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Effects of Forced Egg Retention on the Temporal Progression of West Nile Virus Infection in *Culex pipiens quinquefasciatus* (Diptera: Culicidae)

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Abstract

Environmental factors that impact the biology of mosquito vectors can have epidemiological implications. Lack of oviposition sites facilitated by environmental factors such as temperature and drought can often force *Culex* spp. mosquitoes to retain their eggs. *Culex pipiens quinquefasciatus* Say were fed blood meals containing West Nile virus (WNV; family *Flaviviridae*, genus *Flavivirus*) and either allowed to oviposit or forced to retain their eggs through different time points postinfection (9, 13, 20, 27 d) at 28°C. Oviposition status did not signiÞcantly affect rates of WNV infection (% with virus-positive bodies), dissemination (% with virus-positive legs), or transmission (% with virus-positive saliva) for any of the tested time points. As expected, WNV titers in bodies and legs were significantly ($P < 0.05$) higher at late time points compared with early time points. No significant differences were observed in WNV titers in saliva between time points. There were no significant effects of oviposition status on virus titers of bodies, legs, or saliva. However, we found that egg retention may increase vector competence at early and late time points after infection and that a single oviposition event may decrease vector competence, possibly by activating an immune response against the virus. Environmental changes that influence mosquito biology are important determinants of virus transmission, and further studies are needed to assess the effects of drought on virus transmission risk and how these interactions affect our interpretation of field data.

Keywords

West Nile virus; *Culex*; egg retention

Most female mosquitoes must feed on blood for the development of their eggs. Protein-rich blood meals are converted to amino acids needed for egg development (Clements 2000). If no environmental perturbations (such as abnormal temperatures or drought) occur, female mosquitoes will oviposit at the completion of bloodmeal digestion (Eldridge 1968, Clements 2000). The gonotrophic cycle, the period from blood feeding to oviposition, usually takes 3 d, but this duration may vary because of environmental factors and mosquito species (Garcia-Rejon et al. 2008). Furthermore, a variety of factors can delay oviposition, thereby extending the gonotrophic cycle and impacting the distribution and abundance of future mosquito

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generations (Clements 2000). Factors that most commonly prevent or delay oviposition in mosquitoes include: failure to mate (El-Akad and Humphreys 1988), decreased temperature (Mayne 1926, Eldridge 1968), and the absence of oviposition sites (Woke 1955). *Culex* spp. mosquitoes are also likely to retain or resorb their eggs if they imbibe a partial bloodmeal that is not sufficient for development of eggs, a phenomenon that increases with mosquito age (Nayer and Knight 1981, Awahmukalah and Brooks 1985). *Culex* spp. mosquitoes retain their eggs until a suitable oviposition habitat is available (Provost 1969). Disruption of oviposition stimuli (Bentley and Day 1989), as well as oviposition repellents, such as nonanoic acids (C9) (Schultz et al.1981) or the synthetic pyrethroid cypermethrin (Verma 1986) also may influence oviposition, thereby forcing the gravid mosquito to retain her eggs until more suitable conditions can be found. Drought induces gravid *Culex nigripalpus* Theobald female mosquitoes to retain their eggs, sometimes for up to 5 mo (Day and Edman 1988, Day and Curtis 1989).

Drought is an important environmental factor that can prevent or delay oviposition in *Culex* spp. mosquitoes because water is required for oviposition. Drought may force potentially infectious mosquitoes and hosts to come in contact more frequently around dwindling water sources, and this may increase virus amplification as hypothesized to explain St. Louis encephalitis virus (SLEV; family *Flaviviridae*, genus *Flavivirus*) infection and transmission patterns in Florida (Shaman et al. 2005). The same study showed that a dry spring followed by a wet summer, in combination with the close contact between *C. nigripalpus* and amplification hosts, favors epizootic cycling and amplification of SLEV, and can result in increased virus transmission to sentinel chickens and humans (Shaman et al. 2005).

West Nile virus (WNV; family *Flaviviridae*, genus *Flavivirus*) is a mosquito-borne pathogen that emerged in the northeastern United States in 1999 and has since spread across the country into 48 states (Reisen and Brault 2007). In the southeast, *Culex pipiens quinquefasciatus* Say is one of the primary vectors of WNV (Sardelis et al. 2001, Godsey et al. 2005) because it feeds on the birds that are amplifying hosts and also humans (Zinser et al. 2004). The mosquito–virus interaction is affected by environmental factors such as temperature, and these factors affecting mosquito biology may impact vector competence (Richards et al. 2007).

A mosquito's ability to become infected with and subsequently transmit a virus, i.e., vector competence, is influenced by the ease at which the virus infects and escapes the midgut and infects and escapes the salivary glands (Kramer et al. 1981, DeFoliart et al. 1987). The time required for a virus to complete this cycle and be transmitted to a host is referred to as the extrinsic incubation period (EIP). Typically, the mosquito must first blood feed on an infectious host, complete the EIP, oviposit, and subsequently feed on a second host in order for virus transmission to occur. Egg retention in virus-infected mosquitoes may allow time for infectious agents to complete the EIP necessary for virus transmission, thus increasing the chances of transmission to hosts near the oviposition site during subsequent blood feedings (Day et al. 1990).

We studied the effects of egg retention on temporal progression of infection rates, dissemination rates, transmission rates, and virus titers in bodies, legs, and saliva for *C. p. quinquefasciatus* infected with WNV. These relationships must be characterized to understand the epidemiological impact of these environmental factors on the virus transmission cycle.

Materials and Methods

Mosquito Rearing

C. p. quinquefasciatus from an established laboratory colony originating from Alachua County in north-central Florida in 1995 was used for these experiments. The colony was provided a

20% sucrose solution and maintained under standard conditions as described elsewhere (Richards et al. 2009).

Experimental Design

Twenty-four hours before blood feeding, adult females were transferred to 1-liter cardboard cages with a mesh screen top, sucrose was removed, and water was provided ad libitum. Five-day-old mosquitoes were allowed to feed on pledgets soaked with previously frozen WNV-infected (WNFL03p2–3 strain, originally isolated from a *C. nigripalpus* pool collected in Indian River County in 2005) (Doumbouya 2007) citrated bovine blood (Hemostat, Dixon, CA) according to standard methods described previously (Richards et al. 2007, 2009). Two 0.1-ml samples of the bloodmeal were added to separate tubes containing 1.0 ml BA-1 diluent (Richards et al. 2007) and stored at −80°C until tested to determine the virus titers.

Subsequent to feeding, mosquitoes were immobilized on ice, and five freshly fed fully engorged mosquitoes were frozen at −80°C until tested to determine the amount of virus initially imbibed. Mosquitoes were divided into two groups. One group of ~150 mosquitoes was forced to retain their eggs (egg retention) and the second group of ~150 mosquitoes was allowed to oviposit (oviposition). Individual mosquitoes were treated as experimental units. All fully engorged specimens from the egg retention group were transferred to a single 1-liter cardboard cage with a mesh screen top because they would not be allowed to oviposit. Conversely, each fully engorged specimen from the oviposition group was transferred singly to individual 0.5-liter cardboard cages with a mesh screen top so that we could visually verify the presence of an egg raft, indicating that oviposition had occurred for each mosquito. Small containers were used in the oviposition group so that individual mosquitoes were forced into close contact with the ovi-position source to improve the egg laying success. These cages containing single mosquitoes had an empty 30-ml plastic cup affixed to the bottom of the cage with nontoxic glue that would later serve as an oviposition source. Mosquitoes in both groups were provided 20% sucrose ad libitum and maintained in an incubator at 28°C until sampled at time points of 9, 13, 20, or 27 d after infection. Four days after infection, 15 ml of hay infusion was added to the plastic cups in the cages of the oviposition group, and mosquitoes were allowed to oviposit. The number of days before mosquitoes were allowed to oviposit was determined based on reports of the length of the initial gonotrophic cycle in *Culex* (Awahmukalah and Brooks 1985, Akoh et al. 1992, Garcia-Rejon et al. 2008) and also because the mosquito colony was typically given 4 d for egg development. Mosquitoes that did not lay an egg raft into the oviposition media within 48 h were removed from the experiment so that females would not be separated in oviposition status by >2 d.

Approximately 50% of mosquitoes offered an ovi-position substrate failed to oviposit and were removed from the study. Sample sizes at each time point were selected based on biological relevance and mosquito survival rates. Twelve mosquitoes were sampled after 9 d because we did not expect WNV transmission to occur this early in infection. Twelve mosquitoes were also sampled after 27 d because of low survival rates and starting sample numbers. The largest number of mosquitoes (n = 24) were sampled at each of the 13-and 20-d time points because these are biologically relevant for transmission cycles in natural populations. At each time point, mosquitoes from each treatment were placed into a −20°C freezer for 45 s and transferred to ice to anesthetize them so that their legs and wings could be safely removed with forceps. Cross-contamination was prevented by using separate forceps for bodies and legs. Forceps were soaked in 70% ethanol and flamed between processing of each mosquito. Mosquito legs were placed into a tube containing 1.0 ml BA-1 with two 4.5-mm zinc-plated beads (BB-caliber air gun shot; Daisy, Rogers, AR). Live mosquitoes were gently placed onto an adhesive surface and forced to salivate into capillary tubes filled with immersion oil (Carl Zeiss, Oberkochen, Germany) for 45 min as in Smith et al. (2005). After salivation, bodies were placed into

individual tubes containing 1.0 ml BA-1 with two beads. Saliva was expelled into tubes containing 1.0 ml BA-1. All samples were stored at −80°C until tested to determine virus titer as described previously (Richards et al. 2007, 2009). Briefly, mosquitoes were homogenized for 3 min at 25 Hz (TissueLyser; Qiagen, Valencia, CA) and centrifuged at 3,148*g* for 4 min at 4°C. RNA was extracted with the MagNa Pure LC Total Nucleic Acid Kit using the MagNa Pure Compact Instrument (Roche, Mannheim, Germany). RNA was quantified using the Superscript III One-Step qRT-polymerase chain reaction (PCR) system (Invitrogen, Carlsbad, CA) as described previously (Lanciotti et al. 2000, Richards et al. 2007).

Data Analysis

We used Fisher exact tests (SAS Institute, Cary, NC) to analyze differences in rates of infection, dissemination, or transmission between the oviposition and egg retention groups at each time point. The infection rate was the percentage of all mosquitoes tested having infected bodies. The dissemination rate was the percentage of mosquitoes with infected bodies that also had infected legs. The transmission rate was the percentage of mosquitoes with infected bodies that also had infected saliva. We tested the null hypothesis that infection, dissemination, and transmission rates were equal for different treatment groups (oviposition versus egg retention). Analysis of variance (ANOVA; SAS Institute, Cary, NC) was used to test viral titers in bodies, legs, and saliva for differences between time points and oviposition status. If significant differences were observed, a Duncan multiple comparison procedure was used to determine which means were significantly different within each body part (SAS Institute).

Results

Female *C. p. quinquefasciatus* were fed bloodmeals containing (titer ± SE) 7.1 ± 0.01 logs plaque-forming units (pfu) WNV/ml, and virus titers of five freshly fed mosquitoes were determined to be 5.7 ± 0.1 logs pfu WNV/ml. The WNV titers of bodies, legs, and saliva, along with rates of infection, dissemination, and transmission at each time point are shown in Table 1. Results from ANOVA show a significant effect of time after infection on virus titers in body and legs but not on virus titers in saliva (Table 2). Means comparisons show WNV titers in legs are significantly higher in the egg retention versus the oviposition group at the 9-d time point (Table 1). Fisher exact tests showed no significant differences ($P > 0.05$) in infection, dissemination, or transmission rates between the egg retention and oviposition groups, regardless of time point, indicating that the treatment groups were similar in these rates (Table 2). However, the time point and oviposition status interaction shows that WNV titer in legs differed between oviposition status groups (Table 2). Sample sizes, and therefore the power to detect interactions, were low at the 9- and 27-d time points. Comparisons of means for virus titers of bodies and saliva did not show any significant differences between oviposition status groups (Table 1).

Discussion

Arboviral transmission cycles can be driven by infectious mosquitoes; hence, the information provided here may have bearing on risk assessment of mosquito populations for *Culex* spp.–driven virus transmission. Environmental factors such as increased temperature and drought directly affect egg retention in floodwater mosquitoes such as *C. nigripalpus* because these mosquitoes are dependent on oviposition sources in flooded areas such as ditches and ground pools (Nayer 1982). Egg retention affects *C. p. quinquefasciatus* to a lesser extent because this species uses oviposition sources such as artificial containers and ditches with high organic content that may better withstand drought (Barr 1967). However, egg retention may occur in *C. p. quinquefasciatus* in drought periods when oviposition sources are limited. Rainfall events after drought allow mosquitoes to oviposit and synchronizes a cohort of host-seeking

mosquitoes, allowing potentially infective mosquitoes to spill into human populations, thereby creating epidemic conditions (Day et al. 1990).

Oviposition status (egg retention versus oviposition) did not significantly affect WNV infection, dissemination, or transmission rates under the conditions of our test, indicating that these phenotypes were similar between groups. Mosquitoes that were forced to retain their eggs showed significantly higher virus titers in legs early after infection with WNV and trends toward higher virus titers in saliva at later time points compared with mosquitoes allowed to oviposit. This suggests that egg retention may increase vector competence at early and late time points after infection, with little effect during midpoints. This also may suggest a possible immune response against the virus that is stimulated by oviposition (Styer et al. 2007). We observed the lowest virus titers in saliva (1.8 ± 0.5 logs pfu WNV/ml) in the oviposition group at the 27-d time point that may indicate cytopathic effects of WNV on salivary glands as has been observed in *C. p. quinquefasciatus* > 21 d after infection with WNV (Girard et al. 2007). Caution is advised in interpreting these results because of small sample sizes (n = 12) in groups at early and late time points. Also, although care was taken to sample six legs from each mosquito, it is possible that some mosquitoes could have lost a leg during the course of the experiment, thereby potentially reducing virus titer in leg samples. Infected mosquitoes forced to retain their eggs because of limited oviposition sites also may indirectly increase the probability of virus transmission to naïve hosts because the extension of the gonotrophic cycle would allow completion of the EIP required for virus transmission.

Further tests using larger sample sizes are needed to fully assess these relationships for this and other *Culex* spp. mosquitoes and would allow the power to assess interactive effects of time postinfection and oviposition status. The titer similarities between egg retention and oviposition treatments at the intermediate time points indicate that virus dynamics within these mosquitoes are comparable. Virus titers in the saliva are especially important when assessing transmission risk of mosquito populations, and our results show similar risk, regardless of oviposition status or time after infection. We show limited effects of oviposition or egg retention under the conditions of this test. Environmental changes that influence mosquito biology are an important determinant of virus transmission, and further studies are needed to address the effects of drought on virus transmission risk and how these interactions may affect our interpretation of field data.

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References Cited

Akoh JI, Aigbodion FI, Kumbak D. Studies on the effect of larval diet, adult body weight, size of blood meal and age on the fecundity of *Culex quinquefasciatus* (Diptera: Culicidae). Insect Sci. Applic 1992;13:177–181.

Awahmukalah DS, Brooks MA. Viability of *Culex pipiens pipiens* eggs affected by nutrition and apo-symbiosis. J. Invertebr. Pathol 1985;45:225–230. [PubMed: 3981032]

Barr AR. Occurrence and distribution of the *Culex pipiens* complex. Bull. W.H.O 1967;37:293–296. [PubMed: 5300069]

Bentley MD, Day J. Chemical ecology and behavioral aspects of mosquito oviposition. Annu. Rev. Entomol 1989;34:401–421. [PubMed: 2564759]

Clements, AN. Volume 1: development, nutrition and reproduction. CABI Publishing; Oxon, United Kingdom: 2000. The biology of mosquitoes.

Day, JF.; Edman, JD. Host location, bloodfeeding and oviposition behavior of *Culex nigripalpus* (Diptera: Culicidae): their influence on St. Louis encephalitis virus transmission in southern Florida. In: Scott, TW.; Grumpstrup-Scott, G., editors. Proceedings of a symposium: the role of vector-host interactions in disease transmission. Miscellaneous Publication 68. Entomological Society of America; Lanham, MD: 1988. p. 1-8.

Day JF, Curtis GA. Influence of rainfall on *Culex nigripalpus* (Diptera: Culicidae) blood-feeding behavior in Indian River County, Florida. J. Med. Entomol 1989;82:32–37.

Day JF, Curtis GA, Edman JD. Rainfall-directed oviposition behavior of *Culex nigripalpus* (Diptera: Culicidae) and its influence on St. Louis encephalitis virus transmission in Indian River County, Florida. J. Med. Entomol 1990;27:43–50. [PubMed: 2299655]

DeFoliart GR, Grimstad GR, Watts DM. Advances in mosquito-borne arbovirus/vector research. Annu. Rev. Entomol 1987;32:479–505. [PubMed: 2880554]

Doumbouya, A. Microsatellite DNA analysis of four *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) mosquito populations in Florida and their vector competence for West Nile virus. University of Florida; Gainesville, FL: 2007. Ph.D dissertation

El-Akad AS, Humphreys J. Factors affecting oviposition and egg production in laboratory-reared *Anopheles pharoensis* Theobald. Bull. Soc. Vector Ecol 1988;13:243–247.

Eldridge B. The effect of temperature and photoperiod on blood-feeding and ovarian development in mosquitoes of the *Culex pipiens* complex. Am. J. Trop. Med. Hyg 1968;17:133–140. [PubMed: 5688903]

Garcia-Rejon JE, Farfane-Ale JA, Ulloa A, FloresFlores LF, Rosado-Paredes E, Baak-Baak C, LoronoPina MA, Fernandes-Salas I, Beaty BJ. Gonotrophic cycle estimate for *Culex quinquefasciatus* in Merida, Yucatan, Mexico. J. Am. Mosq. Control Assoc 2008;24:344–34. [PubMed: 18939685]

Girard YA, Schneider BS, McGee CE, Wen J, Han VC, Popov V, Mason PW, Higgs S. Salivary gland morphology and virus transmission during long-term cytopathologic West Nile virus infection in *Culex* mosquitoes. Am. J. Trop. Med. Hyg 2007;76:118–128. [PubMed: 17255239]

Godsey MS Jr. Blackmore MS, Panella NA, Burkhalter K, Gottfried K, Halsey LA, Rutledge R, Langevin SA, Gates R, Lamonte KM, et al. West Nile virus epizootiology in the Southeastern United States, 2001. Vector-Borne Zoonot. Dis 2005;5:82–89.

Kramer LD, Hardy J, Presser S, Houk E. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. Am. J. Trop. Med. Hyg 1981;30:190–197. [PubMed: 7212166]

Lanciotti R, Kerst A, Nasci R, Godsey M, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TagMan reverse transcriptase-PCR assay. J. Clin. Microbiol 2000;38:4066–4071. [PubMed: 11060069]

Mayne B. Notes on the influence of temperature and humidity on oviposition and early life of *Anopheles*. U.S. Publ. Health Rep 1926;41:986–990.

Nayer JK, Knight JW. Ovarian development in *Culex nigripalpus* and its implication for disease transmission. Entomol. Exp. Appl 1981;29:49–59.

Nayer JK. Bionomics and physiology of *Culex nigripalpus* (Diptera: Culicidae) of Florida: an important vector of diseases. FAES IFAS Bull 1982;827:1–73.

Provost, MW. St. Louis encephalitis in Florida. Monograph No. 12. Florida State Board of Health; Jacksonville, FL: 1969. The natural history of *Culex nigripalpus*; p. 46-62.

Reisen W, Brault AC. West Nile virus in North America: perspectives on epidemiology and intervention. Pest Manag. Sci 2007;63:641–646. [PubMed: 17373672]

Richards SL, Mores CN, Lord CC, Tabachnick WJ. Impact of extrinsic incubation temperature and virus exposure on vector competence of *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) for West Nile virus. Vector-Borne Zoonot. Dis 2007;7:629–636.

Richards SL, Lord CC, Pesko KA, Tabachnick WJ. Environmental and biological factors influence *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) vector competence for Saint Louis encephalitis virus. Am. J. Trop. Med. Hyg 2009;81:264–272. [PubMed: 19635881]

Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML. Vector competence of selected North American *Culex* and *Coquilletidia* mosquitoes for West Nile virus. Emerg. Infect. Dis 2001;7:1018–1022. [PubMed: 11747732]

SAS Institute. SAS version 8.2. SAS Institute; Cary, NC: 2002-2008

Schultz, GW.; Hwang, Y-S.; Kramer, WL.; Harold, A.; Mulla, MS. Field evaluation of mosquito ovipositional repellents, pp. 98–99; Proceedings of the 49th Annual Conference of the California Mosquito Vector Control Association. California Mosquito and Vector Control Association Inc.; Sacramento, CA. 1981.

Shaman J, Day J, Stieglitz M. Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida. J. Med. Entomol 2005;42:134–141. [PubMed: 15799522]

Smith DR, Carrara AS, Aguilar PV, Weaver SC. Evaluation of methods to assess transmission potential of Venezuelan equine encephalitis virus by mosquitoes and estimation of mosquito saliva titers. Am. J. Trop. Med. Hyg 2005;73:33–39. [PubMed: 16014828]

Styer LM, Meola MA, Kramer LD. West Nile virus infection decreases fecundity of *Culex tarsalis* females. J. Med. Entomol 2007;44:1074–1085. [PubMed: 18047209]

Verma KVS. Deterrent effect of synthetic pyre-throids on the oviposition of mosquitoes. Curr. Sci 1986;55:373–375.

Woke PA. Deferred oviposition in *Aedes aegypti* (Linn.). Ann. Entomol. Soc. Am 1955;48:39–46.

Zinser M, Ramberg F, Willot E. *Culex quinquefasciatus* (Diptera: Culicidae) as a potential West Nile virus vector in Tucson, Arizona: blood meal analysis indicates feeding on both humans and birds. J. Insect. Sci 2004;4:20–22. [PubMed: 15861236]

Table 1

Mean titers ± SE, infection (bodies), dissemination (legs), and transmission (saliva) rates of *C. p. quinquefasciatus* fed West Nile virus and incubated at 28° C

Time (day)	No. tested	No. infected bodies (%)	No. infected legs (%)	No. infected saliva (%)	Body titer[a]	Leg titer[a]	Saliva titer[a]
Oviposition							
9	12	12 (100)	3 (25)	0 (0)	6.7 ± 0.04	1.2 ± 0.1*	—
13	24	23 (96)	18 (78)	2 (9)	6.9 ± 0.1	4.2 ± 0.3	3.5 ± 0.07
20	24	24 (100)	24 (100)	8 (33)	7.0 ± 0.1	4.9 ± 0.2	3.2 ± 0.4
27	12	12 (100)	12 (100)	2 (17)	7.2 ±0.1	5.5 ± 0.1	1.8 ± 0.5
Egg retention							
9	12	12 (100)	6 (50)	1 (8)	6.4 ± 0.2	3.2 ± 0.4[b]	2.6 ± 0
13	24	24 (100)	19 (79)	3 (13)	6.6 ± 0.1	4.2 ± 0.2	1.9 ± 0.4
20	24	24 (100)	24 (100)	9 (38)	7.0 ± 0.1	4.6 ± 0.2	2.5 ± 0.3
27	12	12 (100)	12 (100)	4 (33)	7.3 ± 0.1	5.2 ±0.1	3.0 ± 0.5

—, no virus-positive mosquitoes.

[a] Logs plaque-forming units WNV per milliliter.

[b] Significantly different ($P < 0.05$) between oviposition and egg retention treatments within body part and time point.

Table 2

Effects of time after infection and oviposition status on body, leg, and saliva West Nile virus titers in *C. p. quinquefasciatus*

Effect	Body titer			Leg titer			Saliva titer		
	F	**df**	***P***	***F***	**df**	***P***	***F***	**df**	***P***
Time	9.99	3,135	**<0.0001**	25.45	3,110	**<0.0001**	0.35	3,22	0.789
Oviposition status	1.58	1,135	0.211	2.72	1,110	0.102	0.68	1,22	0.420
Time × oviposition status	1.60	3,135	0.192	3.95	3,110	**0.010**	3.31	3,22	0.056

Analysis of variance (proc GLM).

Bold values are significant ($P < 0.05$).