How do Nutritional Stress and La Crosse Virus Infection Interact? Tests for Effects on Willingness to Blood Feed and Fecundity in *Aedes albopictus* (Diptera: Culicidae)

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Abstract

Evolutionary theory predicts that vector-borne pathogens should have low virulence for their vector because of selection against pathogens that harm the vector sufficiently to reduce transmission. Environmental factors such as nutritional stress can alter vector–pathogen associations by making the vectors more susceptible to pathogens (condition-dependent competence) and vulnerable to the harm caused by pathogen replication (condition-dependent virulence). We tested the hypotheses of condition-dependent competence and condition-dependent virulence by examining the interactive effects of short-term sugar deprivation and exposure to La Crosse virus (LACV) in female *Aedes albopictus* (Skuse). We predicted that infection status interacts with sugar deprivation to alter willingness to blood feed and fecundity in the second gonotrophic cycle (condition-dependent virulence). Sugar deprivation had no effect on body infection or disseminated infection rates. Infection status, sugar treatment, and their interaction had no effect on fecundity. Mosquitoes that had intermittent access to sugar were significantly more willing to take a second bloodmeal compared with those that had continuous access to sugar. Infection status and the interaction with sugar treatment had no effect on blood-feeding behavior. Thus, we found no evidence of short-term sugar deprivation leading to condition-dependent competence for, or condition-dependent virulence of, LACV in *Ae. albopictus*.

Key words: *Aedes albopictus*, La Crosse virus, sugar, blood feeding, fecundity

Environmental effects on larval and adult mosquitoes can impact individual fitness in ways that produce population-level effects. Limited resource availability for larvae or adults can result in reduced larval survivorship and adult life span (Costanzo et al. 2005, Reiskind and Lounibos 2009, Gilles et al. 2011, Alto et al. 2012, Muturi et al. 2012), lower fecundity (e.g., Mostowy and Foster 2004, Vaidyanathan et al. 2008, Xue et al. 2010), and altered immune function (Suwanchaichinda and Paskewitz 1998, Koella and Sorenson 2002, Telang et al. 2012, Ponton et al. 2013). Resource availability for larvae or adults can affect vector competence for viruses, *Plasmodium*, and filarial parasites (Okech et al. 2004, 2007; Vaidyanathan et al. 2008; Alto and Lounibos 2013; Breaux et al. 2014). Such “condition-dependent competence” and effects of resource limitation on vector life-history traits may determine vectorial capacity (Dye 1986, Moller-Jacobs et al. 2014).

Arbovirus infections of mosquito vectors were traditionally thought to be uniformly benign due to selection against pathogens that harm their vector, which is the main route of transmission (Ewald 1994, Hardy et al. 1983, Breaux et al. 2014). Vector-borne

pathogens do, however, exploit the vector to develop or to replicate (Hardy et al. 1983). Vectors are not passive carriers of pathogens, and insect vectors have multiple immune defenses against infection (Murdock et al. 2013, Blair and Olson 2014). It is now established that arboviruses can increase mortality, reduce fecundity, and alter the feeding behavior of females (reviewed by Lambrechts and Scott 2009). Environmental stress can magnify the impact of arboviral infection on the vector, transforming infections from avirulent to virulent (Brown et al. 2000). Such condition-dependent virulence occurs with both nutritional and temperature stresses (Donegan and Lighthart 1989, Brown et al. 2000, Mitchell et al. 2005, Tripet et al. 2008). Little is known about the potential relationship between condition-dependent competence and condition-dependent virulence in mosquito vectors.

La Crosse virus (LACV; Family: Bunyaviridae) is an important agent of pediatric encephalitis in the United States and is maintained in mosquito vectors through horizontal and vertical transmission (Hughes et al. 2006). *Aedes triseriatus* (Say) is the primary vector, and two invasive mosquito species *Aedes albopictus* (Skuse) and

Aedes japonicus (Theobald) are potential secondary vectors (Gerhardt et al. 2001, Erwin et al. 2002, Lambert et al. 2010, Harris et al. 2015, Westby et al. 2015). Condition-dependent competence for LACV in *Ae. triseriatus* can be influenced by larval nutrition (Grimstad and Haramis 1984, Grimstad and Walker 1991, Bevins 2008). Reduced survivorship of LACV-infected overwintering eggs is postulated to be a product of cold-stress-induced condition-dependent virulence (McGaw et al. 1998). LACV infection can alter the blood-feeding behavior of *Ae. triseriatus* and *Ae. albopictus* (Grimstad et al. 1980, Jackson et al. 2012), but it did not affect female survivorship or fecundity in the first gonotrophic cycle for these species under benign laboratory conditions (Costanzo et al. 2013). In this paper, we test whether short-term nutritional stress of adult *Ae. albopictus* induces condition-dependent competence for LACV infection, and whether interactive effects of LACV infection and stress yield condition-dependent virulence for the vector. We focus on nutritional stress caused by sugar deprivation because that stressor causes both condition-dependent competence and condition-dependent virulence in at least one mosquito–arbovirus system (Vaidyanathan et al. 2008).

Materials and Methods

Mosquito Rearing/Experimental Set up

Ae. albopictus in this experiment were from several generations of a colony originating from Effingham, IL, and maintained in the laboratory for 1 yr. Larvae were reared in 10 cohorts of 250 individuals in 1 liter of water with 0.15 g bovine liver powder with regular food spikes. After emergence, adults from each larval pan were assigned to one of four combinations of blood (infections or noninfectious) and sugar (constant access or intermittent access), so that equal numbers of females from each pan were assigned to each treatment daily. Females were housed in groups in 1-liter paperboard cages by date of emergence with an approximately equal number of males ≤ 5 d old. Cages had ad libitum access to a 20% sucrose solution until females reached 4 d old. They were then given water for 48 h prior to being offered a bloodmeal. Larvae and adults were held at 25°C with a photoperiod of 14:10 (L:D) h.

Six-day-old females were offered appropriate bloodmeals for three consecutive days for 40 min/d using a Hemotek membrane feeder Hemotek Limited (Lancashire, United Kingdom), cold anesthetized, and blood-fed females removed and housed in group cages by treatment and feeding date. Sugar treatments began the day of blood feeding. Females in the constant-sugar group had continuous access to 20% sucrose solution. Those in the intermittent-sugar group had access to 20% sucrose for 24 h and then water for the next 72 h. This regimen was repeated until the end of a 15-d extrinsic incubation period (EIP).

At 15 d post bloodmeal, all surviving females were offered a noninfectious bloodmeal for 40 min/d on each two consecutive days and housed individually. After 4 d, females were killed and kept at -80°C until they could be dissected on fresh slides, with flame sterilized forceps, mature eggs counted, and bodies and legs stored separately at -80°C . Total eggs produced in the second gonotrophic cycle was quantified as: eggs laid + mature eggs dissected.

Virus and Bloodmeal Preparation

Infectious bloodmeals were prepared by inoculating 400 μl of LACV stock virus (initial inoculum = 3.94×10^6 plaque-forming units; LACV/human/1960, GenBank EF485030-EF485032) on confluent monolayers of Vero cells in 75-cm² vented tissue culture flasks.

Flasks were incubated for 1 h at 37°C and 5% CO₂ and intermittently rocked to allow virus attachment and entry. Flasks were then topped with 15 ml of Leibovitz's L-15 media (plus 1% penicillin–streptomycin and 10% fetal bovine serum) and incubated for 72 h at 37°C and 5% CO₂ for virus amplification. To determine bloodmeal titer, 500 μl of the media was stored at -80°C . The medium containing virus was mixed in a 1:1 ratio with defibrinated bovine blood, yielding bloodmeals of $3.2 \times 10^6 \pm 1$ pfu per ml (mean $\pm$ SE; plaque assay in Vero cells; Bara and Muturi 2015). The noninfectious bloodmeal was prepared with a 1:1 ratio of uninfected medium and blood.

Mosquito Infection Determination

Bodies and legs were separately homogenized in 200 μl of L-15 medium with one BB using a Retch shaker for 4 min at 380 rpm. Another 800 μl of medium was added to each sample and then centrifuged for 10 min at 1,000 rpm. Infection status was determined by inoculating 200 μl of supernatant onto confluent Vero cell monolayers in 24-well plates with a positive and negative control on each plate. After 72 h, a sample was considered positive if there were visible cytopathic effects. Infection status of females offered an infectious bloodmeal was classified as: 1) exposed but not infected; 2) exposed and infected but not disseminated (body infected, legs not); or 3) exposed and infected and disseminated infection (both body and legs infected).

Statistical Analyses

Total eggs in the second gonotrophic cycle were analyzed as a two-way ANOVA (SAS PROC GLM) with sugar access (constant, intermittent), infection status (not exposed; exposed–not infected; exposed–nondisseminated; exposed–disseminated), and the interaction as the independent variables. Residuals met assumptions of normality and homoscedasticity. Effects of sugar treatment, infection status, and the interaction on the taking of the second bloodmeal (yes, no) were analyzed with a χ^2 test (SAS PROC CATMOD). The effect of sugar treatment on infection status for those females offered an infectious bloodmeal was analyzed with a 2×4 table χ^2 tests with “died before the end of the EIP,” “not infected,” “body infection only,” and “disseminated infection” as the four possible outcomes (SAS PROC CATMOD). All analyses were performed in SAS 9.3 (SAS User's Guide, SAS Institute Inc., 2011).

Results

There were 418 females that took initial bloodmeals. The four treatment groups had roughly equal number of females, and 51 females died prior to completing the EIP (Table 1). Body infection rate (i.e., nondisseminated infection) was 41.2% of those taking an infectious meal (Table 1). The combined disseminated infection rate for the two sugar treatments was 20.9% of those taking an infectious meal (Table 1) or about one third of the total number infected (Table 1). Sugar treatment had no effect on the frequencies of four infection statuses (died before EIP, not infected, body infection only, disseminated infection; $\chi^2 = 1.42$; df = 3; $P = 0.7002$). Infection status had no significant effect on willingness to take the second bloodmeal ($\chi^2 = 4.09$; df = 3; $P = 0.252$), but there was a significant effect of sugar treatment ($\chi^2 = 9.14$; df = 1; $P = 0.0025$), with intermittent sugar yielding greater willingness to take a bloodmeal than did continuous sugar (Fig. 1). The interaction of infection status and sugar was not significant ($\chi^2 = 1.26$; df = 3; $P = 0.739$; Fig. 1). Fecundity in the second gonotrophic cycle was unaffected by LACV infection

Table 1. Number of females that: entered the experiment in each treatment group; died during the EIP; were not successfully infected; had a nondisseminated infection; had a disseminated infection; were offered a second bloodmeal after the EIP; and took the second bloodmeal when offered

LACV	Constant sugar	Number	Died in EIP (% of fed)	Not infected (% of fed)	Body only positive (% of fed)	Legs positive (% of fed)	Offered second blood meal	Took second bloodmeal (% those offered)
Yes	Yes	105	14 (13.3%)	27 (25.7%)	43 (41.0%)	21 (20.0%)	91	19 (20.1%)
Yes	No	106	14 (13.2%)	25 (23.6%)	44 (41.5%)	23 (21.7)	92	38 (41.3%)
LACV total		211	28 (13.3%)	52 (24.6%)	87 (41.2%)	44 (20.9%)	183	57 (31.1%)
No	Yes	106	16 (15.1%)				90	22 (24.4%)
No	No	101	7 (6.9%)				94	33 (35.1%)
No LACV total		207	23 (11.1%)				184	55 (29.9%)
Grand total		418	51 (12.2%)				367	112 (30.5%)

Fig. 1. Fraction of individuals that took the second bloodmeal. There was a significant effect of sugar treatment, but no significant effect of infection status or the interaction between sugar treatment and infection status. Open bars are constant sugar treatment, and grey bars are intermittent sugar treatment.

($\chi^2 = 1.07$; $df = 3$; $P = 0.3655$), sugar ($\chi^2 = 0.12$; $df = 1$; $P = 0.7258$), and the interaction ($\chi^2 = 0.64$; $df = 3$; $P = 0.5903$; Fig. 2).

Discussion

Multiple sublethal stressors can alter infection and transmission rates for vectors (Vaidyanathan et al. 2008; Muturi et al. 2011a,b; Alto et al. 2014) and also alter adult life-history traits associated with population dynamics and vectorial capacity (e.g., development rate, longevity, and fecundity; Delatte et al. 2009; Muturi et al. 2010). Though LACV infection rate in *Ae. triseriatus* is increased when larvae are reared under suboptimal conditions (Grimstad and

Haramis 1984; Grimstad and Walker 1991; Bevins 2007), we did not detect a parallel effect of suboptimal adult nutrition for *Ae. albopictus*. One reason we may have failed to detect an effect of sugar deprivation is that our intermittent sugar treatment may have been insufficiently stressful. Females may have compensated for the 3 d without sugar by imbibing larger sugar meals when they received sugar, an effect we did not quantify. Withholding sugar for only 24 h was sufficient to yield an effect in an *Anopheles-Plasmodium* system (Ferguson and Read 2002), suggesting that 72 h sugar deprivation should have been sufficiently severe. The 20% sugar solution we used may have been sufficiently concentrated for females to store ample reserves to compensate for 72 h of sugar deprivation. Female *Culex pipiens* (L.) fed on 2, 10, 20, and 40% sucrose were equally susceptible to West Nile virus, but oral transmission of virus was

Fig. 2. Least squares means and standard errors of the number of eggs produced. There were no significant effects of infection status, sugar treatment, or the interaction on the number of eggs produced. Open bars are constant sugar treatment, and grey bars are intermittent sugar treatment.

significantly lower at 40% sucrose (Vaidyanathan et al. 2008). Short-term nutritional deprivation alters immune function in insects, but after the stress is alleviated, immune function can recover (Siva-Jothy and Thompson 2002), suggesting that intermittent low sugar stress may have important but transient effects on vector competence.

Infections with several different arboviruses can alter blood-feeding behavior in several *Aedes* species (Grimstad et al. 1980, Platt et al. 1997, Jackson et al. 2012). LACV infection increased probing and decreased engorgement in *Ae. triseriatus* (Grimstad et al. 1980), reduced bloodmeal size in both *Ae. triseriatus* and *Ae. albopictus*, and decreased refeeding rates in *Ae. triseriatus* but not *Ae. albopictus* (Jackson et al. 2012). Our data showed no effect of LACV infection on *Ae. albopictus* willingness to take a second bloodmeal after the 15-d EIP. Our results and those of Jackson et al. (2012) suggest that LACV is more effective at modifying behavior of *Ae. triseriatus* than that of invasive *Ae. albopictus*, a vector with which LACV has little evolutionary history.

Our finding that intermittent access to sugar increases the likelihood of a second bloodmeal is consistent with previous reports that sugar deprivation increases blood-feeding rates in mosquitoes (Straif and Beier 1996, Canyon et al. 1999, Gary and Foster 2001, Braks et al. 2006). Our goal was to test the hypothesis of condition-dependent effects of infection on propensity to blood feed. We predicted that LACV infection would interact with sugar treatment. Our data did not support this prediction. Because of the rarity of investigations of the interactive effects of arbovirus infection and body condition (or other measures of nutrition status) on blood-feeding behavior, we cannot determine whether lack of condition dependence of blood-feeding behavior is a general property of mosquito–arbovirus systems.

The observed lack of effect of infection on egg production in the second gonotrophic cycle is consistent with the absence of an effect of LACV infection on egg production in the first gonotrophic cycle for both *Ae. triseriatus* and *Ae. albopictus* (Costanzo et al. 2013). This finding raises interesting physiological questions because both species take smaller bloodmeals when LACV infected (Jackson et al. 2012). The generality of the relationship between bloodmeal size and number of eggs produced (e.g., Briegel 1990) suggests that LACV infection alters efficiency of converting blood to eggs.

We found no effect of sugar deprivation on egg production in the second gonotrophic cycle for *Ae. albopictus*. This lack of detectable difference is likely not due to sugar-fed females having crops full of sugar, as all females were starved for 48 h prior to the second bloodmeal (Mostowy and Foster 2004). Females in this experiment reached the second gonotrophic cycle at age ≥ 21 d. Our sugar manipulation was imposed on females between days 6–21. Females that reached the second cycle may have had sufficient time and sugar access to recover from any effects of sugar deprivation. We did not measure bloodmeal size or lipid and glycogen stores, so we cannot postulate a mechanism by which females compensated for previous sugar deprivation.

We also found no interactive effect of LACV infection and sugar on egg production. Glucose concentration and infection have interactive effects on survival of *Anopheles stephensi* infected with *Plasmodium* (Lambrechts et al. 2006). Thus interactive, or condition-dependent, effects may only occur in some vector–pathogen systems, perhaps those where infection has a high cost for the vector. Additional tests for interactive effects of infection and nutrition on adult fitness (e.g., survivorship and fecundity) should be a priority for understanding factors influencing vector populations.

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References Cited

- Alto, B. W., and L. P. Lounibos. 2013. Vector competence for arboviruses in relation to the larval environment of mosquitoes. In W. Takken and C.J.M. Koenraadt (eds.), *Ecology of parasite-vector interactions*. Wageningen Academic Press, The Netherlands.
- Alto, B. W., E. J. Muturi, and R. L. Lampman. 2012. Effects of nutrition and density in *Culex pipiens*. *Med. Vet. Entomol.* 26: 396–406.
- Alto, B. W., S. L. Richards, S. L. Anderson, and C. C. Lord. 2014. Survival of West Nile virus-challenged Southern house mosquitoes, *Culex pipiens*

- quinquefasciatus*, in relation to environmental temperatures. J. Vector Ecol. 39: 123–133.
- Bara, J. J., and E. J. Muturi. 2015. Container type influences the relative abundance, body size, and susceptibility of *Ochlerotatus triseriatus* (Diptera: Culicidae) to La Crosse Virus. J. Med. Entomol. 52: 452–460.
- Bevins, S. N. 2007. Timing of resource input and larval competition between invasive and native container-inhabiting mosquitoes (Diptera: Culicidae). J. Vector Ecol. 32: 252–262.
- Bevins, S. N. 2008. Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). Biol. Invasions 10: 1109–1117.
- Blair, C. D., and K. E. Olson. 2014. Mosquito immune responses to arbovirus infections. Curr. Opin. Insect Sci. 3: 22–29.
- Braks, M. 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: 53–59.
- Breaux, J. A., M. K. Schumacher, and S. A. Juliano. 2014. What does not kill them makes them stronger: Larval environment and infectious dose alter mosquito potential to transmit filarial worms. Proc. Biol. Sci. 281: pii.
- Briegleb, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J. Insect Physiol. 36: 165–172.
- Brown, M. J. F., R. Loosli, and P. Schmid-Hempel. 2000. Condition-dependent expression of virulence in a trypanosome infecting bumblebees. Oikos 91: 421–427.
- Canyon, D. V., J. L. K. Hii, and R. Muller. 1999. Effect of diet on biting, oviposition, and survival of *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 36: 301–308.
- Costanzo, K. S., K. Mormann, and S. A. Juliano. 2005. Asymmetrical competition and patterns of abundance of *Aedes albopictus* and *Culex pipiens* (Diptera: Culicidae). J. Med. Entomol. 42: 559–570.
- Costanzo, K. S., E. J. Muturi, A. V. Montgomery, and B. W. Alto. 2013. Effect of oral infection of La Crosse virus on survival and fecundity of native *Ochlerotatus triseriatus* and invasive *Stegomyia albopicta*. Med. Vet. Entomol. 28: 77–84.
- Delatte, H., G. Gimonneau, A. Triboire, and D. Fontenille. 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.
- Donegan, K., and B. Lighthart. 1989. Effect of several stress factors on the susceptibility of the predatory insect, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the fungal pathogen *Beauveria bassiana*. J. Invertebr. Pathol. 54: 79–84.
- Dye, C. 1986. Vectorial capacity: Must we measure all its components? Parasitol. Today 2: 203–209.
- Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. R. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002. La Crosse Encephalitis in Eastern Tennessee: Clinical, environmental, and entomological characteristics from a blinded cohort study. Am. J. Epidemiol. 155: 1060–1065.
- Ewald, P. W. 1994. Evolution of infectious disease. Oxford University Press, New York, NY.
- Ferguson, H. M., and A. F. Read. 2002. Genetic and environmental determinants of malaria parasite virulence in mosquitoes. Proc. Biol. Sci. 269: 1217–1224.
- 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: 22–28.
- 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. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerg. Infect. Dis. 7: 807–811.
- Gilles, J. R. L., R. S. Lees, S. M. Soliban, and M. Q. Benedict. 2011. Density-dependent effects in experimental larval populations of *Anopheles arabiensis* (Diptera: Culicidae) can be negative, neutral, or overcompensatory depending on density and diet levels. J. Med. Entomol. 48: 296–304.
- Grimstad, P. R., and L. D. Haramis. 1984. *Aedes triseriatus* (Diptera: Culicidae) and La crosse virus III. Enhanced oral transmission by nutrition-deprived mosquitoes. J. Med. Entomol. 21: 249–256.
- Grimstad, P. R., and E. D. Walker. 1991. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission. J. Med. Entomol. 28: 379–386.
- Grimstad, P. R., Q. F. Ross, and G. B. Craig. 1980. *Aedes triseriatus* (Diptera: Culicidae) and La Crosse virus II. Modification of mosquito feeding behavior by virus infection. J. Med. Entomol. 17: 1–7.
- Hardy, J. L., E. J. Houk, L. D. Kramer, and W. C. Reeves. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annu. Rev. Entomol. 28: 229–262.
- Harris, M. C., E. J. Dotseth, B. T. Jackson, S. D. Zink, P. E. Marek, S. L. Paulson, L. D. Kramer, and D. M. Hawley. 2015. Detection and isolation of La Crosse virus in field-collected *Aedes japonicus japonicus* (Diptera: Culicidae) in the Appalachian Region, USA. Emerg. Infect. Dis. 21: 646–649.
- Hughes, M. T., J. A. Gonzalez, K. L. Reagan, C. D. Blair, and B. J. Beaty. 2006. Comparative potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to transovarially transmit La Crosse virus. J. Med. Entomol. 43: 757–761.
- Jackson, B. T., C. C. Brewster, and S. L. Paulson. 2012. La Crosse virus infection alters blood feeding behavior in *Aedes triseriatus* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 49: 1424–1429.
- Koella, J. C., and F. L. Sorenson. 2002. Effect of adult nutrition on the melanization immune response of the malaria vector *Anopheles stephensi*. Med. Vet. Entomol. 16: 316–320.
- Lambert, A. J., C. D. Blair, M. D'Anton, W. Ewing, M. Harborth, R. Seiferth, J. Xiang, and R. S. Lanciotti. 2010. La Crosse virus in *Aedes albopictus* mosquitoes, Texas, USA, 2009. Emerg. Infect. Dis. 16: 856–858.
- Lambrechts, L., and T. W. Scott. 2009. Mode of transmission and the evolution of arbovirus virulence in mosquito vectors. Proc. Biol. Sci. 276: 1369–1378.
- Lambrechts, L., J. M. Chavatte, G. Snounou, and J. C. Koella. 2006. Environmental influence on the genetic basis of mosquito resistance to malaria parasites. Proc. Biol. Sci. 273: 1501–1506.
- McGaw, M. M., L. J. Chandler, L. P. Wasieleski, C. D. Blair, and B. J. Beaty. 1998. Effect of La Crosse virus infection on overwintering of *Aedes triseriatus*. Am. J. Trop. Med. Hyg. 58: 168–175.
- Mitchell, S. E., E. S. Rogers, T. J. Little, and A. F. Read. 2005. Host-parasite and genotype-by-environment interactions: Temperature modifies potential for selection by a sterilizing pathogen. Evolution (N. Y.) 59: 70–80.
- Moller-Jacobs, L. L., C. C. Murdock, and M. B. Thomas. 2014. Capacity of mosquitoes to transmit malaria depends on larval environment. Parasit. Vectors 7: 593.
- Mostow, W. M., and W. A. 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.
- Murdock, C. C., L. L. Moller-Jacobs, and M. B. Thomas. 2013. Complex environmental drivers of immunity and resistance in malaria mosquitoes. Proc. Biol. Sci. 280: 2013–2030.
- Muturi, E. J., K. Costanzo, B. Kesavaraju, R. Lampman, and B. W. Alto. 2010. Interaction of a pesticide and larval competition on life history traits of *Culex pipiens*. Acta Trop. 116: 141–146.
- Muturi, E. J., K. Costanzo, B. Kesavaraju, and B. W. Alto. 2011a. Can pesticides and larval competition alter susceptibility of *Aedes* mosquitoes (Diptera: Culicidae) to arbovirus infection? J. Med. Entomol. 48: 429–436.
- Muturi, E. J., C.-H. Kim, B. W. Alto, M. R. Berenbaum, and M. A. Schuler. 2011b. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. Trop. Med. Int. Health 16: 955–964.
- Muturi, E. J., B. F. Allan, and J. Ricci. 2012. Influence of leaf detritus type on production and longevity of container-breeding mosquitoes. Environ. Entomol. 41: 1062–1068.
- Okech, B. A., L. C. Gouagna, E. W. Kabiru, J. C. Beier, G. Yan, and J. I. Githure. 2004. Influence of age and previous diet of *Anopheles gambiae* on the infectivity of natural *Plasmodium falciparum* gametocytes from human volunteers. J. Insect Sci. 4: 1–8.
- Okech, B. A., L. C. Gouagna, G. Yan, J. I. Githure, and J. C. Beier. 2007. Larval habitats of *Anopheles gambiae* s.s. (Diptera: Culicidae) influences vector competence to *Plasmodium falciparum* parasites. Malar. J. 6: 50.

- Platt, K. B., K. J. Linthicum, K. S. Myint, B. L. Innis, K. Lerdthusnee, and D. W. Vaughn. 1997. Impact of dengue virus infection on feeding behavior of *Aedes aegypti*. *Am. J. Trop. Med. Hyg.* 57: 119–125.
- Ponton, F., K. Wilson, A. J. Holmes, S. C. Cotter, D. Raubenheimer, and S. J. Simpson. 2013. Integrating nutrition and immunology: a new frontier. *J. Insect Physiol.* 59: 130–137.
- Reiskind, M. H., and L. P. Lounibos. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. *Med. Vet. Entomol.* 23: 62–68.
- SAS Institute. 2011. Version 9.3 User's Guide. Cary, NC.
- Siva-Jothy, M. T., and J. J. W. Thompson. 2002. Short-term nutrient deprivation affects immune function. *Physiol. Entomol.* 27: 206–212.
- Straif, S. C., and J. C. Beier. 1996. Effects of sugar availability on the blood-feeding behavior of *Anopheles gambiae* (Diptera: Culicidae). *J. Med. Entomol.* 33: 608–612.
- Suwanchaichinda, C., and S. M. Paskewitz. 1998. Effects of larval nutrition, adult body size, and adult temperature on the ability of *Anopheles gambiae* (Diptera: Culicidae) to melanize Sephadex Beads. *J. Med. Entomol.* 35: 157–161.
- Telang, A., A. A. Qayum, A. Parker, B. R. Sacchetta, and G. R. Byrnes. 2012. Larval nutritional stress affects vector immune traits in adult yellow fever mosquito *Aedes aegypti* (*Stegomyia aegypti*). *Med. Vet. Entomol.* 26: 271–281.
- Tripet, F., F. Aboagye-Antwi, and H. Hurd. 2008. Ecological immunology of mosquito-malaria interactions. *Trends Parasitol.* 24: 219–227.
- Vaidyanathan, R., A. E. Fleisher, S. L. Minnick, K. A. Simmons, and T. W. Scott. 2008. Nutritional stress affects mosquito survival and vector competence for West Nile virus. *Vector Borne Zoonotic Dis.* 8: 727–732.
- Westby, K. M., C. Fritzen, D. Paulsen, S. Poindexter, and A. C. Moncayo. 2015. La Crosse virus infection in field-collected *Aedes albopictus*, *Aedes japonicus*, and *Aedes triseriatus* in Tennessee. *J. Am. Mosq. Control Assoc.* 31: 233–241.