THE INFLUENCE OF CARBOHYDRATE REQUIREMENTS ON

ASIAN TIGER MOSQUITO BEHAVIOR AND FITNESS

By

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ABSTRACT OF THE DISSERTATION

The influence of carbohydrate requirements on Asian tiger mosquito behavior and fitness

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Successful and sustainable mosquito control is not possible without knowledge of the species' biology. We must understand the factors affecting mosquito fitness before we can hope to reduce it. Sugar-feeding behavior was once neglected in the literature, but has now emerged as a significant influence on mosquito fitness. I chose to investigate multiple aspects of sugar-feeding behavior in the Asian tiger mosquito, *Aedes albopictus*. This mosquito is of increasing concern due to its aggressive invasive nature and vector status.

In Chapter 1, I examine the effect of diapause cues on female dietary choices. Diapause cues affected female food choice, particularly the initial choice. Long-day females were three times more likely to choose blood at their first feeding opportunity. The frequency of sugar meals was also altered by treatment with short-day females exhibiting a 33% increase. These findings may be beneficial to mosquito control efforts, particularly those based upon sugar attractants.

In Chapter 2, I investigate the importance of sugar availability for adult male mating success. Males provisioned with sugar for long periods after eclosion experience greater longevity, greater sperm transfer, and mated with more females over their

lifetime. Therefore, I suggest that sterile male release initiatives might consider provisioning adult males for multiple days before release.

In Chapter 3, I explore the effect of the adult male diet on their offspring. While there was no change in the fecundity of the male parent, there was a significant change in offspring development time and size. In both sexes pupation time and eclosion time were significantly longer in offspring of starved males. Unexpectedly, female offspring of starved males had longer wing lengths than those fathered by males with access to sucrose. I developed a mathematical model to examine whether the paternal effect I observed might increase offspring fitness under certain conditions. The model demonstrated that offspring benefit from paternal signals when they experience poor sugar resources as adults. This highlights the existence of environmentally mediated paternal effects in mosquitoes, and more broadly in insects, suggesting that male environment needs to be taken into account when control measures seek to alter environmental conditions.

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**Dedication**


For everyone who challenges me

and

For Christopher

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## Introduction

Knowledge of insect behavior is critical for successful management, as well as for predicting population growth and disease transmission. Insects generally have untapped energy reserves, even after they starve to death (Wigglesworth 1982); this is not the case for mosquitoes (Van Handel 1965, Yuval 1992). In his review of mosquito sugar-feeding, Foster (1995) concluded that sugar is occasionally a "limited" resource, but that it was still unclear whether or not it was a "limiting" resource for any species. The specific location, season, and species play a large role in the answer to this question. Multiple studies have shown high variability in the percentage of a given species that are positive for fructose, while others show clear seasonal changes (Foster 1995). Bellini et al. (2013) found no difference in the fitness of males or females when provided with honey versus 10% sucrose solution. Mosquitoes most often feed on floral nectar, which is primarily composed of sucrose, glucose, and fructose, and generally varies between 20-50% concentration (Foster 1995).

### Blood, sugar, and water: A balancing act

Nayar & Pierce (1977) determined that the energy usage of adult mosquitoes on the first day post-eclosion was dependent upon the level of energy reserves available. Somewhat counterintuitively, those with greater reserves had a lower rate of energy consumption. Similarly, Van Handel (1965) found that once the available energy reached a certain level, usage slowed. After consuming a sugar meal, mosquitoes either use the energy directly, or convert the carbohydrates to either glycogen or triglycerides. He also found that male *Aedes sollicitans* would only take up 1.5 µL of sugar solution at one time,

which was less than half that of their female counterparts. Starved males utilize fat (triglyceride) storage more quickly than glycogen; whereas, females use them proportionally. Starved males produce a relatively tiny amount of fat (3%) from a sugar meal, as opposed to females (30%); regardless, both absorb a sugar meal around the same rate. Later studies found that rates of triglyceride utilization differ greatly between species, even within the same genus (Nayar & Sauerman, 1973; Nayar & Pierce, 1977). In *Aedes albopictus*, teneral energy levels increased with body size, in a linear relationship at high temperatures, and a logarithmic relationship at lower temperatures (Briegel and Timmermann 2001). Glycogen stores were below 10% of lipid and protein levels. Gary & Foster (2006) looked at sugar-feeding timing and frequency in male *Anopheles gambiae*, finding that, on average, they fed twice nightly. Feeding was much more likely to occur during the crepuscular period, and then dwindle through the remainder of the night. Chadee et al. (2013) examined the sugar feeding patterns of *Ae. aegypti,* finding a diel pattern in both males and females, with a small peak in the morning and a large peak in the evening. In *Ae. albopictus* males, flight and walking activity peaked at dawn and dusk in a laboratory setting, with dusk being the higher peak (Boyer et al. 2013)

Canyon et al. (2013) demonstrated that water alone was not enough to allow *Ae. aegypti* to survive in arid conditions; however, both blood and sugar feeding significantly decreased mortality rates. Nusrat et al. (2013) determined that the longevity of female *An. stephensi* increased by 12 days when supplied with sugar in addition to blood, but total fecundity was not affected. Xue et al. (2010) determined that regardless of diet, larger *Ae. aegypti* had greater longevity. In both males and females, sugar feeding significantly

increased longevity.  The mean lifespan of larger females were significantly different when fed on water (5.1 d), sugar (44.4 d), sugar and blood (41.4 d), or water and blood (6.7 d). Large males exhibited an average lifespan of 3.2 day on water and 18.8 days on sucrose. Braks et al. (2006) studied the importance of carbohydrates for *Ae. aegypti* and *Ae. albopictus* females. In both, as long as human blood-meals were available, sugar had no effect on lifetime fecundity; however, females that only received blood laid eggs more quickly and had a shorter average lifespan. In contrast, Briegel (2001) concluded that sugar feeding was pivotal for long distance flight in *Ae. aegypti* regardless of size. He also found that sucrose concentrations from 0.5-50% increased longevity in a logarithmic fashion, with diminishing returns for very high concentrations.  Kaufmann et al. (2013) determined that, in *Ae. aegypti* females, larger individuals were able to fly farther as energy supplies increased. They also determined that glycogen and sugar are used for flight, as opposed to lipids. Gu et al. (2011) determined that female *An. sergentii* experienced declines in multiple aspects of their fitness, including longevity, when released into habitats with lower sugar resources. These females were also more likely to resort to plant tissue feeding. Müller and Schlein (2005) observed the occurrence of plant tissue feeding in multiple species over multiple seasons. When nectar was scarce, tissue feeding increased, as more cellulose was detected, except in *Aedes* species. The authors hypothesized that the species may be more efficient at filtering plant material than *Anopheles* or *Culex* species. Qualls et al. (2013) tested *Ae. albopictus* survival in the presence of  non-flowering plants with no sugar source. Both sexes survived better in the presence lucky bamboo (*Dracaena sanderiana*), than a *Guzmania* bromeliad or Moses-

in-the-cradle (*Rhoeo spathacea*) . However, the study only presumes feeding activity on the plants by measuring longevity rather than direct observation.

**Dietary choice and timing in female mosquitoes**

Unlike males, which only feed on sugar, female mosquitoes constantly need to choose between blood and sugar when both are available. Predicting these choices are not as simple as might be expected. Foster (1995) points out that food choice is a serious dilemma for female mosquitoes since they will be allowed only a few feeding opportunities in their lifetime, and this choice greatly impacts their fitness. While a blood meal is primarily used to obtain protein and other nutrients needed for egg production, it may also be used from energy. Similarly, sugar-meals, can be used as an energy source, but can also contribute to an individual's fecundity (Nayar & Sauerman, 1973; Van Handel, 1965). *Anopheles gambiae* females were observed taking a sugar meal approximately once every four days. These sugar meals occured at least two days after blood meals, generally placing them between gonotrophic cycles. When blood or oviposition sites were not readily available, sugar-feeding became significantly more common. Newly emerged females not allowed access to blood took daily sugar meals. There was also a large amount of variation in the choices of individual females, where some strongly preferred blood and others sugar (Gary and Foster 2006).  Gary and Foster (2001) determined that sugar availability led to a decrease in blood meals and extended lifespan, but no difference in lifetime fecundity. Foster and Takken (2004) determined that newly eclosed *An. gambiae* females were more attracted to nectar-related volatiles, but after maturing and sugar feeding, they switched to human volatiles. Damiens et al.

(2013) found that depriving *An. arabiensis* females of sugar seven hours prior to blood feeding increased egg production by 50%. Similarly, newly emerged *An. gambiae* of both sexes show significant attraction to nectar-related volatiles even in the presence of human volatiles (Foster & Takken, 2004). Yee et al. (1992) examined the frequency of blood- and sugar-meals in two *Aedes* species in the field. For both, blood-feeding peaked slightly sooner that sugar-feeding, but overlapped. This might suggest that blood-meals were sought first, and nectar would occasionally serve as a second choice. If blood and sugar were taken in close succession, taking blood first may also have the advantage of increase egg production, while the opposite order may actually decrease it (Foster et al. 1989; Klowden & Chambers, 1989). Specifically, Foster et al. (1989) suggests that, at least in the case of *Ae. aegypti*, females should attempt to increase their energy reserves by sugar feeding, and then take a blood-meal when their crop is empty in order to maximize fecundity. It seems that taking a blood-meal while still in the process of digesting a sugar-meal may prevent mosquitoes from receiving the full benefit of the sugar-meal. Subsequently, *Ae. aegypti* have the highest fecundity if they have high energy reserves and an empty crop when taking their first blood meal; having an empty crop allows for a larger blood meal (Mostowy and Foster 2004). Edman et al. (1992) determined that wild female *Ae. aegypti* in Thailand did not utilize available sugar sources very often, suggesting they relied more heavily on blood for survival and reproduction. Higa et al. (2000) examined the feeding rhythms of *Ae. albopictus* in Japan and found that they were most successful in trapping both males and females during twilight; however their use of CDC light traps and $CO_2$ traps may have skewed the results for males. Yee & Foster (1992) examined the blood- and sugar-feeding patterns of

multiple mosquito species in the field. Inseminated nulliparous *Ae. albopictus* females were held under 16:8 L:D at 27°C. Sugar and blood feeding activities occupied the same periods; both occurred continuously, with a high peak in the evening and a lower peak in the morning. Blood-feeding occurred more consistently through the nighttime period. However, the experiment did not examine any interaction between the two activities. When a rat was present, *Ae. albopictus* and *Ae. aegypti*, but not *An. quadrimaculatus* or *Culex quinquefasciatus* almost completely abandoned sugar-feeding (Yee and Foster 1992). Other studies suggest that mosquitoes are unlikely to divert their sugar-seeking behavior to feed on nearby hosts (Foster 1995). Stone et al. (2011) demonstrated that *An. gambiae* generally prioritize sugar meals before mating and blood meals after mating.

Interestingly, Klowden & Briegel (1994) determined that *Aedes* (including *Ae. albopictus*) but not *Anopheles* species were inhibited from seeking a second blood meal during a single gonotrophic cycle. Xue et al. (2012) examined host seeking in female *Ae. albopictus* and determined that increased body size led to increased sensitivity to human body odor, and that mated females were most likely to land on a host; however, many uninseminated females still exhibited host seeking activity. Briegel and Timmermann (2001) found that *Ae. albopictus* females were able to multiply their glycogen stores by four after a week of sucrose access and their lipid stores ten times after two weeks. In the same study, the number of eggs in females varied from 15-110. The number of eggs was also positively correlated with body size, and therefore, negatively correlated with temperature. Blood meal protein usage was inversely correlated with temperature, ranging from 35-50%. Each oocyte was composed of 5-7 mcal of lipid and 6.3-6.5 mcal of protein. In general, the species increased its lipid stores in its own body, as well as in

its egg yolk lipids. Nayar and Sauerman (1975) reported that starved *Ae. sollicitans*

females needed to consume 2-3 times the blood volume of sugar-fed females in order to

produce an equivalent amount of eggs. Lea and Van Handel (1970) report that lipid

reserves accumulate much slower from blood digestion versus sugar. Female mosquitoes

usually delay egg development if sufficient nutritional reserves are not available (Foster

1995).  Magnarelli (1979) observed that *Ae. cantator and Ae. sollicitans* fed on flowering

plants during the same period when they were more active in taking blood meals. Bother

species focused on plants that were in peak bloom and exhibited a diurnal feeding pattern.

Xue et al. (2008) determined that access to a sugar meal after blood feeding increased

longevity in female *Ae. albopictus*, and that survival increased with a second blood meal.

*Ae. albopictus* with low energy reserves may take a longer time blood feeding (Xue and

Debboun 2014).

In female *Ae. sollicitans*, equivalent caloric intake of protein blood or sugar

solution results in an equivalent rate of fat synthesis. However, the amount of fat

produced from a blood-meal is only 66% of that produced from the equivalent number of

calories of sugar. This may be due to some of the use of proteins for the production of

eggs (Van Handel 1965). In terms of glycogen, blood is able to produce the same level as

sugar, but the production rate is ten times slower from blood (Van Handel 1965).


**Diapause in mosquitoes**

Diapause is defined as "a form of dormancy that is hormonally programmed in

advance of its onset and is not immediately terminated in response to favorable

conditions" (Denlinger and Armbruster 2014). There is evidence that diapause cues can

cause a variety of behavioral changes in adult mosquitoes (Denlinger and Armbruster 2014). For instance, adult *Cx. pipiens* completely stops blood-feeding in preparation for over-wintering, while *Ae. mariae* alters its oviposition tactics. (Coluzzi et al. 1975); females become much less likely to oviposit on water surfaces if they are reared with diapause cues. In *Ae. albopictus*, diapause eggs are more cold and desiccation resistant than normal eggs (Sota and Mogi 1992). These eggs have one third more surface hydrocarbons. The upregulation of fatty acyl coA elongase most likely plays a role in their production (Urbanski et al. 2010). Poor larval diet also increases the incidence of diapause in *Ae. albopictus,* and 21°C is the optimal temperature for inducing diapause (Pumpuni et al. 1992). Only certain strains of *Ae. albopictus* have the ability to diapause. Lounibos et al. (2003) demonstrated that US introductions originated from multiple locations, resulting in different levels of diapause expression, being more prevalent at northern latitudes. Yee et al. (2012) found that *Ae. albopictus* from northern US areas demonstrated slower development and large size when exposed to long light cycles mimicking summer, illustrating changes more subtle than the diapause phenomenon. Reynolds et al. (2012) demonstrated that larvae in diapausing eggs contained 30% more lipids than their non-diapausing counterparts.

**Male fitness and energy reserves**

Swarming, or lekking, is a common behavior in many mosquito species, in which males fly in a group, and females seek out and enter the group in order to mate. This activity requires significant flight time especially for males, and therefore constitutes a significant energy drain. Male *Anopheles freeborni* use over 50% of their energy stores

in a lek, lasting around 30 minutes, each night and consequently seek a sugar-meal (Yuval et al., 1994). While species that regularly make long flights utilize fat storage, species that make shorter flights generally utilize sugars, as is the case with lekking males who use sugars and glycogen. Male Mediterranean fruit flies which participate in lekking are heavier and contain significantly more sugar and protein than their non-lekking counterparts (Yuval et al., 2002; Yuval et al., 1998). Maïga et al. (2012, 2013) determined levels of nutritional reserves and size in mating male *An. gambiae*, determining that 25 minutes of swarming depleted about half of a male's energy reserves, while mating seemingly depleted no energy reserves. Successfully mated males were larger than their unmated peers. This conflicts with the results of Diabaté et al. (2011), which reported no difference in the average wing length of mated male *An. gambiae*. Females are also more likely to retain the sperm of protein fed males; however, these males exhibited decreased longevity (Yuval et al., 2002). Gary et al. (2009) examined the role of sugar feeding in *An. gambiae* male fitness, along with body size and temperature. As expected, males with larger body size survived longer and were able to inseminate more females. Lower temperatures increased survival time, but also increased time until males were ready to mate. Males allowed a single sugar meal had increased survival time compared to those that were starved, but not as much as those with *ad libitum* access. In large cages (over $2m^3$), starved males had almost no successful matings. *An. freeborni* males lek as light intensity decreases in the evening (between 350 and 0.5 lux); however, this range is known to be species specific (Yuval & Bouskila, 1993). Male *Ae. albopictus* usually locate virgin females near hosts, either in small leks or individually (Gubler and Bhattacharya 1972). Boyer et al. (2013) found that the flight speed of empty males had

faster maximum flight speeds than those of recently fed males.  Chadee et al. (2013) determined that, 36 hours post-eclosion, all sugar-fed males' antennal fibrillae were erect, as opposed to 15% of water-fed individuals.

Another aspect of male mating affected by nutrition is sperm production and ejaculate quality. Sbilordo et al. (2011) examined the effect of starvation on multiple aspects of fecundity in red flour beetles, which are highly promiscuous. They determined that the nutritional status of males affected the number of sperm transferred, the number of sperm used per egg by females, sperm offense and defense ability, and the frequency oviposition and number of viable eggs laid.  The ejaculate quality of the first male can be pivotal for success in preventing other males from inseminating a given female. Energy restrictions affect the number of sperm produced in Indian meal moths (Gage and Cook 1994). Lupold et al. (2010) found that male *Drosophila melanogaster* adjust their ejaculate size based upon the female's age, mating status, and size, with younger, non-virgin, and larger individuals receiving more.

The male accessory glands (MAGs) sometimes play in important role in female mating behavior and egg production (Gillott 1977, 2003; Klowden 1999). In *D. melanogaster,* artificial sex peptide injected into virgin females results in increased yolk protein production. In the grasshopper *Melanoplus sanguinipes*, proteins transfer in the MAG secretion directly contribute to oocyte protein content (Friedel and Gillott 1977). In *Ae. albopictus*, the male accessory gland secretions act to prevent future inseminations by other males (Oliva et al. 2013).  Specifically matrone is a pheromone transmitted which acts upon the female's terminal abdominal ganglion, preventing future mating (Gwadz 1972). MAG secretions from male *Ae. albopictus* reduce mobility and increase

refractoriness to further mating attempts in female *Ae. aegypti* (Tripet et al. 2011, Lima-Camara et al. 2013); however, *Ae. aegypti* develops resistance to this phenomenon after multiple generations of exposure to *Ae. albopictus* (Bargielowski and Lounibos 2013). The accessory glands produce these secretions within the first two days of emergence (Foster and Lea 1975). In *Ae. aegypti*, the ejaculate which remains in the spermatheca is absorbed by the female (Spielman 1964). Scharf et al. (2012) suggests that this may be considered equivalent to a nuptial gift; Helinski and Harrington (2011) determined that malnourished females which mated with virgin males displayed increased longevity compared with those mated with sperm-depleted males, suggesting they may derive some nutritional value from the ejaculate. Klowden and Chambers (1991) found that accessory gland secretions stimulated egg production in starved *Ae. aegypti* females, and that ejaculate from starved males was less likely to have this result. Starved and fed females maintained the same levels of energy stores, but starved females showed a higher mortality. Klowden (1993) found that, in *Ae. albopictus*, mating made females more likely to develop eggs from a small blood-meal. Helinski and Harrington (2012) report that twice-mated *Ae. aegypti* females seem to have no fecundity benefit over those only once-mated. These chemicals also seem to cause the female to become reluctant to mate, but this effect occurs 2 or 3 days after the initial insemination. Taken together, it would seem likely that a significant percentage wild females mate multiple times in quick succession, and then discontinue the behavior indefinitely. In An. gambiae, males with larger accessory were more likely to prevent females from mating a second time (Bryan 1972). Two- and three-day-old males were able to influence females to oviposit more successfully than older males (Chambers and Klowden 2001). Boyer et al. (2012) found

that 26% of wild *Ae. albopictus* produced offspring from multiple males, but sample sizes were rather low.  Cator et al. (2010) shows that parous *Ae. aegypti* females are less likely to synchronize their wing-beats with males, which may explain low numbers of parous females of the species that are caught *in copula*.

In adult male *Ae. aegypti*, new sperm are produced from mature spermatocytes (Ponlawat and Harrington 2007).  In *Ae. albopictus*, full rotation of the terminalia may take up to one day (Oliva, Jacquet, et al. 2012). The timing of peak sexual maturity in male mosquitoes varies between species, but generally begins around three or four days after eclosion (Ponlawat and Harrington 2007, Determinedadogo et al. 2013).  Sperm production begins in the larval stage and continues through adulthood until the male has exhausted its spermatogonia, which are undifferentiated stem cells located in the spermatocytes; it is thought that spermatogenesis peaks in the pupal stage (Clements 1999, 2000).  Sperm production increases with age, body size and temperature (Ponlawat and Harrington 2007, Bader and Williams 2012). The amount of sperm transferred also increases with age in *Ae. aegypti* (Ponlawat and Harrington 2009).  Studies on *Ae. albopictus* males do not agree on the number of females a signal male is able to inseminate over his lifetime, although difference may be seen due to variations methodology or strains used. Ali and Rozeboom (1973) determined that *Ae. albopictus* males will mate with an average of 6.7 females in five days, while Boyer et al. (2011) determined 9.6 matings over seven days, resulting in very similar daily averages of 1.3 and 1.4 per male, respectively.  Boyer et al. (2011) also determined that 5.3 females were mated when males had access to two new females for 12 consecutive days, and the an average of 15.5 spermatheca were filled by each male over his lifetime. Oliva et al.

(2013) determined an average of 11 total females mated per male. Foster and Lea (1975) studied the lifetime mating success of male *Ae. aegypti*. The main conclusion was that periods of rest after sperm and accessory gland depletion allowed males to mate again. A single male was able to inseminate 17.6 females on average. However, Yuval (2006) expects that this number may be unimportant as males are likely to have much fewer mating opportunities in the wild.  In Diptera, male-male competition can be very intense in monanderous or near-monanderous species, result in marked fitness loss (Lizé et al. 2014).


**Parental effects (non-genetic inheritance)**

Non-genetic inheritance, sometimes referred to a "parental effects" are effects on offspring's phenotype resultant from transmission of factors other than DNA from parents or previous generations (Bonduriansky and Day 2009).  Mechanisms may include epigenetic effects, transmission of nutrients, and many others. Epigenetics, a fairly new field, deals with information transmitted from parents to offspring by mechanisms other than the order of nucleotides in the DNA. This information can be transmitted in formats such as DNA methylation or changes in histones. Maternal nutrition can play a key role in restructuring the epigenome after conception (Lane et al. 2014).

There are many examples of maternal effects; however, paternal effects are more difficult to discover and measure since, in species with no paternal care, they would need to be transmitted through mating, either with an epigenetic mechanism, or through chemicals transmitted during mating. Some paternal effects may be the result of indirect maternal effects, where a male influences the female's metabolism or egg production in such a

way that it effects the provisioning of the egg, which is a common occurrence in mosquitoes and other insects (Janssen et al. 1988). Certain accessory gland proteins in *Ae. aegypti* appear 24 hours after male eclosion and increase for multiple days, but can become depleted after repeated mating with no rest (Alfonso-Parra et al. 2014). Micro-RNA has been implicated as one of the mechanisms by which mating affects female gene expression in flies (Zhou et al. 2014). In *Drosophila,* exosomes have been implicated in affecting a female's willingness to mate again. Surprisingly, Nayar and Sauerman (1975) reported that starved *Ae. aegypti* and sugar-fed *Ae. taeniorhynchus* laid eggs with significantly more fat reserves. Epigenetic effects can sometimes be dependent upon both the sex of the parent and of the offspring (Bonduriansky and Day 2009). Grech et al. (2007) explored the effect of parental rearing conditions of *An. stephansi* on offspring biology. They discovered that daughters of parents raised in low food conditions unexpectedly produced more offspring than daughters of parents raised in high food conditions. Since both male and female parents were raised with low or high food, it is not possible to determine if this effect was due to the maternal egg provisioning, or to some other factor such as epigenetics. There was no effect on the offspring's emergence time, size, or survival. The authors hypothesized that laying more eggs quickly may be an optimal strategy for females in poor environments. Otti and Sadd (2008) suggest that the poor nutrition may lead to an increase in early fecundity due to decreased longevity.

Male body size may play an important role in mating success in species that utilize swarms. Yuval et al. (1993) showed that larger males were more likely to be mated after swarming, and only 44% of males appeared to have ever mated at least once. It was unclear whether this was due to stronger flight skills, female choice, or stamina to

remain in swarm and return for a second chance to mate. Ponlawat and Harrington (2007) found that, in *Ae. aegypti*, male size was also a good indicator of total spermatozoa production. Determinedadogo et al. (2013) determined that size of male *An. gambiae* caught *in copula* was significantly greater than the average size of males within mating swarms. *Anopheles* females can apparently use the wing-beat frequency of males (which correlates with size (Cator et al. 2010) to choose mates. Gibson and Russell (2006) documented *Toxorhynchites brevipalpis* synchronize the frequency of their wing-beats before copulation. This ability may serve to prevent interspecific mating. Watanabe et al. (2014) has shown that male leafhoppers (Hemiptera: Cicadellidae) exhibit intrasperm vertical transmission of *Rickettsia* symbionts. Wing length is a very good predictor of fecundity in *Ae. albopictus* (Blackmore and Lord 2000, Armbruster and Hutchinson 2002). Male diet effects offspring metabolism in rats, but these effects are largely unexplained (Ng et al. 2010).

In bed bugs, mated females differentially expressed 20 times more genes as a result of nonheritable sperm variation compared to heritable variation (Otti et al. 2014). While studying parental effects, it is important to keep larval rearing conditions constant, since changes in larval density or diet can easily effect a number of aspects of their biology, including size (Jannat and Roitberg 2013).

**Applications of sugar-feeding for mosquito control**

Ferguson et al. (2005) and Howell and Knols (2009) both cite lack of knowledge of male ecology for failures of transgenic mosquito release programs. Only 3 of fifteen early attempts at population reduction through SIT were considered somewhat successful

as of 2005 (Ferguson et al. 2005). These strategies rely on the mating ability of the released males, which has been poorly studied thus far. They also emphasize that knowledge of factors that affect "longevity, energetic resources, dispersal, habitat use and predation risk" are also very necessary.

The sterile insect technique (SIT) involves the release of sterile males into a population in the hopes of preventing females from obtain viable sperm from wild males. Now other approaches follow the same idea. "Heritable" strategies include "self-limiting" strategies (which would require continued releases), such as lethal transgenes, or certain applications *Wolbachia*, as well as "self-sustaining strategies" such as certain applications of *Wolbachia*, "Y drive", population-wide knock-outs and knock-ins(Burt 2014). Many of these new approaches also require the release of lab-reared mosquitoes into the wild. Fitness costs may be derived from either mass-rearing or from sterilization or genetic manipulation (Ferguson et al. 2005). Carvalho et al. (2013) proposes a two-step transgenic control method, whereby the population would first by suppressed, and then the individuals transmitting the new desired gene would be introduced (as a replacement population). Stone (2013) points out that sterile male release programs need to consider mosquito ecology. There have been numerous releases of sterile male *Aedes* mosquitoes already (Alphey et al. 2013, Bellini et al. 2013, Oliva et al. 2013). SIT programs either sterilize males through radiation, use genetic manipulation to prevent progeny survival, or attempt to use *Wolbachia* to the same effect Oliva et al. (2013). Bellini et al. (2013) determined a 72% reduction in the wild *Ae. albopictus* population in Italy after releasing around 900-1,600 males per hectare per week. Harris et al. (2011) describe the first experimental release of transgenic mosquitoes. They showed that

OX513A males were successfully able to mate with females in the wild and remain somewhat competitive with wild males, although they did suffer a significant fitness cost. Massonnet-Bruneel et al. (2013) also described the fitness of males carrying the dominant lethal OX513A gene. They were able to induce refractoriness as well as wild type males, and had similar mating success; however, their lifespan was 18% lower than wild males. Bargielowski et al. (2012) looked at the flight performance and teneral energy reserves in two genetically modified lines of *Ae. aegypti* with a wild-type. There was no difference in teneral energy reserves or body size; however, flight performance was much worse in the two GM strains.

One obstacle to successful SIT programs is the potential for lowering male fitness in laboratory strains. Lacroix et al. (2012) reported on dispersal and survival of both sterilized and wild-type male *Ae. aegypti* following field releases. For both, longevity was around 2 days, and the maximum dispersal distance was 220m. Bellini et al. (2010) reported flight distances for released *Ae. albopictus* males in an urban setting, finding that they could disperse 200-300m in a week. Baeshen et al. (2014) compared laboratory and wild *An. gambiae ss*, finding that laboratory males had decreased sperm length and accessory gland size, suggesting that introducing field-caught individuals to laboratory colonies may be very important for SIT rearing efforts. Paton et al. (2013) determined the overall survival and mating success in *An. gambiae* was not affected by long-term colonization; however, laboratory individuals lost their assortive mating ability (choosing to mate with individuals of the same strain). Additionally, there is a large difference in copulation time of laboratory versus wild *Ae. albopictus*: 45 versus 8 seconds (Gubler and Bhattacharya 1972; Oliva et al. 2013). However, Bellini et al. (2013) did not find

any difference in the mating competitiveness of sterilized male *Ae. albopictus* in semi-field conditions. Hamady et al. (2013) compared the fitness of laboratory and wild male *Ae. albopictus* mated with laboratory and wild females. Laboratory strains were still able to produce a comparable number of eggs. Interestingly, the offspring of wild females mated with laboratory males showed increased longevity. They concluded that *Ae. albopictus* maintained their fitness even after being colonized for 25 years. Yamada et al. (2014) explored the possibility of using X-ray radiation in place of gamma radiation; however, both of these form of sterilization result in decreased longevity in males.

Pereira et al. (2013) summarized recent efforts to optimize the care of sterile male fruit flies post-eclosion. They discuss provisioning with nutritional, hormonal, and semiochemical supplements; along with optimizing holding conditions and release methods. Certainly, there are major difference in the diets of fruit flies and mosquitoes; male mosquitoes generally obtain sugars from nectar, while fruit flies famously feed on rotting fruits, which naturally contain yeasts and other nutrients not found in nectar. Gavriel et al. (2009) and Perez-Staples et al. (2008) determined re-mating in female fruit flies significantly decline when sterile males were given nutritional supplements during their teneral phase. Fruit flies also produce more pheromones and are therefore more attractive to female when they are fed with yeast and carbohydrates (Epsky and Heath 1993).

Optimizing the rearing and release of sterile males has been the subject of many recent studies. Many focus on larval diet (Puggioli et al. 2013, Yahouédo et al. 2013), but often the most success in mating is coupled with lower longevity, causing a dilemma for scientist trying to optimize fitness. Bellini et al. (2013) tested a device meant to provide a

sugar meal to newly eclosed sterile male mosquitoes. The device did increase the amount of nearby sugar-fed males; however, there was no way to prevent wild fertile males from benefiting as well. Previous studies attempted to determine the optimal larval diet for male *Aedes albopictus* (Puggioli et al. 2013); however, no study has specifically examined optimal retention time of newly eclosed males. A review of facilities used to hold and release sterile male fruit flies found that retention of teneral males would cause significant expense; however, it is unclear if this cost would be outweighed by the benefit of better performance (USDA/APHIS, 2008). Altering post-eclosion holding time based upon the amount of time it takes for a given species to become sexual receptive may be appropriate. Interestingly, female fruit flies are more likely to mate again after mating with younger sterile males, compared with male which are just 5 days older (Gavriel et al. 2009). Sperm competition, where multiple males are seeking to fertilize an egg, is divided into two characteristics: defense quality and offense quality (Sbilordo et al. 2011), where defense is the ability of a male's ejaculate to prevent another male from fertilizing a female he has mated with. Improving this defensive trait would be pivotal for the success of any SIT program where polyandry occurs.

Oliva et al. (2013) examined the defense quality of sterile male *Ae. albopictus* at multiple time points after the first mating with a female. They found that females did not use any sperm from the second mating unless it occurred in close succession to the first (40 minutes), and it was still not very likely (%15). They also determined that males would invest more ejaculate than the female was able to retain. This may suggest that males may expect fewer opportunities to mate than would be available in more polyandrous species, and thus invest more heavily in each opportunity. The extra

ejaculate served to temporarily block the bursa inseminalis, and possibly provide

nutrition to the female once it is broken down. Interestingly, they found that females that

mated with either sterilized or untreated males rarely had all three spermatheca filled.

Observations indicated that there was adequate sperm in the ejaculate, but that it was

never transferred from the bursa inseminalis to the spermathecae, which disappeared 24

hours after mating (Oliva et al. 2013). The number of matings did not affect the number

of spermatheca filled, and sperm remained in the spermatheca, even after six gonotrophic

cycles. A male's copulation success decreased over time when mated with multiple

females and then allowed to rest for three days. Untreated males, allowed to rest, were

able to recover, whereas sterilized males were not. All males were unlikely to avoid

copulating, but were only able to fully inseminate 5 females in one day (Oliva, et al.

2013).  Even after resting, the study estimates that sterile males could fully inseminate

seven females versus 11 in their untreated counterparts, and partially inseminate another

eight, versus nine. Young and Downe (1982) determined that female *Ae. aegypti* were

receptive to second inseminations as early as one hour after the first. A specific steroid

hormone, 20E, transferred during mating in *An. gambiae* is a trigger for a switch in

female behavior, which reduces further mating and induces egg-laying behavior (Gabrieli

et al. 2014).  Sperm depleted males can effect not only female behavior, but the behavior

of other males that come in contact with that female (Louâpre et al. 2014).

Stone (2013) suggested that if males suffer high mortality cost upon release, older

males with greater sexual maturity should be released. Oliva et al. (2012) found that

sterilized males were more competitive with wild males after being maintained on 10%

sucrose for 5 days in the laboratory as compared to one day. It seems likely that wild

males do not "expect" to mate with such high numbers of females as they are artificially able to in the laboratory; otherwise, we would expect that they would ration their sperm investment more carefully (Scharf et al. 2012). Smaller or weaker males would, therefore, be expected to invest more in each mating, as future mating are less likely. Alphey et al. (2013) suggests that SIT programs may inadvertently select for females that mate more than once, in *Ae. albopictus*, these females would need to mate twice within 40 minutes (Oliva et al. 2013a). Oliva et al. (2013b) demonstrated that sugar availability combated the mortality that sterile male *Ae. albopictus* would normally experience in the presence of females, making them more competitive with wild males. Yuval (2006) expressed concern that mating studies performed in the laboratory could not be extrapolated to natural conditions; however, Madakacherry et al. (2013) found rather similar results when they compared mating studies done in small cages (30 $cm^3$), large cages (60 $cm^3$), and semi-field cages (1.75 $m^3$).

Recently, there has been growing interest in the use of attractive plants or volatiles as bait. While some work has been done on "attractive toxic sugar baits" or ASTBs, most work has been done on determining if certain plants are more attractive to particular species of mosquitoes. Some work also uses artificial blends of attractants (Nyasembe et al. 2012, Otienoburu et al. 2012). Mosquitoes generally focus on a few plant species when they are searching for nectar (Foster 1995); however, choosiness generally decreases when energy reserves are low (Jepson and Healy 1988) Kevan and Baker (1983) suggest that many Nemotoceran flies are limited in what nectar sources they have access to due to their proboscis length.

Qualls et al. (2012) proposed the use of ASTBs in wells and cisterns, after showing that a significant proportion of individuals were stained with dye in their prototype trap. Beier et al. (2012) determined that the use of boric acid sugar baits on non-flowering plants drastically reduced populations of *An. sergentii* even if there were abundant sugar sources available.

Revay et al. (2013) demonstrated the use of ATSBs for *Ae. albopictus*. Flowering and non-flowering plants were not different in landing rate; however, flowering plants affected over 5.5% of non-targets, while non-flowering plants affected 0.6% when the method was used in Israel. These numbers may be very different if the method is applied in a different setting. Eugenol was used as the toxic chemical. This demonstrates that the use of flowering plants in ATSBs may have undesired effects on non-target populations, which is a serious concern. However, since applications to non-flowering plants still reduced populations significantly, the method still has merit. Another experiment using only 10% sucrose as bait failed to control populations of *Ae. aegypti* and *Ae. taeniorhunchus* (Xue and Ali 2008). Later, Xue et al. (2011) successfully controlled *Ae. albopictus* by spraying boric acid sugar baits onto plants, and found no difference in flowering an non-flowering applications.

*An. gambiae* have higher fecundity on plants they sought out in the field after a single blood meal, but the effect disappeared after multiple blood meals (Manda et al. 2007). Samson et al. (2013) similarly determined that *Ae. albopictus* were more prevalent on plants with higher sugar content. Müller et al. (2011) investigated floral preferences in *Ae. albopictus* in Israel. Six ornamentals, eleven wild flowering plants, and fresh and rotting fruits were used as bait. *Tamarix chinensis, Vitex agnus-castus,*

*Polygonum baldchuanicum, Buddleja davidii, Prosopis farcta, Ziziphus spina-christi, Polygonum equisetiforme,* and *Ceratonia siliqua* were most attractive. Very ripe or damaged fruit was attractive, while fresh fruits were not. Grimstad and DeFoliart (1974) determined that mosquitoes switched from feeding predominantly on one nectar source to another depending upon the age of the flowers. Females preferred glucose-rich composite flowers (such as ox-eye daisy, yarrow, and goldenrod), while males preferred sucrose-rich plants such as common milkweed. In general mosquitoes prefer lighter colored flowers, or darker flowers with a strong odor, while shape seems unimportant; some researchers hypothesize that this is due to better nighttime visibility (Grimstad and DeFoliart 1974, Foster 1995).

Hall-Mendelin et al. (2010) propose using honey-infused cards as an easy way to monitor arboviruses, since the mosquitoes release virus from their salivary gland during feeding. Junnila et al. (2010) explored the use of PCR to detect plant feeding by mosquitoes. They determined that in dry weather, mosquitoes were more likely to rely on tissue-feeding when nectar was presumably scarce. (Samson et al. 2013) made anecdotal observations that *Ae. albopictus* prevalence around humans increased when particular plants were more prevalent. In contrast, Stone et al. (2012) documented higher bite rates when *An. gambiae* were caged with sugar-poor plants. However, it is not known whether mosquitoes would remain near sugar-poor plants in the field.

**The Asian tiger mosquito**

*Aedes albopictus* originated in southeast Asia and the surrounding islands. *Aedes albopictus* has become a very invasive species (Juliano and Lounibos 2005), which is

known to adapt well to new environments and compete well with local species for limited resources.  It was first discovered in the US, in Texas in 1987 (Moore and Mitchell 1997).

The species is a competent vector of at least 22 arboviruses (Gratz 2004). It has been implicated in outbreaks of Dengue (DENV) and Chikungunya (Delatte et al. 2008, Bonizzoni et al. 2013), and is currently of concern in potential future outbreaks in the US (Franco et al. 2010). It is a less efficient vector of DENV than the primary vector, *Ae. aegypti*; however, its prevalence has made it an important player in recent outbreaks. It has recently spread to all continents except Antarctica. It was first found in the continental US in 1985 in Texas. As of 2013, it is found in 23 states. It's distribution is assumed to be human mediated, as it is often transported in tires and plant material (Bonizzoni et al. 2013). *Ae. albopictus* may be able to transmit West Nile as well as *Dirofilaria* (dog heartworm), Eastern Equine Encephalitis, Venezuelan equine encephalitis, Japanese encephalitis, Cache valley virus, Jamestown Canyon virus, and La Crosse virus(Moore and Mitchell 1997, Gerhardt et al. 1999, Turell et al. 2005). BG-Sentinel traps are an important method for monitoring *Ae. albopictus*, as traditional CDC and gravid traps are not very effective (Farajollahi et al. 2009).  Vertical transmission of West Nile virus is possible in this species in the laboratory, but is unknown in the field (Kramer et al. 2008).

The Asian tiger mosquito is an aggressive anthropophilic day-time biter. Many scientists are concerned about its rapid spread due to increased human traffic across the globe and the "ecological plasticity" of this species (Bonizzoni et al. 2013).  The mosquito was originally considered a "rural" species, inhabiting natural water sources in it larval stage in its native range; however, it has quickly adapted to small man-made

vessels. The species also exhibits rapid evolution in the diapausing form, adapting to

daylight regimes in the United States (Lounibos et al. 2003). The species is often highly

competitive with native mosquitoes, as well as other invasive species such as *Ae. aegypti*

(Bonizzoni et al. 2013). It has a very strong preference for human blood-meals, but will

also take blood from other animals if needed (Ponlawat and Harrington 2005).


**Rationale and Hypotheses**

My goal in writing this dissertation was to elucidate multiple aspects of sugar-

feeding behavior in the Asian tiger mosquito, *Aedes albopictus*. The purpose of this work

was not simply theoretical, as information about the biology of a vector species can be

pivotal for successful management. In Chapter 1, I examine the effect of diapause cues on

female dietary choices. I tested the hypothesis that females exposed to diapause cues

would prioritize sugar meals when offered blood and sugar concurrently, because it

would be advantageous to allocate the majority of their energy resources to laying as

many diapausing eggs as possible before winter rather than allocating energy to increased

longevity. In Chapter 2, I investigate the importance of sugar availability for adult male

mating success. I tested the hypothesis that longer periods of sugar provisioning would

have a positive impact on male fitness and therefore, competitiveness. The findings in

these two chapters may prove beneficial to mosquito management efforts, particularly

those based upon sugar attractants and sterile male release initiatives.

In Chapter 3, I explore the effect of the adult male diet on their offspring. I tested

the hypothesis that well provisioned adult *Aedes albopictus* males produce more

offspring than males with only water access, and furthermore, that offspring of well-

provisioned males have greater fitness than offspring of starved males. I also developed a

mathematical model to examine whether the paternal effect I observed might increase

offspring fitness under certain conditions. This chapter examines the existence of

environmentally mediated paternal effects in mosquitoes, and more broadly in insects.

The findings in this chapter have implications for control programs seek to alter

environmental conditions, specifically those related to sugar resources. They also have

broader implications for the field of entomology, since paternal effects have rarely been

documented.

**Chapter 1:**

**Day-length affects dietary choices in female *Aedes albopictus* (Diptera: Culicidae)**

**Abstract**

In temperate regions, female *Aedes albopictus* begin to lay diapausing eggs as day-length decreases in the autumn. These eggs contain more lipids and hydrocarbons, allowing them to withstand desiccation and low temperatures in the winter. We hypothesized that females would increase their carbohydrate intake to favor the production of diapausing eggs when reared in short-day conditions compared to those reared in long-day conditions. We compared food choices of individual adult females, which were exposed to either a long-day (14:10 L:D) or a short-day light regime (10:14 L:D) which induces diapause in this species. For both treatments we offered blood and sugar meals concurrently every three days, and tracked the choice patterns of 40 females. We ensured that diapause was successfully induced by examining the eggs from both treatments. Diapause cues affected female food choice, particularly the initial choice. The great majority of females took only a single blood meal, regardless of treatment. However, the timing of the blood meal was altered: long-day females were 3 times more likely to choose blood at their first feeding opportunity. The frequency of sugar meals was also altered by treatment with short-day females exhibiting a 33% increase. This may suggest that bite-rates per female may be altered in the autumn depending on the age structure of the population, and that sugar meals may become more important at that time. These findings may be beneficial to mosquito control efforts, particularly those based upon sugar attractants.

**Introduction**

*Aedes albopictus*, the Asian tiger mosquito, is an aggressive day-biting container mosquito. This species' invasive nature, ability to transmit Cache Valley, Eastern equine encephalitis, Jamestown Canyon, La Crosse, and West Nile, and strong preference for human blood (Moore and Mitchell 1997, Gerhardt et al. 1999, Turell et al. 2005, Farajollahi and Nelder 2009, Muñnoz et al. 2011) have made it a serious pest in the United States. Elsewhere, *Ae. albopictus* has been largely responsible for outbreaks of Dengue virus and Chikungunya (Delatte et al. 2008, Bonizzoni et al. 2013). Increasing attention is being given to the biology of *Ae. albopictus* with the hope of using new information in control efforts (Bonizzoni et al. 2013).

The Asian tiger mosquito's rapid adaptation to local daylight regimes is one reason for the species' success in the United States (Lounibos et al. 2003). Diapause is "a form of dormancy that is hormonally programmed in advance of its onset and is not immediately terminated in response to favorable conditions" (Denlinger and Armbruster 2014). Only certain strains of *Ae. albopictus* have the ability to diapause. Lounibos et al. (2003) demonstrated that introductions into the Americas originated from multiple locations, resulting in different levels of diapause expression, depending on latitude. Poor larval diet also increases the incidence of diapause in *Ae. albopictus* (Pumpuni et al. 1992). *Ae. albopictus* from the northern US exhibit slower development and large size when exposed to long light cycles mimicking summer, illustrating changes more subtle than the diapause phenomenon (Yee et al. 2012). Reynolds et al. (2012) demonstrated that larvae in diapausing eggs contained 30% more lipids than their non-diapausing counterparts. In *Ae. albopictus*, diapause eggs are more cold and desiccation tolerant

(Sota and Mogi 1992). Such eggs have one third more surface hydrocarbons, likely due to the upregulation of fatty acyl coA elongase (Urbanski et al. 2010).

Changes resulting from diapause cues are not limited to eggs, however. There is evidence that diapause cues can cause behavioral changes in adult mosquitoes as well (Denlinger and Armbruster 2014). For instance, *Cx. pipiens* discontinues blood-feeding in preparation for over-wintering in the adult phase, and *Ae. mariae* alters its oviposition tactics with females becoming much less likely to oviposit on water surfaces when reared with diapause cues (Coluzzi et al. 1975).

Unlike males, which feed solely on sugar, female mosquitoes must choose between blood and sugar meals when both resources are available. Predicting these choices is more challenging than one might expect. Foster (1995) points out that food choice is a serious dilemma for female mosquitoes since they will be allowed only a few feeding opportunities in their lifetime, and this choice greatly impacts their fitness. While a blood meal is primarily used to obtain protein and other nutrients needed for egg production, it may also be used for energy.  Similarly, sugar-meals can be used as an energy source, but can also contribute to an individual's fecundity (Nayar & Sauerman, 1973; Van Handel, 1965). For female *Ae. sollicitans*, equivalent caloric intake of protein blood or sugar solution results in an equivalent rate of fat synthesis. However, fat produced from a blood-meal is only 66% of the amount produced from the equivalent number of calories from sugar.  This may be due to blood-meal proteins being used for the production of eggs. Mosquitoes can use blood to produce the same amount of glycogen as sugar, but the speed of glycogen production is ten times slower from blood (Van Handel 1965).

Our objective was to determine the effect of day-length, and therefore the effect of laying diapausing eggs, on the dietary choices of adult female *Ae. albopictus*. We assessed the food choices of individual females that were raised under either long-day light conditions, such as those a wild mosquito might experience in the summer, or short-day light conditions, known to induce diapause. We hypothesized that females exposed to diapause cues would increase sugar meals when offered blood and sugar concurrently, since more energy is required to produce diapause eggs, and sugar meals are usually more widely available and can be obtained with less risk.

**Methods**

**Rearing and Maintenance.**

Eggs from a laboratory colony of *Ae. albopictus* were used. This colony was established from eggs collected in Mercer County, NJ, U.S.A. in 2008 and supplemented in subsequent years. Eggs were hatched, 250 per 23x33 cm ceramic tray with 1 liter tap water. Each tray was provisioned with 150 mg brewer's yeast-lactoalbumin (50:50 by weight) on alternate days.

**Experimental conditions.**

Pupae were exposed to either a 14:10 or a 10:14 hour light:dark cycle, referred to as long-day (LD) and short-day (SD) treatments, respectively. For both treatments, a temperature of $21^\circ$ C was maintained for the entirety of the experiment, as this is the optimal temperature for inducing diapause in *Ae. albopictus* (Pumpuni et al. 1992, Lounibos et al. 2003). Following eclosion, 30 female and 60 male adults were moved to a 30.4 cm$^3$ screened cage. Test cages remained under conditions consistent with those used

for rearing, and were provisioned with water-soaked cotton dental wicks. After 72 hours

post-eclosion, individual adult females were provided with a restrained bob white quail

concurrently with a 20% sucrose solution for 10 minutes, 1 hour prior to the dark cycle

(Animal Use Protocol #86-129, Rutgers University). Sucrose solution was delivered via

dental wicks. Immediately after food sources were removed, each female was visually

examined and the number of blood-fed, sugar-fed, or empty individuals was recorded. If

a female was in the process of feeding at the end of the choice test, she was allowed to

finish. This procedure was repeated at 3-day intervals until all of the females from one

treatment were dead. The choices of individual females were tracked by keep with

different choice histories in separate cages.

**Checking Diapause Induction.**

Each cage was supplied with oviposition cups whenever females were present. A

0.94 liter black mason jar was filled with approximately 500 mL water and lined with

Whatman #2 filter paper. Filter papers were changed at 3-day intervals, allowed to air dry

for four days, and then flooded. We attempted to hatch the eggs from both treatments to

ascertain if maternal rearing conditions had successfully induced diapause, according to

the methods of Lounibos (2003). A 5% sodium hypochlorite solution was used to

examine un-hatched eggs for embryonation, and the number embryonated was recorded.

**Statistical Analyses.**

A stacked multinomial logistic model was used to compare choices over time

between the two treatments (PROC GLIMMIX, SAS Institute 2012). This test was

chosen since the experiment involved more than two discreet outcomes (females could

chose blood, sugar, or nothing) and had more than one independent variable (day-length

and female age). The effect of treatment and the effect of the interaction treatment*age on choice was assessed.  Each female's initial food choice post-eclosion was also compared.

## Results

### Diapause Induction.

After flooding eggs for the first time, hatch rates of 30.1% and 0% of total eggs were recorded for long-day and short-day treatments, respectively, indicating successful induction of diapause (Table 1.1). Examining unhatched eggs for embryogenesis revealed similar viability rates; 96.4% and 97.9% for long-day and short-day respectively.

### Choice Frequency.

Females almost exclusively took at single blood meal throughout the experiment. A single female out of 40 took two blood meals in each treatment. The age at which females took a blood meal varied by treatment. Long-day females were three times more likely to take a blood meal at the first opportunity compared to short-day females (Fig. 1.1). The total number of blood and sugar meals taken was affected by treatment (Fig. 1.3). Short-day females took 88% more total sugar meals, whereas long-day females were more likely to have chosen not to feed.

### Treatment and Age Effects.

The effect of day-length and female age on diet choice was tested using a stacked multinomial logistic model (PROC GLIMMIX, SAS). We found that day-length was explanatory for choice ($F = 4.26$; $df = 2, 266$; $P < 0.05$). However, we also found that the interaction between female age and day-length was explanatory for choice ($F = 3.42$; $df =$

4, 266; $P < 0.01$). Specifically, within the long-day treatment, female age impacted choice ($F=3.36$; $df= 5, 266$; $P < 0.006$), while age had no effect on female choice in the short-day treatment ($F=1.94$; $df=2, 266$; $P < 0.09$) (Fig. 1.2). The strongest impact of female age on diet choice were observed on days five ($F=3.22$; $df=3, 266$; $P < 0.03$) and 11 ($F=3.85$; $df=3,266$; $P = 0.01$), the first and third feeding opportunities, irrespective of treatment.

**Discussion**

Our study suggests that day-length impacts dietary choices in female *Ae. albopictus.* We observed different feeding strategies depending on day-length, especially at the first feeding opportunity. That is, short-day females sought sugar most often and long-day females sought blood most often. If seasonal changes in dietary preference exist, these should direct the use of management methods which rely on attraction to either blood-meal or sugar-meal cues.

For example, "attractive toxic sugar baits" (ATSBs) have recently been tested for use in the managment of a number of mosquitoes, including *Ae. albopictus* (Xue et al. 2011, Revay et al. 2013). With growing concerns about non-targets, Revay et al. (2013) demonstrated that ATSBs do not need to be applied to flowering plants to be effective since applications to non-flowering plants still significantly reduced populations. The sugar-attraction of *Ae. albopictus* may also have other novel uses in the future; for example, Hall-Mendelin et al. (2010) proposed using honey-infused cards to monitor arboviruses, since the mosquitoes release virus from their salivary gland during feeding. We predict that ATSBs would be more effective during the fall if, as our data suggests,

*Ae. albopictus* demonstrates increased attraction to sugar at that time. Our study also predicts that the proportion of the adult female population attracted to traps simulating a blood-meal will also be altered by the season and the population's age distribution, as the strong initial preference for blood observed in females reared during long days apparently diminished in females laying diapausing eggs.

Order and timing of dietary choices in female mosquitoes has been studied extensively; however, there are notable variations in findings. For instance, *Anopheles gambiae* females take a sugar meal approximately once every four days (Gary and Foster 2006). Timing is such that sugar meals are at least two days after blood meals, generally placing them between gonotrophic cycles. When blood or oviposition sites are not readily available, sugar-feeding becomes more common. Newly emerged females take sugar meals facultatively when blood is unavailable. Interestingly, the study demonstrated notable variation in the choices of individual females, where some strongly preferred blood and others sugar (Gary and Foster 2006). We observed a similar phenomenon, where some females did not follow the general trend in their feeding order. The cause of this variation is not known. However, we hypothesize that this may be triggered by larval diet, genetic variation, or other cues that are less obvious. Foster and Takken (2004) observed that newly eclosed *An. gambiae* females were most attracted to nectar-related volatiles, but after maturing and sugar feeding, switched to targeting human volatiles. Similarly, Stone et al. (2011) demonstrated that *An. gambiae* generally prioritize sugar meals before mating and blood meals after mating. In contrast, we found that under long-day light conditions, most *Ae. albopictus* females chose to take a blood meal first, while individuals receiving diapause cues (short days) exhibited patterns more similar to *An.*

*gambiae*. This may be partially explained by our experimental design. Since adult males and females were caged together, it was not possible for us to determine on what day a given female mated. It is possible that younger females, not yet receptive to mating would have made different choice than those in our experiment, which were three days old when first allowed a food choice.

The energetics involved in digesting blood or sugar are complex in Culicidae and there appears to be variation among species, and even within species. Gary and Foster (2001) found that for *An. gambiae*, sugar availability led to a decrease in blood meals and extended lifespan, but no difference in lifetime fecundity. Similarly, newly emerged *An. gambiae* of both sexes show significant attraction to nectar-related volatiles even in the presence of human volatiles (Foster & Takken, 2004). Yee et al. (1992) examined the timing of blood- and sugar-meals in two *Aedes* species in the field. For both species, blood-feeding peaked slightly sooner that sugar-feeding, but both overlapped. This might suggest that blood-meals were sought first, and nectar would occasionally serve as a second choice. Yee & Foster (1992) also observed the blood- and sugar-feeding patterns of multiple mosquito species in the field, including *Ae. albopictus*. Sugar and blood feeding activities occupied the same time periods; both occurred continuously, with a high peak in the evening and a low peak in the morning. Blood-feeding occurred more consistently through the nighttime period. The study did not examine interactions between the two activities, however, highlighting the importance of laboratory studies where individuals can tracked over their lifetime. Our data conflict with the some of the findings of Yee & Foster (1992); in their study, when a host was present, sugar-feeding was almost completely abandoned in both *Ae. albopictus* and *Ae. aegypti*, but not *An.*

*quadrimaculatus* or *Culex quinquefasciatus*, while we observed *Ae. albopictus* females take over 100 sugar meals in the presence of a blood-source. Magnarelli (1979) observed that *Ae. cantator and Ae. sollicitans* fed on flowing plants during the same time period they were more active in taking blood meals. Xue et al. (2008) saw that access to a sugar meal after blood feeding increased longevity in female *Ae. albopictus*.

Klowden & Briegel (1994) reported that *Aedes* (including *Ae. albopictus*) but not *Anopheles* species were inhibited from seeking a second blood meal during a single gonotrophic cycle. This concurs with our finding that *Ae. albopictus* females took a single blood meal, almost as a rule; there was only a single instance of an individual taking two blood meals in each treatment. Xue et al. (2012) examined host seeking in female *Ae. albopictus* and determined that increased body size led to increased sensitivity to human body odor. Mated females were most likely to land on a host, but many un-inseminated females still exhibited host-seeking activity.

A female mosquito must obtain energy not only for flight activities, but also for the equally important task of producing eggs. Female mosquitoes usually delay egg development if sufficient nutritional reserves are not available (Foster, 1995). However, we propose that this may not be a viable option a female who ecloses in the fall as temperatures decrease. Therefore, these female must perform a "balancing act" making food choices in order to maximize fecundity with a decreased focus on their own longevity. The ideal diet for such a mosquito is not very obvious from the literature. The amount of protein, lipid, and other nutrients supplied by blood or sugar can be dependent on many factors, and this tradeoff undoubtedly plays a role in female diet choices. Lea and Van Handel (1970) report that lipid reserves accumulate much slower from blood

digestion versus sugar. Briegel and Timmermann (2001) found that *Ae. albopictus* females were able to produce a four-fold increase in their glycogen stores after a week of sucrose access and their lipid stores ten-fold after two weeks. The number of eggs produced by these females ranged from 15-110 per individual. Blood meal protein usage was inversely correlated with temperature, ranging from 35-50%. In general, *Ae. albopictus* females increase their own lipid stores concurrently with their egg yolk lipids. Nayar and Sauerman (1975) reported that starved *Ae. sollicitans* females needed to consume 2-3 times more blood volume than sugar to produce an equivalent amount of eggs. Damiens et al. (2013) observed that depriving *An. arabiensis* females of sugar seven hours prior to blood feeding increased egg production by 50%. If blood and sugar are taken in close succession, taking blood first may have the advantage of increase egg production, while the opposite order may actually decrease it (Foster et al. 1989; Klowden & Chambers, 1989). Our data suggest that *Ae. albopictus* do not always follow the same food-choice strategy, particularly with the timing of the blood meal. We observed that long-day females were much more likely to chose a blood meal first, and subsequently took less sugar. Foster et al. (1989) suggested that *Ae. aegypti* females should attempt to increase their energy reserves by sugar feeding, and later, take a blood-meal when their crop is empty in order to maximize fecundity. This is because *Ae. aegypti* has the highest fecundity if they have high energy reserves and an empty crop when they take their first blood meal; having an empty crop allows for a larger blood meal (Mostowy and Foster 2004). In contrast, Edman et al. (1992) observed that wild female *Ae. aegypti* in Thailand infrequently utilize available sugar sources, suggesting they relied more heavily on blood for survival and reproduction. This suggests variation

even within species. *Ae. albopictus* is known for it's plasticity or adaptability to regional conditions; therefore, our finding may not apply to individuals from other regions, but we recommend that this should be the subject of further studies.

While many studies have attempted to elucidate feeding patterns in *Ae. albopictus*, we believe ours is the first to attempt to determine if diapause cues might cause changes in "normal" feeding behavior. We hypothesize that short-day females seek sugar more often in order to obtain sufficient energy diapausing eggs, which have higher lipid and hydrocarbon composition (Urbanski et al. 2010, Reynolds et al. 2012). Altered meal choices have serious implications for many aspects of vector biology including vector competence, the use of bite-rate/landing counts, the use of certain trapping methods and the importance of floral attractants; therefore, we recommend tailoring control programs to seasonal changes in the target population. There is also a need for more extensive studies of the metabolism of *Ae. albopictus*, particularly in relation to diapause, together with field studies to determine if females are truly more attracted to sugar sources in the fall, when light conditions result in the production of diapausing eggs.
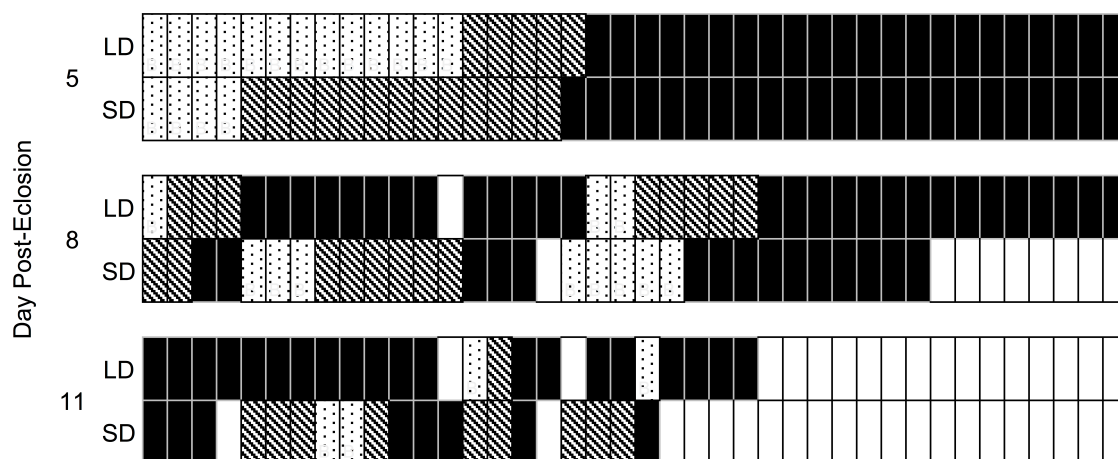
In conclusion, improved understanding of seasonal cues affecting *Ae. albopictus*, and the resulting changes in dietary preferences, may be beneficial to vector control applications, but further laboratory and field studies should be conducted to examine regional variation in the species.

**Acknowledgements**

| Treatment (L:D cycle) | Total Eggs | Total Hatch | % Hatch | Total Un-hatched Embryonated | % Un-hatched Embryonated | Total Viable | % Viable |
|---|---|---|---|---|---|---|---|
| 14:10 | 166 | 50 | 30.1 | 110 | 66.2 | 160 | 96.4 |
| 10:14 | 48 | 0 | 0 | 47 | 97.9 | 47 | 97.9 |

**Table 1.1** F1 generation hatch rates for diapausing and non-diapausing *Aedes albopictus.*

**Fig. 1.1** Diet choice by individual in female *Ae. albopictus* for first three feeding

opportunities. (empty-black; sugar-diagonal bars; blood-dots; dead-unfilled)

**Fig. 1.2** Proportion adult female *Ae. albopictus* food choice by day post-eclosion.

(empty-black; sugar-diagonal bars; blood-grey)

**Fig. 1.3** Proportion life-time meal choices by day-length in adult *Ae. albopictus* females.

(empty-black; fed-white; sugar-diagonal bars; blood-dots)

**Chapter 2:**

**Long-term effects of carbohydrate availability on mating success of newly eclosed
*Aedes albopictus* (Diptera: Culicidae) males**

**Abstract**

Sugar availability varies greatly in nature, and determining how this affects male mosquito fitness is essential for understanding population dynamics. We allowed male *Aedes albopictus* carbohydrate access for increasing intervals of time immediately after eclosion and we evaluated their fitness by comparing mortality, mating success, and sperm transfer. We compared individual male *Aedes albopictus*, which were offered water or 20% sucrose solution for 24, 48, or 72 hrs. We found significant increases in fitness for each additional day of sucrose access. Following sugar exposure, we allowed males daily access to three virgin females. We assessed mating success through observation of spermatozoa in the female spermathecae. When individuals of the same age were compared, males with sugar access exhibited significantly greater mating success than water-treated males in all treatments. The total number of filled spermathecae by males with sugar access in the 48 and 72 hr treatments was also significantly greater on some days; these were 3-5 days post-eclosion in the 48 hr treatment and 5-6 days post-emergence in the 72 hr treatment. The age at which males first had access to females did not effect their mating success. We conclude that extended sugar access at eclosion is important for maximizing fitness in male *Ae. albopictus* and should be applicable to sterile male release efforts, especially when lab-reared males suffer from other disadvantages. We recommend retaining adult males for three days post eclosion prior to release to improve their mating success in male release initiatives.

**Introduction**

Mosquitoes differ in the carbohydrate intake they require as adults depending upon their sex and species. In some instances, adult females may consume blood in the place of plant sugar meals (Braks et al. 2006, Edman et al. 1992); however, males universally require plant sugar meals to increase their low teneral energy supply. For this reason, males will attempt to increase and maintain their energy reserves through repeated sugar feeding immediately following eclosion, even before seeking to mate (Yuval 1992).

Until recently, male mosquito fitness was largely ignored in the context of carbohydrate studies (Foster 1995). Gary et al. (2009) examined the effect of sugar availability on male mosquitoes by quantifying successful mating attempts and concluded that under certain conditions, sugar-deprived *Anopheles* males can inseminate females, but they are unable to compete with males that had sugar access. Verifying which factors influence male mosquito fitness is extremely important for control efforts. Newer literature stresses the importance of gathering knowledge about male mating biology, and points to control failures due to lack of knowledge in this area (Howell and Knols 2009, Ferguson et al. 2010) .

One such control effort, sterile insect technique (SIT), is dependent on male fitness and mating biology. This method relies upon flooding an area with sterile or transgenic males that will attempt to mate with wild females. Ideally, those females will mate only once; and therefore will be unable to produce any viable offspring. In many instances, sterilization techniques and transgenic changes reduce the fitness of the male mosquitoes (Bargielowski et al. 2012).  It has proven to be an effective tool for

controlling other Dipteran populations, and is currently being attempted with several mosquito species including *Aedes albopictus* and *Aedes aegypti* (Oliva et al. 2012, Lacroix et al. 2012). Optimizing the condition of the released males could potentially be a simple way to improve the effectiveness of this method. Increasing energy reserves in newly eclosed males through the use of release stations has been proposed by Bellini et al. (2013); unfortunately, this method limits the researchers' control over the length of time that individuals are exposed to sugar meals.  However, the study indicated that decreased relative humidity and increased temperatures resulted in a decreased number of males testing sugar positive in the release vicinity, suggesting that supplementing males before release may be of increased importance depending upon regional weather conditions.

One popular target of current SIT programs is the increasingly notorious Asian tiger mosquito, *Ae. albopictus*, an invasive container mosquito, which transmits Cache Valley, Eastern equine encephalitis, Jamestown Canyon, La Crosse, and West Nile in the United States (Moore and Mitchell 1997, Gerhardt et al. 1999, Turell et al. 2005). The mosquito is also becoming an increasingly serious nuisance in many parts of the United States, since it's population has multiplied and will bite during the daytime (Moore and Mitchell 1997; Farajollahi and Nelder 2009). Sugar availability appears to be more important than blood for longevity of *Ae. albopictus* females (Xue et al. 2010), but male biology requires more attention if population dynamics and mating success is to be understood.  Female *Ae. albopictus* will occasionally mate more that once (Boyer et al. 2012), highlighting the need to ensure sterile males are competitive. Reisen (2003) pointed out that releases of sterile *Anopheles culicifaces, Culex tritaenirhynchus*, and

*Culex tarsalis* all failed when laboratory strains of released males were unexpectedly not competitive with wild males in the field.

The objective of this study was to determine the effect of sugar provisioning on the fitness of *Ae. albopictus* males. We assessed longevity, mating success, and the relative volume of sperm transfer in males that were given water or carbohydrate access for 24, 48, or 72 hrs, and subsequently starved. We hypothesized that longer periods of sugar provisioning would have a positive impact on male fitness and therefore, competitiveness.

**Material and Methods**

**Rearing and Maintenance.**

Eggs from a laboratory colony of *Ae. albopictus* were used. This colony was established from eggs collected in Mercer County, NJ, U.S.A. in 2008 and supplemented in subsequent years. Eggs were hatched, with 200 per 23x33 cm ceramic tray, with 1 liter of tap water. Each tray was provisioned with 0.12 g brewer's yeast and lactoalbumin (50:50) at two day intervals until the first day pupation was observed. The portion was then reduced to .08 g at two day intervals. Pupae were removed from rearing trays daily and sorted by sex.

**Experimental Conditions.**

Female pupae were placed in a 15x4 cm glass dish filled with 200mL water. The dish was placed in a 3.8 liter cardboard soup cup with a mesh lid with constant access to two sugar-water-soaked cotton dental wicks (20% sucrose concentration). Newly-eclosed adult females were removed from the gallon-sized cage daily and transferred into a

separate 1-liter modified soup cup with access to two 20% sucrose via dental wicks. This guaranteed the ages of all females were known. Only 3-4 day-old adults were used in the experiment, ensuring that they were sexually receptive when introduced to male cages (Gwadz and Craig Jr 1968). Male pupae were placed in identical 3.8-liter containers with constant access to water-wicks. Daily, individual newly-eclosed adult males were moved immediately into the experimental containers, with a single male per container. Excess males were discarded daily.

The experiment occurred in 1-liter modified cardboard soup cups with a 16:8 L:D photoperiod at 25±1°C. Males in sugar treatments were given access to 20% sucrose for 24, 48, or 72 h beginning immediately after their placement into the experimental containers. Males in the 0 h treatments were given only water. Three equivalent water treatments used for comparison, such that the age of the males at the time of first mating opportunity would mirror the three sugar-access treatments.

After males were exposed for the assigned duration, all sugar wicks were replaced with water wicks. Concurrently, three 3-4 day-old females were added to each cage. It is unlikely that a single male would attempt to mate with more than three females in a single night (Boyer et al. 2012). After each 24 hr period, these females were removed, placed into 1.5 mL Eppendorf tubes and stored at -70°C until they could be dissected. Immediately after the exposed females were removed, three fresh females were added to each cage. This was repeated daily until all males in the control treatment had died. Male mortality data was recorded daily. Each treatment was replicated 15 times.

**Spermathecae dissections.**

All spermathecae were dissected from each female and crushed with a cover slip on a

glass microscope slide in 0.5% saline solution. These were examined under light

microscope, and the number containing spermatozoa was recorded. In all instances we

found three spermathecae present.

**Statistical Analyses.**

Analysis of variance (ANOVA) was used to determine the effect of sugar

exposure time on longevity and the average number of females mated per day. Bonferroni

tests were used to determine if there were significant differences in the dependent

variables: longevity mating success, and spermatozoa volume depending on treatment.

A linear regression analysis was used analyze the effect of sugar exposure time on

survivorship. All analyses were conducted using SPSS Statistics v. 21.


**Results**

**Survivorship.**

The length of sugar access had a significant impact upon male survivorship ($F=$

38.7, $df$=89, $P$<0.0001, Fig. 2.1).  Data for all three "water only" treatments were pooled

and labeled as "0 hr" sugar access, since age of first mating opportunity had no

significant effect on survivorship.  Mean survivorship was 4.5, 11.8, 16.0 and 19.8 days

for 0, 24, 48, and 72 hr treatments respectively (Fig. 2.2).  In Bonferroni post hoc tests,

the 0 hr treatment was significantly different from all other treatments ($P$<0.0001); the 24

and 72 hr treatments were different ($P$<0.001), but the 48 hr treatment was not different

from the 24 ($P$=0.221) or 72 hr treatment ($P$=0.35). The linear regression model

demonstrated that for each additional 24 hrs of sugar provisioning, males survived an average of 4.77 additional days (y=0.1988x + 5.67; $R^2$=0.959; $P$=0.02).

**Mating Success.**

Significant differences were seen in mating success between sucrose and water treated males for the 24 ($F$=7.5, $df$=29, $P$<0.01), 48 ($F$=13.6, $df$=29, $P$<0.001), and 72 hr treatments ($F$=31.4, $df$=29, $P$<0.001) when the total number of females inseminated by males of the same age were compared. The length of sugar exposure and age of first mating opportunity did not have an impact on the total number of females inseminated by each male ($F$=3.12, $df$=44, $P$=0.055); the mean number of mated females in sugar treatments were 3.0, 2.6, and 4.0 for 24, 48, and 72 hr treatments, respectively. The age of first mating opportunity did not have an impact on total number of females inseminated by starved males ($F$=2.6, $df$=44, P=0.088). The pooled mean number of mated females was 3.22 per male with sugar access and 1.16 per male with water access (n=45). When analyzing the data by day, there were significant differences in the 24 hr treatments on day 5; the 48 hr treatments on all days; and the 72 hr treatments on all days (Fig. 2.3).

**Relative Spermatozoa Volume.**

Significant differences were also seen in sperm transferred between sucrose and water treated males for the 24 (F=5.7, df=29, P<0.05), 48 (F=13.1, df=29, P=0.001), and 72 hr treatments (F=20, df=29, P=0.0001) when the total number of spermatheca filled by males of the same age were compared. The length of sugar exposure and age of first mating opportunity impacted the total number of spermatheca filled by each male ($F$=6.59, $df$=44, $P$=0.003); the mean number of spermatheca filled in sugar treatments

were 4.2, 2.9, and 6.0 for 24, 48, and 72 hr treatments, respectively. Bonferroni post-hoc analysis demonstrated a difference in the 48 and 72 hr sugar treatments (P=0.002). The age of first mating opportunity did have an impact on total number of spermatheca by starved males (*F*=3.3, *df*=44, P=0.045); however, post-hoc tests did not resolve which treatments were different. The pooled mean number of spermatheca filled was 3.22 per male with water access (n=45). When analyzing the data by day, there were differences in the 24 hr treatments on day 5; the 48 hr treatments on days 3, 4 and 5; and the 72 hr treatments on days 5, 6, and 7 (Fig. 2.4).

**Discussion**

Our study indicates that altering rearing methods for male *Ae. albopictus* can increase fitness, and should therefore be considered in the context of sterile male release initiatives where competition with wild males is of the utmost importance. SIT programs should be able to function more efficiently if each male released survives longer, mates with more wild females, and more often induces in them a reluctance to mate again. We saw male longevity, mating success, and the relative spermatozoa volume transferred notably increase after allowing males to sugar feed for two or three days post-eclosion compared to those males that were starved or only fed for a single day. For the purposes of our study, "mating success" refers to the number of females who were mated as opposed to fecundity, since sterile males would not produce offspring.

Currently, mosquito release initiatives either do not provision adult males, or only do so briefly. Optimizing the rearing and release of sterile males has been the subject of many recent studies. Many focus on larval diet (Puggioli et al. 2013, Yahouédo et al.

2013), but often, increases in mating success are coupled with lower longevity, causing a dilemma for researchers who are trying to optimize fitness. Bellini et al. (2013) have tested a device meant to provide a sugar meal to newly eclosed sterile male mosquitoes. The device did increase the amount of nearby sugar-fed males; however, there was no way to prevent wild fertile males from benefiting as well. Previous studies attempted to determine the optimal larval diet for male *Aedes albopictus* (Puggioli et al. 2013), but no study has specifically examined optimal retention time of newly eclosed males. A review of facilities used to hold and release sterile male fruit flies found that retention of teneral males would be a significant expense; however, it is unclear if this cost would be outweighed by the benefit of better performance (USDA/APHIS, 2008). Altering post-eclosion holding time based upon the amount of time it takes for a given species to become sexually receptive may be appropriate. Looking to other Dipteran groups, female fruit flies are more likely to mate again after mating with younger sterile males, compared with male which are just 5 days older (Gavriel et al. 2009).

We assessed periods of retention for eclosed adults prior to release. It is already well established that male mosquitoes require sugar meals upon eclosion in order to supplement their lower teneral energy reserves (Yuval 1992), and our results generally supported these findings.  We found that male longevity increased by 8 days, from 11.8 to 19.8, after two additional days of sugar feeding.  This would suggest that allowing male feeding for three days could significantly reduce the constraints on longevity due to low teneral energy reserves, even if these adults never found another sugar source after release. We believe it may be worthwhile to investigate provisioning males for over 72 hours in the future. Oliva et al. (2013) also demonstrated that sugar availability combated

the mortality that sterile male *Ae. albopictus* would normally experience in the presence of females, making them more competitive. We expect that this effect would be even greater in locations where there is limited sugar availability in the field, giving the released males an even greater competitive advantage over wild males. This situation is probably not uncommon given the number of studies demonstrating plant tissue feeding in the absence of sugar sources (Gu et al. 2011 Müller and Schlein 2005, Qualls et al. 2013). It is also noteworthy that male mosquitoes require approximately one day post-eclosion for their reproductive organs to fully function, so only two days of potential mating time would be lost if males were held for three days, resulting in an average increase of 6 days alive in the field, excluding other factors such as predation, or additional sugar meals found in the wild.

For the purposes of sterile male release technique, increased longevity alone is not sufficient. The objective in releasing sterile males is to have female mosquitoes mate with these sterile individuals, rather than fertile wild males. Our study suggests that males allowed sugar access for longer periods are also able to inseminate a greater number of females in their lifetimes. Boyer et al. (2012) demonstrated that *Ae. albopictus* females occasionally mate more than once in the field. For SIT to be most effective, females should ideally mate only once per lifetime. There are many factors, both known and unknown, which influence a female mosquitoes decision to mate multiple times; the qualities of the spermatophore that a female receives during mating is very likely to be one of these factors. For this reason, the volume of spermatozoa that a male is able to transfer to female spermathecae may be important for successfully preventing females from mating other males. In general, it is clear that male insect ejaculate quality is

affected by nutrition, and consequently affects the number of sperm used per egg by

females, sperm offense and defense ability, and the frequency oviposition and number of

viable eggs laid (Gage and Cook 1994, Sbilordo et al. 2011, Wedell et al. 2002). In *Ae.*

*albopictus*, the male accessory gland secretions act to prevent future inseminations by

other males (Oliva et al. 2013).  Specifically, matrone is a pheromone transmitted which

acts upon the female's terminal abdominal ganglion, preventing future mating (Gwadz

1972). It is possible that competing wild males may also choose to invest less sperm in a

female if they can detect cues that a female already has sperm stored (Wedell et al. 2002).

We found that well-fed males were able to transfer a greater volume of spermatozoa to

females compared to starved males, with an average of  4.3/9 (47%) spermatheca

containing sperm, in comparison to 1.7/9 (18%) in starved males. The mechanism that

causes this difference is not known; we hypothesize that it may be related to the amount

of time and energy a male is willing to spend in mating with a given female.

Stone (2013) suggested that if males suffer high mortality cost upon release, older

males with greater sexual maturity should be released.  Oliva et al. (2012) found that

irradiated males were more competitive with wild males after being maintained on 10%

sucrose for 5 days in the laboratory as compared to one day. However, it seems unlikely

that wild males have the opportunity to mate with such large numbers of females as they

are able to in the laboratory; otherwise, we would expect that they would ration their

sperm investment more carefully (Scharf et al. 2012).  Smaller or weaker males are

therefore expected to invest more in each mating, as future mating opportunities are less

likely.  Another issue to consider is that, by exposing wild females to so many sterile

males, SIT programs may inadvertently begin to select for females that mate more than

once (Alphey et al. 2013). However, at least in the case of *Ae. albopictus*, these females would need to mate twice within 40 minutes (Oliva et al. 2013a). Oliva et al. (2013b) demonstrated that sugar availability combated the mortality that sterile male *Ae. albopictus* would normally experience in the presence of females, making them more competitive with wild males.
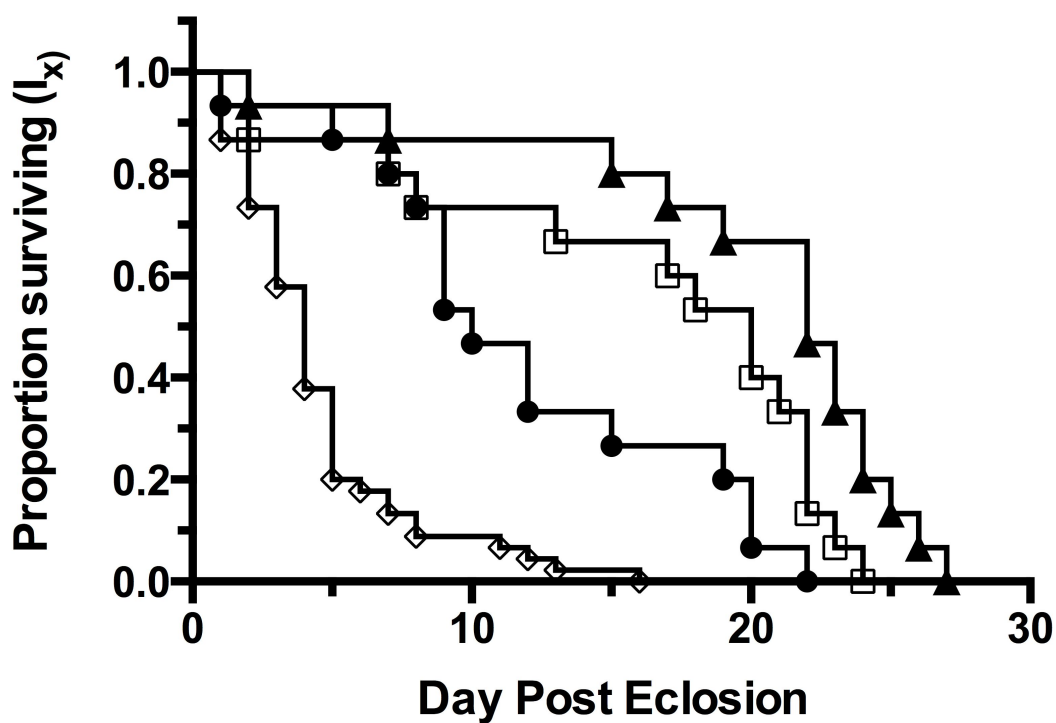
Yuval (2006) expressed concern that mating studies performed in the laboratory, such as the present study, could not be extrapolated to natural conditions; however, Madakacherry et al. (2013) found rather similar results when they compared mating studies done in small cages (30 cm$^3$), large cages (60 cm$^3$), and semi-field cages (1.75 m$^3$).

We recommend retaining and provisioning adult males up to three days post-eclosion to improve their effectiveness in SIT initiatives. This would rule out the usage of any release station device, as proposed in (Bellini, Puggioli, et al. 2013); additionally, this would preclude the use of aerial pupal releases sometimes used in larger SIT operations. Based upon our results, we would expect that the cost associated with retaining the males (i.e. lost field time) will not outweigh the benefits of extended longevity and increased energy reserves. We would also expect that these results are likely hold true for other Culicid species, given the commonality of low teneral energy found in males.
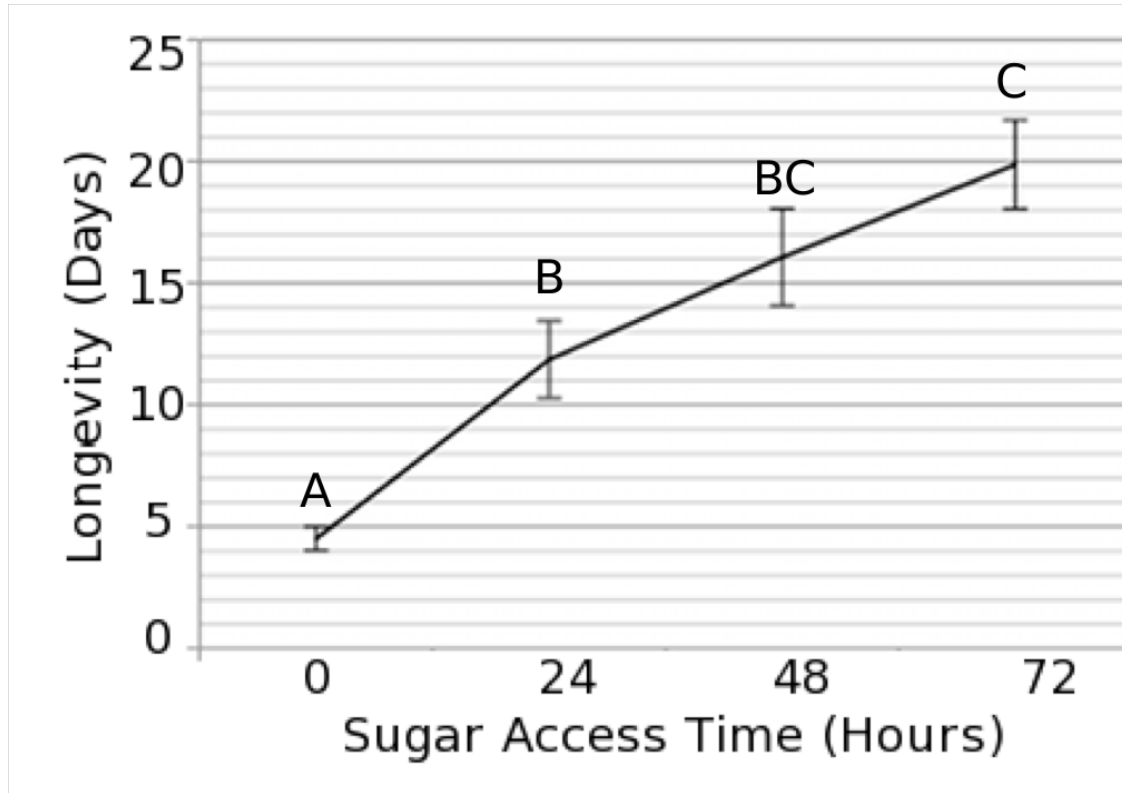
In conclusion, utilizing new knowledge of male mosquito biology has the potential to greatly benefit control programs, specifically those reliant on competition of lab-reared males with wild males.

**Acknowledgements**

**Fig. 2.1** Daily survivorship of *Aedes albopictus*. 24, 48, and 72 h sugar treatments are represented by ●, □, and ▲ respectively; controls were pooled (◇).

**Fig. 2.2** Mean longevity of *Aedes albopictus* males by length of sugar access post-eclosion. Means with different letters are significantly different from each other ($P < 0.05$).

**Fig. 2.3** Mean females mated with sugar-fed (black) and starved (white) *Aedes albopictus* males by days post-eclosion. (A) 24 hr treatment. (B) 48 hr treatment. (C) 72 hr treatment. SEM shown.

**Fig. 2.4** Mean number of spermatheca filled by sugar-fed (black) and starved (white) *Ae. albopictus* males by day post-eclosion. (A) 24 hr treatment. (B) 48 hr treatment. (C) 72 hr treatment. SEM shown.

**Chapter 3:**

**First demonstration of environmentally adaptive paternal effects in mosquitos**

**Abstract**

Paternal effects, non-genetic traits passed through the paternal line, are rarely documented. Examples of adaptive paternal effects mediated by environmental cues are almost non-existent. *Aedes albopictus* is a mosquito vector of great public health significance. We measured male *Aedes albopictus* fitness and offspring traits after exposure to sucrose *ad libitum* or water only throughout adulthood. Adult longevity doubled in the sugar treatment. Females that mated with starved males produced more viable eggs, but the difference was not significant. In both sexes pupation time and eclosion time were significantly longer in offspring of starved males. Unexpectedly, female offspring of starved males had longer wing lengths than those fathered by males with access to sucrose. This increase would likely have long-term population size impacts. We developed a mathematical model using our experimental data to examine whether the paternal effect we observed might increase offspring fitness under certain conditions. The model demonstrated that offspring benefit from paternal signals when they experience poor sugar resources as adults. This study highlights the existence of environmentally mediated paternal effects in mosquitoes, and more broadly in insects, suggesting that male environment needs to be taken into account when control measures seek to alter environmental conditions.

**Introduction**

Non-genetic inheritance, sometimes referred to as "parental effects" are alterations in offspring phenotype resulting from transmission of non-DNA-based information from previous generations (Bonduriansky and Day 2009). Mechanisms for such transmissions may include epigenetic effects, transmission of nutrients, or others. Epigenetics, a comparatively new field, deals with information transmitted from parents to offspring by mechanisms other than the order of nucleotides in DNA such as DNA methylation or changes in histones. Maternal nutrition is one factor that can play a role in restructuring the epigenome after conception (Lane et al. 2014). Examples of parental effects are found in species of many phyla, including members of the Class Insecta. For instance, mated female bed bugs (Cimicidae) differentially express 20 times more genes as a result of nonheritable sperm variation compared to heritable variation (Otti et al. 2014). Micro-RNA has been implicated as one of the mechanisms by which mating affects female gene expression in flies (Zhou et al. 2014). In *Drosophila,* exosomes have been implicated in affecting a female's willingness to mate again.

There are many examples of maternal effects; however, paternal effects are more difficult to discover and measure since, in species with no paternal care, they would need to be transmitted through mating, either with an epigenetic mechanism or through chemicals transmitted during mating [2,4,5]. Reports of parental effects in mosquitos are rare and none are solely paternal, and counterintuitive results are common (Otti and Sadd 2008). Some paternal effects may be the result of indirect maternal effects, where a male influences the female's metabolism or egg production in such a way that it effects the provisioning of the egg, which is a common occurrence in mosquitoes and other insects

(Janssen et al. 1988, Crean and Bonduriansky 2014). Male accessory gland (MAG) proteins lead to some known paternal effects (Crean and Bonduriansky 2014). Certain MAG proteins in *Aedes aegypti* appear 24 hours after male eclosion and increase for multiple days, but can become depleted after repeated mating with no rest (Alfonso-Parra et al. 2014). In a study on effect of parental rearing conditions of *Anopheles stephansi* on offspring biology, daughters of parents raised in low food conditions unexpectedly produced more offspring than daughters of parents raised in high food conditions (Grech et al. 2007). Since both male and female parents were raised with low or high food, it is not possible to determine if this effect was due to the maternal egg provisioning, or to some other factor, such as epigenetics. There was no effect on offspring emergence time, size, or survival. There is no known experimental evidence indicating paternal effects in Culicidae species.

We chose to study *Aedes albopictus*, an invasive container mosquito, able to transmit Cache Valley, Eastern equine encephalitis, Jamestown Canyon, La Crosse, and West Nile in the United States (Moore and Mitchell 1997, Gerhardt et al. 1999, Turell et al. 2005). This mosquito is not only a serious vector, but an increasingly serious nuisance in much of the United States due to rapid population expansion and aggressive anthropophilic feeding tendencies (Moore and Mitchell 1997, Farajollahi and Nelder 2009).

Our objectives were to determine whether carbohydrate access affected the fitness of male mosquitoes and the traits of their offspring. We hypothesize that well provisioned adult *Aedes albopictus* males produce more offspring than males with only water access,

and furthermore, that offspring of well-provisioned males have greater fitness than offspring of starved males.

## Methods

### Rearing and maintenance.

Eggs from a laboratory colony of *Ae. albopictus* were used. This colony was established from eggs collected in Mercer County, NJ, U.S.A. in 2008 and supplemented in subsequent years. Eggs were hatched, 200 eggs per 23x33 cm ceramic tray, with 1 liter of water. Each tray was provisioned with 0.12 g brewer's yeast and lactalbumin (50:50) at two-day intervals until the first day pupation was observed. The food portion was then reduced to 0.08 g at two-day intervals. While studying parental effects, it is important to keep larval rearing conditions constant, since changes in larval density or diet can easily affect a number of aspects of their biology, including size (Jannat and Roitberg 2013).

### Experimental conditions.

All pupae were removed from rearing trays daily, separated by sex and allowed to eclose in 30.5 cm$^3$ cages. The male cage was supplied with a water-soaked cotton dental wick and the female cage with a 20% sucrose-soaked wick.

As male adults eclosed, individuals less than one day old were randomly moved into an experimental cage. Treatment cages were 30.5 cm$^3$ and were populated with 10 males each. Sucrose treatment cages were provisioned with 20% sucrose solution *ad libitum*; water treatment cages were provisioned with only water-soaked wicks. Treatment cages were replicated three times. All rearing and experimentation occurred under constant conditions (15:1:8 hr light: dusk: dark, 25±1°C).

All newly eclosed females were moved to a separate cage daily to track age. All females had been eclosed for a minimum of two days when introduced into experimental cages, ensuring sexual receptivity. Females were provided with 20% sucrose *ad libitum*.

Twenty-four hours after males were placed in experimental cages, females were added for 10 hours, including a 1 hour low-light crepuscular period and a 1 hour dark period. A 1:3 M:F sex ratio was maintained. While females were present, sucrose wicks were removed from experimental cages, so that females mating with sugar-fed males would gain no advantage from extra sugar access.

**Egg collection.**

After removal from treatment cages, females were transferred to new 30.5 cm$^3$ cages with access to 20% sucrose. After 24 hours, females were given access to a restrained bobwhite quail for 30 minutes at 3-day intervals (Animal Use Protocol #86-129, Rutgers University). A 0.94-liter black glass jar with 500 mL water and lined with Whatman #2 filter paper was added to the cage for egg collection. Filter papers were changed at 3-day intervals, allowed to air dry for four days, and then flooded.

**F1 generation fitness.**

Eggs were counted and hatched under the standard conditions described previously. Unhatched eggs were bleached to determine if fertilization occurred according to the methods of Shroyer and Craig (1983). The total number of fertilized eggs, hatch rate, number of larvae and pupae alive and dead, and ecolsion rate of males and females were recorded daily. The wing length of 10 male and 10 female adults randomly selected from each set of egg papers was photographed and measured using Image J software (Abràmoff et al. 2004). Wing length is strongly correlated with

potential fecundity in *Ae. albopictus* (Blackmore and Lord 2000, Armbruster and

Hutchinson 2002).

**Mathematical Model.**

We developed a mathematical model to compare offspring success with and

without a resource signal from the male parent. We defined "resource" as the likelihood

that an individual would locate a sugar meal on a given day. We did the analysis using

two different scenarios for resource fluctuation. In the stochastic version, we used Pop

Tools extension for Microsoft Excel to generate a string of 90 random numbers with

normal distribution, a mean of 50, and a standard deviation of 20 to represent a randomly

fluctuating sugar resource over a period of 90 days (*RS*). In the period version, we

altered the numbers for *RS* to fluctuate in 10-day cycles. To accomplish this, we used the

first number of each cycle as the "cycle factor" (*C*), such that:

$$C_{(t)} = RS_{(t)} \text{ iff } t \bmod 10 = 0 \text{ otherwise } C_{(t-1)}$$

Sugar resource (*RP*) was calculated using the equation:

$$RP_{(t)} = \frac{3C_{(t)} + RS_{(t)}}{4}$$

In this way, we allowed for local variation in resource availability, but only in

modification to longer timescale averages for the cycle. We chose relatively brief cycles

rather than seasonal cycles because *Aedes albopictus* tends to inhabit urban environments

and has short dispersion capabilities. *Ae. albopictus* males are reported to disperse up to

200-300m per week in an urban setting (Bellini et al. 2010). This is a relatively small

distance compared to other nectar foragers such as some bumble bee species, which can

travel over 1 km in one direction for a single foraging trip (Walther-Hellwig and Frankl 2000).

The maternal or female factor (*F*) represents the adult female's resource input and signal to her eggs (the F1 generation). This factor was calculated simply by summing the number of days on which *RS* or *RP* was below 50, during the 10 days prior to egg-laying. This represents the number of days that the female probably starved if she looked for a sugar-meal on that day. We chose to sample 10 days because many females of this species survive into their second gonotrophic cycle, which takes an average of 14 days, and some days must be spent obtaining and digesting a blood meal since this species is not autogenous (Delatte et al. 2009). The paternal male factor (*M*) represents the adult male's signal to his F1 offspring. Similarly, this factor was calculated simply by summing the number of days on which *RS* or *RP* was below 50, taking into account the 3 days prior to mating.

Offspring development time represents the number of days it would take for F1 offspring to develop from egg to adult. The experimental results presented in this paper suggest that both maternal and paternal nutrition can impact larval development time. In our model, good maternal resources are negatively correlated with larval development time because we expected that maternal egg provisioning would result in decreased development time (Table 3.1). In contrast, good paternal resources have no impact on development time, while poorer paternal resources are correlated with increases in larval development time (Table 3.1). Offspring development time was calculated both with (*Dm*) and without paternal impact (*D*). Ef and Em refer to the female and male effect on

development, respectively. *Dm* and *D* were calculated using the equations:

$$Dm_{(t)} = 12 + Ef_{(t)} + Em_{(t)}$$

$$D_{(t)} = 12 + Ef_{(t)}$$

Relative adult size with or without male impact (*Sm*) or (*S*), respectively, was directly correlated with development time and was calculated by dividing development time by five. We choose this factor to make the effect of adult size more proportional to the effect of sugar resources experienced as an adult compared to what would be expected in reality. Success with or without male impact (*Wm*) or (*W*), respectively represents the relative likelihood that the offspring will survive and reproduce (relative fitness). We did not differentiate between male and female offspring. Success was calculated based upon adult size as well as resource availability at eclosion time. High levels of resource availability were considered more important to success than size; however, if resources were poor, larger adults were expected to have an advantage. Sugar resources available to the F1 generation (*Ro*) were simply calculated by taking the sum of the number of days in which *RP* or *RS* was above 50, sampling for five days after adult eclosion. *Wm* and *W* were calculated using the equations:

$$Wm_{(t)} = Sm_{(t)} + Ro_{(t+Dm_{(t)})}$$

$$W_{(t)} = S_{(t)} + Ro_{(t+D_{(t)})}$$

Ten thousand Monte Carlo replicates were generated for both the daily and periodic version.

**Statistical Analyses.**

The total number of viable offspring was calculated by taking the sum of hatched eggs and the number of embryonated eggs. Fecundity was analyzed using ANOVA (SPSS Statistics v21). The treatment variable (diet) was treated as a random variable. Each cage was treated as a single unit. Pupation time, male eclosion time, female eclosion time, and F1 wing length were analyzed using the Mann-Whitney U test for non-parametric data. Potential fecundity was calculated using the formula for laboratory-reared *Ae. albopictus*: $\text{Log}_{10}$ fecundity $= 0.16 + 4.22*(\text{Log}_{10}$ wing length (mm))(Armbruster and Hutchinson 2002). A linear regression model was also used to assess the affect of treatment on survivorship. Within the context of the mathematical model, F1 success was compared with and without paternal effects using a Wilcox signed-rank test. The use of this test was solely to provide a measure of size difference in the dependent variable, since it is possible to overwhelm the power of any statistical test by increasing the number of Monte Carlo realizations.

**Results**

**Survivorship**

Mean survivorship for sugar-fed males (12.7 days) was twice that of starved (water-fed) males (6.3 days) (Fig. 3.1). The slopes determined by the linear regression model was similar (sucrose: $y = -1.5438x + 35.31$ [$R^2 = 0.962$]; water: $y = -1.6563x + 26.235$ [$R^2 = 0.916$]).

**Male fecundity.**

We defined viable offspring as the number of hatched eggs and embryonated eggs. The number of viable offspring produced by females that mated with sucrose-fed or starved males was not significantly different. Females produced 17,602 and 20,201 eggs after mating with sugar-fed and starved males, respectively. The total numbers of viable eggs produced were 17,152 and 19,885 by females mated with sugar-fed and starved males respectively. The proportion of viable eggs produced by females mated with starved males (98.4%) or sugar-fed males was equivalent (98.4 and 97.4%). The hatch rate after initial flooding was also similar for the sugar-fed (8.3% total eggs; 8.6% viable eggs) and starved treatment (6.9% total eggs; 7.1% viable eggs).

**Offspring Traits.**

Offspring of starved males took longer to pupate than offspring of sugar-fed males (U=1,070,033; $n_1$=1,521, $n_2$=1,471; P<0.05; Table 3.2, Fig. 3.2). Male offspring of starved males took longer to eclose as adults than those of sugar-fed males (U=268,085; $n_1$=710, $n_2$=664; P<0.001); however, female offspring of sugar-fed males took longer to eclose than those of starved males (U=202,294; $n_1$=671, $n_2$=673; P=0.001) (Fig. 3.3, 3.4). In all cases, offspring of starved males showed a greater variability in their life history traits.

**Wing length (F1 fecundity).**

Wing length values (mm) were transformed using $\log_{10}$. F1 female wing lengths were significantly different depending on the paternal adult diet (U=6,446; $n_1$=88, $n_2$=126;P<0.05, Fig. 5). Mean $\log_{10}$ wing lengths were 0.440 and 0.450 for females with sugar-fed and starved paternal diets, respectively (Fig. 3.5). Mean potential fecundity

(eggs per female) for these two groups was calculated to be 104.0 and 114.8 respectively (Armbruster and Hutchinson 2002). Male wing lengths were not significantly different depending on paternal diet ($U=5,825$; $n_1=88$, $n_2=121$; $P=0.245$; Fig. 3.5). Mean $\log_{10}$ wing lengths were 0.342 and 0.350 for males with sugar-fed and starved paternal diets, respectively.

**Model Analysis**

For both the stochastic and periodic model, the relative offspring success rate was greater when offspring received information from both parents compared to only the mother ($P<0.0001$; Fig 3.6). When resource varied stochastically, offspring fitness was 9.5% greater if offspring received a paternal signal. When resources varied periodically, offspring fitness was 8.6% greater if offspring received a paternal signal. We also analyzed the mean difference in success for each individual depending on parental signal, which was calculated by subtracting success rate with only maternal signal from success rate with both signals. The mean difference in offspring success was 9.3% greater when resources varied stochastically compared to periodically.

**Discussion**

Our study indicates that adult male diet has demonstrable effects on *Aedes albopictus* F1 offspring and likely affects the F2 generation. Aside from the significance of discovering ways in which a parent's experiences affect their offspring, these paternal effects should be considered in control strategies that affect carbohydrate availability, as well as population size models. We saw the duration of larval and pupal stages increase

in variance when fathers experienced poor conditions as adults. Additionally, we saw an increase in average wing length in adult offspring, which, in itself has implications for the next generation.

Not surprisingly, male longevity doubled when sugar was freely available in adulthood. This supports the finding of previous studies in which male *Ae. albopictus* exhibited increasing longevity in proportion to early adulthood sugar provisioning (Villiard and Gaugler 2015). The other major component to evolutionary fitness, fecundity, was not affected by paternal diet; however, the total fecundity of females mated with starved males was greater than that of females mated with sugar-fed males. In a related study on the effect of adult diet on mosquito fecundity, *An. gambiae* females allowed sugar and blood exhibited increased longevity compared to those allowed only blood; however, total female fecundity was not affected (Gary and Foster 2001). Similarly, we observed no difference in the fecundity of well-fed or starved *Ae. albopictus* males when we quantified the F1 generation, even while longevity had increased. Twice-mated *Ae. aegypti* females seem to have no fecundity benefit over those only mated once (Helinski and Harrington 2012). One explanation for this finding may be that most females mate only once, and in fact, many spermatozoa from a single mating never reach the spermatheca (Jones and Wheeler 1961, Oliva, Damiens, Vreysen, et al. 2013). In this case, an increase in male longevity would not necessarily result in increased female fecundity.

Although the number of viable offspring was not different, we argue that offspring quality, considered in conjunction with quantity, is an equally important measure of fitness; it is of little evolutionary benefit to produce large numbers of

offspring that are unlikely to survive into adulthood and produce their own offspring. Therefore, we also measured offspring hatch rate, time until pupation and adult eclosion, and wing length. The proportion of viable eggs that hatched after a single flooding event was not different. The apparently low hatch rates observed in both treatments were not unexpected; *Ae. albopictus* exhibit a well-documented delayed hatch response, with most eggs hatching only after multiple flooding events (Vitek and Livdahl 2009). Since rates of pupation and eclosion did not exhibit a standard distribution, but exhibited a long tail, we chose to use non-parametric methods to test for differences. Intriguingly, larval development time, and eclosion time males were all longer in offspring with starved fathers. This increase in development may be an evolved response to the poor conditions experienced by the parent. It may benefit the offspring by increasing the probability of experiencing better conditions upon eclosion. Alternatively, increased development time may allow offspring to increase resistance to poor condition in adulthood by taking greater advantage of larval resources. The broader mechanism is not clear; however, slower development in insects may be the result of decreases in ecdysteriod production or sensitivity (Chapman 1998). This is the first demonstration of a strictly paternal effect in Culicidae. Male diet has been shown to effect offspring metabolism in rats; however, these effects are largely unexplained (Ng et al. 2010).

As in many mosquito species, female wing length in *Ae. albopictus* is correlated with body size and fecundity (Armbruster and Hutchinson 2002). We found a small but significant difference in F1 female wing length dependent on the paternal diet. Contrary to expectation, starved males produced female offspring with larger wings than those of well-fed males; an average difference of .01mm. According to formulae developed by

Armbruster and Hutchinson (Armbruster and Hutchinson 2002), this would result in an average of 10.8 more eggs produced by each daughter. This effect would increase exponentially if F2 offspring were considered. Longer development in *Ae. albopictus* can result in larger adult size (Yee et al. 2012); therefore, we hypothesize that this size difference may be a result of the extended development time shown by some of the individuals in the poor paternal diet treatment.

This phenomenon, the production of more offspring to cope with poor environmental conditions, makes sense in the context of evolution. Our findings echo those of Grech et al. who observed that parental larval rearing conditions in *Anopheles stephensi* influenced the fecundity of F1females (Grech et al. 2007). They demonstrated that daughters of parents reared in low food treatments unexpectedly produced more offspring than daughters of parents raised in high food treatments. Given that both male and female parents were raised together, it was not possible to determine if this effect was due to maternal egg provisioning or to some other factor. There was no effect on offspring development time, size, or survival. The authors hypothesized that laying more eggs at a faster rate may be an optimal strategy for females in poor environments. A separate review of this study suggested that poor nutrition may have led to an increase in early fecundity due to decreased longevity (Otti and Sadd 2008).

The only variable in our experiment was the adult male diet, suggesting a potential epigenetic effect. A recent review of the phenomenon of paternal effects points out that these effects are likely to have unique and important impacts in our understanding of evolutionary ecology (Crean and Bonduriansky 2014). Furthermore, they give suggestions about experimentation meant to demonstrate the presence of

paternal effects, particularly when these may be mediated by maternal effects. Specifically, in our case, where eggs are fertilized internally and no parental care is given to offspring (a Type C paternal effect) (Crean and Bonduriansky 2014), it is difficult to determine whether an effect is caused by the father or by a reaction of the mother to the father. Literature concerning male transfer of spermatophores suggests that the paternal effect we observed is not simply a result of differential resource allocation based on their mate's quality, but that the male provides nourishment and other beneficial chemicals within spermatophore. Future studies might wish to assess the F2 generation to determine if this effect is passed on through the F1 generation. Perhaps starvation delays sexual maturity, giving males more time to build up male accessory gland (MAG) secretions, produced in the first few days of adulthood in *Ae. albopictus* (Foster and Lea 1975). In the grasshopper *Melanoplus sanguinipes*, proteins transfer in the MAG secretion directly contribute to oocyte protein content (Friedel and Gillott 1977). In *Ae. albopictus*, the MAG secretions deter future inseminations by other males (Oliva, Damiens, Vreysen, et al. 2013). Matrone is a pheromone transmitted by male mosquitoes during copulation that affects the female's terminal abdominal ganglion, preventing future mating (Gwadz 1972). MAG secretions from male *Ae. albopictus* have been shown to reduce mobility and increase refractoriness to further mating attempts in female *Ae. aegypti* (Tripet et al. 2011, Lima-Camara et al. 2013). In *Ae. aegypti*, the ejaculate which remains in the spermatheca is absorbed by the female (Spielman 1964). Scharf et al. suggests that this may be considered equivalent to a nuptial gift (Scharf et al. 2012). Furthermore, Helinski and Harrington observed that malnourished females which mated with virgin males displayed increased longevity compared with those mated with sperm-depleted males,

suggesting they may derive some nutritional value from the ejaculate (Helinski and Harrington 2011). Alternatively, perhaps weak males purposely provide more MAG substances during their first mating as mating opportunities are quite rare for small under-fed males (Gary et al. 2009); other Dipteran species such as *Drosophila melanogaster* adjust their ejaculate size based upon the female's characteristics (Lupold et al. 2010).  A specific steroid hormone, 20E, transferred during mating in *An. gambiae* has been identified as a trigger for a switch in female behavior, which reduces further mating and induces egg-laying behavior (Gabrieli et al. 2014). Starved *Ae. aegypti*  and sugar-fed *Ae. taeniorhynchus* lay eggs with significantly more fat reserves (Nayar and Sauerman 1975), demonstrating that predicting female resource allocation may not be as intuitive as previously expected. Regardless of the mechanism, the system appears to be a feedback loop in which poor food conditions experienced by adults result in longer larval development time in a portion of their offspring, which in turn results in larger adults with presumably larger teneral energy reserves.  The F1 generation then, will be more suited to a sugar-poor environment if it persists. Another important consideration in the future may be to determine whether the effect we observed increases the fitness of both the male and female parent, or whether increased female investment in eggs might be detrimental to her overall fitness by harming her future clutches (Crean and Bonduriansky 2014). Since many *Ae. albopictus* females only mate once, we would tentatively hypothesize that the effect is equivalent for both the male and the female, and therefore, both positive.

Insect body size is affected by many factors, but can be a determining factor for fitness potential. Adult longevity increases with body size under poor resource conditions

(Lehmann et al. 2006). Male body size is *Ae. aegypti* is good indicator of total spermatozoa production (Ponlawat and Harrington 2007). Body size also plays an important role in mosquito mating success in species that form mating swarms; in one study, larger males were more likely to successfully mate in a swarm, and only 44% of all males they sampled showed evidence of ever mating. Whether this was due to stronger flight skills, female choice, or stamina to remain in swarm and return for a second chance to mate was unclear (Yuval et al. 1993). Other studies have demonstrated the size of male *An. gambiae* captured *in copula* was significantly greater than the average size of males within mating swarms (Sawadogo et al. 2013) and that *Anopheles* females apparently can use the wing-beat frequency of males, which correlates with size, to choose mates (Cator et al. 2010). Since *Ae. albopictus* commonly mates in swarms (Gubler and Bhattacharya 1972), increased male body size may be biologically significant.

The concept that a male parent could adaptively send cues to offspring to increase their fitness according to current environmental conditions is extremely new. Depending on density, sea squirt males (Ascidiacea) can increase their offspring fitness by sending environmental cues that allow offspring to perform better in environments similar to those they themselves experienced (Crean et al. 2013). Our findings now provide an example of this phenomenon in Insecta. Using our experimental findings, we developed a mathematical model to further examine whether the paternal effect we observed are advantageous to offspring fitness depending on environmental conditions. The model demonstrated that male signal could be beneficial to offspring that will experience poor resources as adults. This to a unique finding since poor adult conditions altered offspring phenotype to adapt to those conditions by increasing offspring adult size. Future studies

may wish to investigate additional variables that might impact the benefit that offspring might receive from such a signal. They should also consider potential draw-backs to extended development time. For instance, we predict that males eclosing later will loose some competitive advantage to those who emerge earlier when competing for virgin females.

Our findings have far reaching implications in the field of entomology, as well as the broader field of evolutionary ecology. The idea that a parent passes on information to its offspring is not new; however, mechanisms for transmitting information about their environment were previously thought to be limited to genetic traits, or otherwise passing on learned behaviors. Specifically, paternal involvement was thought to be limited to supplying genetic information, while maternal effects were slightly more extensive, being directly involved in egg production (Crean and Bonduriansky 2014). In the past, male insects that offered no parental care to their offspring had traditionally been considered simply as suppliers of "cheap" sperm (Dawkins 1989). In contrast our study demonstrates that male parents may be providing more information than that contained in genes. In entomology, and specifically vector biology, novel control methods rely on the availability of accurate information about species' development and behavior. Awareness of variables that impact fitness is often the determinant in successful insect management. "Attractive toxic sugar baits" (ATSBs) have recently been tested for use in the control of a number of mosquitoes, including *Ae. albopictus* (Xue et al. 2011, Revay et al. 2013). We recommend that future control programs that seek to alter or treat adult sugar sources should account for potential unintended consequence of paternal effects on the next generation of insects. We also recommend that future studies should investigate the

occurrence of paternal effects in other insect species, particularly those of importance to human health.

| Parent | Parental Resource Value | *Ef* or *Em* |
|--------|-------------------------|--------------|
| Female | Good (0-3) | -1 day |
| | Medium (4-6) | +0 days |
| | Poor (7-10) | +2 days |
| Male | Good (0) | +0 days |
| | Medium (1) | +1 day |
| | Poor (2-3) | +4 days |

**Table 3.1** Values used for calculating *Ef* and *Em* by paternal resource experience.

| Treatment | Mean larval development time (days ± SE) | Mean eclosion Time (days ± SE) | |
|---|---|---|---|
| | | Male | Female |
| Sugar | 9.55 ± 0.06 | 11.37 ± 0.08 | 12.99 ± 0.10 |
| Water | 9.60 ± 0.08 | 11.90 ± 0.11 | 12.72 ± 0.11 |

**Table 3.2** F1 development time in *Aedes albopictus* by paternal diet.

**Fig 3.1** Daily survivorship of male *Aedes albopictus* by adult diet.

**Fig. 3.2** F1 generation *Aedes albopictus* larval development time (total number new pupae) post-flood by paternal diet. Y-axis scale is logarithmic.

**Fig 3.3** Mean adult *Aedes albopictus* eclosion time post flood by paternal diet. SEM shown.

**Fig 3.4** F1 male *Aedes albopictus* adult eclosion time post-flood by paternal diet. Y-axis scale is logarithmic.

**Fig 3.5** F1 female *Aedes albopictus* adult eclosion time post-flood by paternal diet.
Y-axis scale logarithmic.

**Fig 3.6** Mean wing length of *Aedes albopictus* by sex and paternal diet; sugar-fed (filled circle), starved (line). SEM shown.

**Fig 3.7** Mean relative offspring success by parental signal and resource variation. SEM shown.

**Chapter 4:**

**General Conclusions and Implications**

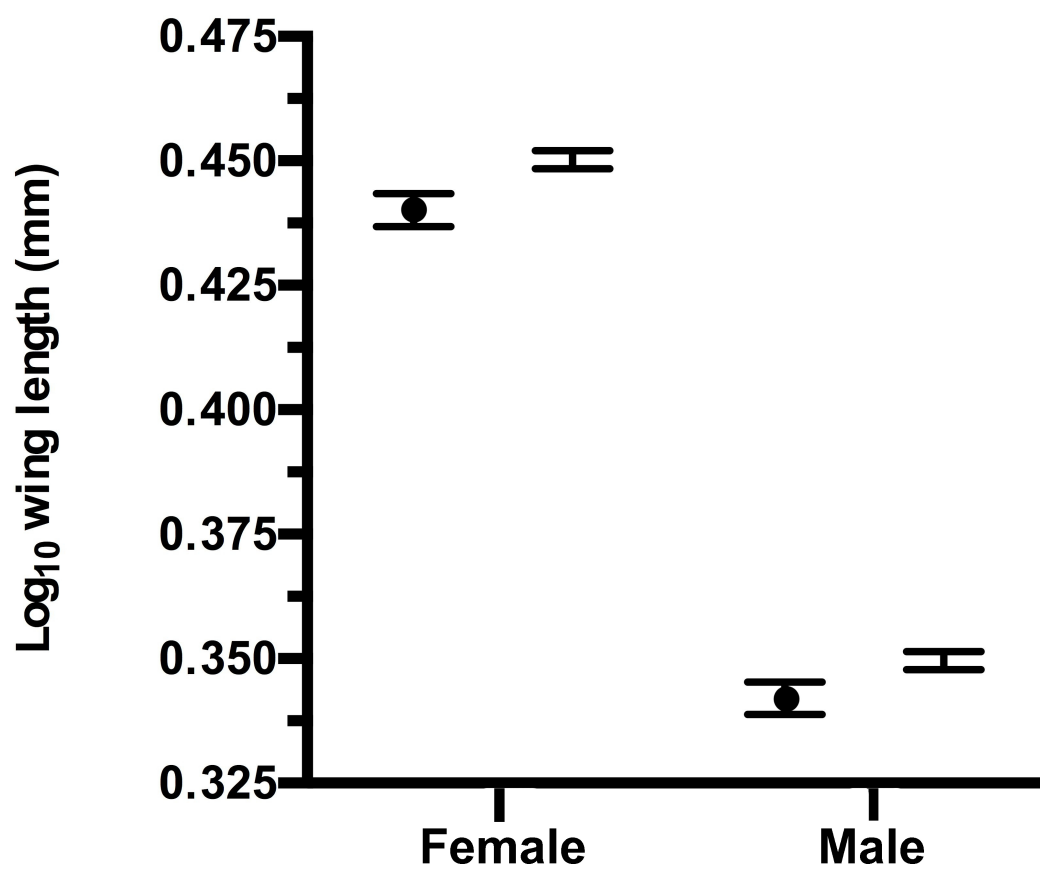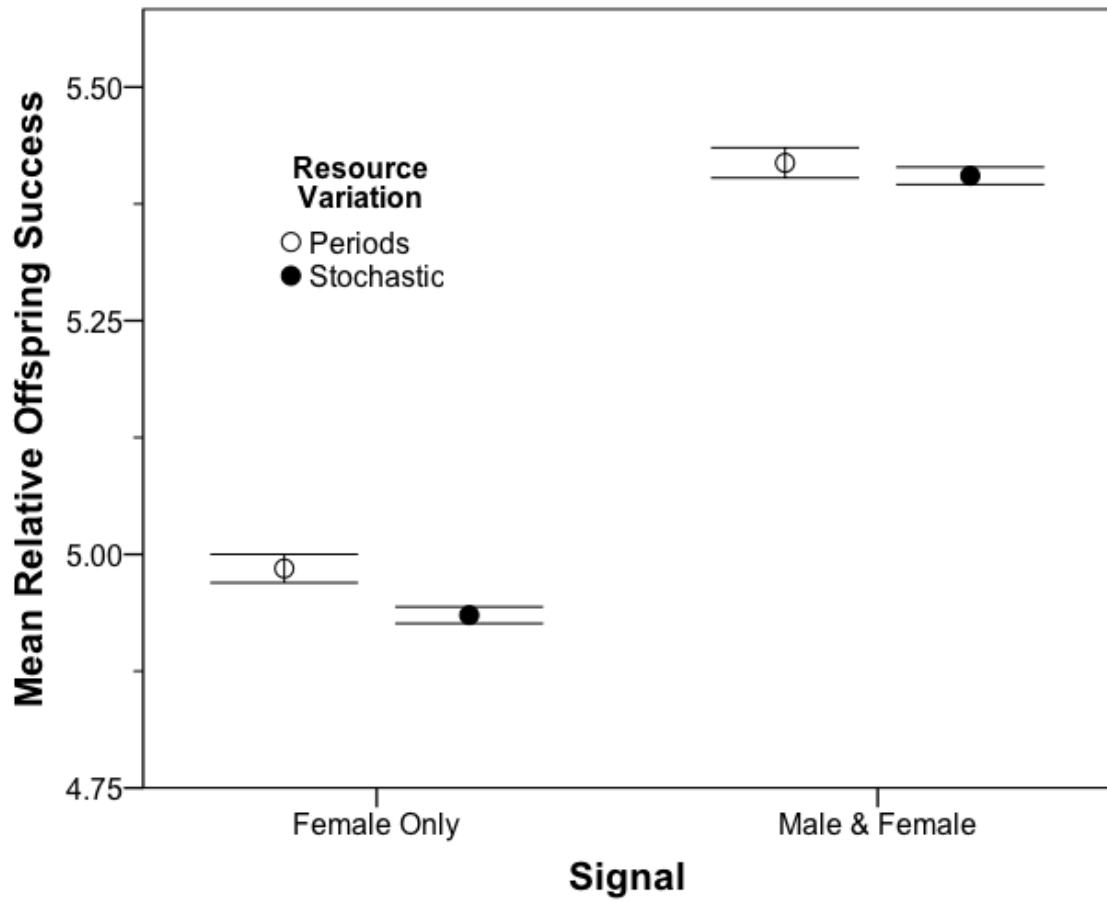Whenever mosquito biologists attempt to explain a failed control program, they almost inevitably point to lack of knowledge of some aspect of the target species' biology or its interactions with the environment. My goal in writing this dissertation was to investigate multiple aspects of sugar-feeding behavior in both the male and female Asian tiger mosquito, *Aedes albopictus*. I chose to work with this particular species because of its local relevance as a newly invasive species in the northeastern United States, in addition to its importance as vector species on a global scale. My focus on sugar-feeding stemmed from a recent surge in interest in the topic in the literature.

In Foster's 1995 review paper on the subject, he states that the then current view was that "…the pervasiveness of mosquito sugar feeding…is ubiquitous and essential, or it is facultative and incidental" (Foster 1995). After this, there emerged a renewed interest in the topic, with the goal of defining what circumstances gave rise to each of these views. Subsequently, when they found circumstances where the it was "ubiquitous" or "essential", entomologists have sought to find methods of using this knowledge for mosquito control.

The development of "attractive toxic sugar baits," or "ATSBs," is a good example of this phenomenon. This technique implements plants, plant volatiles, sugar solutions, or a combination of these as bait, combined with a lethal chemical. Alternatively, scientists have proposed that sugar baits might be used as a non-lethal trapping method, for monitoring populations or other uses. My work described in chapters 1 and 3 may have

consequences for the application of these methods on *Ae. albopictus*. Chapter 1 describes an increase in the female mosquito's proclivity to seek sugar meals when she is exposed to diapause cues during her development. If this is true, it should be taken into account when using sugar or plant mimics as bait. Perhaps ATSBs would be more effective in the fall, and perhaps if researchers observe higher sugar-bait trap counts in the fall they should not assume that this directly relates to a population increase. Chapter 3 describes a previously unknown phenomenon in mosquitoes wherein the offspring of males deprived of sugar are larger than the offspring of starved males. Since larger size in female mosquitoes results in greater fecundity, this implies that environmental sugar resources could influence F2 generation population size. Consequently, any program seeking to alter sugar access should consider this.

Sterile insect technique (SIT), is a control strategy that requires its applicator to possess a good understanding of the target species biology. The strategy relies on flooding local female populations with sterilized males, thus preventing them from producing offspring; therefore, scientists must sterilize, rear, and release males in ways that minimize loss of mating-competitiveness. Chapter 2 describes a method by which entomologists might increase male fitness before release by provisioning them with sugar for a number of days before release. Since male *Ae. albopictus* are not able to mate immediately following eclosion, this may be a simple and practical way to increase released males' chances of mating without sacrificing time in the field.

In addition to more direct, practical applications, the findings that I present in chapter 3 also have broader implications in the fields of entomology and evolutionary ecology. Paternal effects have not been previously documented in mosquitoes, and more

specifically, environmentally adaptive paternal effects have rarely been documented in any species.  The idea that a parent can pass on information to its offspring is not new; however, mechanisms for transmitting information about the local environment were previously thought to be limited to genetic traits or learned behaviors. If such paternal effects exist in other insects or indeed other animals, uncovering them may shed light on previously unexplained phenotypic variation.

While I have attempted to shed light upon a number of facets of *Ae. albopictus'* sugar-feeding behavior, I have inevitably uncovered questions that future studies may wish to address. Most broadly, the applicability of these findings to other species, even within the genus *Aedes*, remains unknown. Many other questions have sprung from this work, which I present in the discussion section of each chapter.

**Literature Cited**

**Abràmoff, M. D., P. J. Magalhães, and S. J. Ram**. **2004**. Image processing with ImageJ. Biophotonics Int. 11: 36–42.

**Alfonso-Parra, C., F. W. Avila, P. Deewatthanawong, L. K. Sirot, M. F. Wolfner, and L. C. Harrington**. **2014**. Synthesis, depletion and cell-type expression of a protein from the male accessory glands of the dengue vector mosquito *Aedes aegypti*. J. Insect Physiol. 70: 117–124.

**Ali, S. R., and L. E. Rozeboom**. **1973**. Comparative laboratory observations on selective mating of *Aedes* (Stegomyia) *albopictus* Skuse and *A*. (S.) *polynesiensis* Marks. Mosq. News. 33: 23–28.

**Alphey, L., A. McKemey, D. Nimmo, M. Neira Oviedo, R. Lacroix, K. Matzen, and C. Beech**. **2013**. Genetic control of *Aedes* mosquitoes. Pathog. Glob. Health. 107: 170–9.

**Armbruster, P., and R. A. Hutchinson**. **2002**. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus* (Diptera: Culicidae ). J. Med. Entomol. 39: 699–704.

**Bader, C. A., and C. R. Williams**. **2012**. Mating, ovariole number and sperm production of the dengue vector mosquito *Aedes aegypti* (L.) in Australia: broad thermal optima provide the capacity for survival in a changing climate. Physiol. Entomol. 37: 136–144.

**Baeshen, R., N. E. Ekechukwu, M. Toure, D. Paton, M. Coulibaly, S. F. Traoré, and F. Tripet**. **2014**. Differential effects of inbreeding and selection on male reproductive phenotype associated with the colonization and laboratory maintenance of *Anopheles gambiae*. Malar. J. 13:19.

**Bargielowski, I., C. Kaufmann, L. Alphey, P. Reiter, and J. Koella**. **2012**. Flight performance and teneral energy reserves of two genetically-modified and one wild-type strain of the yellow fever mosquito *Aedes aegypti.* Vector borne zoonotic Dis. 12: 1053–8.

**Bargielowski, I., and L. P. Lounibos**. **2013**. Rapid evolution of reduced receptivity to interspecific mating in the dengue vector *Aedes aegypti* in response to satyrization by invasive *Aedes albopictus*. Evol. Ecol. 28: 193–203.

**Beier, J. C., G. C. Müller, W. Gu, K. L. Arheart, and Y. Schlein**. **2012**. Attractive toxic sugar bait (ATSB) methods decimate populations of *Anopheles* malaria vectors in arid environments regardless of the local availability of favoured sugar-source blossoms. Malar. J. 11: 31.

Bellini, R., A. Albieri, F. Balestrino, M. Carrieri, D. Porretta, S. Urbanelli, M. Calvitti, R. Moretti, and S. Maini. 2010. Dispersal and survival of *Aedes albopictus* (Diptera: Culicidae) males in Italian urban areas and significance for sterile insect technique application. J. Med. Entomol. 47: 1082–1091.

Bellini, R., F. Balestrino, A. Medici, G. Gentile, and R. Veronesi. 2013. Mating competitiveness of *Aedes albopictus* radio-sterilized males in large enclosures exposed to natural conditions. J. Med. Entomol. 50: 94–102.

Bellini, R., A. Medici, A. Puggioli, F. Balestrino, and M. Carrieri. 2013. Pilot field trials with *Aedes albopictus* irradiated sterile males in Italian urban areas. J. Med. Entomol. 50: 317–25.

Bellini, R., A. Puggioli, F. Balestrino, P. Brunelli, A. Medici, S. Urbanelli, and M. Carrieri. 2013. Sugar administration to newly emerged *Aedes albopictus* males increases their survival probability and mating performance. Acta Trop. 1–8.

Blackmore, M. S., and C. C. Lord. 2000. The relationship between size and fecundity in *Aedes albopictus*. J. Vector Ecol. 25: 212–7.

Bonduriansky, R., and T. Day. 2009. Nongenetic inheritance and its evolutionary implications. Annu. Rev. Ecol. Evol. Syst. 40: 103–125.

Bonizzoni, M., G. Gasperi, X. Chen, and A. A. James. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends Parasitol. 29: 460–468.

Boyer, S., J. Gilles, D. Merancienne, G. Lemperiere, and D. Fontenille. 2011. Sexual performance of male mosquito *Aedes albopictus*. Med. Vet. Entomol. 25: 454–9.

Boyer, S., L. Maillot, L. C. Gouagna, D. Fontenille, D. D. Chadee, and G. Lemperiere. 2013. Diel activity patterns of male *Aedes albopictus* in the laboratory. J. Am. Mosq. Control Assoc. 29: 74–7.

Boyer, S., C. Toty, M. Jacquet, G. Lempérière, and D. Fontenille. 2012. Evidence of multiple inseminations in the field in *Aedes albopictus*. PLoS One. 7: e42040.

Braks, M. A. H., S. A. Juliano, and L. P. Lounibos. 2006. Superior reproductive success on human blood without sugar is not limited to highly anthropophilic mosquito species. Med. Vet. Entomol. 20: 53–9.

Briegel, H. 2001. *Aedes aegypti:* size, reserves, survival, and flight potential. J. Vector Ecol. 26: 21–31.

**Briegel, H., and S. E. Timmermann**. **2001**. *Aedes albopictus* (Diptera: Culicidae): physiological aspects of development and reproduction. J. Med. Entomol. 38: 566–571.

**Bryan, J. H. 1972**. Further studies on consecutive matings in the *Anopheles gambiae* complex. Nature. 239: 519–520.

**Burt, A. 2014**. Heritable strategies for controlling insect vectors of disease. Philos. Trans. R. Soc. B. 369: 20130432.

**Canyon, D. D., R. Muller, and J. L. K. Hii**. **2013**. *Aedes aegypti* disregard humidity-related conditions with adequate nutrition. Trop. Biomed. 30: 1–8.

**Carvalho, D. O., A. L. Costa-da-Silva, R. S. Lees, and M. L. Capurro**. **2013**. Two step male release strategy using transgenic mosquito lines to control transmission of vector-borne diseases. Acta Trop. 1–8.

**Cator, L. J., K. Ng'habi, R. R. Hoy, and L. C. Harrington**. **2010**. Sizing up a mate : variation in production and response to acoustic signals in *Anopheles gambiae*. Behav. Ecol. 21(5): 1033-1039.

**Chadee, D. D., J. M. Sutherland, and J. R. L. Gilles**. **2013**. Diel sugar feeding and reproductive behaviours of *Aedes aegypti* mosquitoes in Trinidad: with implications for mass release of sterile mosquitoes. Acta Trop. 1–5.

**Chambers, G. M., and M. J. Klowden**. **2001**. Age of *Anopheles gambiae* Giles male mosquitoes at time of mating influences female oviposition. J. Vector Ecol. 26: 196–201.

**Chapman, R. F. 1998**. The insects: structure and function, Fouth Edit. ed. Cambridge Univeristy Press.

**Clements, A. N. 1999**. The biology of mosquitoes, vol. 2: sensory reception and behaviour. Cambridge Univeristy Press, Cambridge, United Kingdom.

**Clements, A. N. 2000**. The biology of mosquitoes. development, nutrition and reproduction, vol. 1. Cambridge Univeristy Press, Cambridge, United Kingdom.

**Coluzzi, M., M. Di Deco, and A. Gironi**. **1975**. Influenza del fotoperiodo sulla scelta del luogo di ovideposizione in *Aedes mariae* (Diptera, Culicidae). Parasitologia. 17: 121–130.

**Crean, A. J., and R. Bonduriansky**. **2014**. What is a paternal effect? Trends Ecol. Evol. 29: 554–559.

**Crean, A. J., J. M. Dwyer, and D. J. Marshall**. **2013**. Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. Ecology. 94: 2575–2582.

**Damiens, D., S. M. Soliban, F. Balestrino, R. Alsir, M. J. B. Vreysen, and J. R. L. Gilles**. **2013**. Different blood and sugar feeding regimes affect the productivity of *Anopheles arabiensis* colonies (Diptera: Culicidae). J. Med. Entomol. 50: 336–343.

**Dawkins, R. 1989**. The selfish gene. Oxford University Press Inc., New York.

**Delatte, H., G. Gimonneau, and A. Triboire**. **2009**. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. J. Med. Entomol. 46: 33–41.

**Delatte, H., C. Paupy, J. S. Dehecq, J. Thiria, A. B. Failloux, and D. Fontenille**. **2008**. *Aedes albopictus*, veceur des virus du chikungunya et de la dengue a la Reunion: biologie et controle. Parasite. 15: 3–13.

**Denlinger, D. L., and P. A. Armbruster**. **2014**. Mosquito diapause. Annu. Rev. Entomol. 59: 73–93.

**Diabaté, A., A. S. Yaro, A. Dao, M. Diallo, D. L. Huestis, and T. Lehmann**. **2011**. Spatial distribution and male mating success of *Anopheles gambiae* swarms. BMC Evol. Biol. 11: 184.

**Edman, J., D. Strickman, P. Kittayapong, and T. Scott**. **1992**. Female *Aedes aegypti* (Diptera: Culicidae) in Thailand rarely feed on sugar. J. Med. Entomol. 29: 1035–1038.

**Epsky, N. D., and R. R. Heath**. **1993**. Food availability and phermone production by males of *Anastrepha suspense* (Diptera: Tephritidae). Physiol. Chem. Ecol. 22: 942–947.

**Farajollahi, A., B. Kesavaraju, D. C. Price, G. M. Williams, S. P. Healy, R. Gaugler, and M. P. Nelder**. **2009**. Field efficacy of BG-Sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. J. Med. Entomol. 46: 919–25.

**Farajollahi, A., and M. P. Nelder**. **2009**. Changes in *Aedes albopictus* (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. J. Med. Entomol. 46: 1220–4.

**Ferguson, H. M., A. Dornhaus, A. Beeche, C. Borgemeister, M. Gottlieb, M. S. Mulla, J. E. Gimnig, D. Fish, and G. F. Killeen**. **2010**. Ecology: a prerequisite for malaria elimination and eradication. PLoS Med. 7: e1000303.

**Ferguson, H. M., B. John, K. Ng'habi, and B. G. J. Knols**. **2005**. Redressing the sex imbalance in knowledge of vector biology. Trends Ecol. Evol. 20: 202–9.

**Foster, W. A. 1995**. Mosquito sugar feeding and reproductive energetics. Annu. Rev. Entomol. 40: 443–474.

**Foster, W. A., W. M. Mostowy, and R. G. Hancock**. **1989**. Pre-blood-meal energy status of *Aedes aegypti:* Effect on blood meal size and on allocation of carbohydrares adn lipids among eggs and reserves, pp. 131–137. *In* Host Regul. Dev. Mech. Vector Arthropods Proceeding Second Symp.

**Foster, W. A., and W. Takken**. **2004**. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. Bull. Entomol. Res. 94: 145–157.

**Foster, W., and A. Lea**. **1975**. Renewable fecundity of male *Aedes aegypti* following replenishment of seminal vesicles and accessory glands. J. Insect Physiol. 21: 1085–1090.

**Franco, C., N. A. Hynes, N. Bouri, and D. A. Henderson**. **2010**. The dengue threat to the United States. Biosecur. Bioterror. 8: 273–6.

**Friedel, T., and C. Gillott**. **1977**. Contribution of male-produced proteins to vitellogenesis in *Melanoplus sanguinipes*. J. Insect Physiol. 23: 145–151.

**Gabrieli, P., E. G. Kakani, S. N. Mitchell, E. Mameli, E. J. Want, A. Mariezcurrena Anton, A. Serrao, F. Baldini, and F. Catteruccia**. **2014**. Sexual transfer of the steroid hormone 20E induces the postmating switch in *Anopheles gambiae*. Proc. Natl. Acad. Sci. U. S. A.

**Gage, M., and P. A. Cook**. **1994**. Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). Funct. Ecol. 8: 594–599.

**Gary, R. E., J. W. Cannon, and W. A. Foster**. **2009**. Effect of sugar on male *Anopheles gambiae* mating performance, as modified by temperature, space, and body size. Parasit. Vectors. 2: 19.

**Gary, R. E., and W. A. Foster**. **2001**. Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera: Culicidae). J. Med. Entomol. 38: 22–8.

**Gary, R. E., and W. A. Foster**. **2006**. Diel timing and frequency of sugar feeding in the mosquito *Anopheles gambiae*, depending on sex, gonotrophic state and resource availability. Med. Vet. Entomol. 20: 308–16.

**Gavriel, S., Y. Gazit, and B. Yuval**. **2009**. Remating by female Mediterranean fruit flies (*Ceratitis capitata*, Diptera: Tephritidae): temporal patterns and modulation by male condition. J. Insect Physiol. 55: 637–42.

**Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci**. **1999**. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerg. Infect. Dis. 7: 807–11.

**Gibson, G., and I. Russell**. **2006**. Flying in tune: sexual recognition in mosquitoes. Curr. Biol. 16: 1311–6.

**Gillott, C. 1977**. Fecundity enhancing and receptivity inhibiting substances produced by male insects: a review. Adv. Invertebr. Reprod. 1: 199–218.

**Gillott, C. 2003**. Male accessory gland secretions: modulators of female reproductive physiology and behavior. Annu. Rev. Entomol. 48: 163–84.

**Gratz, N. G. 2004**. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18: 215–27.

**Grech, K., L. A. Maung, and A. F. Read**. **2007**. The effect of parental rearing conditions on offspring life history in *Anopheles stephensi*. Malar. J. 9: 1–9.

**Grimstad, P., and G. DeFoliart**. **1974**. Nectar sources of Wisconsin mosquitoes. J. Med. Entomol. 11: 331–341.

**Gu, W., G. Müller, Y. Schlein, R. J. Novak, and J. C. Beier**. **2011**. Natural plant sugar sources of *Anopheles* mosquitoes strongly impact malaria transmission potential. PLoS One. 6: e15996.

**Gubler, D. J., and N. C. Bhattacharya**. **1972**. Swarming and mating in *Aedes* (S.) *albopictus* in nature. Mosq. News. 23: 219–223.

**Gwadz, R. 1972**. Neuro-hormonal regulation of sexual receptivity in female *Aedes aegypti*. J. Insect Physiol. 18: 259–266.

**Gwadz, R. W., and G. B. Craig Jr**. **1968**. Sexual receptivity in female *Aedes aegypti*. Mosq. News. 28: 586–593.

**Hall-Mendelin, S., S. A. Ritchie, C. a Johansen, P. Zborowski, G. Cortis, S. Dandridge, R. A. Hall, and A. F. van den Hurk**. **2010**. Exploiting mosquito sugar feeding to detect mosquito-borne pathogens. Proc. Natl. Acad. Sci. U. S. A. 107: 11255–9.

**Hamady, D., N. B. Ruslan, A. H. Ahmad, C. S. M. Rawi, H. Ahmad, T. Satho, F. Miake, W. F. Zuharah, Y. Fukumitsu, A. R. Saad, S. Rajasaygar, R. E. M. Vargas, A. H. A. Majid, N. Fadzly, I. A. Ghani, and S. Abubakar**. **2013**. Colonized *Aedes albopictus* and its sexual performance in the wild: implications for SIT technology and containment. Parasit. Vectors. 6: 206.

**Van Handel, E. 1965**. The obese mosquito. J. Physiol. 181: 478–486.

**Harris, A. F., D. Nimmo, A. R. McKemey, N. Kelly, S. Scaife, C. A. Donnelly, C. Beech, W. D. Petrie, and L. Alphey**. **2011**. Field performance of engineered male mosquitoes. Nat. Biotechnol. 29: 1034–7.

**Helinski, M. E. H., and L. C. Harrington**. **2011**. Male mating history and body size influence female fecundity and longevity of the dengue vector *Aedes aegypti*. J. Med. Entomol. 48: 202–211.

**Helinski, M. E. H., and L. C. Harrington**. **2012**. The role of male harassment on female fitness for the dengue vector mosquito *Aedes aegypti*. Behav. Ecol. Sociobiol. 66: 1131–1140.

**Higa, Y., Y. Tsuda, N. Tuno, and M. Takagi**. **2000**. Tempo-spatial variation in feeding activity and density of Aedes albopictus (Diptera: Culicidae) at peridomestic habitat in Nagasaki, Japan. Med. Entomol. 51: 205–209.

**Howell, P. I., and B. G. J. Knols**. **2009**. Male mating biology. Malar. J. 8 Suppl 2: S8.

**Jannat, K. N.-E., and B. D. Roitberg**. **2013**. Effects of larval density and feeding rates on larval life history traits in *Anopheles gambiae* s.s. (Diptera: Culicidae). J. Vector Ecol. 38: 120–6.

**Janssen, G. M., G. de Jong, E. N. G. Joosse, and W. Scharloo**. **1988**. A negative maternal effect in springtails. Evolution (N. Y). 42: 828–834.

**Jepson, P. C., and T. P. Healy**. **1988**. The location of floral nectar sources by mosquitoes: an advanced bioassay for volatile plant odours and initial studies with *Aedes aegypti* (L.) (Diptera: Culicidae). Bull. Entomol. Res. 78: 641–650.

**Jones, J. C., and R. E. Wheeler**. **1961**. Studies on spermathecal filling in *Aedes aegypti* (Linnaeus).

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

**Junnila, A., G. C. Müller, and Y. Schlein**. **2010**. Species identification of plant tissues from the gut of *An. sergentii* by DNA analysis. Acta Trop. 115: 227–33.

**Kaufmann, C., C. Reim, and W. U. Blanckenhorn**. **2013**. Size-dependent insect flight energetics at different sugar supplies. Biol. J. Linn. Soc. 108: 565–578.

**Kevan, P. G., and H. G. Baker**. **1983**. Insects as flower visitors and pollinators. Annu. Rev. Entomol. 28: 407–453.

**Klowden, M., and H. Briegel**. **1994**. Mosquito gonotrophic cycle and multiple feeding potential: contrasts between *Anopheles* and *Aedes* (Diptera: Culicidae). J. Med. Entomol. 31: 618–622.

**Klowden, M., and G. Chambers**. **1991**. Male accessory gland substances activate egg development in nutritionally stressed *Aedes aegypti* mosquitoes. J. Insect Physiol. 37: 721–726.

**Klowden, M. J.** **1993**. Mating and nutritional state affect the reproduction of *Aedes albopictus* mosquitoes. J. Am. Mosq. Control Assoc. 9: 169–73.

**Klowden, M. J.** **1999**. The check is in the male: male mosquitoes affect female physiology and behavior. J. Am. Mosq. Control Assoc. 15: 213–220.

**Klowden, M. J., and G. M. Chambers**. **1989**. Ovarian development and adult mortality in *Aedes aegypti* treated with sucrose, juvenile hormone and methoprene. J. Insect Physiol. 35: 513–517.

**Kramer, L. D., L. M. Styer, and G. D. Ebel**. **2008**. A global perspective on the epidemiology of West Nile virus. Annu. Rev. Entomol. 53: 61–81.

**Lacroix, R., A. R. McKemey, N. Raduan, L. Kwee Wee, W. Hong Ming, T. Guat Ney, S. Rahidah A A, S. Salman, S. Subramaniam, O. Nordin, N. Hanum A T, C. Angamuthu, S. Marlina Mansor, R. S. Lees, N. Naish, S. Scaife, P. Gray, G. Labbé, C. Beech, D. Nimmo, L. Alphey, S. S. Vasan, L. Han Lim, N. Wasi A, and S. Murad**. **2012**. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS One. 7: e42771.

**Lane, M., R. L. Robker, and S. A. Robertson**. **2014**. Parenting from before conception. Science 345: 756–760.

**Lea, A. O., and E. Van Handel**. **1970**. Suppression of glycogen synthesis in the mosquito by a hormone from the medial neurosecretory cells. J. Insect Physiol. 16: 319–21.

**Lehmann, T., R. Dalton, E. H. Kim, E. Dahl, A. Diabate, R. Dabire, and J. P. Dujardin**. **2006**. Genetic contribution to variation in larval development time, adult size, and longevity of starved adults of *Anopheles gambiae*. Infect. Genet. Evol. 6: 410–6.

**Lima-Camara, T. N., C. T. Codeço, N. A. Honório, R. V. Bruno, A. A. Peixoto, and L. P. Lounibos**. **2013**. Male accessory gland substances from *Aedes albopictus* affect the locomotor activity of *Aedes aegypti* females. Mem. Inst. Oswaldo Cruz. 108 Suppl : 18–25.

**Lizé, A., T. A. R. Price, C. Heys, Z. Lewis, and G. D. D. Hurst**. **2014**. Extreme cost of rivalry in a monandrous species: male−male interactions result in failure to acquire mates and reduced longevity. Proc. R. Soc. Biol. 281: 20140631.

**Louâpre, P., S. Llopis, V. Martel, and J. van Baaren**. **2014**. Sperm-depleted males influence the reproductive behaviour of conspecifics. Naturwissenschaften. 101: 875–82.

**Lounibos, L., R. L. Escher, and R. Lourenço-de-Oliveira**. **2003**. Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of *Aedes albopictus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 96: 512–518.

**Lupold, S., M. K. Manier, O. Ala-Honkola, J. M. Belote, and S. Pitnick**. **2010**. Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. Behav. Ecol. 22: 184–191.

**Madakacherry, O., R. S. Lees, and J. R. L. Gilles**. **2013**. *Aedes albopictus* (Skuse) males in laboratory and semi-field cages: Release ratios and mating competitiveness. Acta Trop. 1–6.

**Magnarelli, L. A. 1979**. Diurnal nectar-feeding of *Aedes canator* and *A. sollicitans* (Diptera: Culicidae). Environ. Entomol. 8: 949–955.

**Maïga, H., R. K. Dabiré, T. Lehmann, F. Tripet, and A. Diabaté**. **2012**. Variation in energy reserves and role of body size in the mating system of *Anopheles gambiae*. J. Vector Ecol. 37: 289–297.

**Maïga, H., A. Niang, S. P. Sawadogo, R. K. Dabiré, R. S. Lees, J. Gilles, F. Tripet, and A. Diabaté**. **2013**. Role of nutritional reserves and body size in *Anopheles gambiae* males mating success. Acta Trop. 6–11.

**Manda, H., L. C. Gouagna, W. A. Foster, R. R. Jackson, J. C. Beier, J. I. Githure, and A. Hassanali**. **2007**. Effect of discriminative plant-sugar feeding on the survival and fecundity of *Anopheles gambiae*. Malar. J. 6: 113.

**Massonnet-Bruneel, B., N. Corre-Catelin, R. Lacroix, R. S. Lees, K. P. Hoang, D. Nimmo, L. Alphey, and P. Reiter**. **2013**. Fitness of transgenic mosquito *Aedes aegypti* males carrying a dominant lethal genetic system. PLoS One. 8: e62711.

**Moore, C. G., and C. J. Mitchell**. **1997**. *Aedes albopictus* in the United States: ten-year presence and public health implications. Emerg. Infect. Dis. 3: 329–34.

**Mostowy, W., and W. Foster**. **2004**. Antagonistic effects of energy status on meal size and egg-batch size of *Aedes aegypti* (Diptera: Culicidae). J. Vector Ecol. 29: 84–93.

**Müller, G. C., R.-D. Xue, and Y. Schlein**. **2011**. Differential attraction of *Aedes albopictus* in the field to flowers, fruits and honeydew. Acta Trop. 118: 45–9.

**Müller, G., and Y. Schlein**. **2005**. Plant tissues: the frugal diet of mosquitoes in adverse conditions. Med. Vet. Entomol. 19: 413–22.

**Muñnoz, J., R. Eritja, M. Alcaide, T. Montalvo, R. C. Soriguer, and J. Figuerola**. **2011**. Host-feeding patterns of native *Culex pipiens* and invasive *Aedes albopictus* mosquitoes (Diptera: Culicidae) in urban zones from Barcelona, Spain. J. Med. Entomol. 48: 956–960.

**Nayar, J. K., and D. M. Sauerman**. **1973**. A comparative study of flight performance and fuel utilization as a function of age in females of Florida mosquitoes. J. Insect Physiol. 19: 1977–1988.

**Nayar, J. K., and D. M. Sauerman**. **1975**. The effects of nutrition on survival and fecundity in Florida mosquitoes. Part 3. Utilization of blood and sugar for fecundity. J. Med. Entomol. 12: 220–5.

**Nayar, J., and P. Pierce**. **1977**. Utilization of energy reserves during survival after emergence in Florida mosquitoes. J. Med. Entomol. 14: 54–59.

**Ng, S.-F., R. C. Y. Lin, D. R. Laybutt, R. Barres, J. a Owens, and M. J. Morris**. **2010**. Chronic high-fat diet in fathers programs β-cell dysfunction in female rat offspring. Nature. 467: 963–6.

**Nusrat, J., M. S. Sarwar, and A. Qadoos**. **2013**. Effects of available sugar on reproductive fitness and survival potential of laboratory reared *Anopheles stephensi*. Biol. 59: 173–182.

**Nyasembe, V. O., P. E. a Teal, W. R. Mukabana, J. H. Tumlinson, and B. Torto**. **2012**. Behavioural response of the malaria vector *Anopheles gambiae* to host plant volatiles and synthetic blends. Parasit. Vectors. 5: 234.

**Oliva, C. F., D. Damiens, and M. Q. Benedict**. **2013**. Male reproductive biology of *Aedes* mosquitoes. Acta Trop. 132: 1–8.

**Oliva, C. F., D. Damiens, M. J. B. Vreysen, G. Lemperière, and J. Gilles**. **2013**. Reproductive strategies of *Aedes albopictus* (Diptera: Culicidae) and implications for the sterile insect technique. PLoS One. 8: e78884.

**Oliva, C. F., M. Jacquet, J. Gilles, G. Lemperiere, P.-O. Maquart, S. Quilici, F. Schooneman, M. J. B. Vreysen, and S. Boyer**. **2012**. The sterile insect technique for controlling populations of *Aedes albopictus* (Diptera: Culicidae) on Reunion Island: mating vigour of sterilized males. PLoS One. 7: e49414.

**Oliva, C. F., M. J. Maier, J. Gilles, M. Jacquet, G. Lemperiere, S. Quilici, M. J. B. Vreysen, F. Schooneman, D. D. Chadee, and S. Boyer**. **2012**. Effects of irradiation, presence of females, and sugar supply on the longevity of sterile males *Aedes albopictus* (Skuse) under semi-field conditions on Reunion Island. Acta Trop. 125: 287–93.

**Otienoburu, P. E., B. Ebrahimi, P. L. Phelan, and W. A. Foster**. **2012**. Analysis and optimization of a synthetic milkweed floral attractant for mosquitoes. J. Chem. Ecol. 38: 873–81.

**Otti, O., P. R. Johnston, G. J. Horsburgh, J. Galindo, and K. Reinhardt**. **2014**. Female transcriptomic response to male genetic and nongenetic ejaculate variation. Behav. Ecol. 00: 1–8.

**Otti, O., and B. M. Sadd**. **2008**. Parental guidance? Trans-generational influences on offspring life history in mosquitoes. Trends Parasitol. 24: 197–9.

**Paton, D., M. Touré, A. Sacko, M. B. Coulibaly, S. F. Traoré, and F. Tripet**. **2013**. Genetic and environmental factors associated with laboratory rearing affect survival and assortative mating but not overall mating success in *Anopheles gambiae* sensu stricto. PLoS One. 8: e82631.

**Pereira, R., B. Yuval, P. Liedo, P. E. A. Teal, T. E. Shelly, D. O. McInnis, and J. Hendrichs**. **2013**. Improving sterile male performance in support of programmes integrating the sterile insect technique against fruit flies. J. Appl. Entomol. 137: 178–190.

**Perez-Staples, D., A. M. T. Harmer, S. R. Collins, and P. W. Taylor**. **2008**. Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. Agric. For. Entomol. 10: 255–262.

**Ponlawat, A., and L. C. Harrington**. **2005**. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. J. Med. Entomol. 42: 844–849.

**Ponlawat, A., and L. C. Harrington**. **2007**. Age and body size influence male sperm capacity of the dengue vector *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 44: 422–6.

**Ponlawat, A., and L. C. Harrington**. **2009**. Factors associated with male mating success of the dengue vector mosquito, *Aedes aegypti*. Am. J. Trop. Med. Hyg. 80: 395–400.

**Puggioli, A., F. Balestrino, D. Damiens, R. S. Lees, S. M. Soliban, O. Madakacherry, M. L. Dindo, R. Bellini, and J. R. L. Gilles**. **2013**. Efficiency of three diets for larval development in mass rearing *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 50: 819–825.

**Pumpuni, C. B., J. Knepler, and G. B. Craig**. **1992**. Influence of temperature and larval nutrition on the diapause inducing photoperiod of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 8: 223–7.

**Qualls, W. A., R. De Xue, J. C. Beier, and G. C. Müller**. **2013**. Survivorship of adult *Aedes albopictus* (Diptera: Culicidae) feeding on indoor ornamental plants with no inflorescence. Parasitol. Res. 112: 2313–8.

**Qualls, W. A., R. Xue, E. E. Revay, S. A. Allan, and G. C. Muller**. **2012**. Implications for operational control of adult mosquito production in cisterns and wells in St. Augustine, FL using attractive sugar baits. Acta Trop. 124: 158–161.

**Reisen, W. K. 2003**. Ecological aspects for application of genetically modified mosquitoes, pp. 25–32. *In* Takken, W., Scott, T. (eds.), Ecol. Asp. Appl. Genet. Modif. Mosquitoes. Springer.

**Revay, E. E., G. C. Müller, W. a Qualls, D. L. Kline, D. P. Naranjo, K. L. Arheart, V. D. Kravchenko, Z. Yefremova, A. Hausmann, J. C. Beier, Y. Schlein, and R.-D. Xue**. **2013**. Control of *Aedes albopictus* with attractive toxic sugar baits (ATSB) and potential impact on non-target organisms in St. Augustine, Florida. Parasitol. Res. 113: 73–79.

**Reynolds, J. A., M. F. Poelchau, Z. Rahman, P. a Armbruster, and D. L. Denlinger**. **2012**. Transcript profiling reveals mechanisms for lipid conservation during diapause in the mosquito, *Aedes albopictus*. J. Insect Physiol. 58: 966–73.

**Samson, D. M., W. A. Qualls, D. Roque, P. Diana, T. Alimi, K. L. Arheart, G. C. Müller, C. John, S. T. Augustine, D. P. Naranjo, and N. C. Mu**. **2013**. Resting and energy reserves of *Aedes albopictus* collected in common landscaping vegetation in St. Augustine, Florida. J. Am. Mosq. Control Assoc. 29: 231–236.

**Sawadogo, S. P., A. Diabaté, H. K. Toé, T. Lefevre, T. Baldet, J. Gilles, F. Simard, G. Gibson, S. Sinkins, R. K. Dabiré, and H. K. Toe**. **2013**. Effects of age and size on *Anopheles gambiae* s.s. male mosquito mating success. J. Med. Entomol. 50: 285–293.

**Sbilordo, S. H., V. M. Grazer, M. Demont, and O. Y. Martin**. **2011**. Impacts of starvation on male reproductive success in *Tribolium castaneum*. Evol. Ecol. Res. 13: 347–359.

**Scharf, I., F. Peter, and O. Y. Martin**. **2012**. Reproductive trade-offs and direct costs for males in arthropods. Evol. Biol. 40: 169–184.

**Sota, T., and M. Mogi**. **1992**. Survival time and resistance to desiccation of diapause and non-daipause eggs of temperate *Aedes* (*Stegomyia*) mosquitoes. Entomol. Exp. Appl. 63: 155–161.

**Spielman, A. 1964**. The mechanics of copulation in *Aedes aegypti*. Biol. Bull. 127: 324–344.

**Stone, C. M. 2013**. Transient population dynamics of mosquitoes during sterile male releases: modelling mating behaviour and perturbations of life history parameters. PLoS One. 8: e76228.

**Stone, C. M., I. M. Hamilton, and W. A. Foster**. **2011**. A survival and reproduction trade-off is resolved in accordance with resource availability by virgin female mosquitoes. Anim. Behav. 81: 765–774.

**Stone, C. M., B. T. Jackson, and W. a Foster**. **2012**. Effects of plant-community composition on the vectorial capacity and fitness of the malaria mosquito *Anopheles gambiae*. Am. J. Trop. Med. Hyg. 87: 727–36.

**Tripet, F., L. Lounibos, D. Robbins, and J. Moran**. **2011**. Competitive reduction by satyrization? Evidence for interspecific mating in nature and asymmetric reproductive competition between invasive mosquito vectors. Am. J. Trop. Med. Hyg. 85: 265-270.

**Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. Oguinn, T. G. Andreadis, and J. a Blow**. **2005**. An update on the potential of north American mosquitoes (Diptera: Culicidae) to transmit West Nile Virus. J. Med. Entomol. 42: 57–62.

**Urbanski, J. M., J. B. Benoit, M. R. Michaud, D. L. Denlinger, and P. Armbruster**. **2010**. The molecular physiology of increased egg desiccation resistance during diapause in the invasive mosquito, *Aedes albopictus*. Proc. Biol. Sci. 277: 2683–92.

**USDA/APHIS. 2008**. Final report: United States, Mexico, and Guatemala fruit fly emergence and release facilities review.

**Villiard, A., and R. Gaugler**. **2015**. Long-term effects of carbohydrate availability on mating success of newly eclosed *Aedes albopictus* (Diptera: Culicidae) males. J. Med. Entomol. tjv030.

**Vitek, C. J., and T. Livdahl**. **2009**. Hatch plasticity in response to varied inundation frequency in *Aedes albopictus*. J. Med. Entomol. 46: 766–71.

**Walther-Hellwig, K., and R. Frankl**. **2000**. Foraging distances of *Bombus muscorum*, *Bombus lapidarius*, and *Bombus terrestris* (Hymenoptera, Apidae). J. Insect Behav. 13: 239–246.

**Watanabe, K., F. Yukuhiro, Y. Matsuura, T. Fukatsu, and H. Noda**. **2014**. Intrasperm vertical symbiont transmission. Proc. Natl. Acad. Sci. U. S. A. Early Edit: 2–6.

**Wedell, N., M. J. Gage, and G. A. Parker**. **2002**. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17: 313–320.

**Wigglesworth, V. 1982**. The principles of insect physiology, 7th ed. Springer.

**Xue, R., and A. Ali**. **2008**. Field evaluation of boric acid- and fipronil-based bait stations against adult mosquitoes. J. Am. Mosq. 24: 415–418.

**Xue, R., A. Ali, and D. R. Barnard**. **2012**. Mating status and body size in *Aedes albopictus* (Diptera: Culicidae) affect host finding and deet repellency. Florida Entomol. 95: 268–272.

**Xue, R., and M. Debboun**. **2014**. Fatigue from multiple host-seeking activity of *Aedes albopictus* and its effects on bloodfeeding behavior and deet repellency. J. Am. Mosq. Control. 30: 116–118.

**Xue, R., G. Müller, D. L. Kline, and D. R. Barnard**. **2011**. Effect of application rate and persistence of boric acid sugar baits applied to plants for control of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 27: 56–60.

**Xue, R.-D., A. Ali, and D. R. Barnard**. **2008**. Host species diversity and post-blood feeding carbohydrate availability enhance survival of females and fecundity in *Aedes albopictus* (Diptera: Culicidae). Exp. Parasitol. 119: 225–8.

**Xue, R.-D., D. R. Barnard, and G. C. Muller**. **2010**. Effects of body size and nutritional regimen on survival in adult *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 47: 778–782.

**Yahouédo, G. A., L. Djogbénou, J. Saïzonou, B. S. Assogba, M. Makoutodé, J. Gilles, H. Maïga, K. Mouline, B. K. Soukou, and F. Simard**. **2013**. Effect of three larval diets on larval development and male sexual performance of *Anopheles gambiae* s.s. Acta Trop. 1–6.

**Yamada, H., A. G. Parker, C. F. Oliva, F. Balestrino, and J. R. L. Gilles**. **2014**. X-ray-induced sterility in *Aedes albopictus* (Diptera: Culicidae) and male longevity following irradiation. J. Med. Entomol. 51: 811–816.

Yee, D. A., S. A. Juliano, and S. M. Vamosi. 2012. Seasonal photoperiods alter developmental time and mass of an invasive mosquito, *Aedes albopictus* (Diptera: Culicidae), across its north-south range in the United States. J. Med. Entomol. 49: 825–32.

Yee, W. L., and W. A. Foster. 1992. Diel sugar-feeding and host-seeking rhythms in mosquitoes (Diptera: Culicidae) under laboratory conditions. J. Med. Entomol. 29: 784–791.

Yee, W. L., W. A. Foster, M. J. Howe, and R. G. Hancock. 1992. Simultaneous field comparison of evening temporal distributions of nectar and blood feeding by *Aedes vexans* and *Ae. trivittatus* (Diptera: Culicidae) in Ohio. J. Med. Entomol. 29: 356–360.

Young, A. D. M., and A. E. R. Downe. 1982. Renewal of sexual receptivity in mated female mosquitoes, *Aedes aegypti*. Physiol. Entomol. 7: 467–471.

Yuval, B. 1992. The other habit: sugar feeding by mosquitoes. Bull. Soc. Vector Ecol. 17: 150–156.

Yuval, B. 2006. Mating systems of blood-feeding flies. Annu. Rev. Entomol. 51: 413–40.

Yuval, B., and A. Bouskila. 1993. Temporal dynamics of mating and predation in mosquito swarms. Oecologia. 95: 65–69.

Yuval, B., M. L. Holliday-Hanson, and R. K. Washino. 1994. Energy budget of swarming male mosquitoes. Ecol. Entomol. 19: 74–78.

Yuval, B., R. Kaspi, S. Field, S. Blay, and P. Taylor. 2002. Effects of post-teneral nutrition on reproductive success of male mediterranean fruit flies (Diptera: Tephritidae). Florida Entomol. 85: 165–170.

Yuval, B., R. Kaspi, S. Shloush, and M. S. Warburg. 1998. Nutritional reserves regulate male participation in Mediterranean fruit fly leks. Ecol. Entomol. 23: 211–215.

Yuval, B., J. W. Wekesa, and R. K. Washino. 1993. Effect of body size on swarming behavior and mating success of male *Anopheles freeborni*. J. Insect Behav. 6: 333–342.

Zhou, S., T. F. Mackay, and R. R. Anholt. 2014. Transcriptional and epigenetic responses to mating and aging in *Drosophila melanogaster*. BMC Genomics. 15: 927.

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