Effects of Temperature on the Transmission of West Nile Virus by *Culex tarsalis* (Diptera: Culicidae)

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ABSTRACT *Culex tarsalis* Coquillett females were infected with the NY99 strain of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) and then incubated under constant temperatures of 10–30°C. At selected time intervals, transmission was attempted using an in vitro capillary tube assay. The median time from imbibing an infectious bloodmeal until infected females transmitted WNV (median extrinsic incubation period, EIP_{50}) was estimated by probit analysis. By regressing the EIP rate (inverse of EIP_{50}) as a function of temperature from 14 to 30°C, the EIP was estimated to require 109 degree-days (DD) and the point of zero virus development (x-intercept) was estimated to be 14.3°C. The resulting degree-day model showed that the NY99 WNV strain responded to temperature differently than a lineage II strain of WNV from South Africa and approximated our previous estimates for St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV). The invading NY99 WNV strain therefore required warm temperatures for efficient transmission. The time for completion of the EIP was estimated monthly from temperatures recorded at Coachella Valley, Los Angeles, and Kern County, California, during the 2004 epidemic year and related to the duration of the *Cx. tarsalis* gonotrophic cycle and measures of WNV activity. Enzootic WNV activity commenced after temperatures increased, the duration of the EIP decreased, and virus potentially was transmitted in two or less gonotrophic cycles. Temperatures in the United States during the epidemic summers of 2002–2004 indicated that WNV dispersal and resulting epicenters were linked closely to above-average summer temperatures.

KEY WORDS *Culex tarsalis*, West Nile virus, transmission, temperature, degree-days

THE TIME FROM INGESTION of an infectious bloodmeal until a mosquito is capable of transmitting an acquired arbovirus infection (the extrinsic incubation period, EIP) is an important parameter in measures of vectorial capacity and the rate of virus transmission (Smith 1987, Reisen 1989). Because mosquito host temperature approaches ambient conditions (Meyer et al. 1990), the rate of virus replication within a susceptible mosquito vector and therefore the duration of the EIP can be defined by a degree-day (DD) temperature model generated from experimental infection data by using a regression function (Reisen et al. 1993). When combined with degree-day estimates of the duration of the mosquito gonotrophic cycle (i.e., the rate of host contact) (Reisen et al. 1992b), temporal changes in the effectiveness of transmission essentially delineate the seasonality of virus activity.

West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) is now widely distributed throughout tropical and temperate regions of the world (Hayes 1989, 2001). In North America, it has been recorded from New York to California and from Canada into tropical America (Komar 2003, Cruz et al. 2005), and therefore potentially it must be able to be transmitted under a variety of temperature regimens. Detailed temperature studies have been done with strains of WNV from South Africa and New York, presumably representatives of lineages II and Ia, respectively (Lanciotti et al. 2002, Guthrie et al. 2003). The South African strain of WNV (H442) has had a long evolutionary history with its mosquito vectors and was found to replicate well in and be transmitted by *Culex univitattus* Theobald at temperatures as low as 14°C after 30 d postinfection (dpi) (Cornel et al. 1993). In contrast, the strain of WNV invading North America has had a short transmission history by the local guilds of *Culex* mosquitoes and replicated poorly in *Culex pipiens pipiens* L. at

The collection and infection of wild birds was done under Protocol 11184 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit No. 801049-02 from the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0 from the Department of the Interior. Animal Use and Care Administrative Advisory Committee Protocol No. 11187 approved procedures for using wild birds and chickens for mosquito infection experiments. Use of arboviruses was approved under Biological Use Authorization #0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit #47901.

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temperatures as warm as 18°C, with <50% of females developing a disseminated infection at 32 dpi (Dohm et al. 2002). In a companion study, the NY99 strain did not replicate in *Cx. p. pipiens* at 10°C (Dohm and Turell 2001), perhaps indicating that the thermal threshold for replication for WNV may lie between 10 and 14°C.

Culex tarsalis Coquillett has emerged as the primary rural vector of WNV throughout its distribution from Canada to Mexico (Darsie and Ward 1981, Turell et al. 2005), an area where this mosquito also is the primary vector of endemic western equine encephalomyelitis (family *Togaviridae*, genus *Alphavirus*, WEEV) and St. Louis encephalitis (family *Flaviviridae*, genus *Flavivirus*, SLEV) viruses (Mitchell et al. 1980, Reisen and Monath 1989, Reisen 2003). Historically, the distribution of mosquito-borne encephalitis outbreaks in North America has been delineated by temperature, with WEEV predominating as the etiological agent in the north and SLEV in the south (Hess et al. 1963). This distribution was supported well by DD models of extrinsic incubation period in *Cx. tarsalis*, with WEEV requiring 67 DD and SLEV 115 DD; minimal developmental thresholds were estimated to be 10.9 and 14.9°C, respectively (Reisen et al. 1993). These estimates for SLEV were slightly cooler than observed for an eastern strain of SLEV in *Culex pipiens quinquefasciatus* Say (Hurlbut 1973).

In the current article, we explore the notion that the wide distribution of WNV in time, space, and elevation is possible because this virus replicates and is effectively transmitted under a range of ambient conditions or that climatic variation has provided suitable conditions for epidemics to occur in temperate regions. Our approach was to develop a DD model for WNV transmission by *Cx. tarsalis* and then use this model to investigate variation in transmission efficiency in time and space.

Materials and Methods

Virus and Mosquito Strains. The NY strain of WNV isolated in 1999 from a Flamingo that died in the Bronx Zoo (strain 35211 AAF 9/23/99) was passaged twice in Vero cells and had a titer of 8.6 $\log_{10}$ plaque-forming units (PFU)/ml. The Kern National Wildlife Refuge strain of *Cx. tarsalis* from Kern County, California, was colonized in 2002 and was used in our infection experiments. This relatively recent laboratory strain was moderately susceptible to WNV in previous vector competence studies (Reisen et al. 2005b).

Mosquito Infection and Transmission. To simulate natural infection, females reared in an insectary at 22–24°C and a photoperiod of 18:6 (L:D) h were held until 3–5 d of age and then were offered a restrained passeriform bird for 1 to 2 h during the birds' peak viremia period. Because midsummer photoperiods and warm conditions were used during immature development, females were reproductively active and became gravid at all incubation temperatures. Originally, we used house sparrows, *Passer domesticus*, and house finches, *Carpodacus mexicanus*, on 2 to 3 dpi by subcutaneous inoculation with 3 to 4 $\log_{10}$ PFU of WNV. Unfortunately, the viremia response in these donor birds was variable, and resulting infection rates were low when titers fell below 6.5 $\log_{10}$ PFU/ml (Fig. 1). Therefore, some temperature and time combinations were repeated using white-crowned sparrows (*Zonotrichia leucophrys*) that produced a consistent high titered viremia (Reisen et al. 2005).

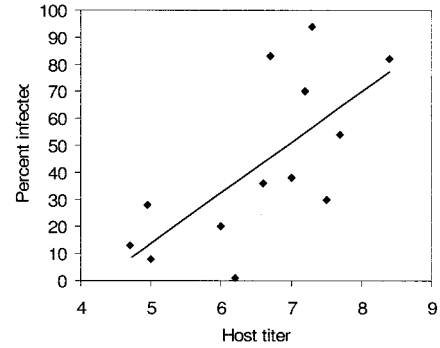

Fig. 1. Percentage of KNWR *Cx. tarsalis* females infected of total surviving incubation at 14–30°C (n = 60–151 females per bird) plotted as a function of donor passeriform bird viremia titers ($\log_{10}$ PFU/ml) measured at mosquito feeding 2 to 3 d postinfection. Plotted is the significant regression function y = 18.7x –79.7. F = 10.1; df = 1, 11; P = 0.009; R^2 = 0.48.

Evaluation methods were similar to those used previously (Reisen et al. 1993). Immediately after feeding, fully engorged females were sorted into 0.67 liters paper cartons and transferred to incubators set at 10, 14, 18, 22, 26 or 30°C with an 18:6 h L:D photoperiod. Females were offered 10% sucrose on cotton pads that were changed at 2-d intervals and were not permitted to oviposit. On six to 10 occasions extending from 2 to 110 dpi depending upon incubation temperature, 20 females were anesthetized with triethylamine, their legs were removed, and their proboscis was inserted into a capillary tube containing ≈10 μl of a 1:1 by volume solution of fetal bovine serum and 10% sucrose. Recovery of virus from the capillary tube contents was considered a measure of transmission (Aitken 1977). The frequency of transmission assessment varied as a function of temperature, and times were selected to bracket when 50% of infected females transmitted virus based on previous studies (Cornel et al. 1993, Goddard et al. 2003). After ≈15 min, expectorate samples were expelled into 300 μl of virus diluent (phosphate buffered saline, 20% fetal calf serum, and antibiotics), and the body and expectorate solution were frozen separately at −80°C. At the end of the 110-d incubation period at 10°C, surviving females were transferred to 26°C and then offered the opportunity to oviposit and blood feed as a group on restrained house sparrows for three consecutive days. Females dying or blood feeding were tested for infection, whereas those remaining alive for >5 d but not refeeding were tested for infection and transmission by the capillary tube method. House sparrows

were tested for viremia at 2 to 3 dpi to determine whether transmission occurred.

Mosquito bodies were triturated in 1000 μl of diluent and clarified by centrifugation. Then, 100 μl of body supernatant and expectorate solutions was tested for infectious virus by plaque assay on Vero cells (Kramer et al. 2002). Virus titers were corrected for dilution and expressed as total PFU per body or expectorate. Twenty females from the 10°C group that tested negative by plaque assay were retested for WNV RNA by real-time reverse transcription-polymerase chain reaction (RT-PCR) by using extraction methods and primers described previously (Chiles et al. 2004).

Statistics. The infection rate was calculated as the percentage of bodies tested that contained virus, whereas the transmission rate was calculated as the percentage of females with positive bodies that expectorated virus. The median extrinsic incubation period (EIP_{50}) for each temperature was calculated as the time from ingestion of the infectious bloodmeal until 50% of infected females were capable of transmission. EIP_{50} values were estimated by probit analysis (Hintze 1998). The rate of the virus development for each temperature was calculated as the inverse of the EIP_{50}. Degree-days and virus developmental zero points were estimated by regression of the EIP rate as a function of incubation temperature. Total degree-days were estimated from the inverse of the slope, whereas the temperature at which zero development occurred was calculated by solving the regression equation for $y = 0$ (Reisen et al. 1993).

Results

Infection. Overall, 1,364 females took a replete bloodmeal from a viremic passeriform donor bird and survived the extrinsic incubation period, of which 592 (43%) were infected, i.e., contained two or more PFU. The percentage of *Cx. tarsalis* females infected after incubation at temperatures from 14 to 30°C increased as a linear function of the viremia titer of the donor bird at the time of blood feeding (Fig. 1). Although some females were infected during every blood feeding, infection rates >50% were not realized until the host blood titer exceeded 6.5 $\log_{10}$ PFU/ml. To produce adequate numbers of infected females for transmission assessments, some temperature–time combinations had to be repeated once, so that 20–40 females were tested per group depending upon the infection rate. The final number infected averaged 15.5 females per temperature–time combination (range 6–25; n = 38 transmission attempts).

Although females in the 10°C group fed on house finches with viremia titers ranging from 6.7 to 7.7 $\log_{10}$ PFU/ml, none of the 120 females tested positive for infectious WNV by plaque assay during the 110-d incubation period. Twenty females also tested negative for WNV RNA by RT-PCR at 71 dpi. After 110 d, the surviving 33 females were transferred to 26°C for a day and then offered the opportunity to oviposit collectively over a subsequent 7-d period. On days 2–4 after transfer, seven females oviposited and nine (including some of the now parous females) blood fed on restrained house sparrows. Of these, one blood-fed female was infected, with a WNV titer of 3.2 $\log_{10}$ PFU. All three house sparrows remained negative for WNV when bled 2 to 3 d after these females fed as a group on them. In addition, capillary tube transmission was attempted for 19 gravid females on day 7 after transfer to 26°C; none transmitted, but one tested positive, with a WNV titer of 3.6 $\log_{10}$ PFU. Data from the 10°C group was not used in any of the subsequent statistical analyses.

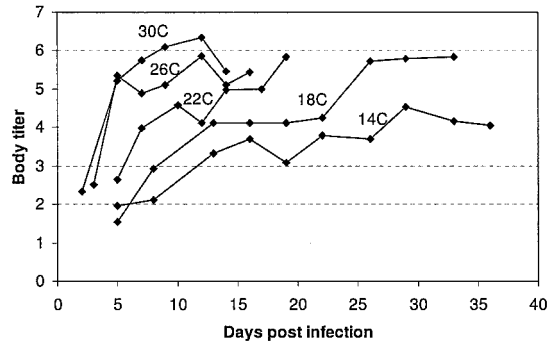

Fig. 2. Mean NY99 WNV titers ($\log_{10}$ PFU) in the bodies of infected *Cx. tarsalis* females incubated at 14–30°C plotted as a function of time in days postinfection.

When tested by a two-way analysis of variance (ANOVA), the mean titer of WNV per infected female increased significantly as a function of time ($F = 15.6$; df = 17, 502; $P < 0.001$) and incubation temperature ($F = 19.8$; df = 4, 502; $P < 0.001$) (Fig. 2). Mean titer per females over time was relatively consistent among temperature groups, because the interaction term in the two-way ANOVA was not significant ($P > 0.05$). Females held at warm temperatures 22–30°C had significantly (Fisher's least significant range test, $P < 0.05$) higher WNV titer in mosquito bodies than those held at cool temperatures 14–18°C, reflecting the accelerated growth of virus at warm temperatures.

Transmission. Of the 592 females that were positive for viral infection, expectorate samples from 512 were assayed for virus, of which 144 were positive. The remaining infected females with low WNV titer (<3 $\log_{10}$ PFU) were scored as transmission negative, because only females with WNV titers >3.5 $\log_{10}$ PFU were capable of transmission (Fig. 3). As expected, the mean ± SE WNV titer in infected females transmitting virus (6.02 ± 0.59; n = 144) was significantly greater ($t = 16.9$, $P < 0.0001$) than the titer for females failing to transmit virus (4.45 ± 1.09; n = 368). The percentage of infected females that transmitted virus increased as a function of time after infection (Fig. 4). Females held at 14°C first transmitted at 36 dpi, whereas females held at 18°C first transmitted at 22 dpi. In contrast, a few females held at both 26 and 30°C transmitted virus as early as five dpi. The quantity of virus expectorated by transmitting females varied markedly (mean 1.89; 95% CL = 1.78–2.01; range 0.8–

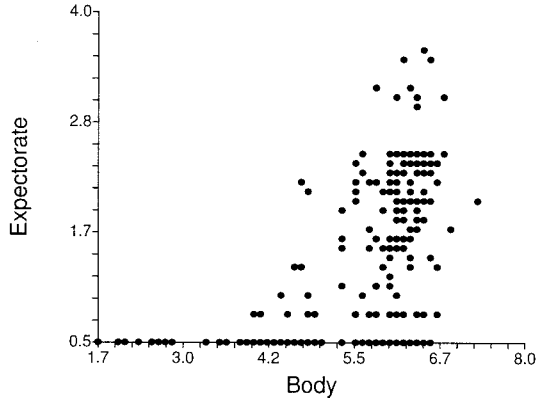

Fig. 3. NY99 WNV titers ($\log_{10}$ PFU) in expectorate samples plotted as a function of WNV virus titer in mosquito bodies for infected females incubated at 14–30°C.

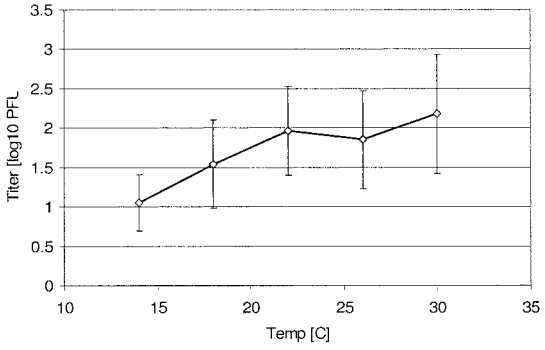

Fig. 5. Mean ± SD quantity of virus ($\log_{10}$ PFU) within the expectorate of females transmitting virus plotted as a function of incubation temperature (°C).

3.6 $\log_{10}$ PFU; $n = 144$) and increased significantly ($F = 4.8$; df = 4, 139; $P = 0.001$) as a function of incubation temperature (Fig. 5). Therefore, not only did females have a shorter EIP at 30°C but also they contained greater titer of WNV and expectorated more virus than females held at cooler temperatures.

The median extrinsic incubation period in dpi was estimated by probit analysis for each incubation temperature and decreased as a negative function of increasing temperature (Fig. 6). The rate of virus development (inverse of the EIP_{50}) was regressed as a function of temperature to estimate the 0 virus developmental temperature and the number of DD required for virus extrinsic incubation (Table 1). The regression function was significant ($F = 450.5$; df = 1, 3; $P < 0.001$) and explained 99% of the variability among the five data points. The 0 developmental point was calculated to be 14.3°C, agreeing with our observation that only 11% of females surviving 36 dpi at 14°C were able to transmit. Recalculating the degree-model based on the time it took until 10% of the females were capable of transmission (EIP_{10}) also provided a significant linear fit (y = −0.117 + 0.0093y; df = 1, 3; $R^2 = 0.92$), reduced estimate of the 0 developmental point to 12.6°C, and the DD to 107.6. Because the y-intercept and the slope of this function did not differ statistically from those using EIP_{50} data (i.e., 95% confidence intervals about these estimates overlapped), we considered these values to be similar.

Discussion

Inter- and Intraspecific Comparisons. WNV within lineages I and II (Lanciotti et al. 2002) may respond to temperature differently (Table 1; Fig. 7). This conclusion ignores the potential confounding effects of vector–virus coevolution. Regression statistics were used to calculate DD functions for *Cx. univittatus* infected with the H442 strain from South Africa (Cornel et al. 1993), and, for comparison, *Cx. tarsalis* infected with SLEV and WEEV from California (Reisen et al. 1993). Results from South Africa were unexpected in that the EIP rate estimates for 30°C were less than values for 26°C, perhaps indicating slower viral growth at warm temperatures. With four data points, this variability prevented a significant regression fit ($F = 6.3$; df = 1, 2; $P = 0.13$) and the calculated function only explained 76% of the variability in the

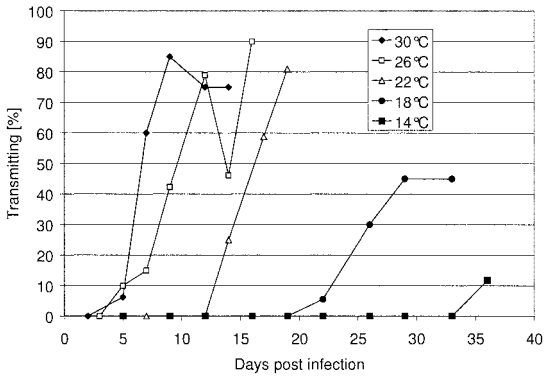

Fig. 4. Percentage of infected *Cx. tarsalis* females transmitting NY99 WNV plotted as a function of time in days postinfection.

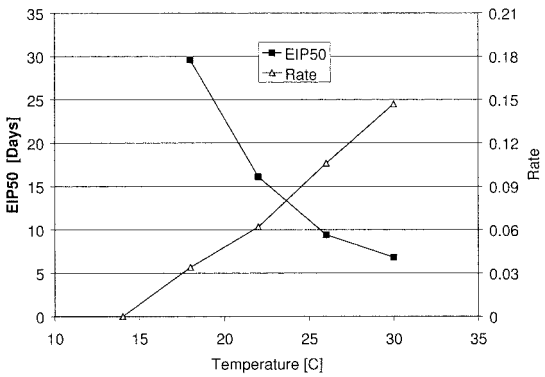

Fig. 6. Median extrinsic incubation period in days (EIP_{50}) and the EIP rate (rate = $1/EIP_{50}$) plotted as a function of the incubation period.

Table 1. Regressions of the extrinsic incubation rate as a function of the incubation temperature for the NY99 (WNV-NY) and South African (WNV-SA) strains of West Nile virus, the BFS 1750 strain of St. Louis encephalitis virus (SLEV), and the BFS1703 strain of western equine encephalomyelitis virus (WEEV)

Parameter	WNV-NY	WNV-SA	SLEV	WEEV
Slope	0.0092*	0.0037ns	0.0087*	0.0135*
L 95% CL	−0.0078	−0.0026	0.0083	0.0078
U 95% CL	0.0105	0.0100	0.0091	0.0192
Intercept	−0.132	−0.012	−0.130	−0.147
DD	108.7	270.3	114.9	74.2
0 growth	14.3	3.3	14.9	10.9
R^2	0.99	0.78	0.99	0.95

Cx. tarsalis was the host for WNV-NY, SLEV, and WEEV, whereas *Cx. univitattus* was the host for WNV-SA. CL, upper (U) and lower (L) 95% CL about the slope; DD = 1/slope; 0, growth, value of X when Y = 0.

* *F* for slope significant, $P < 0.001$; NS, not significant, $P = 0.13$.

data. Regardless of these regression estimates, these data clearly indicated that the H442 strain replicated much faster under cool temperatures than the NY99 strain we used in the current study. H442 was readily transmitted at 14°C, whereas the NY99 WNV strain exhibited a response to temperature very similar to SLEV (95% CL for slopes overlapped). Both WNV and SLEV replicated much more slowly at cool temperatures in comparison with WEEV (Table 1; Fig. 7).

Comparative temperature data with the NY99 strain of WNV were not available for other species of North American mosquitoes. The impact of temperature on *Cx. p. pipiens* infected with a NY99 strain of WNV has been described; however, in this study dissemination (i.e., positive legs) rather than transmission rates were recorded, precluding direct comparison with our data and the calculation of the EIP_{50} (Dohm et al. 2002). Based on our findings in the current and previous studies, only females with elevated WNV titers were capable of transmission and that in time and temperature studies (Kramer et al. 1983), females develop

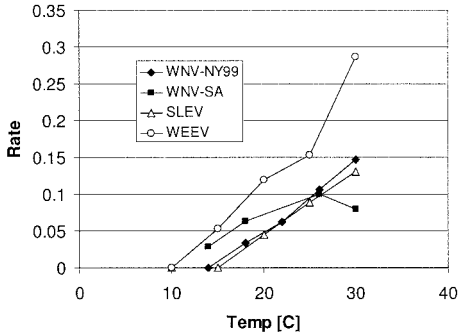

Fig. 7. Rate of virus development (EIP rate) for New York (WNV-NY) and South African (WNV-SA) strains of West Nile virus, the BFS 1750 strain of St. Louis encephalitis virus (SLEV), and the BFS1703 strain of western equine encephalomyelitis virus (WEEV) plotted as a function of incubation temperature. *Cx. tarsalis* was the host for WNV-NY, SLEV, and WEEV, whereas *Cx. univitattus* was the host for WNV-SA.

disseminated infections before being able to transmit. In addition, <50% of infected *Cx. p. pipiens* females had disseminated infections or WNV titer in bodies >5 $\log_{10}$ when incubated at 18 or 20°C (Dohm et al. 2002), indicating few may have been capable of transmission and supporting our contention that the Lineage I NY99 WNV requires warm incubation periods within the mosquito host for efficient replication and transmission.

The EIP at 28°C was estimated for four species of California *Culex* (Goddard et al. 2003) by using the capillary tube method (Aitken 1977). Although possibly affected by differential vector competence (Goddard et al. 2002), estimates of the EIP_{50} were relatively similar: 5–7 d for *Cx. tarsalis* from Yolo County, 9 d for *Cx. p. pipiens* from Shasta County, 11 to 12 d for *Cx. stigmatosoma* from San Bernardino County, and 12 to 13 d for *Cx. p. quinquefasciatus* Say from Kern County; initial transmissions were detected on days 5, 6, 8, and 7, respectively. Data for the Yolo County strain of *Cx. tarsalis* were similar to current observations for the Kern County population estimated by interpolating from the curves in Figs. 4 and 6. Estimates for *Cx. p. quinquefasciatus* infected with a Texas strain of WNV also were consistent, with virus detected within the lumen of the salivary glands after 14 dpi, but not at 7 dpi (Girard et al. 2005). Collectively, these data indicated that the growth of the lineage I strain of WNV within various North American *Culex* species may be relatively similar and allow the extrapolation of our degree-day model to understand WNV transmission patterns in North America.

Importance of Climate Variation in Virus Activity. By dividing the number of degree-days by mean ambient temperature above the thermal minimum, it was possible to estimate the duration of the EIP and the duration of *Cx. tarsalis* gonotrophic cycles 1 and 2 after blood feeding. The DD function for the gonotrophic cycle was estimated previously by holding blood-engorged *Cx. tarsalis* females under constant laboratory temperatures until oviposition and then adding a day to account for host-seeking on the next night (Reisen et al. 1992b). These estimates were verified by oviposition rhythms observed during laboratory life table studies (Reisen 1995, Mahmood et al. 2004) and field mark–release–recapture studies (Reisen et al. 1992a, Reisen and Lothrop 1995). To illustrate the importance of temperature in WNV temporal dynamics, daily ambient temperatures for Mecca in Coachella Valley, Riverside County, and Bakersfield, Kern County, California, during the WNV epidemic year 2004 were downloaded from the California IPM project (http://www.ipm.ucdavis.edu/) and the time in days for gonotrophic cycles 1 and 2 and the EIP calculated and plotted as a function of time in months (Fig. 8). Also included within each panel were the monthly infection rates for *Cx. tarsalis* per 1000 calculated by a maximum likelihood procedure (Biggerstaff 2003) and the enzootic transmission rate measured by sentinel chicken seroconversions per flock. Infection rate data were limited to *Cx. tarsalis*, because although *Cx. p. quinquefasciatus* frequently became

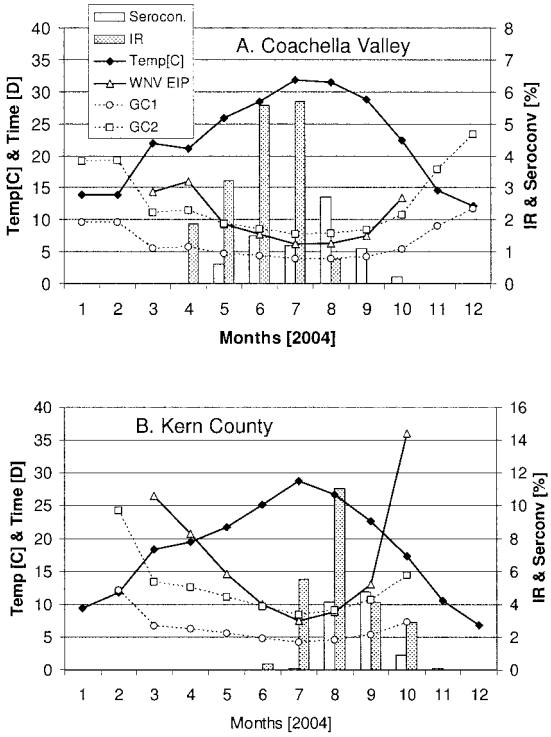

Fig. 8. Mean monthly ambient temperature, the duration of the EIP, and the first and second gonotrophic cycles (GC-1 and GC-2) and measures of virus activity plotted as a function of time in months during 2004 in (A) Coachella Valley and (B) Kern County, California. Measures of WNV activity included the *Cx. tarsalis* WNV infection rate per 1,000 tested (n = 945 pools in Coachella and 475 pools in Kern) and the mean sentinel chicken seroconversion rate per flock of 10 hens per month with seropositive birds replaced (n = 10 flocks per site).

infected at urban and suburban Kern County locations, *Cx. tarsalis* was the primary rural vector and most chicken flocks were positioned at these rural locations. Virus activity did not commence until the EIP decreased in duration to a point where the virus could be transmitted within two gonotrophic cycles (i.e., when the female returned for her third bloodmeal). Virus commenced more rapidly than expected in Coachella Valley, but this was attributed to the daily high maxima achieved under desert conditions, which may disproportionately expedite virus growth under cycling temperature regimens (Cornel et al. 1993). Virus amplified markedly during May–June in Coachella and July–August in Kern and then decreased during September and October as temperatures cooled. Transmission effectiveness in both areas during 2004 was enhanced by temperatures that remained from 2 to 5°C above the 30-yr average. Apparent lag periods in surveillance measures within both areas were related to sampling as well as virus dynamics. Infections within host-seeking females could not be detected until the completion of at least one gonotrophic cycle (>4 d), and seroconversions in sentinel chickens require at least 12 d. However, because of the 2-wk bleeding schedule, some conversions may not be detected until 14–28 dpi.

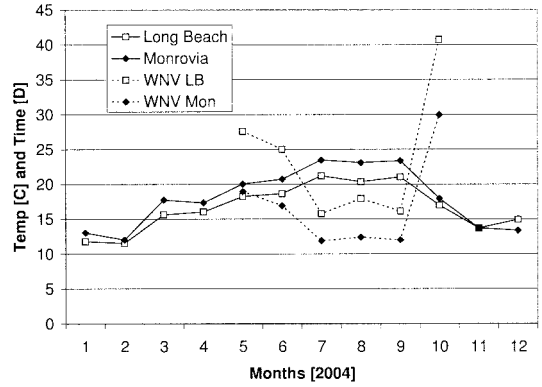

Fig. 9. Mean monthly ambient temperature in the Los Angeles basin and the duration of the EIP plotted as a function of time in months during 2004 by using data from coastal Long Beach and upland Monrovia weather stations.

Temperatures that vary spatially over relatively small distances also may affect transmission dynamics by altering the EIP. For example, temperatures along coastal Los Angeles at Long Beach averaged 1.3°C cooler than upland temperatures at Monrovia throughout 2004 (Fig. 9). These differences were significant (F = 54.9; df = 1, 702; $P < 0.0001$) and most pronounced during summer. Potential differences in the EIP ranged from 4 d during July to 10 d during October. Because the duration of infective life decreases as an exponential function of the EIP, even these seemingly minor differences may have marked consequences in the probability of transmission. For example, if daily survivorship was 0.9 (Reisen et al. 1991, 1992b) and the EIP in August was 17.9 and 12.4 d in Long Beach and Monrovia, then the duration of infective life (IL) would be $IL = P^n/-\ln P$ or 1.4 and 2.6 d, respectively. Therefore, the risk of infection may have been doubled in Monrovia, because infective life was effectively doubled.

Temperature also seems to have impacted the dispersal and amplification of WNV throughout North America (Fig. 10). Based on data reported to the CDC ArboNET system (http://www.cdc.gov/ncidod/dvbid/westnile/index.htm) and mapped by the U.S. Geological Survey (http://westnilemaps.usgs.gov/), it was possible by inspection to discern foci of WNV human disease during 2002, 2003 and 2004 as WNV invaded central and western North America. Cases clustered in Illinois and Indiana and Louisiana during 2002, Colorado and Nebraska during 2003, and Arizona and southern California during 2004. Maps (Fig. 10B, C) depicting temperature deviations from the 30-yr mean during summer (Fig. 10A) indicated that WNV always dispersed into new areas during years with above-normal temperatures and that amplification during the following year occurred during summers with above- or normal temperatures. Subsequent cool summers were associated with decreased or de-

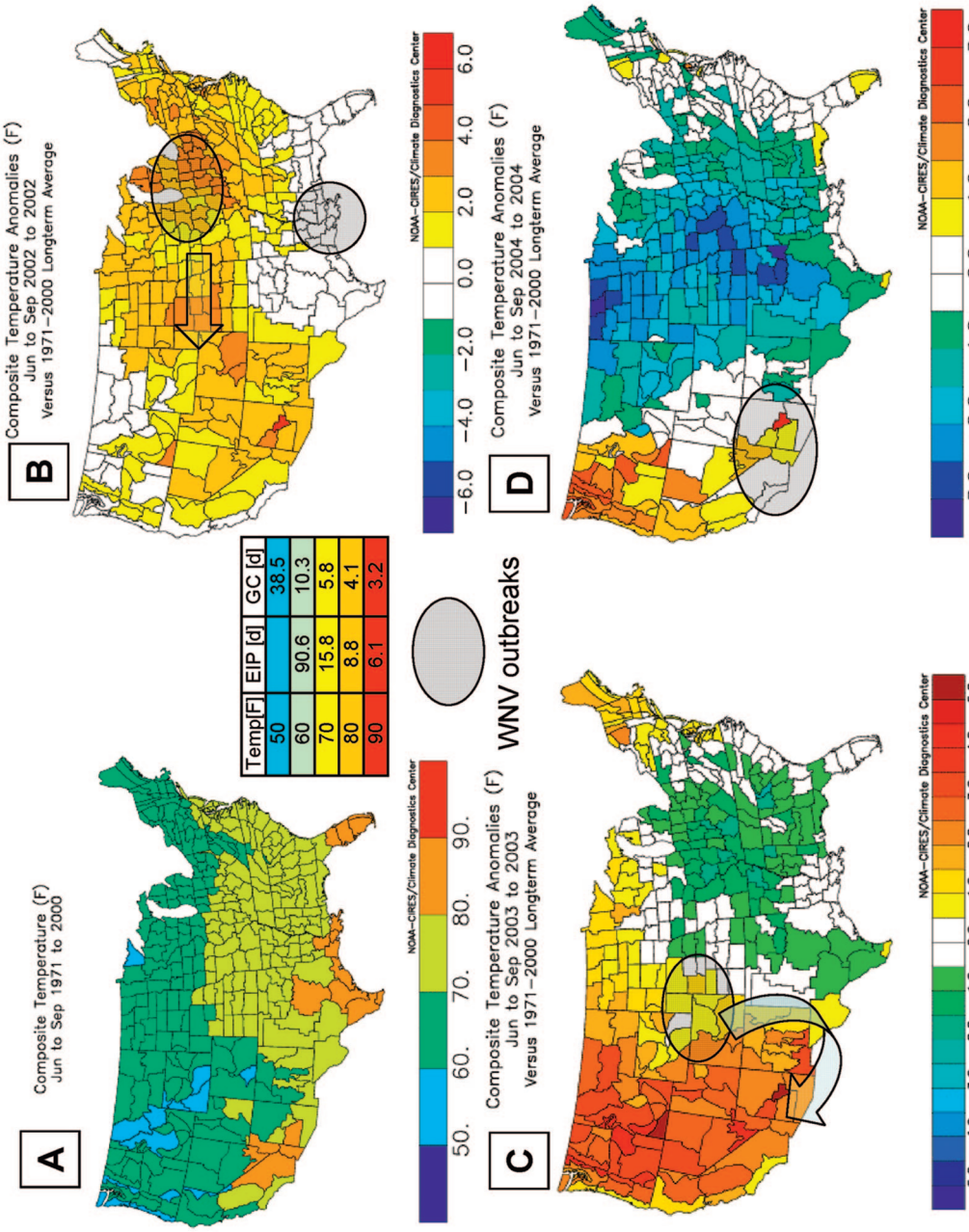

Fig. 10. Mean ambient summer temperatures (June–September) in the United States for the 1971–2000 period (A) and deviations from these means during summers 2002 (B), 2003 (C), and 2004 (D). Indicated are the approximate locations of each WNV epicenter and the direction of virus dispersal into the next epidemic area. The inset shows the approximate time for NY99 WNV extrinsic incubation and the duration of the gonotrophic cycle for *Cx. tarsalis* as a function of temperature increments.

layed virus activity, especially at northern latitudes. Interestingly, outbreaks at southern latitudes were not associated with above normal temperatures, probably because average summer temperatures in areas such as Louisiana and Arizona were sufficient to allow effective transmission (Fig. 10A).

Overwintering. Although not intended as an investigation into the overwintering ecology of WNV, our study did provide information relevant to virus persistence. At southern latitudes field observations indicated that WNV may be transmitted at low levels during winter (Tesh et al. 2004, Reisen et al. 2006, Shaman et al. 2005). Our data indicated that at mean temperatures of 14°C (slightly below our calculated thermal minimum of 14.3°C), females would become infected and could eventually transmit WNV, agreeing with data from South Africa (Cornel et al. 1993). However, in the current study females incubated immediately at 10°C after imbibing an infectious bloodmeal failed to become infected, despite feeding on donor birds with a sufficiently elevated viremia. In contrast, *Cx. p. pipiens* imbibing a viremic bloodmeal at room temperature and maintained at 18°C for 7 d before transfer to 10°C for 21–42 d, became infected but WNV failed to replicate and was not detectable by plaque assay (Dohm and Turell 2001). Infection here was confirmed by heating females to 26°C and then allowing them to blood feed before testing by plaque assay. Similar results were reported previously for diapausing *Cx. tarsalis* infected by intrathoracic inoculation with WEEV and SLEV (Reisen et al. 2002). In this study, WEEV could not be detected by plaque assay when incubated at 10°C, but it was recovered by RT-PCR in some females, although infection rates were lower than realized after terminating diapause and heating the mosquitoes to 26°C. Similarly, field winter infections of *Cx. p. pipiens* with SLEV only were detected after diapause was terminated experimentally and females blood fed (Bailey et al. 1978). These data indicated that at southern latitudes where species such as *Cx. p. quinquefasciatus* may blood feed during winter, females may not become infected unless temperatures approach 14°C and that the duration of the EIP during winter may be greatly elongated and transmission dependent upon intermittent warm periods.

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