

# The Effect of Temperature on Life History Traits of *Culex* Mosquitoes

ALEXANDER T. CIOTA,<sup>1</sup> AMY C. MATAACCHIERO,<sup>1</sup> A. MARM KILPATRICK,<sup>2</sup>  
AND LAURA D. KRAMER<sup>1,3,4</sup>

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**ABSTRACT** Climatic changes forecasted in the coming years are likely to result in substantial alterations to the distributions and populations of vectors of arthropod-borne pathogens. Characterization of the effect of temperature shifts on the life history traits of specific vectors is needed to more accurately define how such changes could impact the epidemiological patterns of vector-borne disease. Here, we determined the effect of temperatures including 16, 20, 24, 28, and 32°C on development time, immature survival, adult survival, mosquito size, blood feeding, and fecundity of both field and colonized populations of the *Culex* mosquitoes *Culex pipiens* L., *Culex quinquefasciatus* Say, and *Culex restuans* Theobald. Our results demonstrate that temperature significantly affects all of these traits, yet also that the extent of this effect is at times incongruent among temperatures, as well as being population and species-specific. Comparisons of colonized mosquitoes with field populations generally demonstrate decreased adult and immature survival, increased blood feeding and egg production, and significant variation in the effects of temperature, indicating that such colonies are not fully representative of natural populations. Results with field populations in general indicate that increases in temperature are likely to accelerate mosquito development, and that this effect is greater at temperatures below 24°C, but also that temperature significantly increases mortality. Among field populations, *Cx. restuans* were most affected by temperature increases, with decreased longevity relative to other species and significant increases in adult and immature mortality measured with each incremental temperature increase. Despite the unique climates characteristic of the geographic ranges of *Cx. quinquefasciatus* and *Cx. pipiens*, evidence of significant species-specific adaptation to temperature ranges was not seen. Taken together, these results indicate that geographic region, as well as species and population differences, must be considered when measuring the effect of temperature on vector populations.

**KEY WORDS** temperature, *Culex*, life history traits

The Intergovernmental Panel on Climate Change has predicted continued and accelerating global warming, resulting in a 2–4°C rise in mean global temperature over the next century (Intergovernmental Panel on Climate Change [IPCC 2007]). These changes in climate are likely to result in significant changes to ecological landscapes and patterns of infectious disease. Vector-borne pathogens, which are transmitted by ectothermic arthropods, are likely to be particularly sensitive to increases in global temperatures (Kilpatrick and Randolph 2012). Although it is generally well-established that increased temperatures have the potential to significantly increase proliferation of pathogens in arthropods (Dohm et al. 2002, Reisen et al. 2006, Kilpatrick et al. 2008), the relationship between temperature and life history traits of arthropod vectors is less clear. Defining this relationship is therefore necessary if we are to predict how a warming

climate could impact the distribution, abundance, and vectorial capacity of disease vectors.

Mosquitoes in the *Culex pipiens* species complex, *Cx. pipiens* L. and *Culex quinquefasciatus* Say, as well as *Culex restuans* Theobald, have worldwide distributions, close association with humans, and are frequently vectors of pathogens of both human and animal diseases (Farajollahi et al. 2011). In N. America, *Culex* mosquitoes are primary vectors of West Nile virus and other arboviral pathogens (Bernard and Kramer 2001, Turell et al. 2002, Kilpatrick et al. 2005, Diaz-Badillo et al. 2011), yet vector competence has been shown to differ among and within-species, and to be temperature-dependent (Kilpatrick et al. 2008, Kilpatrick et al. 2010). In addition, there are a wide variety of behavioral, physiological, and morphological variations among local populations of *Culex* mosquitoes that could potentially impact vectorial capacities (Gunay et al. 2011).

Environmental conditions have been associated with significant variation in both adult and immature stage characteristics of insects, including larval growth rates, development times, body size, fecundity, and longevity (Shelton 1973, Loetti et al. 2011). Temperature is a particularly important abiotic factor for mos-

<sup>1</sup> Wadsworth Center, New York State Department of Health, 5668 State Farm Road, Slingerlands, NY 12159.

<sup>2</sup> Department of Ecology and Evolutionary Biology, 1156 High Street, University of California, Santa Cruz, CA 95064.

<sup>3</sup> School of Public Health, Department of Biomedical Sciences, SUNY, One University Place, Albany, NY 12201.

<sup>4</sup> Corresponding author, e-mail: [ldk02@health.state.ny.us](mailto:ldk02@health.state.ny.us).

quitoes and other arthropods, as it directly affects mortality, life span, and development rates that can cause changes in morphology (Su and Mulla 2001, Debat et al. 2003, Gunay et al. 2011). The majority of insects function optimally within a narrow range of temperatures, and deviations from this range can cause stress during development, which may result in developmental inconsistencies (Mpho et al. 2002). In addition, there are often trade-offs between development time and both adult size and fitness, which can have significant downstream effects on survival, feeding behavior, and fecundity (Shelton 1973, Mohammed and Chadee 2011). Alterations to these life history traits can lead to substantial variations in vectorial capacity of mosquitoes that harbor and transmit pathogens (Dye 1992, Delatte et al. 2009).

It is now well-documented that colonized and field populations can differ significantly in genetic and phenotypic diversity, resulting in variability in larval and adult life-history traits (Mpho et al. 2002, Aguilar et al. 2005). While field conditions are highly dynamic and diverse, the homogeneous conditions common to laboratory colonies may result in the loss of allelic diversity and overall population fitness (Aguilar et al. 2005). Defining these differences is essential for interpreting studies of vector populations, which often rely on highly colonized populations to assess the effects of environmental conditions on vectorial capacity and fitness.

Here, we determined the effect of temperature on life history traits of *Cx. pipiens*, *Cx. quinquefasciatus*, and *Cx. restuans* mosquitoes. Specifically, we measured temperature-dependent variation in development time, immature survival, adult survival, mosquito size, blood-feeding, and fecundity both among species and between colonized and field-derived populations. Our results demonstrate the significant effects changing temperature has on these traits and identify critical population and species-specific differences, which have broad importance in contributing to our understanding of how changing climates will impact pathogen transmission.

## Materials and Methods

**Mosquitoes.** All colonized *Culex* mosquitoes were maintained in 30.5-cm<sup>3</sup> cages in an environmental chamber at 27 ± 2°C with a relative humidity of 45–65% and a photoperiod of 16:8 (L:D) h before the collection of experimental egg rafts. *Cx. pipiens* colony mosquitoes were originally collected in Pennsylvania in 2004 (courtesy of M. Hutchinson) and have been highly colonized at the Wadsworth Center Arbovirus laboratory. *Cx. quinquefasciatus* colony mosquitoes were derived from a laboratory colony provided by D. Fonseca (Rutgers University, New Brunswick, NJ) and initially derived from egg rafts from a highly colonized line from Benzon Research Inc. (Carlisle, PA). *Cx. pipiens* and *Cx. restuans* field egg rafts were collected in August, 2010 from Takoma Park, MD, near Washington, DC, and reared in an environmental chamber maintained at 25–27°C, with a photoperiod of 16:8 (L:D) h for a single generation. Field *Cx. quinquefascia-*

*tus* egg rafts were collected in Orange County, CA (courtesy of R. Cummings). We confirmed species identifications and the absence of hybridization using morphological identification of larvae, and molecular identification using species-specific primers (Smith and Fonseca 2004, Bahnck and Fonseca 2006).

**Mosquito Development.** After hatching of individual groups, 400 larvae per population were counted and equally distributed into two transparent holding pans with 1 liter of filtered water each. Larvae were then placed in environmental chambers (Therma, San Jose, CA) and subjected to a photoperiod of 16:8 (L:D) h under five constant temperatures (16, 20, 24, 28, and 32 ± 1°C). Water dishes were placed inside the chambers to maintain humidity, and temperatures were monitored and recorded daily. Larvae were fed a 1:1:1 ratio of ground koi food, ground rabbit pellets, and bovine liver powder (MP Biomedicals, Solon, OH) in increasing quantities as follows: 60 mg—first instar, 100 mg—second instar, 120 mg—third instar, and 160 mg—fourth instar. Developing larvae were monitored daily for mortality, and dead larvae were counted, recorded, and discarded. Daily pupation also was recorded, and pupae were placed in distilled water in emergence jars (BioQuip Products Inc., Rancho Dominguez, CA) in preparation for eclosion.

**Mosquito Survival, Feeding, and Fecundity.** Newly emergent mosquitoes were sexed, counted, and housed at corresponding temperatures in 3.8-L mesh top paper cups provided with cotton pads soaked in 10% sucrose ad libitum. Survival of all groups was monitored and recorded daily. Dead adults were removed and frozen at –20°C. Wings from ≈20 adults per group per temperature were removed and placed on slides with double-sided tape and were subsequently measured from the alular notch to the distal margin, excluding the fringe using a Zeiss microscope, Axiocam camera, and Axiovision software (Carl Zeiss Microscopy, Gottingen, Germany) to estimate mosquito size (Dodson et al. 2011). Adults were grouped and housed as they emerged in 3-d intervals and held for 5–7 d to allow for mating. Mosquitoes were starved overnight for 12–24 h and offered defibrinated goose blood (Hema Resources, Aurora, OR) with 2.5% sucrose for 1-h. Mosquitoes were then anesthetized using CO<sub>2</sub>, and blood-fed females were sorted, counted, and separated for housing into individual holding cups. Unfed females were kept in the original cup with males, and offered a second bloodmeal 5–7 d later. Oviposition dishes were placed in holding cups containing blood-fed females and were checked daily for the presence of egg rafts. Statistical analyses were performed using GraphPad Prism 4.0 and Statsplus 9.0.

## Results

**Immature Development and Survival.** We found significant differences in development time among species, population (field vs. colony), and temperature (Fig. 1). In general, increased temperatures accelerated development for all groups, yet this effect diminished with temperatures >24°C (Fig. 1;

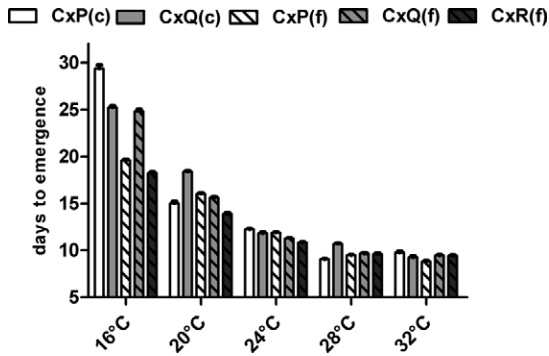


Fig. 1. Development time in mean days to emergence  $\pm$  SEM of field (f) and colony (c) *Cx. pipiens* (CxP), *Cx. quinquefasciatus* (CxQ), and *Cx. restuans* (CxR) at various temperatures.

Kruskal–Wallis test;  $P < 0.05$ ). Specifically, the rate of development increased by an average of 2.9-fold with temperature increases from 16 to 24°C ( $P < 0.001$ ) and an average of 2.3-fold with increases from 24 to 32°C ( $P < 0.001$ ). When considered separately, development times of individual groups were significantly different when comparing rearing temperatures from 16 to 28°C (Dunn’s multiple comparison test;  $P < 0.05$ ). Increases from 28 to 32°C were also associated with decreased development times of colony-derived *Cx. quinquefasciatus* (Mann–Whitney test;  $P < 0.0001$ ), yet did not significantly alter development time of other groups. The largest effect of temperature on development time was measured for *Cx. pipiens* colony mosquitoes, for which mean time to emergence ranged from 29 d at 16°C to 9 d at 28°C, the temperature closest to colony rearing temperatures (26–28°C). Development times of field-derived *Cx. pipiens* were significantly shorter than colonized *Cx. pipiens* at 16°C (Mann–Whitney test;  $P < 0.0001$ ). Although differences between field and colony populations of *Cx. quinquefasciatus* were not as large as those measured at 16°C in *Cx. pipiens*, significantly shorter development times were also measured in field-derived populations relative to colonized *Cx. quinquefasciatus* at 16, 20, and 28°C (Mann–Whitney test;  $P < 0.05$ ).

Species-specific differences in development time of field populations were also observed at all temperatures other than 28°C (Fig. 1). Specifically, *Cx. quinquefasciatus* and *Cx. restuans* emerged an average of 0.7 d later than *Cx. pipiens* at 32°C and 0.9 d earlier at 24°C (Kruskal–Wallis test;  $P < 0.0001$ ). Differences measured at lower temperatures were greater, with shorter development times measured in *Cx. quinquefasciatus* relative to *Cx. pipiens* at 16°C (5 d) and with *Cx. restuans* relative to both other species at 16 and 20°C. Although still significant (Kruskal–Wallis test;  $P < 0.0001$ ), the smallest range in development time was measured in *Cx. restuans*, for which development time spanned from 18 d (16°C) to 8 d (32°C; Fig. 1).

Because proportions of larvae and pupae surviving were similar within groups, data were combined and

Table 1. Proportion of *Culex* (*Cx. pipiens* (P), *quinquefasciatus* (Q), and *restuans* (R) surviving immature stages of development at various temperatures

Group	CxP	$P$ (pop) <sup>a</sup>	CxQ	$P$ (pop) <sup>a</sup>	CxR	$P$ (species) <sup>b</sup>
16°C						
Field	0.92	0.0592	0.96	<0.0001	0.83	<0.0001
Colony	0.87		0.81		—	0.012
20°C						
Field	0.87	0.0007	0.84	0.0358	0.82	0.2460
Colony	0.77		0.89		—	<0.0001
24°C						
Field	0.87	<0.0001	0.86	<0.0001	0.81	0.0434
Colony	0.61		0.70		—	0.0090
28°C						
Field	0.82	0.7730	0.95	<0.0001	0.61	<0.0001
Colony	0.83		0.53		—	<0.0001
32°C						
Field	0.83	<0.0001	0.77	<0.0001	0.34	<0.0001
Colony	0.46		0.38		—	0.0181
$P$ (temp) <sup>c</sup>	<0.0001		<0.0001		<0.0001	

<sup>a</sup>  $\chi^2$  test tests measuring the effect of temperature on immature survival among field or colony populations of the same species.

<sup>b</sup>  $\chi^2$  test tests measuring the effect of species on immature survival for individual populations and temperatures.

<sup>c</sup>  $\chi^2$  test tests comparing immature survival of field and colony populations of individual species and temperatures.

depicted as proportions surviving to adult (Table 1; Fig. 2A). Temperature, population, and species were all associated with significant differences in immature survival, yet this trait overall varied less than both development time and adult survival. Immature survival was highest at 16°C for all groups, yet the degree to which increasing temperatures altered survival varied among species and populations (Table 1; Fig. 2A). Field populations of *Cx. pipiens* and *Cx. quinquefasciatus* were more robust than colonized mosquitoes, showing only modest fluctuations in immature survival resulting from temperature variation. Despite this, the proportion of field-derived *Cx. quinquefasciatus* surviving at 28°C (0.95) was significantly higher than field-derived *Cx. pipiens* (0.85;  $\chi^2$  test,  $P = 0.009$ ). In contrast to the other groups, there was a significant negative correlation between temperature and immature survival for field-derived *Cx. restuans*, as well as colony *Cx. quinquefasciatus*, with <40% of immatures surviving at 32°C for both groups (linear regression analyses,  $r^2 = 0.85$ ;  $P = 0.025$ ; Fig. 2A). Colony *Cx. pipiens* again demonstrated adaptation to the temperature closest to that which they are reared (28°C), for which proportion surviving was significantly higher than both the 24 and 32°C groups ( $\chi^2$  test;  $P < 0.01$ ; Table 1). The low proportion of colony *Cx. pipiens* surviving at 24°C (0.61) can be attributed primarily to male death, as male/female ratio of emerged adults was significantly skewed toward females relative to other groups ( $\chi^2$  test;  $P < 0.0001$ ). No other significant variations in sex ratios were observed among groups.

**Adult Size and Survival.** Average wing length was used to assess the relationship between temperature and body size of *Culex* populations (Fig. 3). With the exception of the colonized *Cx. pipiens*, a negative correlation between body size and temperature was mea-

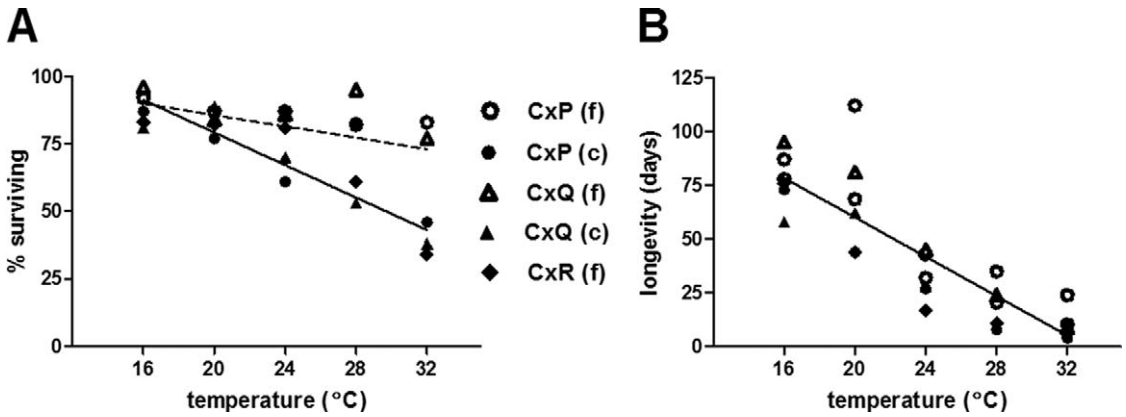


Fig. 2. Relationship between temperature and survival of field-derived (f) and colonized (c) *Culex* (Cx) mosquitoes, *Cx. pipiens* (P), *Cx. quinquefasciatus* (Q), and *Cx. restuans* (R). (A) Relationship between temperature and proportion of larvae surviving to adult. Statistically similar relationships are depicted by individual lines. A significant correlation was identified for *Cx. restuans* and colonized *Cx. quinquefasciatus* (solid line; linear regression analysis,  $r^2 = 0.85$ ;  $P = 0.025$ ;  $y = 139.5 - 3.01x$ ), but not for *Cx. pipiens* or field-derived *Cx. quinquefasciatus* (dotted line;  $r^2 = 0.57$ ;  $P = 0.139$ ). (B) Relationship between temperature and adult longevity. Slopes were statistically similar for all groups (linear regression analysis of mean,  $y = 151.6 - 4.57x$ ;  $r^2 = 0.94$ ;  $P = 0.006$ ), but intercepts were significantly different among groups ( $F = 4.9$ ;  $P = 0.003$ ).

sured, correlating with the increased development time in each group (Fig. 3; linear regression analyses;  $P < 0.001$ ). Although the size of colony *Cx. pipiens* was generally less affected by temperature than other groups, mean wing length of mosquitoes reared at 28°C was significantly larger than at all other temperatures ( $t$ -test;  $P < 0.001$ ). Field-derived *Cx. pipiens* wing lengths were significantly larger than *Cx. quinquefasciatus* and *Cx. restuans* at all temperatures and colony-derived *Cx. pipiens* at 16, 20, and 24°C ( $t$ -tests;  $P < 0.01$ ). No differences in wing length were identified between field and colony populations of *Cx. quinquefasciatus*.

Median female longevity decreased with temperature and differed between species and populations, with modest two- and three-way interactions among variables contributing significantly to the measured variation in longevity (three-factor analysis of variance [ANOVA];  $P < 0.001$ ). Temperature alone ac-

counted for 45.1% of the variation in longevity ( $F = 7328.1$ ;  $P < 0.0001$ ), and the combined effect of all three factors accounted for 61.5% of the variation ( $F = 291.9$ ;  $P < 0.0001$ ), indicating a significant effect of species and population but also variation in longevity among individuals independent of these factors. For all groups, there was a significant negative correlation between temperature and longevity (Fig. 2B; linear regression analysis;  $r^2 = 0.94$ ;  $P = 0.006$ ) for which slopes (i.e., the effect of temperature) were not significantly different among groups ( $P = 0.65$ ) despite differences in longevity (Fig. 4). The largest decreases in median survival resulted from temperature increases from 28 to 32°C for both colonized populations and field-derived *Cx. quinquefasciatus*, and for temperature increases from 20 to 24°C for field populations of *Cx. pipiens* and *Cx. restuans*. Field populations of both *Cx. pipiens* and *Cx. quinquefasciatus* survived significantly longer than *Cx. restuans* at all tempera-

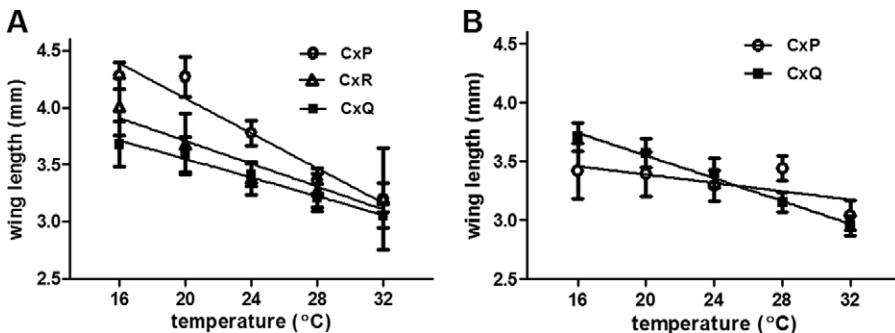


Fig. 3. Mean wing length (millimeters) of (A) field-derived and (B) colonized *Culex* (Cx) mosquitoes at various rearing temperatures. A negative correlation between temperature and winglength was measured in all groups with the exception of colony *Cx. pipiens* (linear regression analysis,  $P = 0.205$ ). Relationships were as follows: *Cx. pipiens* (P; field)  $y = 5.62 - 0.08x$ ;  $r^2 = 0.94$ ;  $P = 0.0064$ ; *Cx. quinquefasciatus* (Q; field)  $y = 4.37 - 0.04x$ ;  $r^2 = 0.99$ ;  $P = 0.0006$ ; *Cx. restuans* (R; field)  $y = 4.72 - 0.05x$ ;  $r^2 = 0.91$ ;  $P = 0.016$ ; *Cx. quinqs* (colony)  $y = 4.52 - 0.05x$ ;  $r^2 = 0.99$ ;  $P = 0.0005$ .

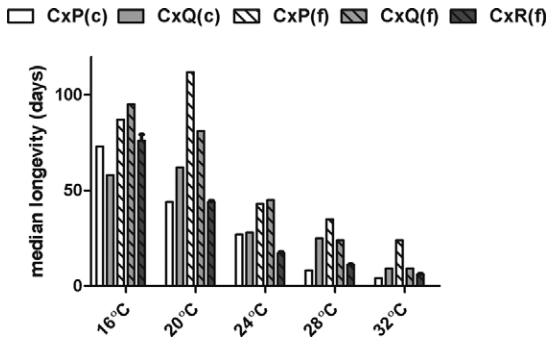


Fig. 4. Median longevity  $\pm$  SEM of field (f) and colonized (c) *Cx. pipiens* (CxP), *Cx. quinquefasciatus* (CxQ), and *Cx. restuans* (CxR) at various temperatures.

tures, and survival of field-derived *Cx. pipiens* was greater than both other species at all temperatures but 16 and 24°C (Mantel-Cox test;  $P < 0.001$ ). With the exception of *Cx. quinquefasciatus* at 28 and 32°C, field populations generally outlived colony populations by highly significant margins (Gehan-Breslow-Wilcoxon test;  $P < 0.001$ ). The most significant of these differences was observed with *Cx. pipiens* at 20°C, for which median survival for the field-derived population was 112 d, as compared with just 44 d for the colony-derived population (Fig. 4).

**Blood Feeding and Fecundity.** Temperatures, population, and species significantly altered *Culex* blood feeding (Fig. 5). The colony populations had significantly higher proportion blood fed relative to field-derived populations at all temperatures for *Cx. quinquefasciatus* and all but 28 and 32°C for *Cx. pipiens*, for which field population feeding was significantly higher ( $\chi^2$  test;  $P < 0.001$ ). In fact, individuals from field populations of *Cx. quinquefasciatus* fed only at 24 and 28°C (Fig. 5A). Species comparisons of colony-derived mosquitoes demonstrates significantly higher proportions of *Cx. pipiens* fed at the lowest temperature (16°C; Fig. 5A;  $\chi^2$  test,  $P = 0.0021$ ), yet significantly higher proportions of *Cx. quinquefasciatus* fed at the two highest temperatures (28 and 32°C; Fig. 4A;

$\chi^2$  test,  $P < 0.001$ ). Only a single *Cx. restuans* female fed during the study, emphasizing the difficulty of colonizing this species and the general reluctance of field-derived populations to feed from artificial systems.

Although the relatively small number of fed females in many groups made assessing fecundity differences difficult, some significant variation among groups was identified (Fig. 5B). Overall, egg raft production among blood-fed females was highest for both populations of *Cx. pipiens* at 20°C and both populations of *Cx. quinquefasciatus* at 24°C. In addition, with the exception of 24°C, for which proportions were statistically similar, a higher proportion of blood-fed field *Cx. pipiens* laid egg rafts than did colony *Cx. pipiens* at all temperatures. In contrast, blood-fed colony *Cx. quinquefasciatus* mosquitoes produced proportionally more egg rafts than field *Cx. quinquefasciatus* at all temperatures, and combined egg raft output was highest for this population (Fig. 5B;  $\chi^2$  test,  $P < 0.001$ ).

Discussion

Although many studies have demonstrated a role for temperature in mosquito development and population dynamics of individual species (Lachmajer and Hien 1975, Rueda et al. 1990, Reisen 1995), quantifying the specific relationships between temperature and life history traits as well as direct comparison of the relative importance of temperature among medically important mosquito vectors is critical to our understanding of how climate shifts could ultimately alter vectorial capacities and patterns of disease transmission (Dye 1992; Rogers and Randolph 2000, 2006). Here, we provide a unique comparison of the effect of temperature on both field-derived and colonized *Cx. pipiens* and *Cx. quinquefasciatus*, as well as a field population of *Cx. restuans*. Our results demonstrate that temperature significantly affects rates of immature development, survival of immature stages, adult size, adult longevity, blood feeding, and fecundity of *Culex* mosquitoes, and that both species and colonization are additional factors significantly altering these life history traits and their susceptibility to temperature shifts.

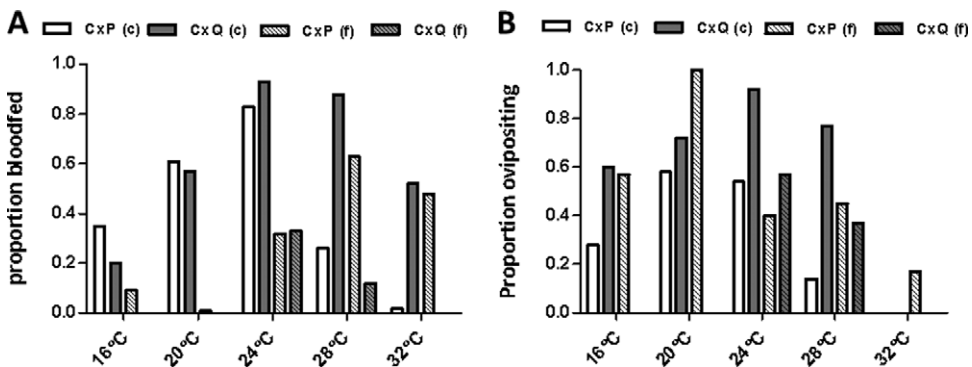


Fig. 5. Blood feeding and fecundity of *Culex* mosquitoes of field (f) and colonized (c) *Cx. pipiens* (CxP), *Cx. quinquefasciatus* (CxQ), and *Cx. restuans* (CxR) at various temperatures. (A) Proportion of blood-fed *Culex* mosquitoes at various temperatures. (B) Proportion of blood-fed *Culex* mosquitoes laying egg rafts at various temperatures.

In concordance with previous studies with other mosquito species, results here demonstrate a positive correlation between temperature and development rate (Lachmajer and Hien 1975, Rueda et al. 1990, Bayoh and Lindsay 2003), indicating that increasing temperatures could generally lead to a more rapid proliferation of *Culex* populations. Despite this, temperatures  $>24^{\circ}\text{C}$  had more modest effects on development time than increases below this temperature, demonstrating that rising mean temperatures in milder regions may have lesser effects on the rate of *Culex* proliferation than would increases in regions where mean summer temperatures range from 16 to  $24^{\circ}\text{C}$ . In addition, the increased mortality observed at higher temperatures in all groups could result in an overall decline in the reproductive output of *Culex* mosquitoes despite modestly accelerated development. Indeed, only field *Cx. pipiens* produced egg rafts at  $32^{\circ}\text{C}$ . Similar effects of high temperature on adult longevity have been observed in other studies (Hawley 1985), including in *Cx. quinquefasciatus* (Rueda et al. 1990) and *Culex tarsalis* (Reisen 1995).

Although temperature was responsible for the majority of the variation in life history traits measured here and all groups were significantly affected by rearing temperatures, important species and population differences were also identified. Others have acknowledged differences in fitness of field and colonized mosquito populations (Tabachnick 2003), yet results here indicate that species and population differences likely require defining such differences for individual studies. Among the most distinct differences was the sensitivity of colonized larvae and pupae to variable temperatures, as opposed to the robustness of field *Cx. pipiens* and *Cx. quinquefasciatus*, for which little effect of temperature on immature survival was measured. The largest effect of temperature was observed with colonized *Cx. pipiens* mosquitoes, for which mean development time at  $16^{\circ}\text{C}$  was significantly higher than all other groups. Furthermore, colonized *Cx. pipiens* was the only group for which the shortest development time was measured at  $28^{\circ}\text{C}$  rather than  $32^{\circ}\text{C}$ . A lack of phenotypic variability was also observed with winglength of colony *Cx. pipiens*, where only modest variation in size was measured with variable temperatures, with  $28^{\circ}\text{C}$  producing the largest mosquitoes. For all other groups, a significant negative correlation was measured between size and temperature (i.e., development time) as has been shown in previous studies (Rueda et al. 1990, Chadee and Beier 1997, Gunay et al. 2011, Mohammed and Chadee 2011). In addition, survival of both immature stages and adults was significantly lower for colonized populations, and overall blood feeding and egg production were generally higher relative to field mosquitoes. These differences, which are consistent with previous studies investigating the effects of colonization (Aguilar et al. 2005, Vitek and Livdahl 2006), are likely a reflection of selection events resulting from long-term colonization and rearing at generally constant environmental conditions, indicating that the use of colonies to measure the effects of temperature or

other environmental variables on life history traits may at times provide imprecise representations of natural populations.

Measurement of species-specific variation among field populations provided a direct assessment of the potential implications of natural temperature fluctuations among *Culex* mosquitoes. *Cx. restuans* were less sensitive to alterations in development time than *Cx. pipiens* or *Cx. quinquefasciatus*, yet had significantly shorter longevity than both other species at every temperature and were the only field population for which immature survival was significantly decreased at temperatures  $>24^{\circ}\text{C}$ . These results suggest that *Cx. restuans*, which are important vectors of *West Nile virus* (Kilpatrick et al. 2005, Degroote and Sugumaran 2012) and more prevalent than *Cx. pipiens* in some rural areas of the eastern United States at certain times during the transmission season (Ebel et al. 2005, Diuk-Wasser et al. 2006), are likely to be the most susceptible of the studied species to population fluctuations as a result of increasing temperatures. Consistent with these findings, previous studies have indicated that *Cx. restuans* populations tend to peak in late spring and early summer, followed by a decline in the hotter summer months (Reiter 1988, Andreadis et al. 2001).

*Cx. quinquefasciatus* and *Cx. pipiens* are sibling species separated by a hybridization zone stretching from  $\approx 30^{\circ}\text{N}$  and  $40^{\circ}\text{N}$  latitude in N. America (Kothera et al. 2009, Farajollahi et al. 2011, Huang et al. 2011), with pure *Cx. quinquefasciatus* to the south and *Cx. pipiens* to the north. This distribution would suggest that *Cx. quinquefasciatus* are more likely to be adapted to higher temperatures, yet only modest differences in life history traits were measured between the two species. Immature survival was significantly higher for *Cx. quinquefasciatus* at  $28^{\circ}\text{C}$  (but not 24 or  $32^{\circ}\text{C}$ ), and development was slightly accelerated at  $24^{\circ}\text{C}$ , yet median longevity for *Cx. pipiens* was similar to *Cx. quinquefasciatus* at  $24^{\circ}\text{C}$  and in fact significantly higher than *Cx. quinquefasciatus* at both 28 and  $32^{\circ}\text{C}$ . This suggests that *Cx. quinquefasciatus*, as they currently reside in areas with mean summer temperatures generally between 24 and  $28^{\circ}\text{C}$  (www.noaa.gov), are likely to experience decreased survival as a result of rising temperatures. Because development rate is only modestly increased at temperatures  $>24^{\circ}\text{C}$ , this increased mortality would decrease population size and, potentially, vectorial capacity of *Cx. quinquefasciatus*. *Cx. pipiens*, as they reside in areas with mean summer temperatures between 16 and  $24^{\circ}\text{C}$ , could also experience decreases in mean longevity with rising temperatures, but this negative effect on the population size could be compensated by the fact that greater increases in development rate occur with temperature increases within this range. Consistent with mortality results, field-derived *Cx. pipiens* blood feeding was also significantly greater than *Cx. quinquefasciatus* at temperatures  $>24^{\circ}\text{C}$ , which could serve to further enhance differences in vectorial capacities. However, when considering the effect of temperature on vectorial capacity, the likelihood of increased pathogen proliferation at higher temperature, and therefore in-

creased vector competence, must also be considered (Dohm et al. 2002, Reisen et al. 2006, Kilpatrick et al. 2008). The fact that the field populations of *Cx. pipiens* were derived from the southern end of this species' range (Washington DC area) may partially explain why decreased fitness at higher temperatures was not observed, and is consistent with the presence of significant population level variation. In support of this, colonized *Cx. pipiens*, originally derived from Pennsylvania, were indeed more susceptible to adult mortality and displayed decreased blood feeding at temperatures  $>24^{\circ}\text{C}$  when compared with colonized *Cx. quinquefasciatus*, yet the potential effects of colonization make these differences difficult to interpret. Genetic diversity identified among populations and individuals of these species, particularly in the case of *Cx. pipiens* (Fonseca et al. 2004, Kilpatrick et al. 2007, Kothera et al. 2009, Huang et al. 2011), together with identification of substantial variation in vector competence for *West Nile virus* among *Culex* populations (Kilpatrick et al. 2010), support the idea of significant population-level phenotypic variation in life history traits and in susceptibility to environmental fluctuations, yet regardless of population variation, these results suggest that differences in mosquito fitness or in the capacity for adaptation to warmer temperatures cannot by itself explain the differences in geographic distributions of these sibling species. Because *Cx. quinquefasciatus* do not have the capacity to enter true diapause as *Cx. pipiens* do (Hayes 1975), the inability to successfully overwinter may explain why *Cx. quinquefasciatus* cannot establish themselves in the northern United States, yet the explanation for the lack of *Cx. pipiens* moving south requires further study.

A potentially important variable not considered in these studies is the fluctuating daily temperature experienced by natural *Culex* populations. Although constant temperatures may provide an accurate representation of the effects of daily mean temperature on mosquito development (Milby and Meyer 1986), some species and populations may be more likely to experience changes in life history traits as a result of diurnal fluctuations (Paaijmans et al. 2010, Lambrechts et al. 2011, Mohammed and Chadee 2011). Laboratory rearing containers are also poor representations of natural adult habitats, and differences in mating success, blood-feeding behavior, and overall reproduction output could differ significantly in natural settings. In addition, the largest effect of climate change on *Culex* populations could result from rising temperatures during breaks in transmission. Milder fall or winter months could serve to significantly alter the size and success of overwintering populations, and milder spring months may advance or accelerate the commencement of pathogen transmission (Ciota et al. 2011). Despite these caveats, our results suggest life history traits of *Culex* mosquitoes are governed, to a large degree, by temperature and to a lesser extent by species and population differences and that these differences could have profound effects on the influence of climate change on the epidemiological patterns of vector-borne disease.

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