Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination

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Abstract
How are organisms responding to climate change? The rapidity with which climate is changing suggests that, in species with long generation times, adaptive evolution may be too slow to keep pace with climate change, and that alternative mechanisms, such as behavioural plasticity, may be necessary for population persistence. Species with temperature-dependent sex determination may be particularly threatened by climate change, because altered temperatures could skew sex ratios. We experimentally tested nest-site choice in the long-lived turtle Chrysemys picta to determine whether nesting behaviour can compensate for potential skews in sex ratios caused by rapid climate change. We collected females from five populations across the species’ range and housed them in a semi-natural common garden. Under these identical conditions, populations differed in nesting phenology (likely due to nesting frequency), and in nest depth (possibly due to a latitudinal cline in female body size), but did not differ in choice of shade cover over the nest, nest incubation regime, or in resultant nest sex ratios. These results suggest that choice of nest sites with particular shade cover may be a behaviourally plastic mechanism by which turtles can compensate for change in climatic temperatures during embryonic development, provided that sufficient environmental variation in potential nest microhabitat is available.

Keywords
Chrysemys picta, common garden, geographic variation, painted turtle, phenology, sex ratio

Disciplines
Ecology and Evolutionary Biology | Evolution | Population Biology | Terrestrial and Aquatic Ecology | Zoology

Comments
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Behavioral plasticity compensates for climate change in a long-lived reptile with temperature-dependent sex determination

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Running title: Nest-site choice and climate change

Abstract

How are organisms responding to climate change? The rapidity with which climate is changing suggests that, in species with long generation times, adaptive evolution may be too slow to keep pace with climate change, and that alternative mechanisms, such as behavioral plasticity, may be necessary for population persistence. Species with temperature-dependent sex determination may be particularly threatened by climate change, because altered temperatures could skew sex ratios. We experimentally investigated nest-site choice in the long-lived turtle Chrysemys picta to determine whether nesting behavior can compensate for potential skews in sex ratios caused by rapid climate change. We collected gravid females from five source populations and housed

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them in a semi-natural common garden. Under these identical conditions, populations differed in nesting phenology due to nesting frequency, and in nest depth due to a latitudinal cline in female body size, but did not differ in choice of shade cover over the nest or in resultant nest sex ratios. These results suggest that selection of nest sites with particular shade cover may be a behaviorally plastic mechanism by which turtles can compensate for change in climatic temperatures during embryonic development, provided that sufficient environmental variation in potential nest microhabitat is available.

Key words: *Chrysemys picta*, geographic variation, painted turtle, phenology, sex ratio

Numerous studies have documented effects of climate change on natural systems, including range shifts, changes in phenology, altered species interactions, and disrupted community structure (reviewed in Parmesan 2006). A growing question is whether, and to what extent, organisms may be able to adjust to climate change, and by what mechanism(s) such adjustment may be possible. Adaptive genetic changes in response to climate change have been observed in a few cases (*e.g.*, Bradshaw and Holzapfel 2001; Umina et al. 2005). However, due to the rapid pace at which global climate change is occurring, non-genetically-based mechanisms such as behavioral plasticity may be more feasible for acclimatization to a different climate, at least until allele frequencies can “catch up” (*e.g.*, Berteaux et al. 2004). Long-lived species could be particularly vulnerable to climate change, as their generation times may be too long to keep pace with rapid environmental shifts through adaptive genetic changes (Dunham and Overall 1994; Pen et al. 2010).
Many effects of climate change on biota are difficult to predict because they involve indirect effects of environmental changes on a multitude of organisms and occur via complex pathways. However, some thermally-sensitive traits exist that are directly impacted by climate and have demographic consequences for populations. Temperature-dependent sex determination (TSD), in which offspring sex is irreversibly determined by the temperature experienced by developing embryos, is such a trait. TSD is common among long-lived reptile groups (Janzen and Paukstis 1991), and the extreme sensitivity of the sex-determining pathway to temperature (Ewert et al. 1994) means that a small change in environmental temperature could dramatically alter offspring sex ratio, and potentially result in populations consisting of predominantly one sex (Janzen 1994a; Nelson et al. 2004; Wapstra et al. 2009; Schwanz et al. 2010).

Reptiles with TSD have survived periods of past tumultuous climate change. Two solutions have been identified to explain the continued persistence of such taxa despite numerous periods of global warming and cooling (Bulmer and Bull 1982): 1) changes in the specific incubation temperatures producing each sex, and 2) changes in maternal nest-site choice, which affects the microclimate Experienced by developing embryos. Although both traits are likely to exert an impact over evolutionary timescales, low heritability suggests that shifts in thermal sensitivity of sex determination are unlikely to compensate for skewed sex ratios resulting from rapid climate change (Janzen 1994a; but see McGaugh and Janzen 2011). Moreover, seemingly minimal phenotypic variation in this sex-ratio reaction norm between populations suggests that this trait has provided an insufficient target for selection to compensate for climatic differences (Morjan 2003a). At the same time, micro-evolutionary responses of nest-site choice to a rapidly changing climate appear to be context-specific at best (McGaugh et al. 2010) and ineffective at worst (Morjan 2003b). Therefore, if long-lived reptiles with TSD are to avoid skewed sex ratios
produced by temperatures changing at unprecedented rates, behavioral phenotypic plasticity in nest-site choice (i.e., the ability of individual females to intra-generationally alter the location or other characteristics of nests in response to environmental conditions) may be the likely immediate mechanism (Morjan 2003b). Importantly, such a result would contrast with the genetically-based adaptation to climate change observed in short-lived taxa (e.g., Bradshaw and Holzapfel 2001; Umina et al. 2005; Balanyá et al. 2006; Pen et al. 2010).

We examined nest-site choice in a model long-lived turtle with TSD, *Chrysemys picta*, using a common-garden design (e.g. Clausen et al. 1939) to determine whether local adaptation of key elements of nest-site choice is more intrinsically- (e.g., genetically-) or environmentally-based. In several reptiles with TSD, maternal choice of oviposition site exhibits local adaptation, with females selecting different nest sites to match incubation conditions to their latitude (Ewert et al. 2005; Doody et al. 2006a). However, whether patterns of geographic variation in nest-site choice are driven by inheritance of specific nesting behavior, or by phenotypic plasticity, is unknown. Our experimental design, by examining patterns of variation in nest-site choice across space, allows us to infer the processes of such variation through time, and thereby predict a species’ capacity to respond to climate change (Doody 2009).

**Methods**

The painted turtle is widely distributed in freshwater habitats across the U.S. and southern Canada. Four subspecies are generally recognized; the western subspecies, *C. p. bellii*, primarily occurs west of the Mississippi River. Female painted turtles emerge from wetlands between May and July to nest in a variety of open habitats ranging from beaches to lawns. Individual females nest from 1 to 3 times per season, depending on population. The species
exhibits Type Ia TSD, with females produced at constant incubation temperatures above 29°C and males produced at constant temperatures below 27°C (Ewert et al. 1994).

We captured adult *C. p. bellii* between 27 April and 27 May 2009 from 5 populations across the subspecies’ range: Socorro Co., New Mexico; Carroll Co., Illinois; Grant Co., Nebraska; Story Co., Iowa; and Skamania Co., Washington (Appendix A). We obtained 5 males and 13, 15, 6, 15, and 11 adult females from the NM, IL, NE, IA, and WA populations, respectively, using a variety of aquatic trapping methods. We measured all turtles (straight and curved carapace length and width; plastron length and width; length of rear limbs) and marked individuals by filing a unique combination of notches into the marginal scutes.

We housed turtles in a series of identical, outdoor, semi-natural ponds at Iowa State University’s Aquatic Research Facility (Story Co., IA; Appendix B). Each population occupied a separate 15 x 40 m pond, each of which was surrounded by a 0.5 m-high drift fence to prevent escape of experimental turtles or entrance of local turtles. Ponds graded from 2 to 3 m deep from north to south, and were filled with water from an adjacent lake approximately 3 weeks before release of the experimental turtles to allow colonization by local aquatic plants, invertebrates, and anurans. Turtles had *ad libitum* access to these food items, and were not provided with supplementary food (we observed no evidence of reduction in food availability throughout the study). Two basking logs were present in each pond. The drift fencing around each pond was set back ~8 m from the shoreline on all sides of the ponds to provide nesting habitat, which ranged from completely open banks to completely shaded areas with dense understory vegetation, thus giving all populations access to a full range of shade cover under which to nest.

We monitored the ponds for nesting activity from 27 May – 3 July 2009. We patrolled the perimeters of all ponds hourly from 0600-1000 h and 1500-2100 h, the times of peak painted
turtle nesting activity. Turtles observed nesting (Appendix C) were monitored from a distance to prevent nest abandonment due to disturbance. Upon completion of nesting, we briefly detained females for identification, and then released them back into their ponds. We excavated all nests within 24 h of construction to determine clutch size and measure nest depth; we also recorded the date of construction and took a hemispherical photograph over the nest site to quantify shade cover using Gap Light Analysis software (as in Doody et al. 2006b). Nests then remained in situ for the duration of the incubation period.

In October 2009 (after hatching but before emergence from the nest), we excavated all nests to retrieve hatchlings. We calculated hatching success as the number of live hatchlings retrieved, divided by the known clutch size. To assess sex, we sacrificed hatchlings with a pericardial overdose of 0.5 mL of 1:1 sodium pentobarbital:water and examined the gonads (Janzen 1994b): individuals lacking oviducts and possessing short gonads were classified as males (1), those with complete oviducts and long gonads were classified as females (0), and individuals possessing both male and female gonads were classified as intersexes (0.5). After sexing, we preserved all specimens in 70% ethanol.

We acquired climate data for each collecting site from the National Climate Data Center (www.ncdc.noaa.gov). We calculated mean May air temperature from 1979 – 2009 for each site as an index of each population’s home climate just before the nesting season. While the 30-year mean May air temperature generally decreased with latitude (Fig. 1), the NE site was cooler than the IA site despite being farther south. Therefore, we used the 30-year mean May temperature, rather than latitude, when regressing experimental parameters against home site (see below).

We performed all statistical analyses using SAS 9.2 (SAS Institute, Cary, North Carolina). We analyzed among-population differences in nesting date, per cent shade cover over
nests, and hatching success using analysis of variance in Proc MIXED with female identity nested within population as a random effect; we analyzed nest depth similarly except that we included female size as a covariate. Importantly, because females in some populations nested multiple times while others nested only once, we analyzed nesting phenology using only the first nest constructed by each female. We included all nests in other analyses, again with female identity nested within population as a random effect. We compared among-population differences in nest sex ratios using a chi-square goodness-of-fit test (Wilson and Hardy 2002). We employed Proc GENMOD to model nest sex ratio with nest date, shade cover, nest depth, population, and all interactions as possible predictors, and ranked candidate models using Akaike’s Information Criterion corrected for small sample size ($\text{AIC}_c$; Burnham and Anderson 2002). We considered the best-supported model and all models with $\Delta\text{AIC}_c < 2.0$ to be competing models.

Results

Overall, 26 females (4 from NM, 8 from IL, 3 from NE, 5 from IA, and 6 from WA) constructed a total of 34 nests in the common-garden experiment. An unidentified IL female constructed one nest. Two IA females and four IL females nested twice, and one IA female nested three times. All females from NM, NE, and WA nested only once. Female size, measured as plastron length, was strongly correlated with mean rear limb length, with larger females having longer rear limbs ($R^2 = 0.59, P < 0.0001$; Appendix D). Female plastron size was positively correlated with home latitude ($R^2 = 0.71, P < 0.0001$). Clutch size was also positively correlated with home latitude ($R^2 = 0.46, P = 0.001$), but when standardized for female size,
populations did not differ in clutch size (ANCOVA with plastron length as a covariate; \( F_{4,33} = 0.90, P = 0.51 \)).

The date on which females constructed their first nest differed among the five populations, with IA and IL females nesting earlier than NM, NE, and WA females (\( F_{4,21} = 4.11, P = 0.01 \); Fig. 2). Although shade cover over randomly-selected locations around each pond ranged from 7 - 70% (data not shown), populations did not differ in the amount of shade cover over sites selected by females for nesting (\( F_{4,33} = 0.83, P = 0.55 \); Table 1; Fig. 3), and there was no significant correlation between shade cover and mean home climate (\( R^2 = 0.016, P = 0.48 \)). Nest depth was positively correlated with female rear limb length (\( R^2 = 0.39, P < 0.0001 \)) and, as female size increased with home latitude, nest depth also increased with home latitude (\( R^2 = 0.136, P = 0.03 \)). When standardized for female size, populations did not differ in mean nest depth (\( F_{4,33} = 0.10, P = 0.98 \); Fig. 4); thus, differences in nest depth among populations were caused by among-population differences in female size. Nest depth was inversely correlated with mean home climate (\( R^2 = 0.16, P = 0.02 \)), with females from warmer climates constructing shallower nests than females from cooler climates.

Hatching success did not differ among populations when all nests were included (\( F_{4,6} = 4.06, P = 0.06 \)), but when only first clutches were included, nests from NE and WA females had lower hatching success than nests from NM, IA, and IL females (\( F_{4,24} = 2.90, P = .04 \)). Similarly, hatching success tended to be positively correlated with mean home climate (\( R^2 = 0.19, P = 0.10 \)).

We observed one intersex hatchling, which emerged from a WA nest and possessed one ovary and one testis. NE was excluded from analyses of sex ratio due to low survival; however, the single surviving NE nest yielded a sex ratio of 0.5 and, along with a nest from NM, had the
smallest proportion of male hatchlings in this study. Excluding NE, the sex ratio produced by females did not differ among populations ($\chi^2 = 2.49, P = 0.65$; Fig. 5). Including NE, the nest-site choice model for predicting nest sex ratio with the lowest AICc included the parameters Population and Nesting date; the model containing Nesting date and Shade cover was within 2 $\Delta$AICc. No other models were competitive.

Discussion

The rapid rate of global climate change may preclude long-lived organisms from keeping pace through adaptive cross-generational genetic changes. Instead, mechanisms such as genetically-based phenotypic plasticity and/or non-genetic forms of plasticity may be necessary. Our study showed that behavioral phenotypic plasticity in female choice of shade cover over the nest site may comprise an immediate mechanism by which long-lived reptiles with TSD can avoid skews in sex ratio potentially caused by rapid climate change. Indeed, we hypothesize that phenotypic plasticity should be a general mechanism by which organisms with long generation times respond immediately to accommodate rapid changes in phenotypic selection (sensu Price et al. 2003).

We found that populations from across the geographic range of C. picta differed in nesting date and nest depth despite experimental exposure to a common environment. Nesting phenology is likely constrained by the number of clutches produced per year, which varies in this species: populations in which females nest multiple times in a season likely have less plasticity in nesting date than populations where females nest only once in a season. Indeed, in this study, the populations that constructed their first nests earlier were also those that nested multiple times; populations that nested later also nested once. Interestingly, while climate change may increase
the length of the nesting season and thereby allow some populations to nest an additional time in a single season, sex ratio skew may paradoxically increase because the thermosensitive period of the extra nest is likely to occur during cooler conditions (Schwanz and Janzen 2008; Tucker et al. 2008). Regardless of its effects on sex ratio, however, timing of nesting has low heritability in C. picta (McGaugh et al. 2010) and therefore shifts in nesting phenology are likely to be behaviorally plastic rather than inherited (Schwanz and Janzen 2008).

Nest depth affects the incubation regime and thereby nest sex ratio (Refsnider, unpubl data). Reptiles that construct subterranean nests by digging with their front limbs, such as lizards and tuatara, can vary nest depth by simply digging more deeply (Nelson et al. 2004). Freshwater turtles, however, construct nests using their back limbs, which suggests that maximum nest depth is constrained by rear limb length. In our study, females from southern populations were smaller and dug shallower nests than females from more northern populations. In their home environment, however, NM females dug deeper nests than those from IL (Morjan 2003a), suggesting that NM populations are currently constructing nests of maximum depth for their body size, and likely have less adaptive potential to shift nest depth than populations in which mean nest depth is currently shallower than the maximum depth possible based on female size. While increased adult mortality can cause rapid shifts in growth rate and adult size in reptiles (e.g. Sasaki et al. 2008; Wolak et al. 2010), indirect selection for increased female body size through selection for females with the ability to increase nest depth is likely much weaker. Therefore, increases in female body size will probably occur only gradually, and compensation for rapid climate change through selection for deeper nests is likely to be evolutionarily constrained by relatively weak selection for increased female size.
Populations in this field experiment did not differ in the amount of shade cover over nests, despite all females having a full range of shade cover options from which to choose a nest site. Shade cover is a reliable predictor of nest sex ratio in the IL population (Janzen 1994b; Schwanz et al. 2010), and females may adjust choice of nest shade cover in order to match a nest site’s future incubation regime to prevailing environmental conditions (Doody et al. 2006a). The lack of among-population difference in nest shade cover observed in our study suggests that females from a range of local climates adjusted their choice of nest shade cover in response to environmental conditions in the common-garden experiment. Moreover, the selection of similar amounts of nest shade cover among females resulted in similar incubation regimes (Appendix E) and similar nest sex ratios across populations. These results suggest that choice of nest shade cover is a behaviorally plastic mechanism by which female turtles can influence the sex ratio of their offspring. Importantly, however, a diversity of shade cover must be available to nesting turtles in order for this plasticity to be expressed. Behavioral plasticity in traits such as maternal choice of nest shade cover, which can be adjusted based on immediate environmental conditions, may be important mechanisms in allowing species with long generation times to adjust to rapid climate change when adaptive changes in allele frequencies occur too slowly to compensate.

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Literature cited


Table 1. Population means of nest-site choice parameters and nest sex ratio for *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Populations are listed in order of decreasing latitude. Sex ratio was not calculated for NE due to low sample size. All values are means ± standard deviation.

<table>
<thead>
<tr>
<th>Popn</th>
<th>N</th>
<th>Nesting date</th>
<th>% shade cover</th>
<th>Nest depth (mm)</th>
<th>Nest sex ratio (proportion male)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WA</td>
<td>6</td>
<td>20 June ± 6.1</td>
<td>19.0 ± 6.6</td>
<td>103.33 ± 24.01</td>
<td>0.87 ± 0.18</td>
</tr>
<tr>
<td>IA</td>
<td>8</td>
<td>12 June ± 11.0</td>
<td>26.9 ± 14.6</td>
<td>96.25 ± 4.43</td>
<td>0.92 ± 0.16</td>
</tr>
<tr>
<td>NE</td>
<td>3</td>
<td>20 June ± 7.0</td>
<td>35.0 ± 21.1</td>
<td>91.67 ± 12.58</td>
<td>--</td>
</tr>
<tr>
<td>IL</td>
<td>13</td>
<td>6 June ± 7.8</td>
<td>25.8 ± 10.1</td>
<td>89.17 ± 9.73</td>
<td>0.89 ± 0.12</td>
</tr>
<tr>
<td>NM</td>
<td>4</td>
<td>20 June ± 2.6</td>
<td>27.8 ± 17.5</td>
<td>83.75 ± 11.09</td>
<td>0.88 ± 0.25</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Mean May air temperature from 1979 – 2009 at each population’s site of collection. Climate data are from the National Climate Data Center (www.ncdc.noaa.gov) and were collected at Bosque del Apache (NM site), Clinton, IA (IL site), Hyannis, NE (NE site), Ames 8 WSW (IA site), and Skamania Fish Hatchery (WA site). Data were unavailable for some sites in some years. Populations are listed in the legend in order of increasing latitude.

Figure 2. Nesting phenology of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Only the dates of each female’s first nest are included. Populations differed in mean date of first nesting ($F_{4,21} = 4.11, P = 0.01$).

Figure 3. Percent shade cover over nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Populations did not differ in % shade cover ($F_{4,33} = 0.83, P = 0.55$).

Figure 4. Depth of nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Nest depth was positively correlated with female rear limb length ($R^2 = 0.39, P < 0.0001$) and increased with latitude of home population ($R^2 = 0.136, P = 0.03$). Red = NM, orange = IL, green = NE, blue = IA, purple = WA.

Figure 5. Sex ratios of nests of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. Sex ratio did not differ among populations (NE excluded due to low sample size; $\chi^2 = 2.49, P = 0.65$).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Appendices

Appendix A. Range of the painted turtle, *Chrysemys picta* (from Ernst *et al.* 1994). Outline encloses the range of the western subspecies, *C. p. bellii*. Black circles indicate locations from which turtles were collected for use in this experiment (Bosque del Apache National Wildlife Refuge, Socorro County, NM; Thomson Causeway Recreation Area, Carroll County, IL; Hyannis, Grant County, NE; Ames, Story County, IA; and Pierce National Wildlife Refuge, Skamania County, WA).

Appendix B. Series of experimental ponds in which *Chrysemys picta bellii* were housed at Iowa State University’s Aquatic Research Facility, Story County, IA. The pond in the foreground is the western-most pond, with ponds situated in parallel from west to east and oriented with the long axis of each pond running from north (foreground) to south. Ponds are 15 m (west-east axis) by 40 m (north-south axis). Note the drift fencing surrounding all ponds to prevent escape of experimental turtles and entry of local turtles.

Appendix C. A female painted turtle, *Chrysemys picta bellii*, nesting in the outdoor common-garden experiment at Iowa State University’s Aquatic Research Facility, Story County, IA. This female is in the process of arranging eggs within the nest cavity with her left rear limb.

Appendix D. Population means of female size and nest characteristics of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. All values are means ± standard deviation.

Appendix E. Population means of nest incubation regime parameters of *Chrysemys picta bellii* collected from 5 populations that nested in a common-garden in Story Co., IA in 2009.
All values are means in °C ± standard deviation. Thermosensitive period (TSP) was considered days 16 – 46 of the 60-day incubation period.
Appendix D

Population means of female size and nest characteristics of *Chrysemys picta bellii* collected from 5 populations that nested in a semi-natural common-garden experiment in Story Co., IA in 2009. All values are means ± standard deviation.

<table>
<thead>
<tr>
<th>Popn.</th>
<th>Female rear limb length (mm)</th>
<th>Female plastron length (mm)</th>
<th>Clutch size</th>
<th>Egg mass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NM</td>
<td>80.4 ± 2.6</td>
<td>138.5 ± 3.7</td>
<td>7.5 ± 1.7</td>
<td>6.42 ± 0.68</td>
</tr>
<tr>
<td>IL</td>
<td>80.9 ± 4.3</td>
<td>155.8 ± 4.5</td>
<td>9.9 ± 1.6</td>
<td>6.03 ± 0.84</td>
</tr>
<tr>
<td>NE</td>
<td>87.2 ± 4.5</td>
<td>153.7 ± 4.0</td>
<td>11.3 ± 2.1</td>
<td>5.84 ± 0.76</td>
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<td>IA</td>
<td>87.7 ± 3.2</td>
<td>165.4 ± 8.4</td>
<td>11.4 ± 2.1</td>
<td>6.99 ± 0.71</td>
</tr>
<tr>
<td>WA</td>
<td>96.5 ± 7.5</td>
<td>173.7 ± 13.0</td>
<td>13.5 ± 3.0</td>
<td>6.42 ± 0.73</td>
</tr>
</tbody>
</table>
Appendix E

Population means of nest incubation regime parameters of *Chrysemys picta bellii* collected from 5 populations that nested in a common-garden in Story Co., IA in 2009. All values are means in °C ± standard deviation. Thermosensitive period (TSP) was considered days 16 – 46 of the 60-day incubation period.

<table>
<thead>
<tr>
<th></th>
<th>WA (n = 2)</th>
<th>IA (n = 3)</th>
<th>NE (n = 2)</th>
<th>IL (n = 6)</th>
<th>NM (n = 3)</th>
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</thead>
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<tr>
<td>incubation period</td>
<td>23.54 ± 0.09</td>
<td>23.05 ± 0.34</td>
<td>22.96 ± 0.63</td>
<td>23.65 ± 0.63</td>
<td>23.65 ± 0.36</td>
</tr>
<tr>
<td>temp</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TSP temp</td>
<td>23.06 ± 0.02</td>
<td>22.51 ± 0.63</td>
<td>22.63 ± 0.54</td>
<td>23.60 ± 0.83</td>
<td>23.09 ± 0.21</td>
</tr>
<tr>
<td>minimum incubation</td>
<td>17.75 ± 0.35</td>
<td>17.17 ± 1.04</td>
<td>17.75 ± 0.25</td>
<td>16.83 ± 2.44</td>
<td>18.00 ± 0.00</td>
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<tr>
<td>temp</td>
<td></td>
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<tr>
<td>maximum incubation</td>
<td>31.75 ± 0.35</td>
<td>30.67 ± 0.58</td>
<td>30.25 ± 1.25</td>
<td>32.50 ± 2.05</td>
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<td>temp</td>
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<tr>
<td>Daily temp range,</td>
<td>7.32 ± 0.08</td>
<td>6.02 ± 0.36</td>
<td>6.07 ± 0.78</td>
<td>7.02 ± 2.35</td>
<td>7.38 ± 0.76</td>
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<td>incubation period</td>
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<tr>
<td>Daily temp range,</td>
<td>7.76 ± 0.06</td>
<td>6.03 ± 0.52</td>
<td>6.44 ± 0.94</td>
<td>7.53 ± 2.06</td>
<td>7.60 ± 0.60</td>
</tr>
<tr>
<td>TSP temp</td>
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