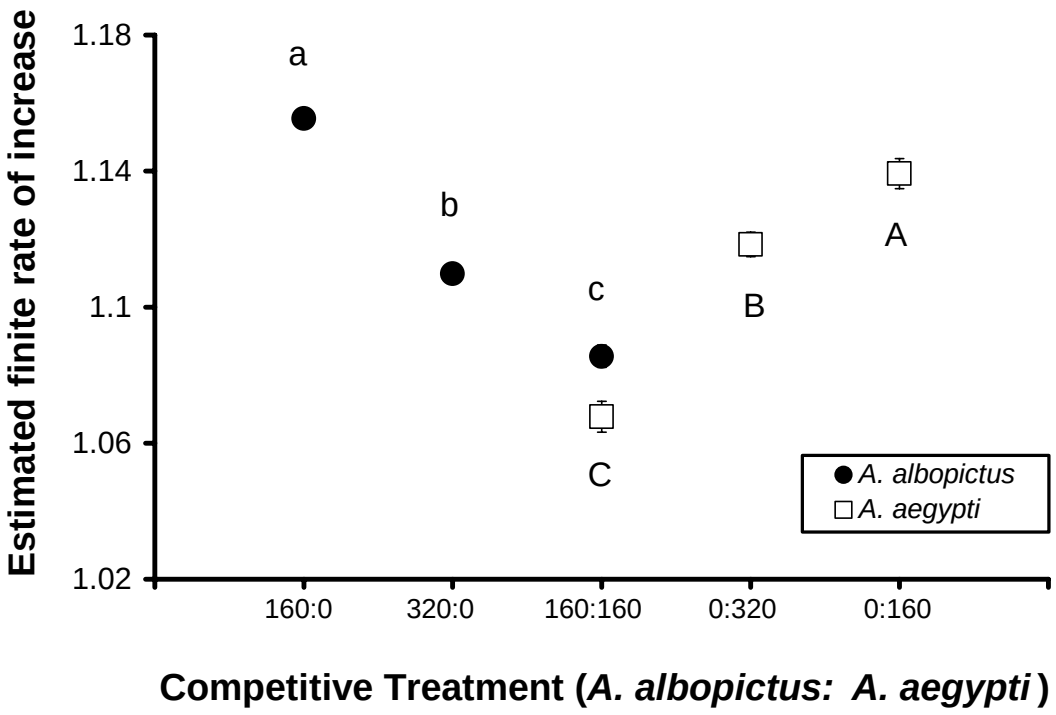

Figure 3-3. Least squares means (± SE) for estimated finite rate of increase, λ′, for *Aedes albopictus* and *A. aegypti*. Points without bars have standard errors too small to appear on the graph. Different lowercase and uppercase letters indicate significant differences between means for *A. albopictus* and *A. aegypti*, respectively.

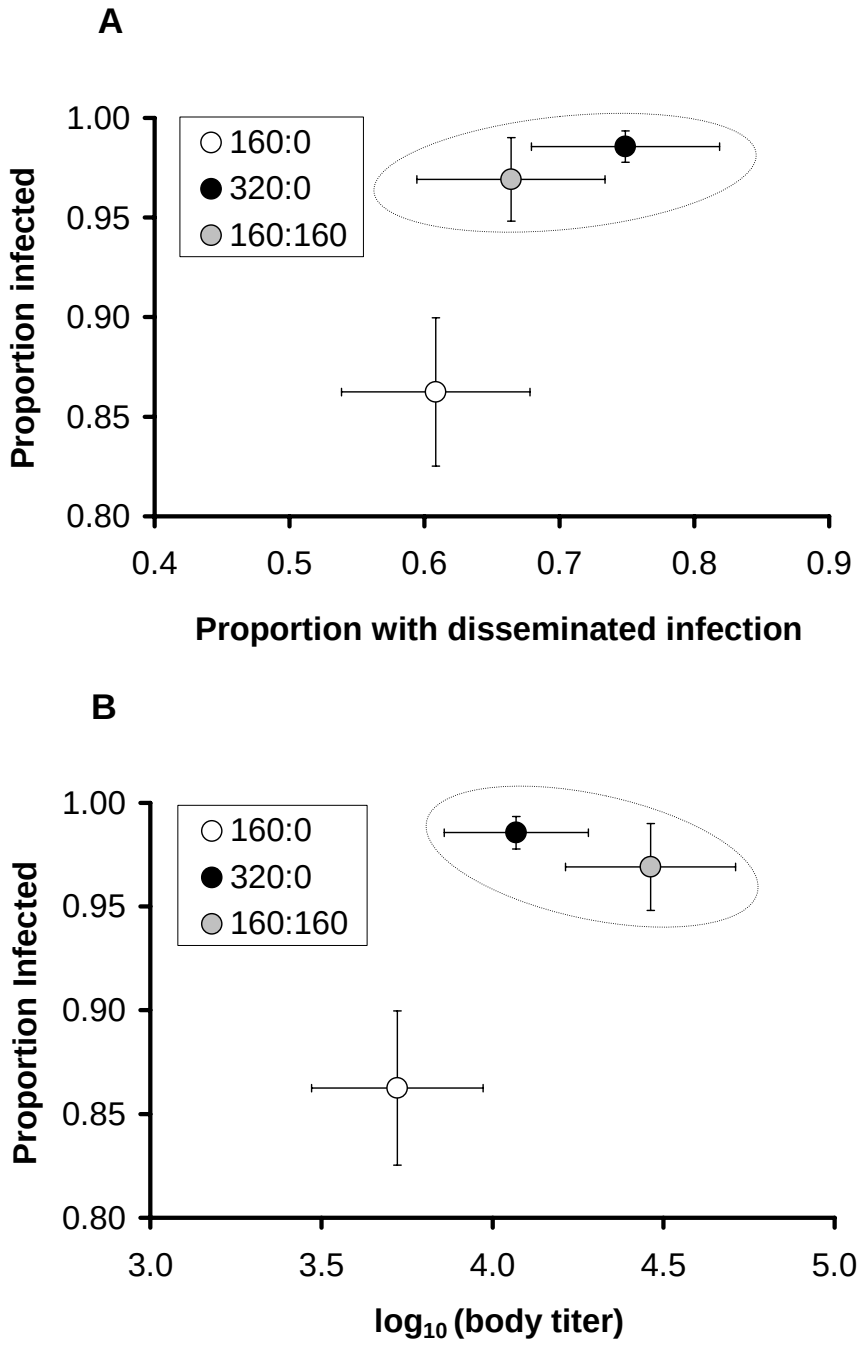

Figure 3-4. Bivariate plots of least squares means (± SE) for three dependent variables for *Aedes albopictus* females fed on a Sindbis virus blood meal. (A) Proportion of infected females vs. proportion with disseminated infection. (B) Proportion of infected females vs. body titer. In both graphs, the dashed ellipse indicates multivariate means that are not significantly different. Numbers in the figure key represent the ratio of *A. albopictus* to *A. aegypti.*

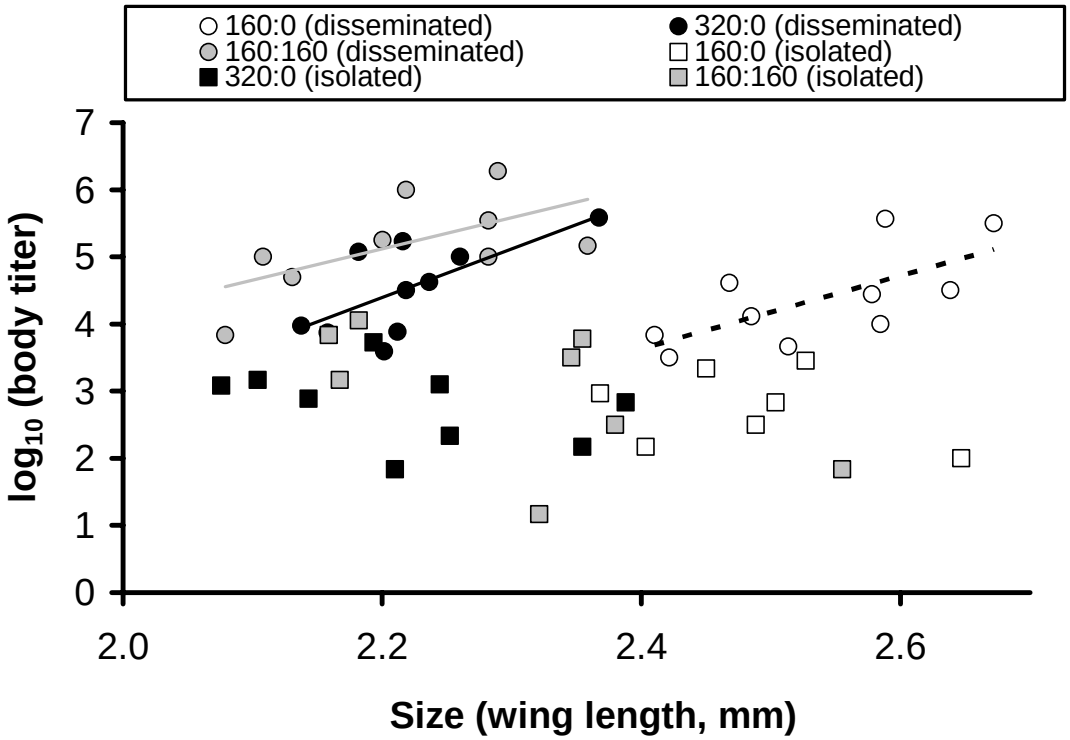

Figure 3-5. Least squares means for body titer and size of adult *Aedes albopictus* females with disseminated (i.e., infection spread beyond the midgut, infecting secondary target organs such as body, legs) and isolated (i.e., infection limited to the midgut) Sindbis virus infections. The size effect on females with disseminated infections gives a slope of 5.48 (SE = 1.28). Solid and dased lines drawn through bivariate means show the best fit for *A. albopictus* with disseminated infections in three competitive treatment conditions. Numbers in the figure key represent the ratio of *A. albopictus* to *A. aegypti*.

Table 3-1. Multivariate ANOVA for main effects and multivariate pairwise contrasts of competitive treatment effects on female *Aedes albopictus* and *A. aegypti* population growth measurements: time to emergence, survivorship to emergence, and adult size.

Comparison	df	Pillai's trace	P	Standardized Canonical Coefficients		
				Time	Surv.	Size
A. albopictus						
Competitive treatment	6	1.02	< 0.0001	- 0.79	1.19	1.97
160,0 vs. 320,0	3	0.90	< 0.0001	- 0.88	1.18	1.89
160,0 vs. 160,160	3	0.91	< 0.0001	- 0.73	1.19	2.02
320,0 vs. 160,160	3	0.13	0.3274			
Error df	26					
A. aegypti						
Competitive treatment	6	0.93	0.0006	- 1.11	2.01	0.27
0,160 vs. 0,320	3	0.88	< 0.0001	- 1.12	1.99	0.28
0,160 vs. 160,160	3	0.76	< 0.0001	- 1.05	2.09	0.20
0,320 vs. 160,160	3	0.14	0.4472			
Error df	19					

Table 3-2. ANCOVA for the effects of competitive treatment and size covariate on body titer for *Aedes albopictus* and *A. aegypti* females with disseminated infections.

Source	df	F	P
A. albopictus, disseminated			
Size	1	18.38	0.0002
Competitive treatment	2	15.20	< 0.0001
Size x competition	2	0.26	0.7750
Error df	25		
A. aegypti, disseminated			
Size	1	1.74	0.2170
Competitive treatment	2	0.74	0.5036
Size x competition	2	2.62	0.1336
Error df	11		

Table 3-3. Product moment correlation coefficients ($r_{1,2}$) for the relationship between population growth measurements (time to emergence, survivorship, size, and λ′) and infection parameters. Infection parameters include infection, body titer of females with isolated infection, and dissemination for *Aedes albopictus* (df=25) and *A. aegypti* (df=12). An infection limited to the midgut is called an "isolated infection," whereas an infection spread beyond the midgut, infecting secondary target organs (e.g., salivary glands, head, legs), is called a "disseminated infection." Asterisks denote significant correlation coefficients (* $P < 0.05$; ** $P < 0.001$). No $r_{1,2}$ values are reported for *A. aegypti* dissemination and body titer (isolated) since all infected individuals had disseminated infections.

Infection parameters and growth parameters	*A. albopictus*	*A. aegypti*
Infection		
Time to Emergence	0.50 **	- 0.08
Survivorship	- 0.50 **	- 0.07
Size	- 0.64 **	0.17
λ′	- 0.55 **	0.10
Body Titer (disseminated)		
Time to Emergence	0.26	- 0.24
Survivorship	- 0.38 *	0.38
Size	- 0.00098	0.13
λ′	- 0.40 *	0.19
Dissemination		
Time to Emergence	- 0.04	.
Survivorship	- 0.22	.
Size	- 0.20	.
λ′	- 0.13	.

CHAPTER 4
LARVAL COMPETITION AND SUSCEPTIBILITY OF *Aedes aegypti* AND *Aedes albopictus* TO INFECTION BY DENGUE VIRUS

Introduction

Dengue virus (DENV) is an arthropod borne (arbo) virus. There are four different serotypes of DENV that are the cause of morbidity and mortality throughout much of the tropical world. Approximately 50-100 million cases of dengue fever (DF) occur annually with hundreds of thousand of cases of dengue hemorrhagic fever (DHF), a life-threatening form of dengue. In Southeast Asia, range expansion of the primary vector *Aedes aegypti* (L.) and human migration have contributed to hyperendemicity (co-circulation of multiple serotypes in a single location) and associated epidemic DF and DHF (Gubler 2002). Regions with hyperendemic DENV are expanding, and recent introductions of Southeast Asian genotypes of DENV to the Western Hemisphere pose an increased risk of transmission in the tropical Americas resulting in greater number of cases of severe DHF (Cologna et al. 2005, Cologna and Rico-Hesse 2003, Rico-Hesse et al. 1997, Lewis et al. 1993).

The yellow fever mosquito *A. aegypti* and Asian tiger mosquito *A. albopictus* (Skuse) are considered the primary and secondary vectors of DENV, respectively (Rodhain and Rosen 1997). However, the relative importance of these *Aedes* species in DENV transmission in nature is difficult to determine, especially in regions where they coexist. *Aedes aegypti* and *A. albopictus* have sympatric and allopatric breeding sites in Southeast Asia as well as in many locations in the Western Hemisphere where dengue is

endemic and a serious health risk to humans. Geographic strains of both these *Aedes* species vary in their susceptibility to DENV infection (e.g., Bennett et al. 2002, Failloux et al. 2002, Vazeille-Falcoz et al. 1999, Boromisa et al. 1987, Gubler et al. 1979, Gubler and Rosen 1976). Additionally, mosquito infection parameters may be altered by different serotypes and strains of DENV (e.g., Moncayo et al. 2004, Armstrong and Rico-Hesse 2003, 2001, Rosen et al. 1985, Whitehead et al. 1971). For example, Southeast Asian DENV-2 strains, which are more virulent than American genotypes, consistently show significantly greater disseminated infection in *A. aegypti* compared to American DENV-2 strains (Cologna et al. 2005, Armstrong and Rico-Hesse 2003, 2001), but no comparable data exist for *A. albopictus*. Contrasting outcomes of dengue infection have been obtained for these two *Aedes* species in laboratory and field studies. Studies using well-established laboratory mosquito strains, suggested that DENV (serotypes 1, 2, 3, 4) were less efficient at infecting and causing disseminated infections in *A. aegypti* compared to other *Aedes* species, including *A. albopictus* (Rodhain and Rosen 1997, Rosen et al. 1985, Gubler et al. 1979). Similarly, a greater proportion of *A. albopictus* had disseminated DENV-2 virus infection compared to sylvatic *A. aegypti formosus* (Vazeille et al. 2001). In contrast laboratory experiments showed that a greater proportion of *A. aegypti* (F_1-F_2 generation) had disseminated DENV-2 infections compared to *A. albopictus*, although the proportion of *A. albopictus* with disseminated infections increased with subsequent laboratory generations (Vazeille et al. 2003). In summary, research to date is equivocal whether *A. albopictus* or *A. aegypti* is the superior vector. Rather, it is likely that the genetic background of both the virus and mosquito

species play important roles in determining vector competence for dengue (Tabachnick 1994).

The establishment of *A. albopictus* in new regions, especially where *A. aegypti* is absent, also spreads the risk for DENV transmission since human movement and transport may place the reservoir, virus, and vector in close proximity to one another. In the southern U.S., the introduction and spread of *A. albopic*tus was associated with declines in resident *A. aegypti* (Juliano et al. 2004, O'Meara et al. 1995, Mekuria and Hyatt 1995, Hornby et al. 1994, Hobbs et al. 1991). The two *Aedes* species occupy similar container habitats, and interspecific competition among the larval stages is well-documented and a likely contributor to the observed decline of *A. aegypti* (e.g., Costanza et al. 2005a, Braks et al. 2004, Juliano et al. 2004, Lounibos et al. 2002, Barrera 1996). Competition, due to resource limitation or high larval density, has been demonstrated for many mosquito species and is usually reflected by an increase in larval development time and mortality, and decrease in adult size (e.g., Alto et al. 2005ab, Juliano and Lounibos 2005, Peck and Walton 2005, Braks et al. 2004, Juliano et al. 2004, Ye-Ebiyo et al. 2003, Gimnig et al. 2002, Lounibos et al. 2002, Schneider et al. 2000, Teng and Apperson 2000, Léonard and Juliano 1995, Broadie and Bradshaw 1991).

The effects of larval competition likely impact the adult stage, and therefore may also influence adult susceptibility to pathogens (e.g., arboviruses), including DENV (Vazeille et al. 2003, Black et al. 2002, Sumanochitrapon et al. 1998). However, this field of investigation has not been well explored. Small *O. triseriatus* adults derived from competitive larval environments under laboratory conditions or from field collections had similar La Crosse virus (LACV) infection rates compared to large adults,

but greater dissemination and transmission (Grimstad and Walker 1991, Paulson and Hawley 1991, Grimstad and Haramis 1984). Large *A. aegypti* adults, reared with abundant resources and low larval density, had greater incidence of disseminated DENV-2 viral infection compared to smaller adults (Sumanochitrapon et al. 1998). Explicit examination of the effects of intra- and interspecific competition between *A. albopictus* and *A. aegypti* on vector competence has only been examined in a model using SINV (Alto et al. 2005a). These experiments demonstrated that competition resulted in increased development time to emergence and decreased survivorship, size, and a performance index (λ′) for both species, and enhanced *A. albopictus* SINV infection parameters, but not *A. aegypti* parameters. Specifically, competitively stressed *A. albopictus* had greater infection, disseminated infection, and body titer than unstressed individuals, and proportion infected contributed the most to these significant effects (Alto et al. 2005a). While the results of this previous work were compelling, *A. aegypti* and *A. albopictus* are not vectors of SINV in nature. Therefore, the artificial nature of the virus-vector association limits applying these results more generally. To examine the generality of patterns observed in this previous work, and to use a virus of primary importance to human health, I conducted an experiment to test whether competition among larvae affects DENV infection parameters, and whether population growth measurements are associated with DENV infection parameters, for *A. aegypti* and *A. albopictus*. For this study, infection parameters refer to proportion of females infected with DENV, proportion of females with disseminated infection, and DENV body titer.

Materials and Methods

Competition Study

Mosquitoes used the experiment were the same continuously propagated colony strains described elsewhere (Alto et al. 2005a). *Aedes albopictus* Lake Charles strain and *A. aegypti* Rockefeller strain represent long-standing laboratory colonies (Nasci et al. 1989, Craig and Vandehey 1962). The current experimental setup was similar to that used to investigate the affects of larval competition on adult *Aedes* infection with SINV (Alto et al. 2005a). A similar experimental design was desirable because it facilitated comparison between experiments to evaluate whether competition has similar affects on *Aedes* infection parameters for arboviruses in two different families (i.e., SINV in Togaviridae, DENV in Flaviviridae).

Larval food resources used in the competition experiment were identical to those described by Alto et al. (2005a), except that supplemental resources were added on day 13 instead of day 10. Briefly, larval rearing vessels consisted of 5-L plastic containers filled with 4000 ml tap water, 500 ml oak leaf infusion water (O'Meara et al. 1989), and 0.2 g larval food (1:1 albumin: yeast). Food resources were allowed to incubate for 5 d before newly hatched (<24 h old) mosquitoes were added to experimental containers. Three days after adding the larvae, a supplemental 500 ml oak infusion and 0.2 g larval food were added. Thirteen days later, 50% of the liquid was removed, except larvae, and 0.1 g larval food, 250 ml oak leaf infusion water, and 2250 ml tap water were added. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti* (i.e.., 160:0, 320:0, 160:160, 0:320, and 0:160). Ten replicates were used for each treatment, except for 320:0 and 0:320, which had 11 replicates. Containers were maintained under constant environmental conditions (28°C ± 1°C, 14:10 L:D

photoperiod). Pupae were removed from containers daily and stored in 20-ml water-filled vials until adult emergence. Larval rearing in the competition experiment lasted until all pupae emerged as adults or larvae had died.

Measurements on individuals and cohorts were used to evaluate competitive treatment effects on *A. albopictus* and *A. aegypti* population growth. Mean female size (wing length in mm) and mean time to adult emergence (days) were determined for each treatment replicate. Wing length was measured as the distance from the axillary incision to the distal point on the lateral margin of the wing, excluding the wing fringe. Female survivorship per replicate was calculated as (number of adult females) / (total number of original larvae) of a given species. Estimated finite rate of increase (λ′) was calculated for each replicate:

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

where λ′ is a composite index of performance based on a transformation of r′ (Juliano 1998, Livdahl 1984, 1982, Livdahl and Sugihara 1984). N_o is the initial number of females in a cohort (assumed to be 50 %), A_x is the number of females emerging on day x, and w_x is mean female size on day x. D is the time (in days) from emergence to oviposition. For *A. albopictus* and *A. aegypti,* D is assumed to be 14 and 12 d, respectively (Livdahl and Willey 1991, Juliano 1998). Mean female size per day, used to calculate λ′, was obtained from all females assayed for viral infection as well as all unfed

females obtained from the entire duration of the experiment. Number of eggs produced by a female was estimated from female size based on a regression function $f(w_x)$. The following fecundity-size relationships ($f(wx)$) were used to calculate λ':

A. aegypti (Briegel 1990)*:*

$f(w_x) = 2.50(w_x^3) - 8.616$

$r^2 = 0.875$, $N = 206$, and $P<0.001$

A. albopictus (Lounibos et al. 2002)*:*

$f(w_x) = 78.02\ (w_x) - 121.24$

$r^2 = 0.713$, $N = 91$, and $P<0.001$

In both cases w_x is wing length in millimeters. Population growth measurements (time to emergence, size of females assayed for infection, survivorship) were analyzed, separately for *A. albopictus* and *A. aegypti*, by Multivariate Analyses of Variance (MANOVA) to quantify the effect of competition. Thus, MANOVA used sizes of females assayed for DENV infection and λ' was calculated based on sizes of females assayed for infection as well as all unfed females obtained over the duration of the experiment. Significant effects were further analyzed by all possible contrasts of pairs of main effect multivariate means using the sequential Bonferroni method (experimentwise $\alpha = 0.05$). Standardized canonical coefficients (SCC) were used to describe the relative contribution of each population growth measurement to significant multivariate effects as well as their relationship to each other (e.g., positive or negative: SAS Institute 2002, Scheiner 2001). Competitive treatment effects on *A. albopictus* and *A. aegypti* λ' were analyzed by separate one-way ANOVA, and significant effects were further analyzed by

pairwise comparisons of main effect means (Ryan-Einot-Gabriel-Welsch test, SAS Institute 2002). Raw data adequately met assumptions of univariate normality and homogeneous variances for all population growth measurements in MANOVA and ANOVA, except *A. albopictus* development time, which showed departure from normality. No common transformations improved normality, however, MANOVA, using Pillai's trace, is robust to departures from normality (Scheiner 2001). Also, the highly significant treatment effects and similar direction of competitive effects suggest that the departure from normality had little effect in determining the results.

Infection Study

Viral propagation

A Southeast Asian genotype of DENV-2 was originally isolated from a patient in Thailand in 1974. This virus isolate had been passed once in the mosquito *Toxorhynchites amboinensis* (Doleschall), 3 times in Vero cells, twice in *A. albopictus* C6/36 cells, and 3 additional passages in Vero cells (S. Fernandez, pers. comm.). Subsequently, the DENV stock was passed twice in Vero cells. T-75 cm^2 flasks with confluent monolayers of Vero cells were individually inoculated with 3 ml media (Leibovitz L-15 media, 10% fetal bovine serum (FBS), 50 µg/ml gentamicin) containing 200µl DENV-2 stock. T-75 cm^2 flasks were rocked for 1 h at 37ºC, to allow for adsorption, after which media were added to bring the total volume to 10 ml and incubated at 35ºC. Media in T-75 cm^2 flasks was renewed on days 4 and 8 and harvested for infectious bloodmeals on day 11. Freshly recovered media-virus suspension (i.e., unfrozen) from day 11 was used as the source virus for use in infectious bloodmeals offered to mosquitoes (Miller et al. 1982). Infectious bloodmeals using previously frozen

(-80°C) DENV-2 stock showed significantly lower infection and dissemination in both *Aedes* species compared to fresh grown virus, even at similar titers (*unpublished data*), in agreement with observations for other arboviruses, including DENV-2 (Turell 1988, Miller 1987, Miller et al. 1982). All procedures involving DENV-2 were performed in a biosafety level-3 facility.

Oral infection of mosquitoes

Adult mosquitoes from the larval competition experiment were housed by treatment replicate and species in wax-coated cardboard containers (14 cm high x 11 cm diameter) and provided with an oviposition cup, 20% sucrose, and water. Sucrose and water were renewed every 48 h. Sucrose, but not water, was removed from mosquitoes 24 h prior to blood feeding. Infectious blood meals were offered to 4-7 d old females using a silicon membrane feeder system (Alto et al. 2005a, 2003, Butler et al. 1984). Citrated bovine blood was combined with DENV-2 stock in a 4 to 1 ratio, respectively to provide a blood meal titer of 6.2 $\log_{10}$ PFU/0.2 ml. Membrane feeders with infectious blood were heated at 37ºC in an incubator for 20 min. and offered to *Aedes* females for 30 min. An aliquot of infectious blood was immediately frozen at -80ºC and later tested by plaque assay to determine the titer of blood meals offered to *Aedes* females. Next, mosquitoes were cold anesthetized and fully bloodfed females were identified, using a stereo microscope within a glove box, isolated, and held for a 12 d extrinsic incubation period at 28°C ± 1°C and provided with sucrose, water and a 14:10 h light:dark photoperiod regime. Sucrose and water were renewed every 48 h. Mosquitoes that survived the extrinsic incubation period were individually stored in vials at -80ºC and, subsequently, their wings were removed and measured as an indicator of female size (see above).

Interspecific (*A. aegypti* versus *A. albopictus*) differences in DENV-2 susceptibility to infection were evaluated by MANOVA and SCC on the response variables proportion infected, and proportion with disseminated infection. A one-way ANOVA tested for interspecific differences in body titer of *Aedes* females with disseminated DENV-2 infections. Next, individual one-way MANOVAs and SCC, for each *Aedes* species, were used to determine competitive treatment effects on proportion infected, and proportion with disseminated infection. Significant effects were further analyzed by all possible pairwise contrasts of pairs of bivariate means using the sequential Bonferroni method (experimentwise $\alpha = 0.05$). Raw data adequately met assumptions of univariate normality and homogeneous variances for analyses, except for proportion *A. albopictus* infected, which showed departure from normality. No common transformations, including arcsine square root, improved normality. The sensitivity of departure from normality was assessed by analyzing the proportion *A. albopictus* infection using a Kruskal-Wallis nonparametric test which is a weaker test but does not assume normality. Results of the nonparametric test gave the same conclusions as parametric analyses, thus I am confident that effects on proportion *A. albopictus* infected are not artifacts produced through departure in normality. Further, MANOVA, using Pillai's trace, is robust to departures in normality (Scheiner 2001).

The effects of mean female size on proportion infected, proportion with disseminated infection, and body titer of females with disseminated infection were assessed by treating size as a covariate in an analysis of covariance (ANCOVA), with competition treatment and competition x size interactions as categorical variables (SAS Institute 2002). ANCOVAs involving body titer used size based on wing length

measurements of females with disseminated DENV-2 infections. ANCOVAs involving proportion infected and proportion with disseminated infection used size based on wing length measurements of all females assayed for DENV-2 infection. Initially all ANCOVAs tested for equality of slopes for each size by competitive treatment (i.e., each competitive treatment has its own slope estimate). ANCOVAs determined to have common slopes (i.e., no significant size x competitive treatment interaction) were re-tested for the equality of the intercepts. ANCOVAs determined to have similar intercepts were re-tested as ANOVAs with competitive treatment and no size covariate. Significant effects were further analyzed by all possible pairwise comparisons of treatment means (Tukey-Kramer adjustment of experimentwise $\alpha = 0.05$, SAS Institute 2002). Raw data adequately met assumptions of univariate normality, homogeneous variances, and linearity, however, the proportion *A. albopictus* infected with DENV showed some departure from normality. No common transformations, including arcsine square root, improved normality.

Product-moment correlation coefficients ($r_{1,2}$) were used to describe the relationship between population growth measurements (time to emergence, survivorship, size, λ') and infection parameters: proportion infected, proportion with disseminated infection, and body titer of females with disseminated infection among competitive treatments of the two *Aedes* species. Thus, these analyses pool all competitive treatments. The correlation analyses of body titer and size were based on sizes of females with disseminated DENV-2 infections. Correlation coefficients quantify the strength of the relationship (positive or negative) between population growth measurements and infection parameters.

Blood meal plaque assay

Titrations of DENV-2 infectious blood meals were performed by plaque assays in duplicate 6-well plates of confluent monolayers of Vero cells maintained with Leibovitz L-15 media, 10% FBS, and 50 μg/ml gentamicin. 10-fold serial dilutions of infectious blood meal samples were made by combining a 0.2 ml DENV-2 blood meal sample with 1.8 ml media (2X Eagle's Minimum Essential Medium (EMEM) containing Earle's Basic Salt Solution, 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin), thus creating a 10^{-1} dilution. This process was repeated to yield a full range of dilutions from 10^{-1} to 10^{-9}. At the time of viral inoculation, media covering cell monolayers in the wells was removed and wells were individually inoculated with 0.2 ml of the serial dilutions. Six-well plates were gently rocked for 1 h incubation at 35ºC and a 5% CO_2 atmosphere.

Following incubation, the first overlay of agarose was applied to the cell monolayer. The first and second overlays of agarose described here provided sufficient reagents to complete ten 6-well plate plaque assays. Briefly, 1.8 g Seaplaque low melting agarose (FMC Biotechnology) was added to 100 ml of double distilled water. The solution was heated until completely melted and then cooled to 40ºC. In a separate flask, 10 ml FBS was combined with 2 ml non-essential amino acid solution, 100U/ml penicillin and 100 μg/ml streptomycin. In another separate flask, 1 ml of L-glutamine and 250 μg/ml of Amphotericin B were added to 100 ml of 2X EMEM. Next, the EMEM mixture was added to the agarose followed by the FBS mixture. Each well received 3 ml of the first overlay of reagents. Six-well plates remained motionless for 5 min. to allow for the agarose to gel and then well-plate covers were removed for 15 min.

to facilitate drying. Finally, well plate covers were replaced and the 6-well plates were incubated for 6 d at 35ºC and a 5% CO_2 atmosphere.

The second overlay of agarose was applied on the 6th day of incubation. Briefly, 1.8 g Seaplaque low melting agarose was combined with 2.0 g sodium chloride and 200 ml double distilled water. The solution was heated until completely melted and then cooled to 40ºC. Next, 9 ml neutral red solution (Sigma Cat: N2889) was added to the solution and each well received 3 ml of the second overlay reagents. Six-well plates were treated similar as the first overlay except that plates were incubated for 24 h at 35ºC and 5% CO_2 atmosphere. Plaques were counted and expressed in plaque forming units (PFU) per 0.2 ml of test inoculum.

Mosquito homogenization, plaque assay, and RNA extraction

For each mosquito, wings and legs were separated from bodies using forceps sterilized with 70% ethanol followed by intense flaming (Turell et al. 1984). Wings were measured and used as an indicator of female size for λ´ calculations. Bodies were assayed to determine infection and whole body viral titer, whereas legs were assayed as an indicator of disseminated infection (Turell et al. 1984). Bodies and legs were homogenized separately in 2 ml flat bottom vials containing 1 ml media (Leibovitz L-15 media, 10% FBS, 100 U/ml of penicillin, 100 μg/ml streptomycin, and 250 μg/ml Amphotericin B) and 2 zinc plated steel BBs (Daisy®). Homogenization was performed by placing vials into a TissueLyser (Qiagen) for 6 min. at 25 Hz followed by centrifugation at 3148 x g for 4 min. and 4ºC. Body infection and disseminated infection were determined by plaque assays using undiluted body and leg stock solutions. Plaque assays were performed similarly to assays of blood meal titer, except that 12-well plates

were used instead of 6-well plates and additional antibiotics were added. A single well was inoculated for each body and leg stock solution of each tested female. Only females determined to have disseminated DENV-2 infections (i.e., positive infection in legs) were subsequently assayed for body titer. Plaque assays for female bodies and legs were scored as positive or negative with no attempt to count number of plaques. Subsequently, body homogenates of females with disseminated infections were thawed and DENV-2 RNA was extracted from 140 µl of the sample using QIAamp viral RNA Mini Kits (Qiagen) and then assayed by quantitative real-time (RT)-PCR (Armstrong and Rico-Hesse 2003, 2001).

Quantitative RT-PCR

Quantitative RT-PCR allowed for determination of the relative amounts of virus in the body (viral titer), as measured by cDNA amplification, standardized with a plaque assay (Richardson et al. 2006, Bustin 2000). A commercially available quantitative RT-PCR kit, SuperScript™ III Platinum® one-step quantitative RT-PCR system (Invitrogen™), and fluorogenic probe hydrolysis (TaqMan®) technology was used to detect DENV-2 RNA. DENV-2 virus specific primers targeted the capsid gene (Forward 237-251 bp, Reverse 305-284 bp) (Callahan et al. 2001). Primer sequences were: Forward (5′-CAT GGC CCT KGT GGC G- 3′) and Reverse (5′-CCC CAT CTY TTC AGT ATC CCT G-3′) (Callahan et al. 2001). The DENV-2 specific dual-labeled fluorogenic oligonucleotide probe included a 5′-reporter dye and a 3′-quencher dye (250 nm DLB 5′ 6-FAM / 3′ BHQ-2 (5′-TCC TTC GTT TCC TAA CAA TCC-3′) (Callahan et al. 2001).

Reactions used a thermostable enzyme, *Taq* DNA polymerase, derived from a bacterium *Thermus aquaticus* (Holland et al. 1991). The oligonucleotide probe anneals to the target RNA sequence downstream from a primer site. Under these conditions, the reporter and quencher dyes are in close proximity so the quencher inhibits fluorescence emission. The 5′ nuclease activity of *Taq* DNA polymerase cleaves the probe during extension so the reporter and quencher dyes separate and results in a detectable fluorescence signal which is recorded by the lightcycler. PCR products are detected by the generation of a fluorescent signal, and the intensity of the signal is directly related to product accumulation. Regular cycling of temperature allows for denaturation, annealing, and extension steps that are repeated resulting in exponential growth of the target amplicon (DENV-2 cDNA).

Each reaction included: 0.4 µl SuperScript™ III RT/Platimum® *Taq* mix, 10 µl 2X reaction mix (a buffer system, MgSO4, dNTPs and stabilizers), 1 µl forward primer (10µM), 1 µl reverse primer (10µM), 0.5 µl fluorogenic probe (10µM), 4.2 µl DEPC treated H_2O, and 2 µl test sample (positive or negative for DENV-2 RNA). Reactions were performed in glass capillary tubes in a thermocycler, LightCycler 2.0 Instrument equipped with LightCycler software version 3.5 (Roche Molecular Biochemicals). The thermal cycle included: RT, 30 min. at 48ºC; Denaturing, 2 min. at 95ºC; followed by 45 cycles of PCR, 15 s at 95ºC, 1 min. at 60ºC. Each lightcyler set of reactions included a negative control (water) and positive control standard (DENV-2 stock RNA, 10^{-5} dilution). The positive control was an indicator of cDNA synthesis and served as a known standard of DENV-2 RNA used to produce cDNA. Plaque forming units (PFU) were calculated by a standard curve method that compared cDNA synthesis to *in vitro*

PFU for the same full range of positive DENV-2 RNA stock virus titrated in parallel by quantitative RT-PCR and plaque assay (Richardson et al. 2006, Bustin 2000). Quantitative RT-PCR estimates crossing points which are cycle numbers that correspond to the point at which exponential growth of the target amplicon occurs. Positive and dilute test samples have high crossing points, since there was little initial RNA, whereas concentrated test samples have low crossing points since there were greater amounts of initial RNA. A standard curve was generated by assaying a full range of 10-fold serial dilutions of DENV-2 stock (7.2 $\log_{10}$ PFU/0.2 ml) by plaque assay (see blood meal plaque assay) to quantify PFU, as well as by quantitative RT-PCR testing DENV-2 cDNA synthesis. Three replicates were used for each dilution assayed by quantitative RT-PCR (slope = -3.007, intercept = 34.54, $r^2 = 0.9604$). Plaque assays determined that 2.2 $\log_{10}$ PFU/0.2 ml corresponded to the 10^{-5} dilution. These estimates were used to transform crossing points to PFU in determining body titer for *Aedes* females with disseminated DENV-2 infections.

Species by Competition Comparison

An additional set of analyses were used as alternative methods to address mosquito species by competitive treatment effects. The intention of the design of the competition experiment should be primarily thought of as two experiments; One experiment for intra- and interspecific competition for *A. albopictus* (160:0, 320:0, and 160:160) and another experiment for intra- and interspecific competition for *A. aegypti* (0:160, 0:320, and 160:160). However, it is possible to analyze subsets of the experimental treatments to further isolate species and competition effects on population growth measurements and infection parameters. Population growth measurements (time to emergence, size of females assayed for infection, survivorship) were analyzed by a two-way MANOVA and

SCC with mosquito species (*A. albopictus* and *A. aegypti*) and competitive treatment (160:0, 320:0, 0:160, and 0:320) as factors. Treatments involving both species present (e.g., 160:160) were intentionly omitted, thus isolating treatment effects. Similarly, infection parameters (proportion infected, proportion with disseminated infection) were analyzed by a two-way MANOVA and SCC with mosquito species and competitive treatment as factors. Species and competitive treatment effects on body titer were analyzed using a two-way ANOVA and significant effects were further analyzed by all possible pairwise comparisons of treatment means (Tukey-Kramer adjustment of experimentwise $\alpha = 0.05$, SAS Institute 2002). Additionally, separate one-paired t-tests were used to address species effects on proportion infected, proportion with disseminated infection, and body titer in the interspecific competitive treatment (160:160).

Results

Competition Study

For both *A. albopictus* and *A. aegypti*, competitive treatments significantly affected population growth measurements (Table 4-1) in the pattern 160 larvae < 320 larvae = 160:160 larvae (Figs. 4-1, 4-2). Greater competition consistently resulted in significantly smaller adult size, longer time to emergence, and lower survivorship (*A. albopictus* mean ± SE proportion surviving; 160:0, 0.42 ± 0.03, 320:0, 0.27 ± 0.03, 160:160, 0.32 ± 0.03; *A. aegypti* mean ± SE proportion surviving; 0:160, 0.36 ± 0.02, 0:320, 0.31 ± 0.01, 160:160, 0.33 ± 0.02) than all less intense competitive treatments (Figs. 4-1, 4-2). For both *Aedes*, SCC showed that differences in adult size and time to emergence contributed the most to the significant competition effect as well as to subsequent treatment differences (Table 4-1). For both species, competitive treatments significantly affected λ' (*A. albopictus*, $F_{2, 28} = 90.44$, $P < 0.0001$; *A. aegypti*, $F_{2, 28} = 150.84$, $P < 0.0001$) and

λ' was significantly greater in the pattern: 160 larvae > 320 larvae > 160+160 larvae (Fig. 4-3).

Infection Study

The infection study produced 2508 mosquitoes that successfully completed the extrinsic incubation period. Six infectious blood meals were given over the course of the experiment and plaque assays showed some variation between the blood meal viral titer used in the experiment (mean ± SE; 6.47 ± 0.098 $\log_{10}$ PFU/ 0.2ml, 6.2-6.8 $\log_{10}$ PFU/0.2 ml range). It was desirable to compare competitive treatments for females exposed to identical blood meal titers. Logistic constraints precluded determining whether different bloodmeal viral titers produced differences in mosquito infection parameters. Further, different numbers of females were available for each bloodfeeding trial with many treatments having few females available to bloodfeed. This presented a problem since it prevented comparisons of all competitive treatments within a given bloodfeeding trial. Thus, females were compared using those that completed the extrinsic incubation period from the first bloodfeeding (6.2 $\log_{10}$ PFU/0.2ml) which represented ca. 50 % of the total number of females available to assay. Assaying less than all available females for DENV-2 infection assumes that females from the first bloodfeeding are representative of all females from the competition experiment. Similar outcomes of competition-induced changes in infection parameters from a previous study support this assumption (Alto et al. 2005a). Mean ± SE females assayed per treatment replicate were; 160:0 (16.56 ± 1.41), 320:0 (12.5 ± 1.60), 160:160 (*A. albopictus*) (8.29 ± 2.07), 160:160 (*A. aegypti*) (17.90 ± 0.98), 0:320 (19.54 ± 0.45), and 0:160 (20.00 ± 0).

Interspecific differences (i.e., *A. albopictus* versus *A. aegypti*) in susceptibility to infection with DENV-2 were compared and proportions infected and proportions with disseminated infection were significantly different between the two *Aedes* species (Pillai's trace $_{2, 56} = 0.58$, $P < 0.0001$). Proportion with disseminated infection (SCC = 1.19) contributed more to the overall interspecific difference than proportion infected (SCC = -0.88). The opposite signs of the SCCs showed a negative relationship between infection and dissemination, from the fact that *A. albopictus* had a greater proportion of infected females but a lower proportion of disseminated infections compared to *A. aegypti* (Fig. 4-4). Body titer was significantly different between species ($F_{1, 47} = 85.26$, $P < 0.0001$) and *A. albopictus* (mean ± SE, 4.6 ± 0.06 $\log_{10}$ PFU/0.2 ml) had greater body titer compared to *A. aegypti* (mean ± SE, 3.9 ± 0.04 $\log_{10}$ PFU/0.2 ml).

Competition had significant effects on the proportion infected and proportion with disseminated infections for *A. albopictus* (Table 4-2). Proportion infected provided the largest contribution to bivariate differences among treatments and proportion with disseminated infection contributed less (Table 4-2). *Aedes albopictus* at low density had significantly lower proportion infected and disseminated infection compared to high density intra- and interspecific competition (Table 4-2, Fig. 4-5). The two high density treatments did not differ significantly (Table 4-2, Fig. 4-5). Competitive treatments resulted in similar trends of infection and dissemination for *A. aegypti*, however, there were no significant effects (Table 4-2, Fig. 4-6).

Separate analyses of covariance with mean female size as a covariate and mean body titer, proportion infected, and proportion with disseminated infection showed no significant competitive treatment or covariate effects on body titer for *A. albopictus*

(Table 4-3, Fig. 4-7). There were marginally significant effects of size x competition interaction, as shown by regression lines for the three competitive treatments, and significant competition effects for proportion of *A. albopictus* infected (Table 4-3, Fig. 4-8). Pairwise comparisons of LS means among competitive treatments showed a significantly greater proportion of *A. albopictus* infected at both high density intra- and interspecific competitive treatments than low density treatment (LS means shown in Fig. 4-5). Proportions infected were not significantly different between high density intra- and interspecific competitive treatments (LS means shown in Fig. 4-5). There was no significant size effect for proportion of *A. albopictus* infected (Table 4-3, Fig. 4-8). There were marginally significant effects of competition on *A. albopictus* proportion with disseminated infection (Table 4-3). Pairwise comparisons of LS means among competitive treatments showed marginally significant differences ($P = 0.0501$) in proportion of *A. albopictus* with disseminated infection between low density versus high density interspecific competition with no other significant effects (LS means shown in Fig. 4-5). There was no significant size effect for proportion of *A. albopictus* with disseminated infection (Table 4-3, Fig. 4-9). For *A. aegypti*, there were no significant competitive treatment or covariate effects (Table 4-3, Figs. 4-10, 4-11, 4-12).

Product-moment correlations showed significant relationships between the proportions of *A. albopictus* infected and with disseminated DENV-2 infections and time to emergence and size (Table 4-4). Time to emergence, positively associated with intra- and interspecific competition, was positively correlated with the proportion infected and disseminated infection whereas size was negatively correlated with the proportion infected and disseminated infection (Table 4-4).

Species by Competition Comparison

A two-way MANOVA showed significant species (*A. albopictus* and *A. aegypti*) and competitive treatment (160:0, 320:0, 0:160, 0:320) effects on population growth measurements, but no species x competitive treatment interaction (Table 4-5). SCC showed that differences in adult size and time to emergence contributed the most to the significant species and competitive treatment effects (Table 4-5). *Aedes aegypti* had significantly shorter time to emergence (mean ± SE d; 12.81 ± 0.29 and 13.74 ± 0.29 for *A. aegypti* and *A. albopictus*, respectively) and larger body size (mean ± SE mm; 2.83 ± 0.02 and 2.74 ± 0.02 for *A. aegypti* and *A. albopictus*, respectively) than *A. albopictus*. Crowded larval conditions (320 larvae) resulted in smaller size (mean ± SE mm; 2.65 ± 0.02 and 2.92 ± 0.02 for 320 and 160 larvae, respectively) and longer time to emergence (mean ± SE d; 15.49 ± 0.29 and 11.07 ± 0.30 for 320 and 160 larvae, respectively).

A two-way MANOVA showed significant species (*A. albopictus* and *A. aegypti*) and competitive treatment (160:0, 320:0, 0:160, 0:320) effects on proportion infected and proportion with disseminated infection, but no species x competitive treatment interaction (Table 4-6). SCC showed that differences in the proportion of females with disseminated infections contributed the most to the significant species effect, whereas differences in the proportion infected contributed the most the significant competitive treatment effect (Table 4-6). *Aedes aegypti* had lower infection rates (mean ± SE proportion infected; 0.75 ± 0.02 and 0.88 ± 0.02 for *A. aegypti* and *A. albopictus*, respectively) but higher dissemination rates (mean ± SE proportion with disseminated infection; 0.60 ± 0.03 and 0.32 ± 0.03 for *A. aegypti* and *A. albopictus*, respectively) than *A. albopictus.* Crowded larval conditions (320 larvae) resulted in higher infection rates (mean ± SE proportion infection; 0.88 ± 0.02 and 0.76 ± 0.03 for 320 and 160 larvae, respectively) and

dissemination rates (mean ± SE proportion with disseminated infection; 0.50 ± 0.03 and 0.42 ± 0.03 for 320 and 160 larvae, respectively) than uncrowded conditions (160 larvae).

A two-way ANOVA showed significant species and species x competitive treatment interaction effects on body titer (Table 4-7). *Aedes albopictus* had significantly greater body titer than *A. aegypti* but this interspecific effect depended on competitive treatment (Fig. 4-13). The species x competitive treatment interaction was attributable to less interspecific difference (*A. albopictus* versus *A. aegypti*) in body titer from crowded larval conditions (320 larvae) compared to uncrowded conditions (160 larvae) (Fig. 4-13).

Separate one paired t-tests on infection parameters in the interspecific competitive treatment (i.e., 160:160) showed significant differences in the proportion infected, proportion with disseminated infections, and body titer (All $P < 0.05$). *Aedes albopictus* had significantly greater proportion infected (mean ± SE; *A. albopictus*, *A. aegypti*; 0.93 ± 0.04, 0.82 ± 0.03), lower proportion with disseminated infection (mean ± SE; *A. albopictus*, *A. aegypti*; 0.47 ± 0.09, 0.71 ± 0.05), and greater body titer (mean ± SE; *A. albopictus*, *A. aegypti*, 4.50 ± 0.08, 3.99 ± 0.05) than *A. aegypti*.

Discussion

All population growth measurements showed consistently poorer performance for mosquitoes reared at high larval density (Figs. 4-1, 4-2, 4-3). These results are consistent with those of a similar experiment, using *Aedes* species in tests of competitive treatment effects on adult SINV infection (Alto et al. 2005a). The goal of the SINV and DENV experiments was to maximize adult mosquito production, to assess vector competence, without negating the effects of larval competition. To achieve this, a combination of natural (oak leaf infusion) and artificial (yeast, albumin) larval food resources was used.

Previous laboratory and field research shows contrasting outcomes of competition between these *Aedes* species dependent upon larval resource type, with *A. aegypti* an equal or superior competitor with protein-rich resources (e.g., liver powder, yeast) and *A. albopictus* a superior competitor with plant detritus (e.g., leaves) (Juliano and Lounibos 2005, Braks et al. 2004, Juliano 1998, Barrera 1996, Black et al. 1989). The present study confirms the ability to replicate environmetal variables that provide for larval competition between *A. aegypti* and *A. albopictus*.

Higher levels of intra- and interspecific competition significantly enhanced DENV-2 infection and dissemination for *A. albopictus.* Phenotypic expression of vector competence in adult mosquitoes was significantly altered by competitive conditions of the aquatic larval environment. These results may be the product of norms of reaction of the genes controlling vector competence in these mosquitoes. Other studies on competition and phenotypic expression of a trait have shown that the phenotypic expression of virulence is altered when hosts were exposed to different competitive conditions or nutrient gradients (e.g., Bedhomme et al. 2004, Scheiner 1993). Vector competences studies have shown that there is evidence that a great amount of phenotypic variance in DENV-2 infection in *A. aegypti* is associated with environmental and random experimental effects (e.g., Bosio et al. 2000). The proportion of infected *A. albopictus* females contributed more to the competitive treatment effects than the proportion of females with disseminated infections (Table 4-2). This observation suggests that initial infection in the adult mosquito midgut is most influenced by larval competition.than escaping the midgut and infecting other organs (i.e., dissemination).

The lack of significant covariate (size) effects in the analyses of covariance suggests that size had no direct effect on vector competence within competitive treatments for both species (160, 320, 160:160) (Table 4-3). A marginally significant interaction between the covariate and competitive treatment for proportion *A. albopictus* infected (Table 4-3) suggests that the effect of mosquito size on infection may depend on competition experienced. Among replicates of the low density treatments, which produced females of the largest average sizes, proportion infected declined with increasing mean body size. This decline is not evident in smaller females from high density treatments (Fig. 4-8). Significant or marginally significant effects of competition were observed for *A. albopictus* because more intense competition resulted in greater DENV-2 infection and disseminated infection (Figs. 4-8, 4-9). Thus, differences in *Aedes* size, within competitive treatments make little or no contribution to the differences observed in infection parameters.

Although mean size may contribute little to differences in infection parameters within competitive treatments, mean size was significantly correlated with infection parameters across competitive treatments, perhaps due to competitive effects (Table 4-4, Figs. 4-8, 4-9). Specifically, small adult females, associated with intra- and interspecific competition, had enhanced infection and dissemination rates for *A. albopictus* (Table 4-4). Under the conditions of the current study small *A. albopictus* produced by competitive treatments may pose a greater health risk for DENV transmission compared to larger conspecifics. This effect may be enhanced because small adults may bloodfeed more frequently than larger adults (Scott et al. 2000b). However, competitive treatment differences in size may be correlated with overall competitive stress, making it difficult to

determine whether size alone was correlated with variation in infection parameters between treatment groups. Female longevity, host attack rates, and bloodfeeding success, important contributors to vector potential, are positively related to size (Xue et al. 1995, Willis and Nasci 1994, Nasci 1991, Nasci 1987, Nasci 1986ab, Hawley 1985, Haramis 1985, 1983), so determining the epidemiological importance for DENV transmission of different sized individuals coming from differing competitive environments requires interpretation of multiple life history parameters. Contrary to infection and dissemination results, no significant correlations were observed between size and body titer for either *Aedes* species (Table 4-4), consistent with results using two *A. aegypti* strains and DENV-2 (Bosio et al. 1998). Thus, size of *A. albopictus* mosquitoes is correlated with DENV-2 infection and dissemination, but not body titer. This suggests that different mechanisms are responsible for associations between size and different vector competence traits.

A midgut barrier has been proposed for mosquitoes that become infected but fail to spread arboviruses beyond the midgut to secondary tissues (i.e., disseminate) (Thomas et al. 1993, Houk et al. 1986, Weaver et al. 1984, Hardy et al. 1983). Enhanced dissemination and transmission of LACV by small *O. triseriatus* adults was suggested to be a result of fewer basal lamina layers present in smaller adults, thus weakening the midgut escape barrier (Grimstad and Walker 1991, Paulson and Hawley 1991). Midgut basal laminae thickness was significantly different among three *A. albopictus* strains, suggesting genetic control, and thickness was inversely related to dissemination of DENV-1. However, within an *A. albopictus* strain, basal laminae thickness did not differ between disseminated and nondisseminated female infections (Thomas et al. 1993).

Thus, although basal laminae thickness may vary with adult size and, in part, determine dissemination, other mechanical and physiological factors are also likely involved such as stress-induced midgut perforation or viral modulation. A description of the mechanism(s) responsible for the observed results was beyond the scope of these studies. Similar results were reported for *A. albopictus* vector competence for SINV (Alto et al. 2005a), and may suggest a common physiological (e.g., repressed innate immune function) (Sanders et al. 2005) or mechanical (e.g., leaky midgut hypothesis) (Chandler et al. 1998, Weaver et al. 1991, Weaver 1986, Hardy et al. 1983, Miles et al. 1973, Boorman 1960) mechanism(s) responsible for the observed results.

Correlation analyses suggested that life history traits, in addition to size, were associated with DENV-2 infection parameters (Table 4-4). Similar to size correlations, longer time to emergence for *A. albopictus*, associated with greater intra- and interspecific competition, was associated with enhanced infection and dissemination (Table 4-4). Taken together, results show consistent associations of reduced fitness measurements with higher *A. albopictus* DENV-2 infection parameters, but not *A. aegypti*, suggesting species differences in infection responses. These results agree with other reports investigating competitive stress and infectibility for a variety of systems, where competition in the form of nutrient limitation (Koella and Sørensen 2002, Beck and Levander 2000, Oppliger et al. 1998, Suwanchaichinda and Paskewitz 1998, Morris and Potter 1997, Lively et al. 1995, Matson and Waring 1984, Steinhaus 1958) and other stressors (Lafferty and Holt 2003, Kiesecker and Skelly 2001) increases host susceptibility to infection by pathogens and parasites.

The current experimental results reflect only females assayed from the first infectious bloodfeeding. These females had shorter time to emergence than females given infectious bloodmeals at later dates. This experimental approach assumes that the females assayed for DENV infection are representative, in terms of infection parameters, of all females from the entire duration of the competition experiment. It is not clear how longer time to emergence, and associated differences in subsequent adult physiology, may alter infection parameters. Intuition suggests there may be a positive relationship so that longer time to emergence is associated with enhanced infection parameters, as suggested by the correlation analyses (Table 4-4). However, this reasoning may be simplistic and may not capture temporal differences in competitive interactions among larvae. For example, adult females associated with longer time to emergence may be initially exposed as larvae to intense competition, followed by release from competition when competitors are removed from the environment (mortality, emergence to adulthood). Thus, differences in infection parameters may be associated with temporal differences in intensity of competition. Additional studies in the future will specifically focus on addressing the relationship between time to emergence and infection parameters. Results from these studies will address whether females emerging early in a competitive experiment are similar, in terms of infection parameters, to females with longer time to emergence.

Beyond the effects of competition, an interspecific comparison showed significant differences between *A. aegypti* and *A. albopictus* infection parameters. *Aedes albopictus* had a significantly greater proportion of DENV infected females, but a lower proportion of disseminated females, compared to *A. aegypti*. The mosquitoes used in the current

research were derived from well-established laboratory colonies. Although it is well accepted that laboratory colonization may alter *A. albopictus* and *A. aegypti* susceptibility for DENV and yellow fever virus (YFV) respectively (Vazeille et al. 2003, Lorenz et al. 1984), the current research results do agree with results for multiple mosquito strains (F_{1-2} generation) and different DENV serotypes (DENV-1, mosquitoes $\geq F_5$) (Chen et al. 1993). *A. aegypti* had significantly greater DENV-2 disseminated infection compared to *A. albopictus* using three Southeast Asian DENV-2 strains, including an almost identical strain used in the current experiment (Vazeille et al. 2003). The current experiment represents a detailed comparison of DENV-2 vector competence between *A. aegypti* and *A. albopictus*, including information on species-specific infection, dissemination, and whole body titer. Accurate description of relative viral susceptibility of *A. aegypti* and *A. albopictus* requires a comparison of multiple infection parameters, especially since some pairs of infection parameters yield interspecific differences of opposite direction (e.g., infection: *A. albopictus* > *A. aegypti*, dissemination; *A. albopictus* < *A. aegypti*) (Fig. 4-4). However, it is important to recognize that vector competence is only one compenent in determining DENV transmission in nature (i.e., vectorial capacity) and the roles of these two vector species in DENV transmission may be dynamically changing depending on both genetic and environmental parameters.

Species-specific variation in DENV-2 infection parameters may be viewed as fundamental differences in physiology between these *Aedes* species and the outcome of infection. Genetic studies on DENV-2 have mapped several quantitative trait loci controlling DENV-2 midgut infection (Gomez-Machorro et al. 2004, Bosio et al. 2000) and dissemination (Bennett et al. 2005a) in *A. aegypti*. Similar studies have not been

done on *A. albopictus*. However, quantitative trait loci controlling infection parameters may depend on mosquito and virus strains. In addition to differences in infection and dissemination, *A. albopictus* had on average significantly greater viral body titer compared to *A. aegypti*, yet it disseminated DENV-2 poorly. Bosio et al. (1998) showed that DENV-2 dissemination rates in *A. aegypti* were independent of midgut virus titer. Interspecific differences in body titer of *Aedes* females with disseminated infections suggest that factors limiting viral replication in *A. aegypti* were more efficient compared to *A. albopictus*. These results were unexpected and may represent species-specific differences in viral selection pressure within the vector host. This study represents the most extensive DENV body titer comparison between these two *Aedes* species so it is not clear whether other mosquito and DENV strains show *Aedes*-specific differences in viral load. A more efficient midgut escape barrier in *A. albopictus* than *A. aegypti* may select for greater viral replication, and associated body titer, since failure to escape the midgut results in failure to infect the next host. Alternatively, *A. albopictus* may merely be a better physiological environment for DENV-2 replication than *A. aegypti*, regardless of barriers.

The observed *Aedes*-specific difference in body titer represents a favorable situation to test hypotheses that integrate host-pathogen theory and vectorial capacity. Vectorial capacity, the daily rate at which future pathogen inoculations arise from a current infective case, are sensitive to changes in daily survivorship of the vector. Thus, small changes in DENV-induced adult survivorship (Fernandez et al. 2003) may result in relatively large differences in vectorial capacity (Dye 1986). Although arboviruses are assumed to have little negative effects on their arthropod vectors, many exceptions are

known (Platt et al. 1997, Faran et al. 1987, Turell et al. 1985, Beaty et al. 1980, Tesh 1980, Grimstad et al. 1980, Mims et al. 1966). Host-parasite theory suggests parasite/pathogen load is inversely related to host fitness (e.g., Anderson and May 1978, May and Anderson 1978). Future experiments should address DENV-induced differences in adult *Aedes* longevity and reproductive success and its contribution to the relative importance of *A. aegypti* and *A. albopictus* in DENV transmission (e.g., longevity and vectorial capacity), especially in sympatric locations for these *Aedes* species where dengue is endemic.

The observation that *A. aegypti* had a greater proportion of females with disseminated infections suggest that the midgut escape barrier in *A. aegypti* (Bennett et al. 2005ab, Bosio et al. 2000, 1998) was less efficient at limiting spread of viral infection to secondary tissues compared to *A. albopictus*. These species-specific differences in dissemination are consistent with the observation that *A. aegypti* is the more important vector in DENV transmission to humans. However, in the current study, the actual number of DENV transmitting mosquitoes (assumes dissemination is an indicator of potential to transmit) may be more similar between *A. aegypti* and *A. albopictus* when simultaneously accounting for both proportion infection (*A. albopictus* > *A. aegypti*) and proportion with disseminated infections (*A. albopictus* < *A. aegypti*) (Fig. 4-4). The proportion of potential transmitters are equivalent to the product of the proportion of females infected and the proportion of females with disseminated infection (*A. albopictus*, 0.33; *A. aegypti*, 0.49).

The species by competition comparison analyses (i.e., two-way MANOVAs and two-way ANOVA) showed significant effects of *Aedes* species and competitive

treatments on population growth measurements and infection parameters (Table 4-5, 4-6). Fundamental physiological differences in *Aedes* size and time to emergence were responsible for the species effect (Table 4-5, SCC), so that *A. aegypti* performed better than *A. albopictus* under the experimental conditions. Other authors have demonstrated that *A. aegypti* develop more quickly than *A. albopictus* with nutrient rich resources (e.g, albumin, yeast) (Klowden and chambers 1992). Also, interspecific differences in infection parameters (Table 4-6) show *A. aegypti* was less resistant to limiting the spread of DENV infection, as shown by higher dissemination rates. Similar to the other analyses, competitive treatment effects were realized by longer time to emergence and smaller adult size (Table 4-5). Also, competitive treatment effects enhanced infection parameters (Table 4-6), especially proportion females infected. There were no species x competitive treatment interactions for population growth measurements or infection parameters. These later results were surprising since it suggests that *A. albopictus* and *A. aegypti* infection parameters respond similarly to competition. These results differ compared to results from the analyses done separately for each species (i.e., one-way MANOVAs). However, in these later analyses *A. aegypti* did show a trend, although not significant, for competition-induced differences in infection parameters (Fig. 4-6). Discrepancy between results of different analyses on infection parameters may be due to greater power in the current analyses compared to analyses of each species separately. Species x competitive treatment interactions were found for DENV body titer (Table 4-7) so that differences in *Aedes* species body titers were more similar under crowded larval conditions (Fig. 4-13), perhaps due to enhanced stress. Although these analyses were

interesting, they are limited to capturing only the results from intraspecific competitive treatments.

Competitive interactions among larval stages alter adult *A. albopictus* infection parameters for DENV with the general association of increased larval competition increasing adult susceptibility to DENV infection. This phenomenon suggests the importance of an under explored connection between mosquito larval ecology and the epidemiology of arboviruses. Mathematical models of arthropod-borne infectious diseases have generally ignored the effects of larval environment on vector competence, assuming a "black box" approach to all infection dynamics within the vector (Dye 1986). The results of this study suggest that larval conditions are an important aspect of DENV biology, and should be included in future considerations of dengue epidemiology. Recent studies have begun to relate landscape measures (e.g., vegetation cover, rainfall, temperature, and container density) and vector ecology, specifically in the context of competition at the larval stage (Barrera et al. 2006, Schneider et al. 2004, Morrison et al. 2004). The current research bridges the gap between these studies associating landscape and DENV vector potential (Kolivras 2006, Barrera et al. 2006, Morrison et al. 2004, Schneider et al. 2004, Kuno 1997). In this study, I have shown that environmental-induced differences in adult size, as well as other life history traits, may play an important role in determining vector competence and vectorial capacity (e.g., altered infection parameters, longevity, bloodfeeding frequency) and should be considered in vector intervention methods aimed at reducing dengue and in building realistic, predictive models of dengue epidemiology (e.g., Schneider et al. 2004, Luz et al. 2003, Smith et al. 2004, Focks et al. 2000, 1995).

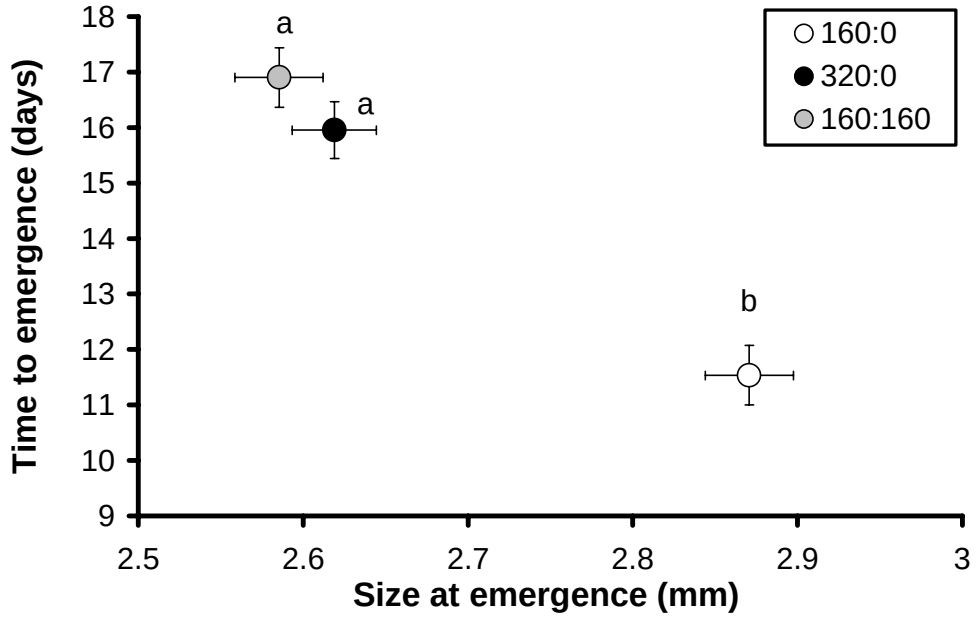

Figure 4-1. *Aedes albopictus* least squares means (± SE) for female size and time to emergence. Different letters indicate significant differences between bivariate means. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti*—160:0, 320:0, 160:160.

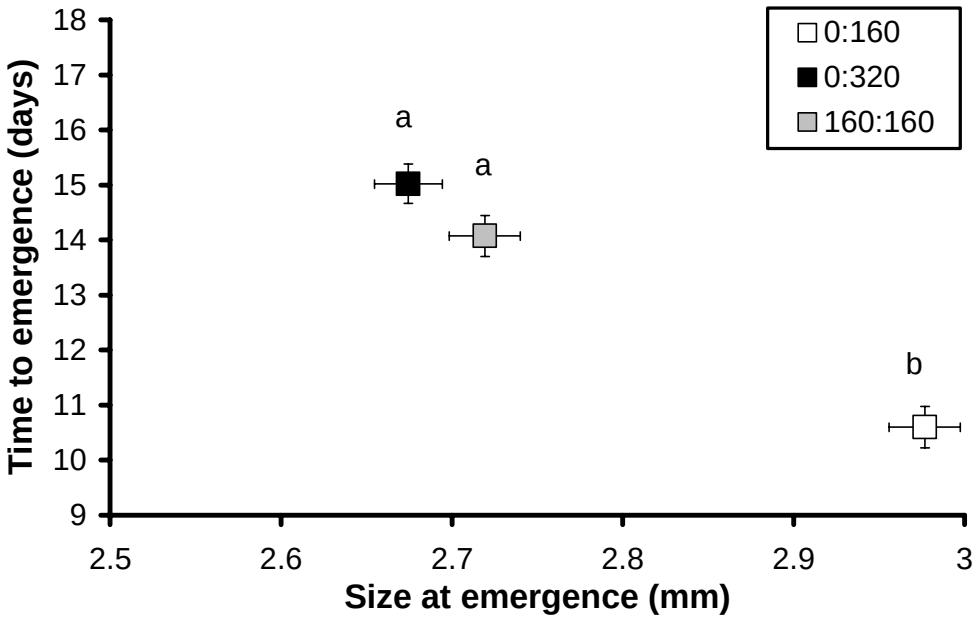

Figure 4-2. *Aedes aegypti* least squares means (± SE) for female size and time to emergence. Different letters indicate significant differences between bivariate means. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti*—0:160, 0:320, 160:160.

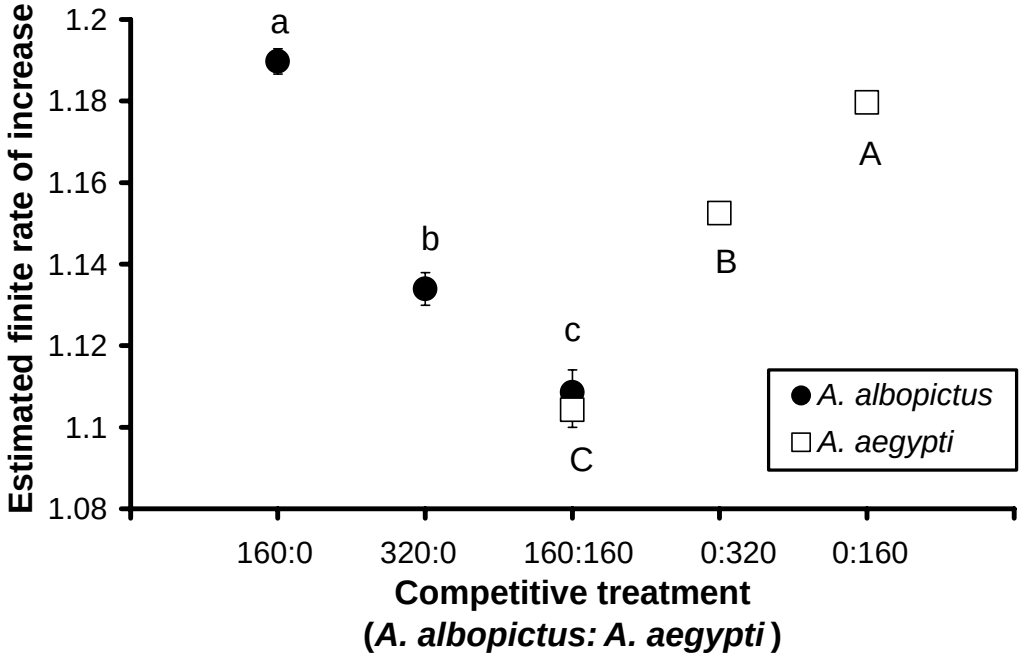

Figure 4-3. Least squares means (± SE) for estimated finite rate of increase, λ′, for *A. albopictus* and *A. aegypti*. Points without bars have standard errors too small to be visible. Different lowercase and uppercase letters indicate significant differences between means for *A. albopictus* and *A. aegypti*, respectively.

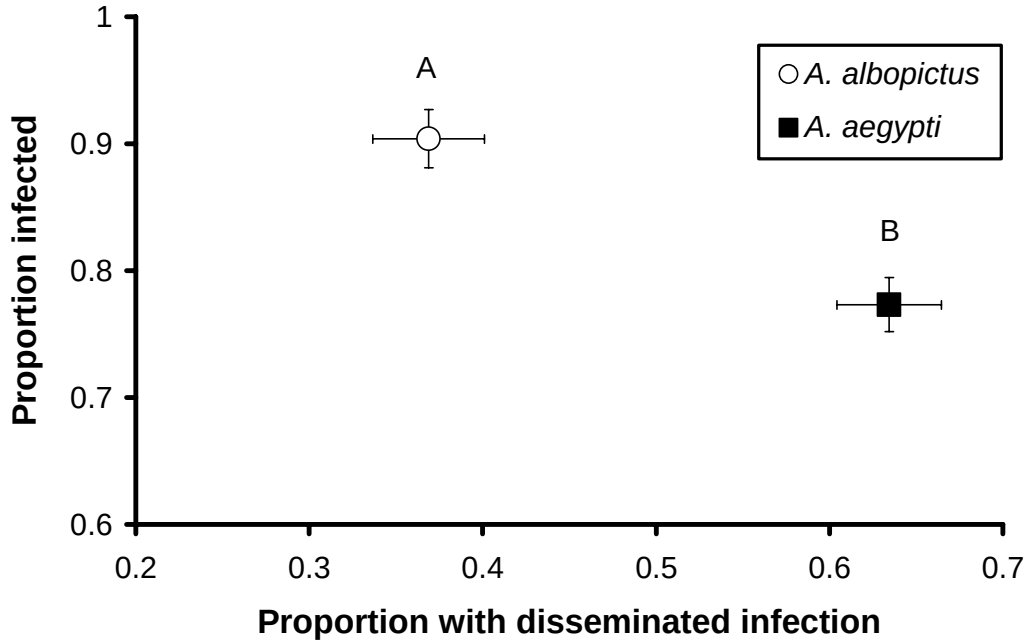

Figure 4-4. Least squares (± SE) for proportion of *A. albopictus* and *A. aegypti* infected and disseminated infections. Uppercase letters indicate significant differences between bivariate means.

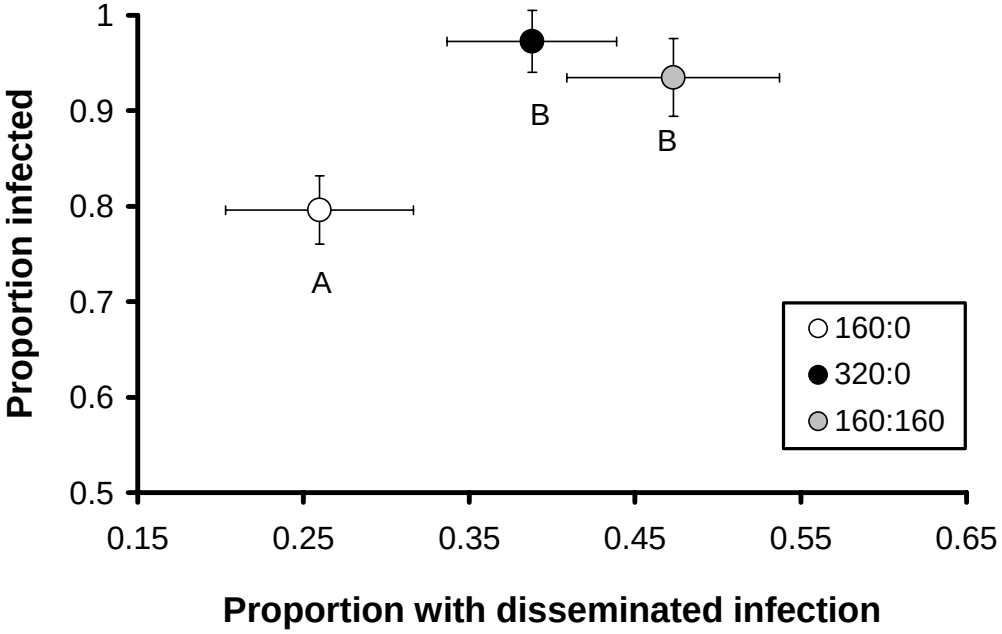

Figure 4-5. Bivariate plots of least squares means (± SE) for proportion of *A. albopictus* infected and disseminated infections. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti* – 160:0, 320:0, 160:160. Different letters indicate significant differences between bivariate means.

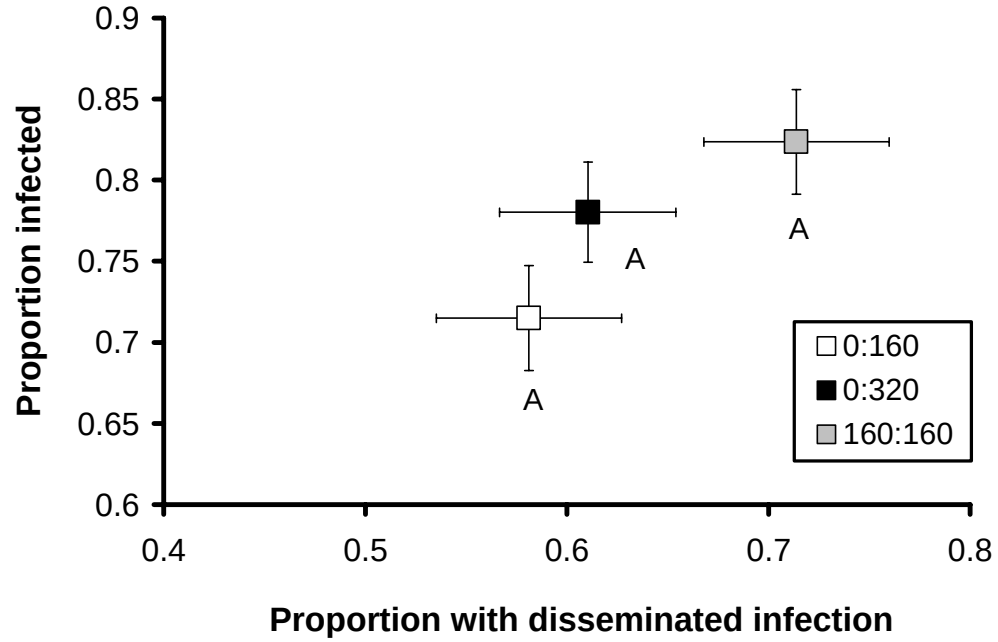

Figure 4-6. Bivariate plots of least squares means (± SE) for proportion of *A. aegypti* infected and disseminated infections. Competition treatments consisted of species density ratios of *A. albopictus*: *A. aegypti* – 0:160, 0:320, 160:160. Absences of different letters indicate no significant differences between bivariate means.

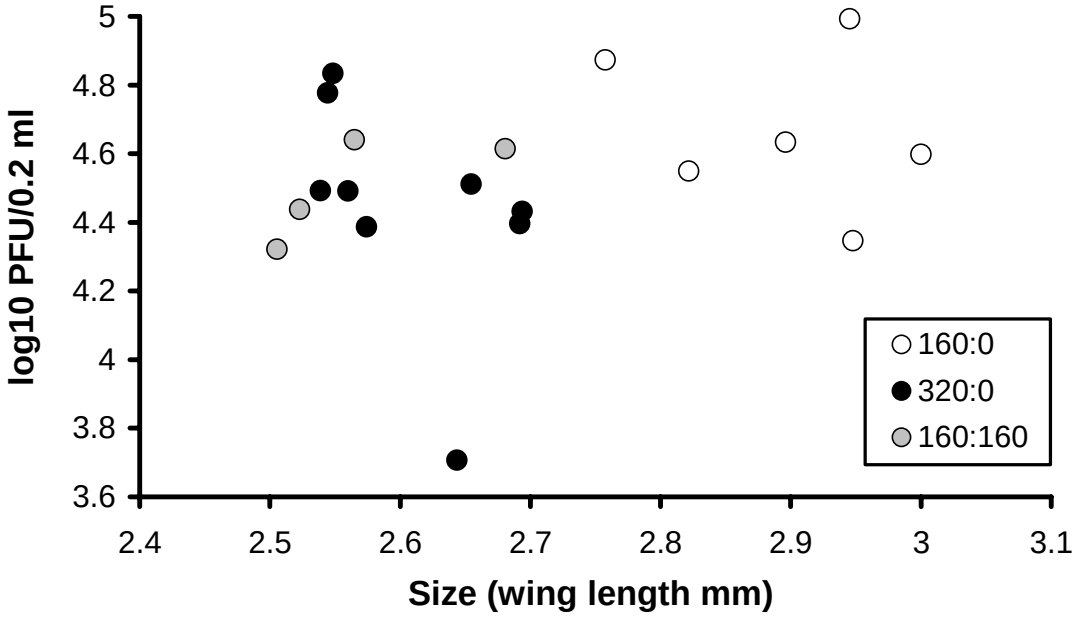

Figure 4-7. Least squares means for body titer and size of adult *A. albopictus* females with disseminated (i.e., infection spread beyond the midgut, infecting secondary target organs) dengue-2 virus infections. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti*.

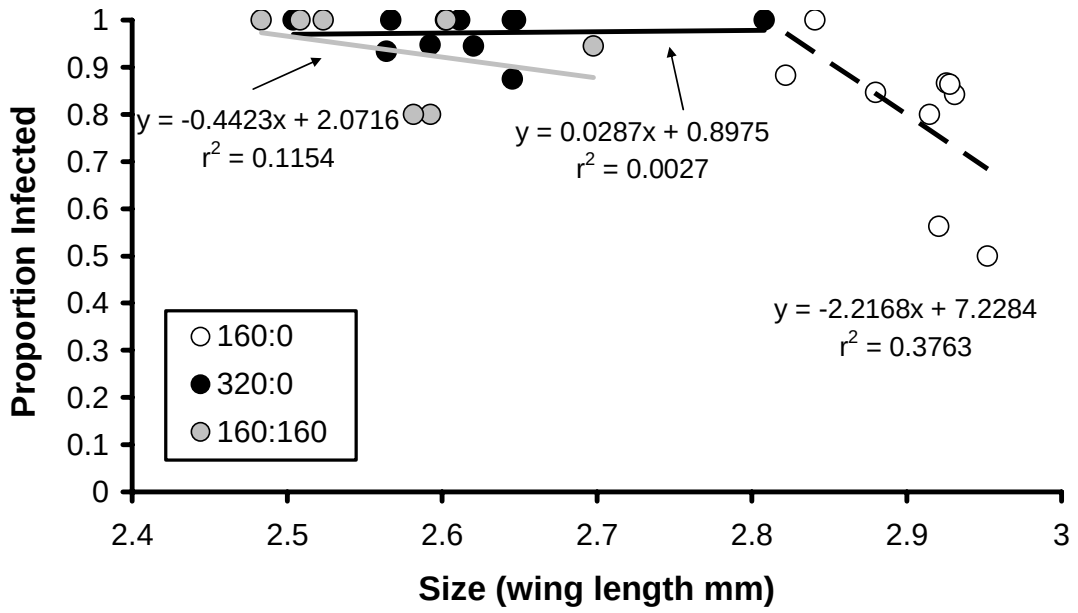

Figure 4-8. Least squares means for proportion infected and size of adult *A. albopictus* females. Solid and dashed lines drawn through bivariate means show the best fit for *A. albopictus* in three competitive treatment conditions. Regression equations, with associated r^2 values, are shown for competitive treatments. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti*.

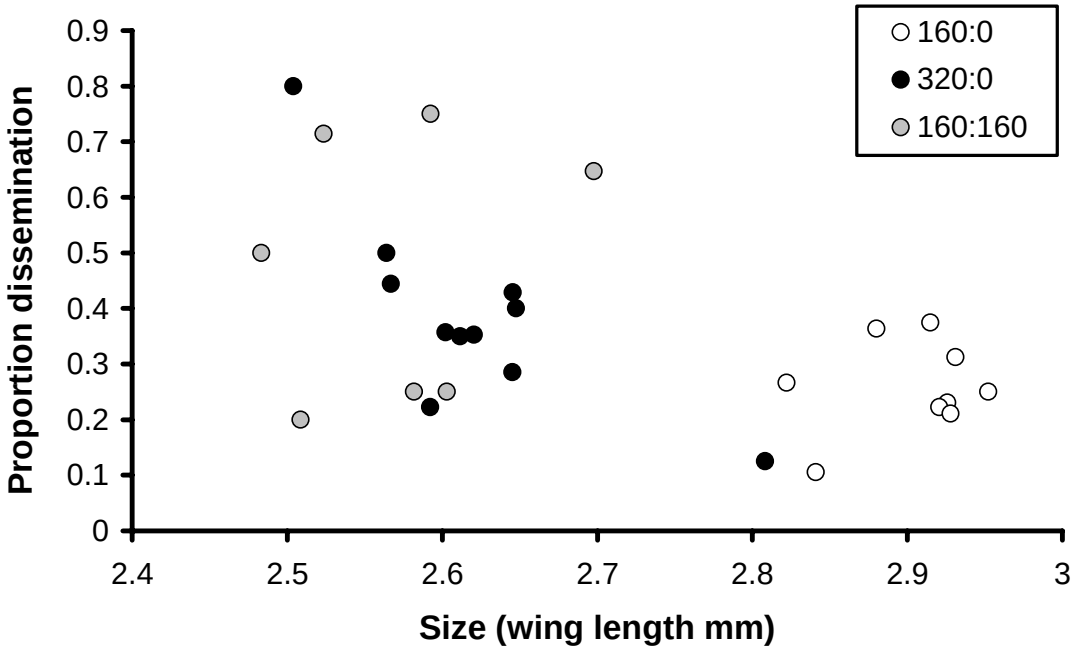

Figure 4-9. Least-squares means for proportion disseminated infections and size of adult *A. albopictus* females. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti*.

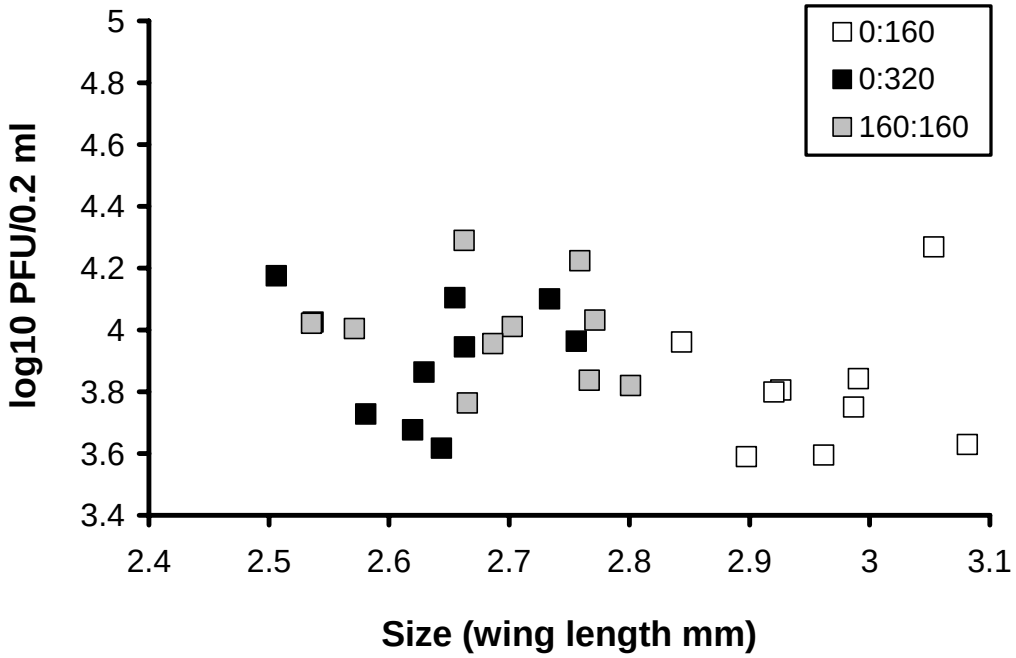

Figure 4-10. Least-squares means for body titer and size of adult *A. aegypti* females with disseminated (i.e., infection spread beyond the midgut, infecting secondary target organs) dengue-2 virus infections. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti.*

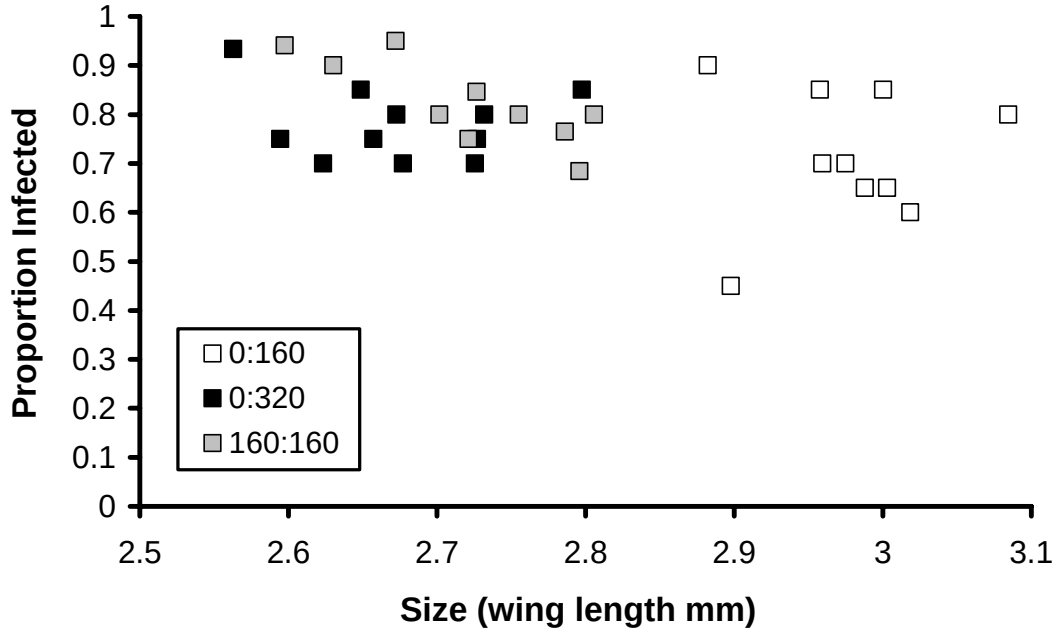

Figure 4-11. Least-squares means for proportion infected and size of adult *A. aegypti* females. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti.*

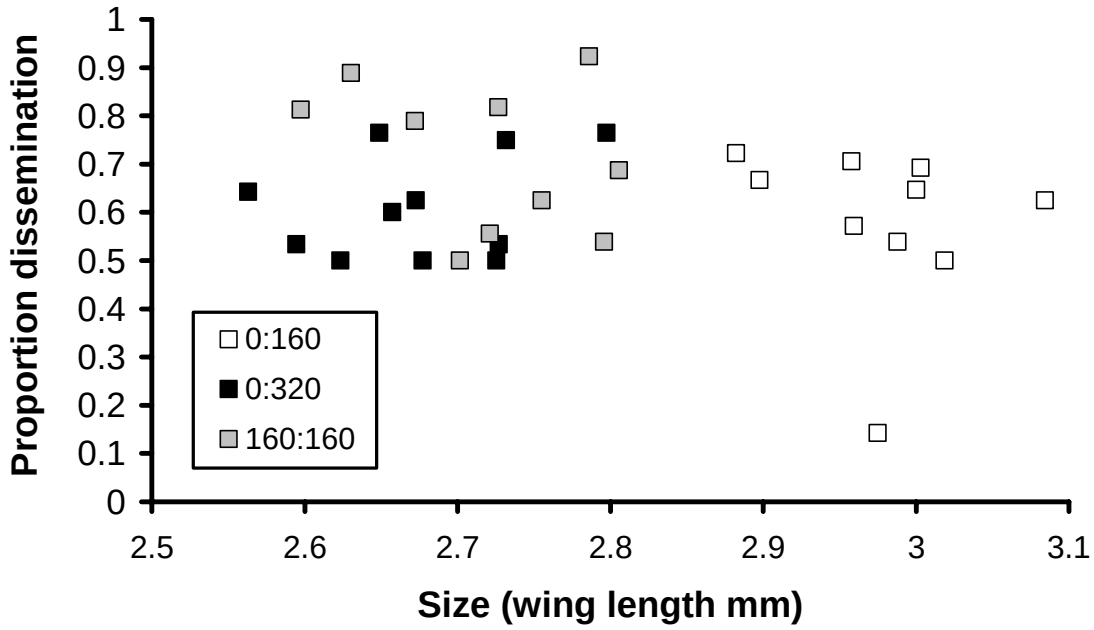

Figure 4-12. Least-squares means for proportion disseminated infections and size of adult *A. aegypti* females. Numbers in the figure key represent the ratio of larval *A. albopictus* to *A. aegypti*.

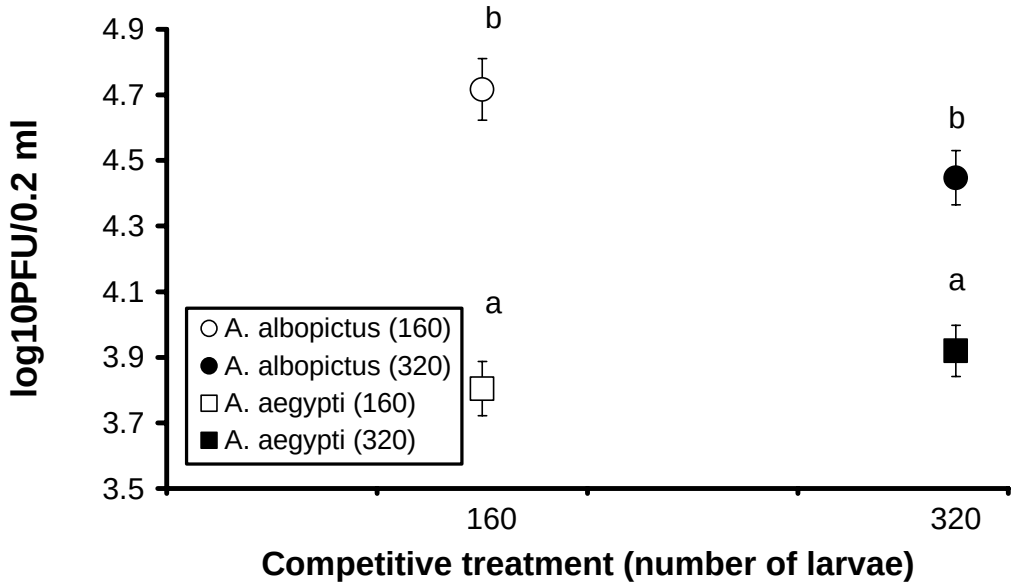

Figure 4-13. Two-way ANOVA for species (*A. albopictus* and *A. aegypti*) and competitive treatment (*A. albopictus*: *A. aegypti*, 160:0, 320:0, 0:160, and 0:320) effects on Least-squares means for body titer.

Table 4-1. MANOVA and multivariate pairwise contrasts of competitive treatment effects on female *Aedes albopictus* and *A. aegypti* population growth measurements: time to emergence, survivorship to emergence, and adult size.

				Standardized Canonical Coefficients		
Comparison	df	Pillai's trace	*P*	Time	Survivorship	Size
A. albopictus						
Competitive treatment	6	0.86	< 0.0001	0.84	- 0.31	- 1.13
160:0 vs. 320:0	3	0.71	< 0.0001	0.76	- 0.43	- 1.13
160:0 vs. 160:160	3	0.74	< 0.0001	0.90	- 0.22	- 1.12
320:0 vs. 160:160	3	0.11	0.3826			
Error df	28					
A. aegypti						
Competitive treatment	6	0.88	< 0.0001	0.96	- 0.27	- 1.62
0:160 vs. 0:320	3	0.85	< 0.0001	0.99	- 0.28	- 1.59
0:160 vs. 160:160	3	0.78	< 0.0001	0.90	- 0.23	- 1.69
0:320 vs. 160:160	3	0.17	< 0.1844			
Error df	28					

Table 4-2. Multivariate ANOVA for main effects and multivariate pairwise contrasts of competitive treatment effects on female *Aedes albopictus* and *A. aegypti* proportion infected and proportion with disseminated infection.

				Standardized Canonical Coefficients	
Comparison	df	Pillai's trace	*P*	Infection	Dissemination
A. albopictus					
Competitive treatment	4	0.51	0.0057	1.04	0.61
160:0 vs. 320:0	2	0.41	0.0022	1.11	0.49
160:0 vs. 160:160	2	0.36	0.0060	0.89	0.77
320:0 vs. 160:160	2	0.06	0.4897		
Error df	24				
A. aegypti					
Competitive treatment	4	0.23	0.1394		
Error df	28				

Table 4-3. ANCOVA (after testing for equality of slopes) for the effects of competitive treatment and size covariate on body titer, proportion infected, and proportion with disseminated infection for *Aedes albopictus* and *A. aegypti* females. Body titer refers only to females with disseminated dengue-2 infections. Size x competition interactions with P > 0.05 are not shown except for marginally significant interactions. Error df's reflect final reduced analyses (ANCOVA or ANOVA).

Source	df	*F*	*P*
A. albopictus			
Titer (infection disseminated)			
Size	1	0.99	0.3335
Competitive treatment	2	1.99	0.1672
Error df	17		
Infection			
Size	1	1.90	0.1812
Competitive treatment	2	7.10	0.0038
Size x competition	2	3.39	0.0529
Error df	21		
Dissemination			
Size	1	1.87	0.1843
Competitive treatment	2	3.24	0.0568
Error df	24		
A. aegypti			
Titer (infection disseminated)			
Size	1	0.01	0.9285
Competitive treatment	2	2.38	0.1123
Error df	26		

Table 4-3 (continued)

Infection			
Size	1	1.71	0.2022
Competitive treatment	2	2.87	0.0737
Error df	28		
Dissemination			
Size	1	0.27	0.6064
Competitive treatment	2	2.32	0.1167
Error df	28		

Table 4-4. Product-moment correlation coefficients ($r_{1,2}$) for the relationship between population growth measurements (time to emergence, survivorship, size, and λ′) and infection parameters for *A. albopictus* (df=25) and *A. aegypti* (df=29). Replicates in which only a single female body titer was measured were excluded from correlations, resulting in 18 and 27 df's for *A. albopictus* and *A. aegypti*, respectively. ** P < 0.01, *** P < 0.001, and **** P < 0.0001 show significant correlation coefficients. A sequential Bonferonni adjustment corrected experimentwise α=0.05 for 12 comparisons for *A. albopictus* and *A. aegypti*, respectively.

Infection Parameter	**Growth Correlates**	*A. albopictus* ($r_{1,2}$)	*A. aegypti* ($r_{1,2}$)
Infection	Time to Emergence	0.50 **	0.38
	Survivorship	- 0.15	- 0.22
	Size	- 0.62 ****	- 0.41
	λ′	- 0.43	- 0.45
Dissemination	Time to Emergence	0.55 ***	0.15
	Survivorship	- 0.04	- 0.39
	Size	- 0.50 **	- 0.22
	λ′	- 0.39	- 0.41
Body Titer (disseminated)	Time to Emergence	- 0.43	0.26
	Survivorship	0.05	- 0.22
	Size	0.28	- 0.29
	λ′	0.31	- 0.37

Table 4-5. Two-way MANOVA of species (*A. albopictus* and *A. aegypti*) and competitive treatment (*A. albopictus*: *A. aegypti*, 160:0, 320:0, 0:160, and 0:320) effects on female population growth measurements: time to emergence, survivorship to emergence, and adult size.

				Standardized Canonical Coefficients		
Comparison	df	Pillai's trace	*P*	Time	Survivorship	Size
Species	3	0.25	0.0133	- 0.82	0.04	1.69
Competitive treatment (trt.)	3	0.86	< 0.0001	1.23	- 0.53	- 1.24
Species x Competitive trt.	3	0.13	0.1523			
Error df	38					

Table 4-6. Two-way MANOVA of species (*A. albopictus* and *A. aegypti*) and competitive treatment (*A. albopictus*: *A. aegypti*, 160:0, 320:0, 0:160, and 0:320) effects on proportion infected and proportion with disseminated infections.

				Standardized Canonical Coefficients	
Comparison	df	Pillai's trace	*P*	Infection	Dissemination
Species	2	0.64	< 0.0001	- 0.86	1.24
Competitive treatment (trt.)	2	0.27	< 0.0035	1.17	0.45
Species x Competitive trt.	2	0.08	0.2136		
Error df	37				

Table 4-7. Two-way ANOVA for species (*A. albopictus* and *A. aegypti*) and competitive treatment (*A. albopictus*: *A. aegypti*, 160:0, 320:0, 0:160, and 0:320) effects on body titer.

Comparison	df	*F*	*P*
Species	1	72.65	< 0.0001
Competitive treatment (trt.)	1	0.83	0.3706
Species x Competitive trt.	1	5.18	0.0299
Error df	31		

CHAPTER 5
COMPETITION, ARBOVIRUS INFECTION, AND FUTURE EXPERIMENTS

Competition and Enhanced Infection

Typically, vector competence studies are performed on adult mosquitoes derived from larvae that developed with a surplus of food and space. Rearing larvae in this manner, particularly container species such as *A. aegypti* and *A. albopictus*, fails to recreate their stressful larval environment. Our lack of knowledge of how larval conditions, particularly competition, interact with vector competence is a gap in understanding the transmission of arboviruses. In the current research, the effects of larval competition, as determined by population growth measurements, were preserved while allowing sufficient adult production to examine quantitatively how larval competition impacted arbovirus infection.

For both larval competition experiments, intense competition was associated with reductions in mosquito fitness measurements (Chapter 3, Figs. 3-1, 3-2, 3-3; Chapter 4, Figs. 4-1, 4-2, 4-3). Previous studies using highly nutritious resources (yeast, liver powder) showed that *A. aegypti* was the superior larval competitor (Barrera 1996, Black et al. 1989). However, the outcome of interspecific competition experiments between *A. albopictus* and *A. aegypti* using natural resources (leaves) showed that *A. albopictus* was the superior larval competitor (Juliano and Lounibos 2005, Braks et al. 2004, Juliano 1998). Supplementing natural resources (leaves) with invertebrate carcasses, a nutrient rich resource, reduces resource limitation and the competitive advantage of *A. albopictus,* and may promote coexistence (Daugherty et al. 2000). A comparative study of leaves

and enriched resources, confirmed these variable outcomes and concluded that the outcome of competition between these species is likely to depend on resource type (Barrera 1996b), as in other systems (e.g., Tilman 1982). In the current studies, interspecific competition between these *Aedes* species as measured by λ' was more intense than intraspecific competition (Chapter 3, Fig. 3-3; Chapter 4, Fig. 4-3). These results suggest an unstable competitive equilibrium, where the outcome of competition depends not only on resources (see above) but also on the initial abundance of the two species, so that the more abundant species competitively excludes the other species (i.e., Grover 1997).

Similar designs in the two competition experiments allowed for robust cross-experiment comparisons of infection parameters in adult mosquitoes. The two competition experiments yielded similar competitive treatment effects on adult infection parameters for the two unrelated viruses. Specifically, reduced mosquito fitness, associated with intense competition, enhanced infection parameters for SINV and DENV (Chapter 3, Table 3-3, Figs. 3-4, 3-5; Chapter 4, Table 4-4, Fig. 4-5). *Aedes albopictus* infection parameters showed strong effects of larval competition, with proportion infected with SINV and DENV contributing the most to competitive treatment effects relative to the other infection parameters measured (Chapter 3, SCC in text; Chapter 4, Table 4-2). *Aedes aegypti* showed no competition-induced changes in infection parameters for the viruses examined suggesting differences in competition-induced responses of *A. aegypti* and *A. albopictus* infection parameters (Tables 3-3, 4-4).

Although general effects of competition were similar for both experiments, population growth measurements showed that the intensity of competition was slightly

less in the DENV experiment (Chapter 3, Figs. 3-1, 3-2, 3-3; cf. Chapter 4, Figs. 4-1, 4-2, 4-3). However, time to emergence in the high density treatments (320 and 160:160) were longer in the DENV experiment than in the SINV experiment (Chapter 3, Fig. 3-2, LS means in text; cf. Chapter 4, Figs. 4-1, 4-2). Adult size and competitive treatments had independent and opposite effects on SINV body titer for *A. albopictus*, so that larger size and more intense competitive conditions were both associated with greater body titer (Chapter 3, Fig. 3-5). The lack of size effects within competitive treatments on DENV infection parameters (Chapter 4, Table 4-3) may suggest virus-specific effects on infection parameters associated with size. Alternatively, lack of size effects within competitive treatments may be related to larger sizes of mosquitoes in the DENV experiment, compared to the SINV experiment. Sizes of *A. albopictus* and *A. aegypti* from uncrowded larval conditions (160 larvae) in the SINV experiment were similar to sizes of the two *Aedes* species from crowed larval conditions (320 and 160:160 larvae) in the DENV experiment (Chapter 3, Fig. 3-1, in text; cf. Chapter 4, Figs. 4-1, 4-2). Larger sized mosquitoes in the DENV experiment may be, in part, a result of sampling females for size and infection only from the first DENV infectious blood feeding (~ 50% of the females). These mosquitoes emerged to adulthood early in the competition experiment when larval food resources may have been more abundant relative to later in the experiment. In addition to size differences, females assayed for the DENV experiment had shorter time to emergence and it is not clear how these factors may alter infection parameters. Differences between experiments in the expression of infection parameters associated with adult size may depend on the range of adult sizes. Size effects on DENV infection parameters were observed between competitive treatments (Chapter 4, Table 4-

4) suggesting that size was related to DENV infection parameters, although over a greater range of sizes than observed in the SINV experiment. However, competitive treatment differences in size may be correlated with other life history traits, as well as overall competitive stress, making it difficult to determine whether size alone was correlated with variation in infection parameters between treatment groups.

Laboratory colonies of these *Aedes* species have traditionally been accepted as representative of their natural populations. However, laboratory colonization may alter infection parameters due to founder effects, genetic drift, and unintentional selection imposed on laboratory colonies (Wallis et al. 1985, Lorenz et al. 1984). For example, laboratory colonies may be established from a small number of individuals and thus there is a higher probability of missing rare alleles compared to a colony derived from a larger number of founders (Munstermann 1994). Laboratory colonization with associated selection and drift, even after only a few generations, may reduce heterozygosity and the number of alleles (Munstermann 1994, 1980). Despite these shortcomings the current experiments on competition and infection used well-established laboratory colonies of *Aedes* mosquitoes for two major reasons. First, it was unclear whether larval competition would have strong, subtle, or any effect on adult infection parameters. Minimizing intra-population variation in response to viral infection, associated with greater genetic variability, should increase the likelihood of detecting competition-induced effects on adult infection parameters. Secondly, the use of identical laboratory mosquito strains, and similar experimental design between experiments, allowed for comparisons of infection parameters for SINV and DENV. The use of F_1 generation *Aedes*, whose parents were collected from natural field populations from different years (i.e., SINV

versus DENV experiments), would have confounded cross experiment comparisons of mosquito infection parameters for SINV and DENV due to undefined genetic variation in the vector populations.

In addition to competitive effects on these *Aedes* species, there were also interspecific differences in infection parameters. A comparison of mosquito species showed that *A. albopictus* had greater infection but lower dissemination of SINV and DENV than *A. aegypti* (Chapter 3, in text; cf. Chapter 4, Fig. 4-4). In contrast, viral body titer was significantly greater in *A. aegypti* for SINV and greater in *A. albopictus* for DENV (Chapter 3, in text; cf. Chapter 4, in text). Similar responses of the two vector species to SINV and DENV infection and dissemination may suggest similar mechanisms controlling these processes. Specifically, midgut infection barriers are more efficient in *A. aegypti* than *A. albopictus* and midgut escape barriers are more efficient in *A. albopictus* than *A. aegypti*. There is a growing literature on the genetic controls of mosquito vector competence, particularly of *A. aegypti* vector competence for DENV. Genetic studies with DENV-2 have identified candidate genes (e.g., early trypsin gene coding for the proteolytic enzyme trypsin) associated with susceptibility to infection (Gorrochotegui-Escalante et al. 2005) and mapped several quantitative trait loci controlling midgut infection (Gomez-Machorro et al. 2004, Bosio et al. 2000) and dissemination in *A. aegypti* (Bennett et al. 2005a), although similar studies are lacking for *A. albopictus*. These studies demonstrate that there is extensive genetic variation in the types of loci and the genes at individual loci that control vector competence of *A. aegypti* for DENV. Species-specific differences in body titer suggest differences in the abilities of these closely related species to limit SINV and DENV replication.

Future Studies

Field-collected Mosquitoes for Infection Experiments

The current experiments showed similar directional effects of competition-induced changes in SINV and DENV infection parameters for *A. albopictus*. It is unclear whether genetically more heterogeneous mosquitoes would have similar infection responses. Further insight may be gained from experiments using mosquitoes more representative of natural populations. Future studies should consider experiments on larval competition and infection parameters using F_1 generation *Aedes* whose parents were collected from natural field populations. The outcome of such additional experiments would answer whether conclusions based on *Aedes* laboratory colonies apply to field populations, and whether trends in infection parameters induced by competitive interactions are similar among more genetically heterogeneous populations.

Laboratory competition experiments using F_1 generation *Aedes* might be coupled with infection experiments using adults obtained from field-collected *A. albopictus* and *A. aegypti* pupae (e.g., Paulson and Hawley 1991). The size of *Aedes* adults emerged from field-collected pupae is an accurate indicator of larval conditions (e.g., competition, temperature) because body size is fixed after emergence to adulthood. Moreover, female size is positively related to fitness. Sizes of *A. aegypti* and *A. albopictus* females in the SINV and DENV experiments were within the range of sizes of field-collected individuals. However, there is a broader range of sizes of both these *Aedes* from field collections (Schneider et al. 2004, Scott et al. 2000b, Willis and Nasci 1994, Nasci 1991, 1990, 1986ab). The proposed experiment will determine whether a broader range of adult *Aedes* sizes are associated with a broader range of infection parameters.

Mechanisms Responsible for Competition-enhanced Infection

The focus of this research was to determine whether larval competition alters adult infection parameters for different arboviruses. An investigation of the mechanisms responsible for the observed results was beyond the scope of these studies. However, it may be useful to consider mechanisms that may be responsible for competition-induced changes in adult infection parameters. Similar effects of competition on *A. albopictus* infection parameters for unrelated arboviruses highlight the importance of previously unappreciated ecological factors in determining mosquito vector potential and may suggest a common physiological mechanism(s) responsible for the observed results (e.g., repressed innate immune system) (Sanders et al. 2005, Dimopoulos 2003). Mounting an immune response (melanization response, antimicrobial peptides) comes at a cost of reduced fecundity in *A. aegypti* and *Anopheles gambiae* (Giles) (Schwartz and Koella 2004, Ahmed et al. 2002), demonstrating a trade-off between host immunity and fitness components (Sheldon and Verhulst 1996). If similar trade-offs occur in the current system, competitively stressed individuals may be less able to mount an effective immune response, or perhaps pay a higher fitness price compared to unstressed individuals.

A simple mechanical mechanism may account for the effects of competition on virus infection and dissemination. It is likely that arboviruses, found within ingested blood meals, enter mosquito midgut cells by membrane fusion or receptor-mediated endocytosis (e.g., Barth and Schatzmayr 1992, Hase et al. 1989, Hardy et al. 1983), followed by several days of midgut replication, and finally dissemination via hemolymph to secondary tissues. Several studies have invoked a "leaky midgut" hypothesis to account for the appearance of arboviruses in the hemolymph and secondary tissues

shortly after (minutes to hours) imbibing an infectious blood meal (Chandler et al. 1998, Weaver et al. 1991, Weaver 1986, Hardy et al. 1983, Miles et al. 1973, Boorman 1960). The leaky midgut hypothesis contends that rapid spread of infectious viruses to secondary tissues is facilitated by physical disruptions in the midgut epithelium and associated tissues (e.g., Weaver et al. 1991) or thru intercellular junctional spaces (Houk and Hardy 1979). Dual infectious bloodmeals, with both microfilarial parasites and arboviruses, showed that physical disruption of the midgut by microfilarial penetration facilitated greater arboviral infection (Vaughan et al. 1999, Zytoon et al. 1993, Turell et al. 1984). Similar results were obtained when perforations were experimentally induced in mosquito midguts (Zytoon et al. 1993). Midgut stress and disruptions have been shown to occur during bloodfeeding, presumably due to midgut distension or over-distension, and to facilitate direct arboviral access to the midgut (Weaver et al. 1991, Houk and Hardy 1979). Thus, midgut stress and disruptions facilitate greater arboviral infection, as well as dissemination, since arboviruses bypass the midgut barriers and arrive in the interior of midgut cells or the mosquito body cavity (hemocoel) without replication in the midgut epithelium. This phenomenon is likely to have important epidemiological significance because it allows for arboviral infection in mosquito species that may otherwise show refractoriness to arboviral infection or transmission, perhaps due to barriers, (i.e., genetic refractoriness) (Hardy et al. 1978), and it reduces the extrinsic incubation period which enhances vectorial capacity. A testable hypothesis is that competition-induced enhancement of mosquito arboviral infection and dissemination may be attributable to midgut stress that allows arboviruses to enter (infect) and leave (disseminate), as well as bypass, the midgut tissues of competitively stressed mosquitoes

with greater ease than less stressed individuals. Support for this hypothesis would come from an experiment showing that radiolabeled virus in bloodmeals can be detected at greater levels in the midgut and hemocoel of competitively stressed mosquitoes than in less stressed individuals shortly after engorgement (Weaver et al. 1991). Additional support would come from morphological detection, via electron microscopy (Grimstad and Walker 1991), of differences in midgut tissues such as perforations or basal laminae thickness, between competitively stressed and unstressed individuals.

Other Epidemiologically Significant Factors: Adult Survival

Aedes albopictus had much greater viral body titer compared to *A. aegypti*, yet it was a poorer disseminator of DENV (Chapter 4, Fig. 4-4). These results were unexpected and may represent species-specific differences in viral selection pressure within the vector host. Host-parasite theory suggests parasite/pathogen load is inversely related to host fitness (Anderson and May 1978, May and Anderson 1978). Although arboviruses are assumed to have little negative effects on their arthropod vectors, many exceptions are known, such as retarded larval development (Beaty et al. 1980, Tesh 1980), reduced adult longevity, including DENV-3 infected *A. aegypti* (e.g., Joshi et al. 2002, Faran et al. 1987), fecundity (e.g., Turell et al. 1985, Tesh 1980), and damage to the salivary glands with reduced ability to re-feed (e.g., Platt et al. 1997, Turell et al. 1985, Grimstad et al. 1980, Mims et al. 1966). No comparative studies exist for the effect of DENV infection on adult *A. aegypti* and *A. albopictus* longevity. *Aedes* species-specific differences in DENV body titer pose new questions and opportunities for understanding the relative roles of *A. aegypti* and *A. albopictus* in DENV transmission. The widespread expansion of *A. albopictus* in the last three decades has increased the number of locations where these *Aedes* species are sympatric and dengue is endemic. A

clearer understanding of the relative importance of these species in DENV maintenance and transmission will improve dengue prediction and control.

It is assumed that the more anthropophilic *A. aegypti* is the more important dengue vector. However, it is likely that other factors are involved in determining vector potential, and the relative importance of *A. aegypti* and *A. albopictus* may depend on ecological conditions, including host preference and availability, larval nutrition, adult temperature and relative humidity. One readily testable hypothesis involves DENV-induced differences in vector longevity. Measurements of vectorial capacity, the daily rate at which future pathogen inoculations arise from a current infective case, are especially sensitive to changes in the: (1) probability a vector feeds on a host in a given day, (2) duration of the extrinsic incubation period, and (3) daily survivorship rate of the vector. Even small changes in DENV-induced adult survivorship (Fernandez et al. 2003) may result in relatively large differences in vectorial capacity estimates for *A. albopictus* and *A. aegypti* (Dye 1986). A testable hypothesis is that *A. aegypti* is, in part, a more efficient DENV transmitter because *Aedes* species-specific differences in viral load lead to species-specific differences in adult survivorship. The first step in addressing this hypothesis would be to establish that a negative relationship exists between dengue viral load and adult survivorship. This hypothesis predicts that the greater viral load carried by *A. albopictus* results in a greater reduction in adult survivorship compared to *A. aegypti*. An experimental test of this hypothesis would involve offering adult *Aedes* bloodmeals containing DENV or lacking DENV (i.e., control). Measurements would be made on adult DENV body titer and daily survivorship. Support for the hypothesis would come from detecting an interaction between species treatment (*A. albopictus* and *A. aegypti*)

and bloodmeal (DENV infected and uninfected) showing that *A. albopictus* was more detrimentally affected by DENV infection than *A. aegypti*.

Other Ecological Interactions in the Larval Stages

These competition experiments attempted to describe the importance of a biological interaction i.e., competition, as a contributor to vector competence. Other potentially important biological interactions that may affect adult mosquito vector competence include larval parasitism (e.g., Bedhomme et al. 2005, Agnew et al. 1999, Washburn et al. 1991), apparent competition (e.g., Juliano and Lounibos 2005), and predation (e.g., Griswold and Lounibos 2005, Kesavaraju and Juliano 2004). Take for example predation, like competition, is widespread and plays an important role in determining mosquito performance and represents an untested ecological factor that may alter mosquito susceptibility to arboviral infection. Reduction in survival is perhaps the most obvious, and readily measurable, outcome of predation. However, other population growth measurements (development time, size at emergence) may be influenced by predation and interact with each other in determining survivorship. For example, predation typically reduces prey development time due to preferential consumption of slowly developing prey as well as release from competition. Future studies on predation should initially establish whether larval predation alters arboviral infection parameters for surviving adult mosquitoes compared to predator-free conditions. These studies would also address whether there were similarities in the associations between population growth measurements between predator- and competition-induced differences in infection parameters.

Relationships between population growth measurements and predation treatments in determining infection parameters are likely to differ for mosquito prey species that

show facultative changes in behavioral responses to predation (e.g., *O. triseriatus*) compared to prey species that show little change in behavior (e.g., *A. albopictus*). A behavioral study on water-borne cues from a predator induced more frequent low-risk behaviors in native mosquito *O. triseriatus* but not invasive *A. albopictus* (Kesavaraju and Juliano 2004). *Aedes albopictus* outcompetes *O. triseriatus*, however, predator-mediated coexistence, attributable to differences in prey behavioral response, may promote coexistence between these two competing species (Griswold and Lounibos 2005, Juliano and Lounibos 2005). A more detailed manipulation of predation, or in combination with competition, treatments might include actual versus perceived predation (e.g., caged predator) which would hold survivorship constant and allow for a more detailed description of the relationship of other population growth measurements (size, development time) and infection parameters. Thus, this later treatment would identify predator-induced indirect effects (e.g., behavioral, morphological: Relyea 2000) that alter mosquito vector infection parameters.

Conclusions

Competition in the larval stages enhanced arboviral infection parameters of adult *A. albopictus* using a model (SINV) and natural (DENV) arbovirus-vector system. These effects may apply generally to mosquito-virus systems suggesting that larval conditions are an important aspect of vector competence and should be included in future considerations of arbovirus transmission. These results, coupled with future experiments, may lead to a clearer understanding of the relationship between larval ecology and adult vector competence and vectorial capacity.

LIST OF REFERENCES

Abrams, P.A. 1995. Implications of dynamically variable traits for identifying, classifying and measuring direct and indirect effects in ecological communities. American Naturalist **146**: 112-134.

Agnew, P., S. Bedhomme, C. Haussy, and Y. Michalakis. 1999. Age and size at maturity of the mosquito *Culex pipiens* infected by microsporidian parasite *Vavraia culicis*. Proceedings of the Royal Society of London Series B **266**: 947-952.

Ahmed, A.M., S.L. Baggott, R. Maingon, and H. Hurd. 2002. The costs of mounting an immune response are reflected in the reproductive fitness of the mosquito *Anopheles gambiae*. Oikos **97**: 371-377.

Alto, B.W., L.P. Lounibos, S. Higgs, and S.A. Juliano. 2005a. Larval competition differentially affects arbovirus infection in *Aedes* mosquitoes. Ecology **86**: 3279-3288.

Alto, B.W., S.P. Yanoviak, L.P. Lounibos, and B.G. Drake. 2005b. Effects of elevated atmospheric $C0_2$ on water chemistry and mosquito growth under competitive conditions in container habitats. Florida Entomologist **88**: 372-382.

Alto, B.W., L.P. Lounibos, and S.A. Juliano. 2003. Age-dependent bloodfeeding of *Aedes aegypti* and *Aedes albopictus* on artificial and living hosts. Journal of the American Mosquito Control Association. **19**: 347-352.

Alto B.W., and S.A. Juliano. 2001a. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. Journal of Medical Entomology **38**: 548-556.

Alto B.W., and S.A. Juliano. 2001b. Precipitation and temperature effects on populations of *Aedes albopictus* (Diptera: Culicidae): implications for range expansion. Journal of Medical Entomology **38**: 646-656.

Altwegg, R. 2002. Trait-mediated indirect effects and complex life-cyles in two European frogs. Evolutionary and Ecology Research **4**: 519-536.

Anderson, R.M. and R.M. May. 1978. Regulation and stability of host-parasite population interactions. I. Regulatory Processes. Journal of Animal Ecology. **47**: 219-247.

Armstrong, P.M., and R. Rico-Hesse. 2003. Efficiency of dengue serotype 2 virus strains to infect and disseminate in *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **68**: 539-544.

Armbruster, P., and R.A. Hutchinson. 2002. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae). Journal of Medical Entomology **39**: 699-704.

Armstrong, P.M., and R. Rico-Hesse. 2001. Differential susceptibility of *Aedes aegypti* to infection by the American and Southeast Asian genotypes of dengue type 2 virus. Vector Borne and Zoonotic Diseases **1**: 159-168.

Baqar, S., C.G. Hayes, and T. Ahmed. 1980. The effect of larval rearing conditions and adult age on the susceptibility of *Culex tritaeniorhynchus* to infection with West Nile virus. Mosquito News **40**: 165-171.

Barrera, R. 1996a. Species concurrence and the structure of a community of aquatic insects in tree holes. Journal of Vector Ecology **21**: 66-80.

Barrera, R. 1996b. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecological Entomology **21**: 117-127.

Barrera, R., M. Amador, and G.C. Clark. 2006. Ecological factors influencing *Aedes aegypti* (Diptera: Culicidae) productivity in artificial containers in Salinas, Puerto Rico. Journal of Medical Entomology **43**: 484-492.

Barth, O.M., and H.G. Schatzmayr. 1992. Brazilian dengue virus type 1 replicating in mosquito cell cultures. Memórias do Instituto Oswaldo Cruz **87**: 1-7.

Beaty, B.J., R.B. Tesh, and T.H.G. Aitken. 1980. Transovarial transmission of yellow fever virus in *Stegomyia* mosquitoes. American Journal of Tropical Medicine and Hygiene **29**: 125-132.

Beck, M.A., and O.A. Levander. 2000. Host nutritional status and its effect on a viral pathogen. The Journal of Infectious Diseases **182** (Suppl 1): S93-S96.

Bedhomme, S., P. Agnew, C. Sidobre, and Y. Michalakis. 2005. Prevalence-dependent costs of parasite virulence. Public Library of Science Biology **3**: 1403-1408.

Bedhomme, S., P. Agnew, C. Sidobre, and Y. Michalakis. 2004. Virulence reaction norms across a food gradient. Proceedings of the Royal Society of London, Series B **271**: 739-744.

Bennett, K.E., D. Flick, K.H. Fleming, R. Jochim, B.J. Beaty, and W.C. Black, IV. 2005a. Quantitative trait loci that control dengue-2 virus dissemination in the mosquito *Aedes aegypti*. Genetics **170**: 185-194.

Bennett, K.E., B.J. Beaty, and W.C. Black, IV. 2005b. Selection of D2S3, an *Aedes aegypti* (Diptera: Culicidae) strain with high oral susceptibility to dengue 2 virus and D2MEB, a strain with a midgut barrier to dengue 2 escape. Journal of Medical Entomology **42**: 110-119.

Bennett, K.E., K.E. Olson, M. de L. Munoz, I. Fernandez-Sala, J. A. Farfan-Ale, S. Higgs, W.C. Black IV, and B.J. Beaty. 2002. Variation in vector competence for dengue 2 virus among 24 collections of *Aedes aegypti* from Mexico and the United States. American Journal of Tropical Medicine and Hygiene **67**: 85-92.

Birch, L.C. 1953. Experimental background to the study of the distribution and abundance of insects. III. The relation between innate capacity for increase and survival of different species of beetles living together on the same food. Evolution **7**: 136-144.

Birungi, J., and L.E. Munstermann. 2002. Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: Evidence for an independent invasion into Brazil and United States. Annals of the Entomological Society of America **95**: 125-132.

Bishop A, and B.M. Gilchrist. 1946. Experiments upon the feeding of *Aedes aegypti* through nimal membranes with a view to applying this method to the chemotherapy of malaria. Parasitology **37**: 85-100.

Black, W.C. IV, K.E. Bennett, N. Gorrochotegui-Escalante, C.V. Barillas-Mury, I. Fernandez-Salas, M. de L. Munoz, J.A. Farfan-Ale, K.E. Olson, and B.J. Beaty. 2002. Flavivirus susceptibility in *Aedes aegypti*. Archives of Medical Research **33**: 379-388.

Black, W.C., IV, K.S. Rai, B.J. Turco, and D.C. Arroyo. 1989. Laboratory study of competition between United States strains of *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **32**: 847-852.

Blackmore, M.S. and C.C. Lord. 2000. The relationship between size and fecundity in *Aedes albopictus*. Journal of Vector Ecology **25**: 212-217.

Boromisa, R.D., K.S. Rai, and P.R. Grimstad. 1987. Variation in the vector competence of geographic strains of *Aedes albopictus* for dengue 1 virus. Journal of the American Mosquito Control Association **3**: 378-386.

Boorman, J. 1960. Observations on the amount of virus present in the hemolymph of *A. aegypti* infected with Uganda S, yellow fever and Semlikie Forest virus. Transactions of the Royal Society of Tropical Medicine and Hygiene **54**: 362-365.

Bosio, C.F., R.E. Fulton, M.L. Salasek, B.J. Beaty, and W.C. Black. 2000. Quantitative trait loci that control vector competence for dengue-2 virus in the mosquito *Aedes aegypti*. Genetics **156**: 687-698.

Bosio, C.F., B.J. Beaty, and W.C. Black. 1998. Quantitative genetics of vector competence for dengue-2 virus in *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **59**: 965-970.

Bosio, C.F., R.E. Thomas, P.R. Grimstad, and K.S. Rai. 1992. Variation in the efficiency of vertical transmission of dengue-1 virus by strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology **29**: 985-989.

Bowen M.F. 1991. The sensory physiology of host-seeking behavior in mosquitoes. Annual Review of Entomology **36**: 139-158.

Bowers, D.F., C.G. Coleman, and D.T. Brown. 2003. Sindbis virus-associated pathology in *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology **40**: 698-705.

Bowers, D.F., B.A. Abell, and D.T. Brown. 1995. Replication and tissue tropism of the Alphavirus Sindbis in the mosquito *Aedes albopictus.* Virology **212**: 1-12.

Bradshaw, W.E., and C.M. Holzapfel. 1986. Geography of density-dependent selection in pitcher-plant mosquitoes. Pages 48-65 *in* F. Taylor and R. Karban, editors. The evolution of insect life cycles. Springer, New York, NY.

Braks, M.A. H., N.A. Honório, L.P. Lounibos, R. Lourenco-de-Oliveira, and S.A. Juliano. 2004. Interspecific competition between two invasive species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. Annals of the Entomological Society of America **97**: 130-139.

Briegel, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. Journal of Insect Physiology **36**: 165-172.

Briegel, H. 1985. Mosquito reproduction: Incomplete utilization of the blood meal protein for oögenesis. Journal of Insect Physiology **31**: 15-21.

Briegel, H., and S.E. Timmermann. 2001. *Aedes albopictus* (Diptera: Culicidae): Physiological aspects of development and reproduction. Journal of Medical Entomology **38**: 566-571.

Broadie, K.S., and W.E. Bradshaw. 1991. Mechanisms of interference competition in the western tree-hole mosquito, *Aedes sierrensis*. Ecological Entomology **16**: 145-154.

Broberg, L., and W.E. Bradshaw. 1995. Density-dependent development in *Wyeomyia smithii* (Diptera, Culicidae) – Intraspecific competiton is not the result of interference. Annals of the Entomological Society of America **88**: 465-470.

Brummer-Korvenkontio, M., O. Vapalahti, P. Kuusisto, P. Saikku, T. Manni, P. Koskela, T. Nygren, H. Brummer-Korvenkontio, and A. Vaheri. 2002. Epidemiology of Sindbis virus infection in Finland 1981-96: possible factors explaining a peculiar disease pattern. Epidemiology and Infection **129**: 335-345.

Brummer-Korvenkontio, M., P. and Kuusisto. 1981. Onko Suomen Länsiosa säästynyt "Pogostalta" (Has western Finland been spared the "Pogosta"?). Suom Lääkäril **32**: 2606-2607.

Buckley, A., A. Dawson, S.R. Moss, S.A. Hinsley, P.E. Bellamy, and E.A. Gould. 2003. Serological evidence of *West Nile virus*, *Usutu virus* and *Sindbis* virus infection of birds in the UK. Journal of General Virology **84**: 2807-2817.

Burgess, L. 1959. Probing behaviour of *Aedes aegypti* (L.) in response to heat and moisture. Nature **184**: 1968-1969.

Bustin, S.A. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. Journal of Molecular Endocrinology **25**: 169-193.

Butler, J.F., W.R. Hess, R.G. Endris, and K.H. Holscher. 1984. *In vitro* feeding of *Ornithodoros* ticks for rearing and assessment of disease transmission. Acarology **VI**: 1075-1081.

Byers, J.E., and L. Goldwasser. 2001. Exposing the mechanism and timing of impact of nonindigenous species on native species. Ecology **82**: 1330-1343.

Callahan, J.D., S-J. L. Wu, A. Dion-Schultz, B.E. Mangold, L.F. Peruski, D.M. Watts, K.R. Porter, G.R. Murphy, W. Suharyono, C-C King, C.G. Hayes, and J.J. Temenak. 2001. Development and evaluation of serotype- and group-specific flourogenic reverse transcriptase PCR (TaqMan) assays for dengue virus. Journal of Clinical Microbiology **39**: 4119-4124.

Carpenter, S.R. 1983. Resource limitation of larval treehole mosquitoes subsisting on beech detritus. Ecology **64**: 219-223.

Carpenter, S.R. 1982a. Stemflow chemistry: Effects on population dynamics of detritivorous mosquitoes in tree-hole ecosystems. Oecologia **53**: 1-6.

Carpenter, S.R. 1982b. Comparisons of equations for decay of leaf litter in tree-hole ecosystems. Oikos **39**: 17-22

Chandler, L.J., C.D. Blair, and B.J. Beaty. 1998. La Crosse virus infection of *Aedes triseriatus* (Diptera: Culicidae) ovaries before dissemination of virus from the midgut. Journal of Medical Entomology **35**: 567-572.

Chatfield, C. 1989. The analysis of time series. An introduction. Chapman and Hall, New York, NY.

Chen, W-J, H-L. Wei, E-L. Hsu, and E-R. Chen. 1993. Vector competence of *Aedes albopictus* and *Ae. aegypti* (Diptera: Culicidae) to Dengue 1 virus on Taiwan: Development of the virus in orally and parenterally infected mosquitoes. Journal of Medical Entomology **30**: 524-530.

Christophers, R.C. 1960. *Aedes aegypti*. The yellow fever mosquito: Its life history, bionomics, and structure. Cambridge University Press, New York, NY.

Clements, A.N. 1999. The biology of mosquitoes, volume 2, sensory reception and behaviour. CABI Publishing, New York, NY.

Cloe, W.W., III, and G.C. Garman. 1996. The energetic importance of terrestrial arthropod inputs to three warm-water streams. Freshwater Biology **36**: 105-114.

Cologna, R., P.M. Armstrong, and R. Rico-Hesse. 2005. Selection for virulent dengue viruses occurs in humans and mosquitoes. Journal of Virology **79**: 853-859.

Cologna, R., and R. Rico-Hesse. 2003. American genotype structures decrease dengue virus output from human monocytes and dendritic cells. Journal of Virology **77**: 3929-3938.

Connell, J.H. 1983. On the prevalence and relative importance of interspecific competition: Evidence from field experiments. American Naturalist **122**: 661-696.

Connell, J.H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology **42**: 710-723.

Costanzo, K.S., B. Kesavaraju, and S.A. Juliano. 2005a. Condition-specific competition in container mosquitoes: The role of noncompeting life-history stages. Ecology **86**: 3289-3295.

Costanzo, K.S., K. Mormann, and S.A. Juliano. 2005b. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). Journal of Medical Entomology **42**: 559-570.

Craig, G.B., Jr., and R.C. Vandehey. 1962. Genetic variability in *Aedes aegypti* (Diptera: Culicidae) I. Mutations affecting color pattern. Annals of the Entomological Society of America **55**: 47-58.

Crombie, A.C. 1947. Interspecific competition. Journal of Animal Ecology **16**: 44-73.

D'Antonio, C.M., and P.M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics **23**: 63-87.

Daugherty, M.P., B.W. Alto, and S.A. Juliano. 2000. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **37**: 364-372.

Davis, E.E. 1984. Development of lactic acid-receptor sensitivity and host-seeking behaviour in newly emerged female *Aedes aegypti* mosquitoes. Journal of Insect Physiology **30**: 211-215.

DeFoliart, G.R., P.R. Grimstad, and D.M. Watts. 1987. Advances in mosquito-borne arbovirus/vector research. Annual Review of Entomology **32**: 479-505.

De Silva, A.M., W.P.J. Dittus, P.H. Amerasinghe, and F.P. Amerasinghe. 1999. Serologic evidence for an epizootic dengue virus infecting Toque Macaques (*Macaca sinica*) at Polonnaruwa, Sri Lanka. American Journal of Tropical Medicine and Hygiene **60**: 300-306.

de Thoisy, B., P. Dussrt, and M. Kazanji. 2004. Wild terrestrial rainforest mammals as potential reservoirs for flaviviruses (yellow fever, dengue 2 and St. Louis encephalitis viruses) in French Guiana. Transactions of the Royal Society of Tropical Medicine and Hygiene **98**: 409-412.

Diallo M., Y. Ba, A.A. Sall, O.M. Diop, J.A. Ndione, M. Mondo, L. Girault, and C. Mathiot. 2003. Amplification of the sylvatic cycle of dengue virus type 2, Senegal, 1999-2000: Entomologic findings and epidemiologic considerations. Emerging Infectious Diseases **9**: 362-367.

Dieng, H, C. Mwandawiro, M. Boots, R. Morales, T. Satho, N. Tuno, Y. Tsuda, and M. Takagi. 2002. Leaf litter decay process and the growth performance of *Aedes albopictus* larvae (Diptera: Culicidae). Journal of Vector Ecology **27**: 31-38.

Dimopoulos, D. 2003. Insect immunity and its implication in mosquito – malaria interactions. Cellular Microbiology **5**: 3-14.

Dohm, D.J., T.M. Logan, J.F. Barth, and M.J. Turell. 1995. Laboratory transmission of Sindbis virus by *Aedes albopictus*, *Ae. aegypti*, and *Culex pipiens* (Diptera: Culicidae). Journal of Medical Entomology **32**: 818-821.

Draper, N.R., and H. Smith. 1966. Applied regression analysis. Wiley, New York, NY.

Dye, C. 1986. Vectorial Capacity: must we measure all its components? Parisitology Today **2**: 203-208.

Dye, C. 1984. Competition amongst larval *Aedes aegypti*: the role of interference. Ecological Entomology **9**: 355-357.

Dye, C. 1982. Intraspecific competition amongst larval *Aedes aegypti*: food exploitation or chemical interference? Ecological Entomology **7**: 39-46.

Eads, R.B. 1972. Recovery of *Aedes albopictus* from used tires shiped to United States ports. Mosquito News **32**:113-114.

Eritja, R., R. Escosa, J. Lucientes, E. Marquès, R. Molina, D. Roiz, and S. Ruiz. 2005. Worldwide invasion of vector mosquitoes: present European distribution and challenges for Spain. Biological Invasions **7**: 87-97.

Espmark, A., and B. Niklasson. 1984. Ockelbo disease in Sweden: epidemiological, clinical, and virological data from the 1982 outbreak. American Journal of Tropical Medicine and Hygiene **33**: 1203-1211.

Failloux, A-B, M. Vazeille, and F. Rodhain. 2002. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. Journal of Molecular Evolution **55**: 653-663.

Faran, M.F., M.J. Turell, W.S. Romoser, R.G. Routier, P.H. Gibbs, T.L. Cannon, and C.L. Bailey. 1987. Reduced survival of adult *Culex pipiens* infected with Rift Valley Fever virus. American Journal of Tropical Medicine and Hygiene **37**: 403-409.

Fernández, Z., A.C. Moncayo, A.S. Carrara, O.P. Forattini, and S.C. Weaver. 2003. Vector competence of rural and urban strains of *Aedes* (Stegomyia) *albopictus* (Diptera: Culicidae) from São Paulo State, Brazil for IC, ID, and IF subtypes of Venezuelan equine encephalitis virus. Journal of Medical Entomology **40**: 522-527.

Fish, D. 1983. Phytotelmata: flora and fauna. Pages 1-27 *in* J.H. Frank and L.P. Lounibos, editors. Phytotelmata: terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey.

Fish, D., and S.R. Carpenter. 1982. Leaf litter and larval mosquito dynamics in tree-hole ecosystems. Ecology **63**: 283-288.

Focks, D.A., R.J. Brenner, J. Hayes, and E. Daniels. 2000. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. American Journal of Tropical Medicine and Hygiene **62**: 11-18.

Focks, D.A, E. Daniels, D.G. Haile, and J.E. Keesling. 1995. A simulation model of the epidemiology of urban dengue fever: Literature analysis, model development, preliminary validation, and samples of simulation results. American Journal of Tropical Medicine and Hygiene **53**: 489-506.

Francy, D.B., T.G.T. Jaenson, J.O. Lundstrom, E.B. Schildt, A. Espmark, B. Henriksson, and B. Niklasson. 1989. Ecological studies of mosquitoes and birds as hosts of Ockelbo virus in Sweden and isolations of Inkoo and Batai viruses from mosquitoes. American Journal of Tropical Medicine and Hygiene **41**: 355-363.

Frank, J.H., and L.P. Lounibos. 1983. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, New Jersey.

Freier, J.E., and L. Rosen. 1988. Vertical transmission of dengue viruses by *Aedes mediovittatus*. American Journal of Tropical Medicine and Hygiene **39**: 218-222.

Gimnig, J.E., M. Ombok, S. Otieno, M.G. Kaufman, J.M. Vulule, and E.D. Walker. 2002. Density-dependent development of *Anopheles gambiae* (Diptera: Culicidae) larvae in artificial habitats. Journal of Medical Entomology **39**: 162-172.

Gleiser, R.M., J. Urrutia, and D.E. Gorla. 2000a. Effects of crowding on populations of *Aedes albifasciatus* larvae under laboratory conditions. Entomologia Experimentalis et Applicata **95**: 135-140.

Gleiser, R.M., J. Urrutia, and D.E. Gorla. 2000b. Body size variation of the floodwater mosquito *Aedes albifasciatus* in central Argentina. Medical and Veterinary Entomology **14**: 38-43.

Gomez-Machorro, C., K.E. Bennett, M. del L. Munoz, and W.C. Black, IV. 2004. Quantitative trait loci affecting dengue midgut infection barriers in an advanced intercross line of *Aedes aegypti*. Insect Molecular Biology **13**: 637-648.

Gorrochotegui-Escalante, N. S. Lozano-Fuentes, K.E. Bennett, A. Molina-Cruz, B.J. Beaty, and W. Black IV. 2005. Association mapping of segregating sites in the early trypsin gene and susceptibility to dengue-2 virus in the mosquito *Aedes aegypti*. Insect Biochemistry and Molecular Biology **35**: 771-788.

Gresikova, M., M. Sekeyova, G. Tempera, S. Guglielmino, and A. Castro. 1978. Identification of a Sindbis virus strain isolated from *Hyaloma marginatum* ticks in Sicily. Acta Virol. (Praha) **22**: 231-232.

Grimstad, P.R., and E.D. Walker. 1991. *Aedes triseriatus* (Dipera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. Journal of Medical Entomology **28**: 378-386.

Grimstad, P.R., S.L. Paulson, and G.B. Craig, Jr. 1985. Vector competence of *Aedes hendersoni* (Diptera: Culicidae) for La Crosse virus and evidence of a salivary-gland escape barrier. Journal of Medical Entomology **22**: 447-453.

Grimstad, P.R. and L.D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. III. Enhanced oral transmission by nutrition-deprived mosquitoes. Journal of Medical Entomology **21**: 249-256.

Grimstad, P.R., Q.E. Ross, and G.B. Craig, Jr. 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus II. Modification of mosquito feeding behavior by virus infection. Journal of Medical Entomology. **17**: 1-7.

Griswold, M.W., and L.P. Lounibos. 2005. Does differential predation permit invasive and native mosquito larvae to coexist in Florida? Ecological Entomology **30**: 122-127.

Grover, J.P. 1997. Resource competition. Chapman and Hall, New York, NY.

Gubler, D.J. 2002. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends in Microbiology **10**: 100-103.

Gubler, D.J. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. Pages 1-22 *in* D.J. Gubler and G. Kuno, editors. Dengue and dengue hemorrhagic fever. CABI Publishing, New York, NY.

Gubler, D.J., and G. Kuno. 1997. Dengue and dengue hemorrhagic fever. CABI Publishing, New York, NY.

Gubler, D.J., W. Suharyono, R. Tan, M. Abidin, and A. Sie. 1981. Viraemia in patients with naturally acquired dengue infection. Bulletin of the World Health Organization **59**: 623-630.

Gubler, D.J., S. Nalim, R. Tan, H. Saipan, and J. Sulianti Saroso. 1979. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene. **28**: 1045-1052.

Gubler, D.J., and L. Rosen. 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. American Journal of Tropical Medicine and Hygiene **25**: 318-325.

Hancock R.G., and Foster W.A. 2000. Exogenous juvenile hormone and methoprene, but not male accessory gland substances or ovariectomy, affect the blood/nectar choice of female *Culex nigripalpus* mosquitoes. Medical Veterinary Entomology **14**: 376-382

Hairston, N.G. 1951. Interspecific competition and its probable influence upon the vertical distribution of Appalachian salamanders of the genus *Plethodon*. Ecology **32**: 266-274.

Hairston, N.G., F.E. Smith, and L.B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist **94**: 421-425.

Haramis, L.D. 1985. Larval nutrition, adult body size, and the biology of *Aedes triseriatus*. Pages 431-437 *in* L.P. Lounibos , J.R. Rey, and J.H Frank, editors. Ecology of mosquitoes: Proceedings of a workshop. Florida Medical Entomology Laboratory, Vero Beach, FL.

Haramis, L.D. 1983. Increased adult size correlated with parity in *Aedes triseriatus*. Mosquito News **43**: 77-79.

Hardy, J.L. 1988. Susceptibility and resistance of vector mosquitoes. Pages 87-126 *in* T.P. Monath, editor. The arboviruses: Epidemiology and ecology. CRC Press, Boca Raton, FL.

Hardy, J.L., E.J. Houk, L.D. Kramer, and W.C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annual Review of Entomology **28**: 229-262.

Hardy, J.L., G. Apperson, S. M. Asman, and W.C. Reeves. 1978. Selection of a strain of *Culex tarsalis* highly resistant to infection following ingestion of Western equine encephalomyelitis virus. American Journal of Tropical Medicine and Hygiene **27**: 313-321.

Harrington, L.A., T.W. Scott, K. Lerdthusnee, R.C. Coleman, A. Costero, G.G. Clark, J.J. Jones, S. Kitthawee, P. Kittayapong, R. Sithiprasasna, and J.D. Edman. 2005. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. American Journal of Tropical Medicine and Hygiene **72**: 209-220.

Hase, T., P.L. Summers, and K.H. Eckels. 1989. Flavivirus entry into cultured mosquito cells and human peripheral blood monocytes. Archives of Virology **104**: 129-143.

Hawley, W.A. 1988. The biology of *Aedes albopictus*. Journal of the American Mosquito Control Association **4** (Supplement): 1-40.

Hawley, W.A. 1985. The effect of larval density on adult longevity of a mosquito, *Aedes sierrensis*: epidemiological consequences. Journal of Animal Ecology **54**: 955-964.

Hawley, W.A., P. Reiter, R.S. Copeland, C.B. Pumpuni, and G.B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable introduction in used tires from Northern Asia. Science **236**: 1114-1116.

Heard, S.B. 1994. Pitcher-plant midges and mosquitoes: a processing chain commensalism. Ecology **75**: 1647-1660.

Higgs, S. 2004. How do mosquito vectors live with their viruses? Pages 103-137 *in* S.H. Gillespie, G.L. Smith, and A. Osbourn, editors. .Microbe-Vector Interactions in Vector-Borne Diseases. Cambridge University Press, Cambridge, UK.

Ho, B.C., A. Ewert, and L. Chew. 1989. Interspecific competition among *Aedes aegypti*, *Ae. albopictus*, and *Ae. triseriatus* (Diptera: Culicidae): larval development in mixed cultures. Journal of Medical Entomology **26**: 615-623.

Hobbs J.H., Hughes E.A., and B.H. Eichold, II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile Alabama. Journal of the American Mosquito Control Association **7**: 488-489.

Holland, P.M., R.D. Abramson, R. Watson, and D.H. Gelfand. 1991. Detection of specific polymerase chain reaction product by utilizing the 5′----3′ exonuclease activity of *Thermus aquaticus* DNA polymerase. Proceedings of the National Academy of Sciences **88**: 7276-7280.

Holway, D.A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive Argentine ant. Ecology **80**: 238-251.

Honório, N.A., W.C. Silva, P.J. Leite, J.M. Gonçalves, L.P. Lounibos, and R. Lourenco-de-Oliveira. 2003. Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an Urban endemic dengue area in the State of Rio de Janeiro, Brazil. Memorias do Instituto Oswaldo Cruz **98**: 191-198.

Hornby, J.A., Moore D.E., and Miller T.W., Jr. 1994. *Aedes albopictus* distribution, abundance, and colonization in Lee County, Florida, and its effect on *Aedes aegypti*. Journal of the American Mosquito Control Association **10**: 397-402.

Houk, E.J., L.D. Kramer, J.S. Hardy, and S.B. Presser. 1986. An interspecific mosquito model for the mesenteronal infection barrier to Western equine encephalomyelitis virus (*Culex tarsalis* and *Culex pipiens*). American Journal of Tropical Medicine and Hygiene **35**: 632-641.

Houk, E.J., and J.L. Hardy. 1979. In vivo negative staining of the midgut continuous junction in the mosquito, *Culex tarsalis* (Diptera: Culicidae). Acta Tropica **36**: 267-275.

Innis, B.L. 1997. Antibody responses to dengue virus infection. Pages 245-271 *in* D.J. Gubler and G. Kuno, editors. Dengue and dengue hemorrhagic fever. CABI publishing, New York, NY.

Jackson, A.C., J.C. Bowen, and A.E.R. Downe. 1993. Experimental infection of *Aedes aegypti* (Diptera: Culicidae) by the oral route with Sindbis virus. Journal of Medical Entomology **30**: 332-337.

Jaenson, T.G.T., and B. Niklasson. 1986. Feeding patterns of mosquitoes (Diptera: Culicidae) in relation to the transmission of Ockelbo disease in Sweden. Bulletin of Entomological Research **76**: 375-383.

Joshi, V., R.C. Sharma, Y. Sharma, S. Adha, K. Sharma, H. Singh, A. Purohit, and M. Singhi. 2006. Importance of socioeconomic status and tree holes in distribution of *Aedes* mosquitoes (Diptera: Culicidae) in Jodhpur, Rajasthan, India. Journal of Medical Entomology **43**: 330-336.

Joshi, V., D.T. Mourya, and R.C. Sharma. 2002. Persistence of dengue-3 through transovarial transmission passage in successive generations of *Aedes aegypti* mosquitoes. American Journal of Tropical Medicine and Hygiene. **67**: 158-161.

Joshi, V., M. Singhi, R.C. Chaudhary. 1996. Transovarial transmission of dengue 3 by *Aedes aegypti*. Transactions of the Royal Society of Tropical Medicine and Hygiene **90**: 643-644.

Juliano, S.A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? Ecology **79**: 255-268.

Juliano, S.A., and L.P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecology Letters **8**: 558-574.

Juliano, S.A., L.P. Lounibos, and G.F. O'Meara. 2004. A field test for competitive effects of *Aedes albopictus* on *Aedes aegypti* in south Florida: differences between sites of coexistence and exclusion? Oecologia **139**: 583-593.

Juliano, S.A., G.F. O'Meara, J.R. Morrill, and M.M. Cutwa. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia **130**: 458-469.

Jupp, P.G., N.K. Blackburn, D.L. Thompson, and G.M. Meenehan. 1986a. Sindbis and West Nile virus infections in the Witwatersrand-Pretoria region. South African Journal of Medical Science **70**: 218-220.

Jupp, P.G., B.M. McIntosh, and N.K. Blackburn. 1986b. Experimental assessment of the vector competence of *Culex* (*Culex*) *neavei* Theobald with West Nile and Sindbis viruses in South Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene **80**: 226-230.

Jupp, P.G., B.M. McIntosh, and D.B. Dickinson. 1972. Quantitative experiments on the vector capability of *Culex* (*Culex*) *theileri* Theobald with West Nile and Sindbis viruses. Journal of Medical Entomology **9**: 393-395.

Jupp, P.G., and B.M. McIntosh. 1970a. Quantitative experiments on the vector capability of *Culex* (*Culex*) *univittatus* Theobald with West Nile and Sindbis viruses. Journal of Medical Entomology **7**: 371-373.

Jupp, P.G., and B.M. McIntosh. 1970b. Quantitative experiments on the vector capability of *Culex* (*Culex*) *pipiens fatigans* Wiedemann with West Nile and Sindbis viruses. Journal of Medical Entomology **7**: 353-356.

Kaufman, M.G., E.D. Walker, T.W. Smith, R.W. Merritt, and M.J. Klug. 1999. Effects of larval mosquitoes (*Aedes triseriatus*) and stemflow on microbial community dynamics in container habitats. Applied and Environmental Microbiology **65**: 2661-2673.

Kay, B.H., J.D. Edman, I.D. Fanning, and P. Mottram. 1989. Larval diet and the vector competence of *Culex annulirostris* (Diptera: Culicidae) for Murray Valley encephalitis virus. Journal of Medical Entomology **26**: 487-488.

Kesavaraju, B., and S.A. Juliano. 2004. Differential behavioral responses to water-borne cues to predation in two container dwelling mosquitoes. Annals of the Entomological Society of America **97**:194-201

Khin, M.M. and K.A. Than. 1983. Transovarial transmission of dengue 2 virus by *Aedes aegypti* in nature. American Journal of Tropical Medicine and Hygiene **32**: 590-594.

Kiesecker, J.M., and D.K. Skelly. 2001. Effects of disease and pond drying on gray tree frog growth, development, and survival. Ecology **82**: 1956-1963.

Kitching, R.L. 1971. An ecological study of water-filled treeholes and their position in the woodland ecosystem. Journal of Animal Ecology **40**: 281-302.

Klowden, M.J., and N.M. Fernandez. 1996. Effects of age and mating on the host seeking behavior of *Aedes aegypti* mosquitoes. Journal of Vector Ecology **21**: 156-158.

Klowden, M.J., and H. Briegel. 1994. Mosquito gonotrophic cycle and multiple feeding potential: Contrasts between *Anopheles* and *Aedes*. Journal of Medical Entomology **31**: 618-622.

Klowden, M.J., and G.M. Chambers. 1992. Reproductive and metabolic differences between *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae). Journal of Medical Entomology **29**:467-471.

Klowden M.J., J.L. Blackmer, and G.M. Chambers. 1988. Effects of larval nutrition on the host-seeking behavior of adult *Aedes aegypti* mosquitoes. Journal of the American Mosquito Control Association **4**: 73-75.

Klowden M.J., and A.O. Lea. 1984. Blood feeding affects age-related changes in the host-seeking behavior of *Aedes aegypti* (Diptera: Culicidae) during oocyte maturation. Journal of Medical Entomology **21**: 274-277.

Klowden M.J., and A.O. Lea. 1979a. Humoral inhibition of host-seeking in *Aedes aegypti* during oocyte maturation. Journal of Insect Physiology **25**: 231-235.

Klowden M.J., and A.O. Lea. 1979b. Abdominal distention terminates subsequent host-seeking behaviour of *Aedes aegypti* following a blood meal. Journal of Insect Physiology **25**: 583-585.

Klowden M.J., and A.O. Lea. 1978. Blood meal size as a factor affecting continued host-seeking by *Aedes aegypti* (L.). American Journal of Tropical Medicine and Hygiene **27**: 827-831.

Koella, J.C., and F.L. Sørensen. 2002. Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*. Medical and Veterinary Entomology **16**: 316-320.

Kolivras, K.N. 2006. Mosquito habitat and dengue risk potential in Hawaii: A conceptual framework and GIS application. The Professional Geographer **58**: 139-154.

Kramer, L.D., J.L. Hardy, S.B. Presser, and E.J. Houk. 1981. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. American Journal of Tropical Medicine and Hygiene **30**: 190-197.

Kuno, G. 1997. Factors influencing the transmission of dengue viruses. Pages 61-88 *in* D.J. Gubler and G. Kuno, editors. Denuge and dengue hemorrhagic fever. CABI Publising, New York, NY.

Kurkela, S., T. Manni, J. Myllynen, A. Vaheri, and O. Vapalahti. 2005. Clinical and laboratory manifestations of Sindbis virus infection: Prospective study, Finland, 2002-2003. Journal of Infectious Diseases **191**: 1820-1828.

Kurkela, S., T. Manni, A. Vaheri, and O. Vapalahti. 2004. Causative agent of Pogosta disease isolated from blood and skin lesions. Emerging Infectious Diseases **10**: 889-894.

Lafferty, K.D., and R.D. Holt. 2003. How should environmental stress affect the population dynamics of disease? Ecology Letters **6**: 654-664.

Laine, M., R. Luukkainen, J. and A. Toivanen. 2004. Sindbis viruses and other alphaviruses as cause of human arthritic disease. Journal of Internal Medicine **256**: 457-471.

Laine, M., R. Luukkainen, J. Jalava, J. Ilonen, P. Kuusisto, and A. Toivanen. 2000. Prolonged arthritis associated with Sindbis-related (Pogosta) virus infection. Rheumatology **39**: 1272-1274.

Leitmeyer, K.C., D.W. Vaughn, D.M. Watts, R. Salas, I. Villalobos de Chacon, C. Ramos, and R. Rico-Hesse. 1999. Dengue virus structural differences that correlate with pathogenesis. Journal of Virology **73**: 4738-4747.

Léonard, P.M., and S.A. Juliano. 1995. Effect of leaf litter and density on fitness and population performance of the hole mosquito *Aedes triseriatus*. Ecological Entomology **20**: 125-136.

Levine, J.M. 1999. Indirect facilitation: Evidence and predictions from a riparian community. Ecology **80**: 1762-1769.

Levine, J.M., M. Vila, C.M. D'Antonio, J.S. Dukes, K. Grigulis, and S. Lavorel. 2002. Mechanisms underlying the impacts of exotic plant invasions. Proceedings of the Royal Society of London B. **270**: 775-781.

Lewis, J.A., G-J Chang, R.S. Lanciotti, R.M. Kinney, L.W. Mayer, and D.W. Trent. 1993. Phylogenetic relationships of Dengue-2 viruses. Virology **197**: 216-224.

Livdahl, T.P. 1984. Interspecific interactions and the r-K continuum: laboratory comparisons of geographic strains of *Aedes triseriatus*. Oikos **42**: 193-202.

Livdahl, T.P. 1982. Competition within and between hatching cohorts of a treehole mosquito. Ecology **63**: 1751-1760.

Livdahl, T.P., and M.S. Willey. 1991. Prospects for an invasion: Competition between *Aedes albopictus* and native *Aedes triseriatus*. Science **253**: 189-191.

Livdahl, T.P., and G. Sugihara. 1984. Non-linear interactions of populations and the importance of estimating per capita rates of change. Journal of Animal Ecology **53**: 573-580.

Lively, C.M., S.G. Johnson, L.F. Delph, and K. Clay. 1995. Thinning reduces the effect of rust infection on jewelweed (*Impatiens capensis*). Ecology **76**: 1859-1862.

Lord, CC., C.R. Rutledge, and W.J. Tabachnick. 2006. Relationships between host viremia and vector susceptibility for arboviruses. Journal of Medical Entomology **43**: 623-630.

Lorenz, L., B.J. Beaty, T.H.G. Aitken, G.P. Wallis, and W.J. Tabachnick. 1984. The effect of colonization upon *Aedes aegypti* susceptibility to oral infection with yellow fever virus. American Journal of Tropical Medicine and Hygiene **33**: 690-694.

Lounibos, L.P. 2002. Invasions by insect vectors of human disease. Annual Review of Entomology **47**: 233-266.

Lounibos, L.P., R.L. Escher, and R. Lourenco-de-Oliveira. 2003a. Asymmetric evolution of photoperiodic diapause in temperature and tropical invasive populations of *Aedes albopictus* (Diptera: Culicidae). Annals of the Entomological Society of America **96**: 512-518.

Lounibos, L.P., G.F. O'Meara, N. Nishimura, and R.L. Escher. 2003b. Interactions with native mosquito larvae regulate the production of *Aedes albopictus* from bromeliads in Florida. Ecological Entomology **28**: 551-558.

Lounibos, L.P., S. Suarez, Z. Menendez, N. Nishimura, R.L. Escher, S.M. O'Connell, and J.R. Rey. 2002. Does temperature affect the outcome of larval competition between *Aedes aegypti* and *Aedes albopictus*? Journal of Vector Ecology **27**: 86-95.

Lounibos, L.P., N. Nishimura, and R.L. Escher. 1993. Fitness of a treehole mosquito: influences of food type and predation. Oikos **66**: 114-118.

Lounibos, L.P., N. Nishimura, and R.L. Escher. 1992. Seasonality of oak litterfall in Southeastern Florida. Florida Scientist **55**: 92-98.

Lundström, J.O. 1999. Mosquito-borne viruses in Western Europe: A review. Journal of Vector Ecology **24**: 1-39.

Lundström, J.O., K.M. Lindström, B. Olsen, R. Dufva, and D.S. Krakower. 2001. Prevalence of Sindbis virus neutralizing antibodies among Swedish passerines indicates that thrushes are the main amplifying hosts. Journal of Medical Entomology **38**: 289-297.

Lundström, J.O. 1994. Vector competence of Western European mosquitoes for arboviruses: A review of field and experimental studies. Bulletin for the Society of Vector Ecology **19**: 23-36.

Lundström, J.O., S. Vene, J-F. Saluzzo, and B. Niklasson. 1993a. Antigenic comparison of Ockelbo virus isolates from Sweden and Russia with Sindbis virus isolates from Europe, Africa, and Australia: Further evidence for variation among Alphaviruses. American Journal of Tropical Medicine and Hygiene **49**: 531-537.

Lundström, J.O., M.J. Turell, and B. Niklasson. 1993b. Viremia in three orders of birds (Anseriformes, Galliformes and Passeriformes) inoculated with Ockelbo virus. Journal of Wildlife Disease **29**: 189-195.

Lundström, J.O., S. Vene, A. Espmark, M. Engvall, and B. Niklasson. 1991. Geographic and temporal distribution of Ockelbo disease in Sweden. Epidemiology and Infection **106**: 567-574.

Lundström, J.O., B. Niklasson, and D.B. Francy. 1990a. Swedish *Culex torrentium* and *Cx. pipiens* (Diptera: Culicidae) as experimental vectors of Ockelbo virus. Journal of Medical Entomology **27**: 561-563.

Lundström, J.O., M.J. Turell, and B. Niklasson. 1990b. Effect of environmental temperature on the vector competence of *Culex pipiens* and *Cx. torrentium* for Ockelbo virus. American Journal of Tropical Medicine and Hygiene **43**: 534-542.

Luo, T., and D. Brown. 1993. Purification and characterization of a Sindbis virus-induced peptide which stimulates its own production and blocks RNA synthesis. Virology **194**: 44-49.

Luz, P.M., C.T. Codeço, E. Massad, and C.J. Struchiner. 2003. Uncertainties regarding dengue modeling in Rio de Janeiro, Brazil. Memórias do Instituto Oswaldo Cruz **98**: 871-878.

Lvov, D.K., T.M. Skvortsova, L.K. Berezina et al. 1984. Isolation of Karelian fever agent from *Aedes communis* mosquitoes. Lancet **2**: 399-400.

Lvov, D.K., T.M. Skvortsova, N.G. Kondashina, et al. 1982. Etiology of Karelian fever, a new arbovirus infection. Voprosy Virus **27**: 690-692.

Maciá, A., and W.E. Bradshaw. 2000. Seasonal availability of resources and habitat degradation for the western tree-hole mosquito, *Aedes sierrensis*. Oecologia **125**: 55-65.

Mack, R.N., D. Simberloff, W.M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications **10**: 689-710.

Madon M.B., M.S. Mulla, M.W. Shaw, S. Kluh, and J.E. Hazelrigg. 2002. Introduction of *Aedes albopictus* (Skuse) into Southern California and potential for its establishment. Journal of Vector Ecology **27**: 149-154.

Matson P.A., and R.H. Waring. 1984. Effects of nutrient and light limitation on mountain hemlock: susceptibility to laminated root rot. Ecology **65**: 1517-1524.

Mattingly, P.F. 1957. Genetical aspects of the *Aedes aegypti* problem. I. Taxonomy and bionomics. Annals of Tropical Medicine and Parasitology. **51**: 392-408.

May, R.M., and R.M. Anderson. 1978. Regulation and stability of host-parasite population interactions. II. Destabilizing processing. Journal of Animal Ecology **47**: 249-267.

McClelland, G.A.H., and B. Weitz. 1963. Serological identification of the natural hosts of *Aedes aegypti* (L.) and some other mosquitoes (Diptera, Culicidae) caught resting in vegetation in Kenya and Uganda. Annals of Tropical Medicine and Parasitology **57**: 214-224.

McIntosh B.M., P.G. Jupp, and I. Dos Santos. 1978. Infection by Sindbis and West Nile viruses in wild populations of *Culex* (C*ulex*) *univittatus* Theobald (Diptera: Culicidae) in South Africa. Journal of the Entomological Society of South Africa **41**: 57-61.

McIntosh B.M., P.G. Jupp, I. Dos Santos, and G.M. Meenehan. 1976. Epidemics of West Nile and Sindbis viruses in South Africa with *Culex* (C*ulex*) *univittatus* Theobald as vector. South African Journal of Medical Science **72**: 295-300.

McIntosh B.M., P.G. Jupp, G.M. McGillivray, and J. Sweetnam. 1967. Ecological studies on Sindbis and West Nile viruses in South Africa. I. Viral activity as revealed by infection of mosquitoes and sentinel fowls. South African Journal of Medical Science **32**: 1-14.

McIntosh B.M., G.M. McGillivray, D.B. Dickinson, H. Malherbe. 1964. Illness caused by Sindbis and West Nile viruses in South Africa. South African Journal of Medical Science **38**:291-294.

Mekuria Y., and M.G. Hyatt. 1995. *Aedes albopictus* in South Carolina. Journal of the American Mosquito Control Association **11**: 468-470.

Meola RW., and R.S. Petralia. 1980. Juvenile hormone induction of biting behavior in *Culex* mosquitoes. Science **209**:1548-1550.

Miles, J.A., J.S. Pillai, and T. Maguire. 1973. Multiplication of Whataroa virus in mosquitoes. Journal of Medical Entomology **10**: 176-185.

Miller, B.R. 1987. Increased yellow fever virus infection and dissemination rates in *Aedes aegypti* mosquitoes orally exposed to freshly grown virus. Transactions of the Royal Society of Tropical Medicine and Hygiene **81**: 1011-1012.

Miller, B.R., B.J. Beaty, T.H. G. Aitken, K. H. Eckels, and P.K. Russell. 1982. Dengue-2 vaccine: Oral infection, transmission, and lack of evidence for reversion in the mosquito, *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **31**: 1232-1237.

Mims, C.A., M.F. Day, and I.D. Marshall. 1966. Cytopathic effect of Semliki Forest virus in the mosquito *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **22**: 332-337.

Mitchell, C.J. 1991. Vector competence of North and South American strains of *Aedes albopictus* for certain arboviruses: A review. Journal of the American Mosquito Control Association **7**: 446-451.

Mogi, M., I. Miyagi, K. Abadi, and Syafruddin. 1996. Inter- and intraspecific variation in resistance to desiccation by adult *Aedes* (*Stegomyia*) spp. (Diptera: Culicidae) from Indonesia. Journal of Medical Entomology **33**: 53-57.

Moncayo, A.C., Z. Fernandex, D. Ortiz, F. Diallo, A. Sall, S. Hartman, C.T. Davis, L. Coffey, C.C. Mathiot, R.B. Tesh, and S.C. Weaver. 2004. Dengue emergence and adaptation to peridomestic mosquitoes. Emerging Infectious Diseases **10**: 1790-1796.

Moore, C.G., 1999. *Aedes albopictus* in the United States: current status and prospects for further spread. Journal of the American Mosquito Control Association **15**: 221-227.

Moore, C.G., and D.M. Whitacre. 1972. Competition in mosquitoes. 2. Production of *Aedes aegypti* larval growth retardants at various densities and nutrition levels. Annals of the Entomological Society of America **65**: 915-918.

Moore, C.G., and B.R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity, and the production of growth retardant. Annals of the Entomological Society of America **62**: 1325-1331.

Morris, J.G., Jr., and M. Potter. 1997. Emergence of new pathogens as a function of changes in host susceptibility. Emerging Infectious Diseases **3**: 435-441.

Morrison, L.W. 1999. Indirect effects of phorid fly parasitoids on the mechanisms of interspecific competition among ants. Oecologia **121**: 113-122.

Morrison, A.C., K. Gray, A. Getis, H. Astete, M. Sihuincha, D. Focks, D. Watts, J.D. Stancil, J.G. Olson, P. Blair, and T.W. Scott. 2004. Temporal and geographic patterns of *Aedes aegypti* (Diptera: Culicidae) production in Iquitos, Peru. Journal of Medical Entomology **41**: 1123-1142.

Munstermann, L.E. 1994. Unexpected genetic consequences of colonization and inbreeding – Allozyme tracking in Culicidae (Diptera). Annals of the Entomological Society of America **87**: 157-164.

Munstermann, L.E. 1980. Distinguishing geographic stains of the *Aedes altropalpus* grwoup (Diptera: Culicidae) by analysis of enzyme variation. Annals of the Entomological Society of America **73**: 699-704.

Murphy, F.A., S.G. Whitfield, W.D. Sudia, and R.W. Chamberlain. 1975. Interactions of vector with vertebrate pathogenic viruses. Pages 25-48 *in* K. Maramorosch and Shope, editors. Invertebrate immunity. Academic Press, New York, NY.

Murray, D.L., L.B. Keith, and J.R. Cary. 1998. Do parasitism and nutritional status interact to affect production in snowshoe hares? Ecology **79**: 1209-1222.

Myles, K.M., D.J. Pierro, and K.E. Olson. 2004. Comparison of the transmission of two genetically distinct Sindbis viruses after oral infection of *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **41**: 95-106.

Nasci, R.S. 1991. Influence of larval and adult nutrition on biting persistence in *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **28**: 522-526.

Nasci, R.S. 1990. Relationship of wing length to adult dry weight in several mosquito species (Diptera: Culicidae). Journal of Medical Entomology **27**: 716-719.

Nasci, R.S. 1987. Adult body size and parity in field populations of the mosquitoes *Anopheles crucians*, *Aedes taeniorhynchus* and *Aedes sollicitans*. Journal of the American Mosquito Control Association **3**: 636-637.

Nasci, R.S. 1986a. The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field. Journal of the American Mosquito Control Association **2**: 61-62.

Nasci, R.S. 1986b. Relationship between adult mosquito (Diptera: Culicidae) body size and parity in field populations. Environmental Entomology **15**: 874-876.

Nasci, R.S., and C.J. Mitchell. 1994. Larval diet, adult size, and susceptibility of *Aedes aegypti* (Diptera: Culicidae) to infection with Ross River virus. Journal of Medical Entomology **31**: 123-126.

Nasci, R.S., S.G. Hare, and F.S. Willis. 1989. Interspecific mating between Louisiana strains of *Aedes albopictus* and *Aedes aegypti* in the field and laboratory. Journal of the American Mosquito Control Association **5**: 416-421.

Naeem, S. 1988. Resource heterogeneity fosters coexistence of a mite and a midge in pitcher plants. Ecological Monographs **58**: 215-227.

Nawrocki, S.J., and W.A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. Journal of the American Mosquito Control Association **3**: 314-317.

Niebylski, M.L., H.M. Savage, R.S. Nasci, and G.B. Craig, Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. Journal of the American Mosquito Control Association. **10**: 447-450.

Niklasson, B. 1989. Sindbis and Sindbis-like viruses. Pages 167-176 *in* T.P. Monath, editor. The arboviruses: epidemiology and ecology, volume 4. CRC, Boca Raton, FL.

Niklasson, B., A. Espmark, and J.O. Lundstrom. 1988. Occurrence of arthralgia and specific IgM antibodies three to four years after Ockelbo disease. Journal of Infectious Diseases **157**: 832-835.

Niklasson, B., and A. Espmark. 1986. Ockelbo disease: arthralgia 3-4 years after infection with a Sindbis virus related agent. Lancet **i**: 1039-1040.

Niklasson, B., A. Espmark, J.W. LeDuc, T.P. Gargan, W. Ennis, R.B. Tesh, and A.J. Main, Jr. 1984. Association of a sindbis-like virus with Ockelbo disease in Sweden. American Journal of Tropical Medicine and Hygiene **33**: 1212-1217.

Norder H., J.O. Lundström, O. Kozuch, and L.O. Magnius. 1996. Genetic relatedness of Sindbis virus strains from Europe, Middle East, and Africa. Virology **222**: 440-445.

Olson, K.E., S. Higgs, P.J. Gaines, A.M. Powers, B.S. Davis, K.I. Kamrud, J.O. Olson, C.D. Blair, and B.J. Beaty. 1996. Genetically engineered resistance to dengue-2 virus transmission in mosquitoes. Science **272**: 884-886.

Olson, K., and D.W. Trent. 1985. Genetic and antigenic variations among geographical isolates of Sindbis virus. Journal of General Virology **66**: 797-810.

O'Meara, G.F., L. F. Evans, Jr., A.D. Gettman, and J.P. Cuda. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. Journal of Medical Entomology **32**: 554-562.

O'Meara, G.F., F.E. Vose, and D.B. Carlson. 1989. Environmental factors influencing oviposition by *Culex* (*Culex*) (Diptera: Culicidae) in two types of traps. Journal of Medical Entomology **26**: 528-534.

Oppliger, A., J. Clobert, J. Lecomte, P. Lorenzon, K. Boudjemadi, and H.B. John-Alder. 1998. Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. Ecology Letters **1**: 129-138.

Osenberg, C.W., G.C. Mittelbach, and P.C. Wainwright. 1992. Two-stage life histories in fish: The interaction between juvenile competition and adult performance. Ecology **73**: 255-267.

Pages, J-P., G. Pache, D. Joud, N. Magnan, and R. Michalet. 2003. Direct and indirect effects of shade on four forest tree seedlings in the French Alps. Ecology **84**: 2741-2750.

Paradise C.J., and K. Kuhn. 1999. Interactive effects of pH and leaf litter on a shredder, the scirtid beetle, *Helodes pulchella*, inhabiting tree-holes. Freshwater Biology **41**: 43-49.

Park, T. 1948. Experimental studies of interspecies competition. I. Competition between populations of the flour beetles, *Tribolium confusum* Duval and *Tribolium castaneum* Herbst. Ecological Monographs **18**: 265-308.

Paulson, S.L., and W.A. Hawley. 1991. Effect of body size on the vector competence of field and laboratory populations of *Aedes triseriatus* for La Crosse virus. Journal of the American Mosquito Control Association **7**: 170-175.

Peck, G.W., and W.E. Walton. 2005. Effect of different assemblages of larval foods on *Culex quinquefasciatus* and *Culex tarsalis* (Diptera: Culicidae) growth and whole body stoichiometry. Environmental Entomology **34**: 767-774.

Petren, K. and T.J. Case. 1996. An experimental demonstration of exploitation competition in an ongoing invasion. Ecology **77**: 118-132.

Petren, K., D.T. Bolger, and T.J. Case. 1993. Mechanisms in the competitive success of an invading sexual gecko over an asexual native. Science **259**: 354-358.

Platt, K.B., J.A. Mangiafico, O.J. Rocha, M.E. Zaldivar, J. Mora, G. Trueba, and W.A. Rowley. 2000. Detection of dengue virus neutralizing antibodies in bats from Costa Rica and Ecuador. Journal of Medical Entomology **37**: 965-967.

Platt, K.B., K.J. Linthicum, K.S.A. Myint, B.L. Innis, B. Lerdthusnee, and D.W. Vaughn. 1997. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **57**: 119-125.

Ponlawat, A., and L.C. Harrington. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. Journal of Medical Entomology **42**: 844-849.

Pumpuni, C.B., J. Knepler, and G.B. Craig, Jr. 1992. Influence of temperature and larval nutrition on diapause inducing photoperiod of *Aedes albopictus*. Journal of the American Mosquito Control Association **8**: 223-227.

Reed, L.J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. American Journal of Hygiene **27**: 493-497.

Reisen, W.K., Y. Fang, and V.M. Martinez. 2006. Effects of temperature on the transmission of West Nile virus by *Culex tarsalis* (Diptera: Culicidae). Journal of Medical Entomology **43**: 309-317.

Reisen, W.K., J.L. Hardy, and S.B. Presser. 1997. Effects of water quality on the vector competence of *Culex tarsalis* (Diptera: Culicidae) for Western Equine encephalomyelitis (Togaviridae) and St. Louis encephalitis (Flaviviridae) viruses. Journal of Medical Entomology **34**: 631-643.

Reiter, P. 1998. *Aedes albopictus* and world trade in used tires, 1988-1995: the shape of things to come? Journal of the American Mosquito Control Association **14**: 83-94.

Reiter, P., and R.F. Darsie. 1984. *Aedes albopictus* in Memphis, Tennesse (USA): An achievement of modern transportation? Mosquito News **44**: 396-399.

Reitz, S.R., and J.T. Trumble. 2002. Competitive displacement among insects and arachnids. Annual Review of Entomology **47**: 435-465.

Relyea, R.A. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. Ecology **81**: 2278-2289.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution **43**: 223-225.

Richardson, J., A. Molina-Cruz, M.I. Salazar, and W.Black IV. Quntitative analysis of dengue-2 virus RNA during the extrinsic incubation period in individual *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **74**: 132-141.

Rico-Hesse, R., L.M. Harrison, R.A. Salas, D. Tovar, A. Nisalak, C. Romas, J. Boshell, M.T.R. de Mesa, R.M.R. Nogueira, and A.T. da Rosa. 1997. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology **230**: 244-251.

Rodhain, F. 1991. The role of monkeys in the biology of dengue and yellow fever. Comparative Immunology, Microbiology and Infectious Disease **14**: 9-19.

Rodhain, F, and L. Rosen. 1997. Mosquito vectors and dengue virus-vector relationships. Pages 45-60 *in* D.J. Gubler and G. Kuno, editors. Dengue and dengue hemorrhagic fever. CABI Publishing, New York, NY.

Rosen, L., L.E. Roseboom, D.J. Gubler, J.C. Lien, and B.N. Chaniotis. 1985. Comparative susceptibility of mosquito species and strains to oral and parenteral infection with Dengue and Japanese Encephalitis viruses. American Journal of Tropical Medicine and Hygiene **34**: 603-615.

Rosen, L., D.A. Shroyer, R.B. Tesh, J.E. Freier, and J.C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **32**: 1108-1119.

Rudnick, A. 1978. Ecology of dengue virus. Asian Journal of Infectious Disease **2**: 156-160.

Rudnick, A. 1965. Studies of the ecology of dengue in Malaysia: a preliminary report. Journal of Medical Entomology **2**: 203-208.

Sammels, L.M., M.D. Lindsay, M. Poidinger, R.J. Coelen, and J.S. Mackenzie. 1999. Geographic distribution and evolution of Sindbis virus in Australia. Journal of General Virology **80**: 739-748.

Sanders, H.R., B.D. Foy, A.M. Evans, L.S. Ross, B.J. Beaty, K.E. Olson, and S.S. Gill. 2005. Sindbis virus induces transport processes and alters expression of innate immunity pathway genes in the midgut of the disease vector, *Aedes aegypti*. Insect Biochemistry and Molecular Biology **35**: 1293-1307.

Sanders, N.J., and D.M. Gordon. 2003. Resource-dependent interactions and the organization of desert ant communities. Ecology **84**: 1024-1031.

SAS Institute. 2002. SAS/STAT user's guide, version 9. SAS Institute, Inc., Cary, NC.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6. SAS Institute, Inc., Cary, NC.

Savage H.M., M.L. Niebylski, G.C. Smith, C.J. Mitchell, and G.B. Craig, Jr. 1993. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. Journal of Medical Entomology **30**: 27-34.

Scheiner, S.M. 2001. MANOVA: multiple response variables and multispecies interactions. Pages 99-115 *in* S.M. Scheiner and J. Gurevitch, editors. Design and analysis of ecological experiments. Second edition. Oxford University Press, Oxford, UK.

Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology and Systematics **24**: 35-68.

Schneider, J.R., A.C. Morrison, H. Astete, T.W. Scott, and M.L. Wilson. 2004. Adult size and distribution of *Aedes aegypti* (Diptera: Culicidae) associated with larval habitats in Iquitos, Peru. Journal of Medical Entomology **41**: 634-642.

Schneider, P., W. Takken, and P. McCall. 2000. Interspecific competition between sibling species larvae of *Anopheles arabiensis* and *An. gambiae*. Medical and Veterinary Entomology **14**: 165-170.

Schoener, T.W. 1983. Field experiments on interspecific competition. American Naturalist. **122**: 240-285.

Schwartz, A., and J.C. Koella. 2004. The cost of immunity in the yellow fever mosquito, *Aedes aegypti* depends on immune activation. Journal of Evolutionary Biology **17**: 834-840.

Scott, T.W. 2001. Are bats really involved in dengue virus transmission? Journal of Medical Entomology **38**: 771-772.

Scott, T.W., A.C. Morrison, L.H. Lorenz, G.G. Clark, D. Strickman, P. Kittayapong, H. Zhou, and J.D. Edman. 2000a. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: population dynamics. Journal of Medical Entomology **37**: 77-88.

Scott, T.W., P.H. Amerasinghe, A.C. Morrison, L.H. Lorenz, G.G. Clark, D. Strickman, P. Kittayapong, and J.D. Edman. 2000b. Longitudinal studies of *Aedes aegypti* (Diptera: Culicidae) in Thailand and Puerto Rico: Blood feeding frequency. Journal of Medical Entomology **37**: 89-101.

Scott, T.W., G.G. Clark, P.H. Amerasinghe, L.H. Lorenz, P. Reiter, and J.D. Edman. 1993a. Detection of multiple blood feeding patterns in *Aedes aegypti* (Diptera: Culicidae) during a single gonotrophic cycle using histological technique. Journal of Medical Entomology **30**: 94-99.

Scott, T.W., E. Chow, D. Strickman, P. Kittayapong, and J.D. Edman. 1993b. Blood feeding patterns of *Aedes aegypti* (Diptera: Culicidae) collected in a rural Thai village. Journal of Medical Entomology **30**: 922-927.

Seabaugh, R.C., K.E. Olson, S. Higgs, J.O. Carlson, B.J. Beaty. 1998. Development of a chimeric Sindbis virus with enhanced *per os* infection of *Aedes aegypti*. Virology **243**: 99-112.

Seaton D.R., Lumsden W.H.R. 1941. Observations on the effects of age, fertilization and light on biting by *Aedes aegypti* (L.) in a controlled microclimate. Annals of Tropical Medicine and Parasitology **35**: 23-36.

Sheldon, B.C. and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology and Evolution **11**: 317-321.

Shirako, Y. B. Niklasson, J.M. Dalrymple, E.G. Strauss, and J.H. Strauss. 1991. Structure of the Ockelbo virus genome and its relationship to other sindbis viruses. Virology **182**: 753-764.

Shroyer, D.A. 1986. *Aedes albopictus* and arboviruses: A concise review of the literature. Journal of the American Mosquito Control Association **2**: 424-428.

Skogh, M., and A. Espmark. 1982. Ockelbo disease: epidemic arthritis-exanthema syndrome in Sweden caused by Sindbis-virus like agent. Lancet **3**: 795-796.

Smith, C.E.G. 1956. The history of dengue in tropical Asia and its probable relationship to the mosquito *Aedes aegypti*. Journal of Tropical Medicine and Hygiene **59**: 243-251.

Smith, D.L., J. Dushoff, and F.E. McKenzie. 2004. The risk of a mosquito-borne infection in a heterogeneous environment. Public Library of Science **2**: 1957-1964.

Sokal R.R., and F.J. Rohlf. 1995. Biometry. W.H. Freeman and Company, New York, NY.

Sota, T., M., Mogi, and K. Kato. 1998. Local and regional-scale food web structure in *Nepenthes alata* pitchers. Biotropica **30**: 82-91.

Sota T, and Mogi M. 1992. Interspecific variation in desiccation survival time of *Aedes* (*Stegomyia*) mosquito eggs is correlated with habitat and egg size. Oecologia **90**: 353-358.

Sprenger, D., and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. Journal of the American Mosquito Control Association **2**: 217-219.

Steinhaus, E.A. 1958. Crowding as a possible stress factor in insect disease. Ecology **39**: 503-514.

Sumanochitrapon, W., D. Strickman, R. Sithiprasasna, P. Kittayapong, and B.L. Innis. 1998. Effect of size and geographic origin of *Aedes aegypti* on oral infection with Dengue-2 virus. American Journal of Tropical Medicine and Hygiene **58**: 283-286.

Suwanchaichinda, C., and S.M. Paskewitz. 1998. Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize Sephadex beads. Journal of Medical Entomology **35**: 157-161.

Swift, M.J., O.W. Heal, and J.M. Anderson. 1979. Decomposition in terrestrial ecosystems. University of California Press, Berkeley, CA.

Tabachnick, W.J. 1994. Genetics of insect vector competence for arboviruses. Pages 93-108 *in* K.F. Harris, editor. Advances in disease vector research, Volume 10. Springer-verlag, New York, NY.

Tabachnick, W.J. 1991. Evolutionary genetics and arthropod-borne disease. The yellow fever mosquito. American Entomologist **37**: 14-24.

Tabachnick, W.J., L.E. Munstermann, and J.R. Powell. 1979. Genetic distinctness of sympatric forms of *Aedes aegypti* in East Africa. Evolution **33**: 287-295.

Takahashi, M. 1976. The effects of environmental and physiological conditions of *Culex tritaeniorhynchus* on the pattern of transmission of Japanese encephalitis virus. Journal of Medical Entomology **13**: 275-284.

Takken W, M.J. Klowden, and G.M. Chambers. 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. Journal of Medical Entomology **35**: 639-645.

Taylor, R.M., H.S. Hurlbut, T.H. Work, J.R. Kingston, and T.E. Frothingham. 1955. Sindbis virus: a newly recognized arthropod-transmitted virus. American Journal of Tropical Medicine and Hygiene **4**: 844-862.

Teng H-J, and C. Apperson. 2000. Development and survival of immature *Aedes albopictus* and *Aedes triseriatus* (Diptera: Culicidae) in the laboratory: Effects of density, food, and competition on response to temperature. Journal of Medical Entomology **37**: 40-52.

Tesh, R.B. 1982. Arthritides caused by mosquito-borne viruses. Annual Review of Medicine **33**:31-40.

Tesh, R.B. 1980. Experimental studies on the transovarial transmission of Kunjin and San Angelo viruses in mosquitoes. American Journal of Tropical Medicine and Hygiene **29**: 657-666.

Thenmozhi, V., S.C. Tewari, R. Manavaian, A. Balasubramanian, and A. Gajanans. 2000. Natural vertical transmission of dengue viruses in *Aedes aegypti* in southern India. Transactions of the Royal Society of Tropical Medicine and Hygiene **94**: 507-.

Thomas, R.E., W.K. Wu, D. Verleye, and K.S. Rai. 1993. Midgut basal lamina thickness and dengue-1 virus dissemination rates in laboratory strains of *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology **30**: 326-331.

Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, NJ.

Trpis, M., and W. Hausermann. 1986. Dispersal and other population parameters of *Aedes aegypti* in an African village and their possible significance in epidemiology of vector-borne diseases. American Journal of Tropical Medicine and Hygiene **35**: 1263-1279.

Turell, M.J. 1988. Reduced Rift Valley Fever virus infection rates in mosquitoes associated with pledget feedings. American Journal of Tropical Medicine and Hygiene **39**: 597-602.

Turell, M.J., M.L. O'Guinn, D.J. Dohm, and J.W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. Journal of Medical Entomology **38**: 130-134.

Turell, M.J., and J.O. Lundström. 1990. Effect of environmental temperature on the vector competence of *Aedes aegypti* and *Ae. taeniorhynchus* for Ockelbo virus. American Journal of Tropical Medicine and Hygiene **43**: 543-550.

Turell, M.J., J.O. Lundström, and B. Niklasson. 1990. Transmission of Ockelbo virus by *Aedes cinereus*, *Ae. communis*, and *Ae. excrucians* (Diptera: Culicidae) collected in an enzootic area in central Sweden. Journal of Medical Entomology **27**: 266-268.

Turell, M.J., T.P. Gargan, II, and C.L. Bailey. 1985. *Culex pipiens* (Diptera: Culicidae) morbidity and mortality associated with Rift Valley Fever virus infection. Journal of Medical Entomology **22**: 332-337.

Turell, M.J. P.A. Rossignol, A. Spielman, C.A. Rossi, and C.L. Bailey. 1984. Enhanced arbovirus transmission by mosquitoes that concurrently ingested microfilariae. Science **225**: 1039-1041.

Turell, M.J., T.P. Gargan II, and C.L. Bailey. 1984. Replication and dissemination of rift valley fever virus in *Culex pipiens*. American Journal of Tropical Medicine and Hygiene **33**: 176-181.

Turunen, M., P. Kuusisto, P.-E. Uggeldahl, and A. Toivanen. 1998. Pogosta disease: Clinical observations during an outbreak in the province of north Karelia, Finland. British Journal of Rheumatology **37**: 1177-1180.

Vaughan. J.A., M. Trpis, and M.J. Turell. 1999. *Brugia malayi* microfilariae (Nematoda: Filaridae) enhance the infectivity of Venezuelan equine encephalitis virus to *Aedes* mosquitoes (Diptera: Culicidae). Journal of Medical Entomology **36**: 758-763.

Vazeille, M., L. Rosen, L. Mousson, and A-B. Failloux. 2003. Low oral receptivity for dengue type 2 viruses of *Aedes albopictus* from Southeast Asia compared with that of *Aedes aegypti*. American Journal of Tropical Medicine and Hygiene **68**: 203-208.

Vazeille, M., L. Mousson, I. Rakatoarivony, R. Villeret, F. Rodhain, J-B Duchemin, and A-B. Failloux. 2001. Population genetic structure and competence as a vector for dengue type 2 virus of *Aedes aegypti* and *Aedes albopictus* from Madagascar. American Journal of Tropical Medicine and Hygiene **65**: 491-497.

Vazeille-Falcoz, M., L. Mousson, R. Rodhain, E. Chungue, and A-B. Failloux. 1999. Variation in oral susceptibility to dengue type 2 virus of populations of *Aedes aegypti* from the islands of Tahiti and Moorea, French Polynesia. American Journal of Tropical Medicine and Hygiene **60**: 292-299.

Walker, E.D., D.L. Lawson, R.W. Merritt, W.T. Morgan, and M.J. Klug. 1991. Nutrient dynamics, bacterial populations, and mosquito productivity in treehole ecosystems and microorganisms. Ecology **72**: 1529-1546.

Walker, E.D., and R.W. Merritt. 1988. The significance of leaf detritus to mosquito (Diptera: Culicidae) productivity from treeholes. Environmental Entomology **17**: 199-206.

Wallis, G.P., T.H.G. Aitken, B.J. Beaty, L. Lorenz, G.D. Amato, and W.J. Tabachnick. 1985. Selection for susceptibility and refractoriness of *Aedes aegypti* to oral infection with yellow fever virus. American Journal of Tropical Medicine and Hygiene. **34**: 1225-1231.

Wang, E., H. Ni, R. Xu, A.D. T., Barrett, S.J. Watowich, D.J. Gubler, and S.C. Weaver. 2000. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. Journal of Virology **74**: 3227-3234.

Washburn, J.O., D.R. Mercer, and J.R. Anderson. 1991. Regulatory role of parasites: Impact on host population shifts with resource availability. Science **253**: 185-188.

Weaver, S.C. 1986. Electron microscopic analysis of infection patterns for Venezuelan equine encephalomyelitis virus in the vector mosquito *Culex* (*Melanoconion*) *taeniopus*. American Journal of Tropical Medicine and Hygiene. **35**: 624-631.

Weaver, S.C., T.W. Scott, L.H. Lorenz, and P.M. Repik. 1991. Detection of Eastern Equine Encephalomyelitis virus deposition in *Culiseta melanura* following ingestion of radiolabeled virus in blood meals. American Journal of Tropical Medicine and Hygiene. **44**: 250-259.

Weaver, S.C., W.F. Scherer, E.W. Cupp, and D.A. Castello. 1984. Barriers to dissemination of Venezuelan encephalitis viruses in the Middle American enzootic vector mosquito, *Culex* (*Melanoconion*) *taeniopus*. American Journal of Tropical Medicine and Hygiene **33**: 953-960.

Werner, E.E. 1992. Individual behavior and higher-order species interactions. The American Naturalist **140**: S5-S32.

Werner, E.E. 1991. Nonlethal effects of a predator on competitive interactions between two anuran larvae. Ecology **72**: 1709-1720.

Werner, E.E., and B.R. Anholt. 1996. Predator-induced behavioral indirect effects: Consequences to competitive interactions in anuran larvae. Ecology **77**: 157-169

White, D.O, and F.J. Fenner. 1994. Medical virology. Academic Press, New Yorik, NY.

Whitehead, R.H. T.M. Yuill, D.J. Gould, and P. Simasathien. 1971. Experimental infection of *Aedes aegypti* and *Aedes albopictus* with dengue viruses. Transactions of the Royal Society of Tropical Medicine and Hygiene **65**: 661-667.

Willis, F.S. and R.S. Nasci. 1994. *Aedes albopictus* (Diptera: Culicidae) population density and structure in Southwest Louisiana. Journal of Medical Entomology **31**: 594-599.

Woodring, J.L., S. Higgs, and B.J. Beaty. 1996. Natural cycles of vector-borne pathogens. Pages 51-72. i*n* B.J. Beaty and W. C. Marquardt, editors. The biology of disease vectors. University Press of Colorado, Niwot, CO.

Wootton, J.T. 1994. The nature and consequences of indirect effects in ecological communities. Annual Review of Ecology and Systematics **25**: 443-466.

Wootton, J.T. 1993. Indirect effects and habitat use in an intertidal community: Interaction chains and interaction modifications. The American Naturalist **141**: 71-89.

Woodall J.P., M.C. Williams, and J.M. Ellice. 1962. Sindbis infection in man. East Afrrican Virus Research **12**: 17.

Xue, R-D. D.R. Barnard, and C.E. Schreck. 1995. Influence of body size and age of *Aedes albopictus* on human host attack rates and the repellency of Deet. Journal of the American Mosquito Control Association **11**: 50-53.

Xiong, C., R. Levis, P. Shen, S. Schlesinger, C.M. Rice, and H.W. Huang. 1989. Sindbis virus: An efficient, broad host range vector for gene expression in animal cells. Science **243**: 1188-1191.

Yanoviak, S. P. 1999. Effects of leaf litter species on macroinvertebrate community properties and mosquito yield in Neotropical tree hole microcosms. Oecologia **120**: 147-155.

Ye-Ebiyo, Y., R.J. Pollack, A. Kiszewski, and A. Spielman. 2003. Enhancement of development of larval *Anopheles arabiensis* by proximity to flowering maize (*Zea mays*) in turbid water and when crowded. American Journal of Tropical Medicine and Hygiene **68**: 748-752.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall, Englewood Cliffs, NJ.

Zytoon, E.M. H.I. El-Belbasi, and T. Matsumura. 1993. Mechanisms of increased dissemination of Chikungunya virus in *Aedes albopictus* mosquitoes concurrently ingesting microfilariae of *Dirofilaria immitis*. American Journal of Tropical Medicine and Hygiene. **49**: 201-207.

BIOGRAPHICAL SKETCH

Barry W. Alto was born in Virginia, MN, in the heart of the Great North Woods. He spent a great deal of his childhood enjoying the outdoors. After graduating from Virginia High School, he moved to St. Paul, MN, and received his Bachelor of Arts degree in biology from University of St. Thomas. His schooling continued in Normal, IL, where he received his Master of Science degree in biology from Illinois State University.