Phenotypic plasticity of nest timing in a post-glacial landscape: how do reptiles adapt to seasonal time constraints?

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Abstract. Life histories evolve in response to constraints on the time available for growth and development. Nesting date and its plasticity in response to spring temperature may therefore be important components of fitness in oviparous ectotherms near their northern range limit, as reproducing early provides more time for embryos to complete development before winter. We used data collected over several decades to compare air temperature and nest date plasticity in populations of painted turtles and snapping turtles from a relatively warm environment (southeastern Michigan) near the southern extent of the last glacial maximum to a relatively cool environment (central Ontario) near the northern extent of post-glacial recolonization. For painted turtles, population-level differences in reaction norm elevation for two phenological traits were consistent with adaptation to time constraints, but no differences in reaction norm slopes were observed. For snapping turtle populations, the difference in reaction norm elevation for a single phenological trait was in the opposite direction of what was expected under adaptation to time constraints, and no difference in reaction norm slope was observed. Finally, among-individual variation in individual plasticity for nesting date was detected only in the northern population of snapping turtles, suggesting that reaction norms are less canalized in this northern population. Overall, we observed evidence of phenological adaptation, and possibly maladaptation, to time constraints in long-lived reptiles. Where present, (mal) adaptation occurred by virtue of differences in reaction norm elevation, not reaction norm slope. Glacial history, generation time, and genetic constraint may all play an important role in the evolution of phenological timing and its plasticity in long-lived reptiles.

Key words: glaciation; individual variation; Jinks-Connolly rule; random regression; range limit; reaction norm; turtle.

INTRODUCTION

Adaptation by genetic differentiation may confer relatively low fitness in variable environments, given that fitness optima change both within and between generations (Levins 1968). However, if environmental cues are used to modify the phenotype, then fitness can be increased across environments by virtue of a better match between the phenotype and adaptive optima (Scheiner 1993, Yamahira and Conover 2002, Pigliucci 2010). Phenotypic plasticity is the ability of a genotype to produce different phenotypes in different environments, and the trait–environment association is described by the norm of reaction (Schmalhausen 1949, Stearns 1989). A reaction norm can be visualized as a regression line, where the elevation (or intercept) of the line represents the mean phenotype expressed in the average environment, and the

slope represents the change in phenotypic value across environments (Stearns and Koella 1986, Pigliucci 2001, Ghalambor et al. 2007, Nussey et al. 2007). Both the elevation and slope of the reaction norm can be heritable and under selection (Scheiner and Lyman 1991, de Jong 2005, Nussey et al. 2005, Pigliucci 2005, Pelletier et al. 2007), such that adaptive phenotypic plasticity might be anticipated in variable environments.

A variety of animals adjust the timing of nesting with respect to spring temperatures (Congdon et al. 1983, Lovich et al. 2012; Charmantier et al. 2008, Telemeco et al. 2009). Temperature–phenology plasticity may be especially important for ectotherms whose fitness is constrained by the limited duration of warm temperatures within seasonal environments (Rowe and Ludwig 1991, Rowe et al. 1994), given that relatively high spring temperatures generally increase the length of the activity season before the subsequent onset of winter (Menzel et al. 2001). Theory suggests that the timing of nesting in seasonal environments represents a trade-off between delaying nesting to acquire additional energy (e.g., greater fecundity or offspring investment)

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vs. nesting early to increase the length of the embryonic and/ or juvenile growth period (Rowe et al. 1994, see also Altwegg 2002). In the absence of strong genetic constraint, phenotypic plasticity of nest timing in response to spring temperature may evolve to balance this trade-off.

Genetic constraint on plasticity may be common: empirical and theoretical studies demonstrate that genes influencing the elevation of the reaction norm can also affect the slope of the reaction norm (Scheiner and Lyman 1991, Czesak et al. 2006, Springate et al. 2011). In laboratory studies, for instance, selection in the direction opposite to that of an environmental influence (counter-gradient selection) often decreases the reaction norm slope even when the slope was not directly under selection (Jinks and Connolly 1975, Roff 1997). Further, the complex genetic architecture of plasticity can slow the response to selection, and the evolution of reaction norm slope might be delayed relative to reaction norm elevation (de Jong 2005). It is therefore difficult to predict how plasticity will evolve in the wild, and to understand plasticity more broadly, more empirical examples of phenotypic plasticity under natural conditions are required.

Long-term, individually based data on reproductive phenology has resulted in renewed enthusiasm for studying phenotypic plasticity in natural settings (reviewed by Brommer 2013). The paradigm emerging from long-term studies is that historical or contemporary differences in the environment have the potential to shape variation in plasticity both among populations, and among individuals within populations (Charmantier et al. 2008, Husby et al. 2010, Porlier et al. 2012). However, despite the importance of time constraints in life-history evolution (e.g., Rowe and Ludwig 1991, Rowe et al. 1994, Ejsmond et al., 2015), the extent to which time constraints affect plasticity for phenological timing has not been studied in a comparative framework.

The present study draws from two long-term studies of painted turtles (*Chrysemys picta marginata*) and snapping turtles (*Chelydra serpentina*), to compare patterns of phenotypic plasticity of nest timing between populations in southeastern Michigan (MI), USA and central Ontario (ON), Canada (Fig. 1a). The ON locale is near the northern range limit of both species (Ernst and Lovich 2009) and the MI locale is located 600 km to the southwest. The activity season is shorter in ON than in MI because ambient temperatures are lower, such that lakes and ponds have fewer ice-free days, and temperatures are too low for activity and growth for a greater proportion of the year (Fig. 1b). Given that embryonic development of both species of turtles must be completed before winter arrives (otherwise embryos perish), there is less time for embryos to develop in ON than in MI, such that the time constraint on embryo development is stronger in ON than in MI. A shortened period for embryo development generally results in low survival of embryos and/or low fitness of juveniles due to limited time at physiologically suitable temperatures (Ewert 1985, Bobyn and Brooks 1994a,b, Lourdais et al. 2004, Parker and Andrews 2007),
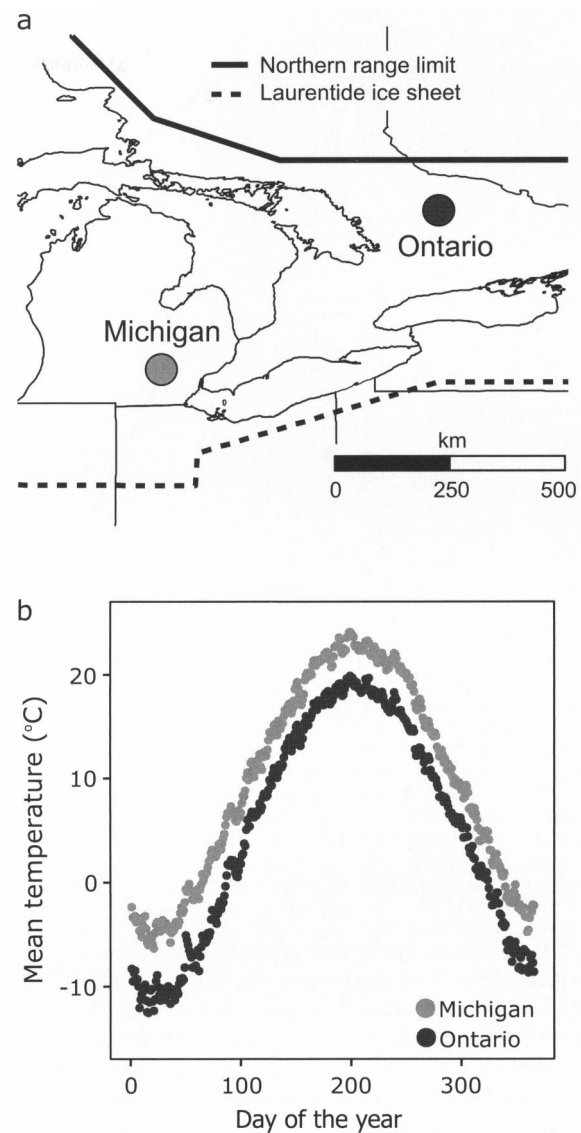

FIG. 1. (a) Location of the study sites in Ontario, Canada and Michigan, USA in relation to the approximate glacial maximum of the Laurentide ice sheet 18,000 years before present and the approximate northern range limit of painted and snapping turtles. (b) Mean temperature over the duration of the studies. [Color figure can be viewed at wileyonlinelibrary.com]

such that adaptation to shortened periods of embryo development likely exist. We therefore used the concept of time constraints to guide our a priori predictions for differences between these populations in temperature–phenology plasticity (Fig. 2).

If time constraints on embryonic development have not affected the evolution of phenotypic plasticity, then recently diverged populations should have similar reaction norm slopes, and a difference in population-mean nest timing could simply be due to a difference in the temperatures experienced by each population (Fig. 2a). However, when time constraints exist, a proportion
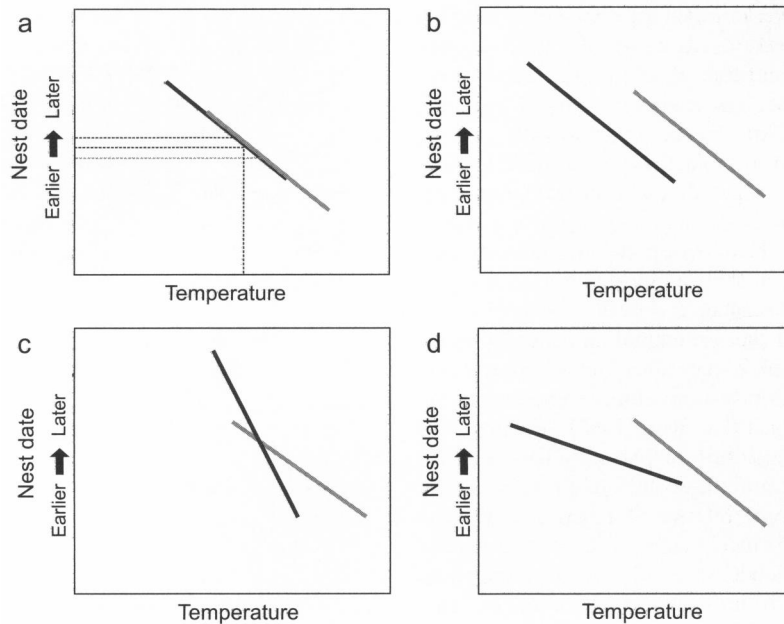
FIG. 2. Conceptual model for temperature–nest-date plasticity in Ontario (blue or dark lines) and Michigan (orange or light lines), from the perspective of stronger directional selection for early phenological timing in Ontario. (a) The null expectation is that no difference exists in reaction norm slope. Here, population-mean nest date differs between populations (horizontal dotted lines), but only because it is warmer in Michigan, i.e., the temperature-corrected mean nesting date (vertical and horizontal dotted line) is identical in Michigan and Ontario. (b) Difference in reaction norm elevation but not slope. Selection for early nest date in Ontario results in relatively lower reaction norm elevation. (c) Difference in reaction norm slopes. Selection targets the slope of the reaction norm, such that the Ontario population exhibits a steeper reaction norm slope. (d) If there is a genetic correlation between the slope and elevation of reaction norm, selection for earlier nesting (i.e., a lower reaction norm elevation) in an environment where temperatures are more conducive to late nesting increases the stability of the genotype by environment interaction. The result is that earlier nest timing and a shallow reaction norm slope is observed in Ontario (the environment with strong counter-gradient selection), a pattern consistent with the Jinks-Connolly rule. [Color figure can be viewed at wileyonlinelibrary.com]

of the variance in annual reproductive success will be attributable to annual variation in the length of time available for embryonic development, such that directional selection for earlier nesting will exist. Therefore, one possible outcome is that reaction norm elevation for phenological traits (e.g., nesting date) will be lower in ON than in MI (Fig. 2b). Alternatively, selection may act primarily on reaction norm slope, in which case we expect the slope to be steeper in ON than in MI, but elevation might be similar between the locales after accounting for differences in temperature (Fig. 2c). Finally, if there is a genetic correlation between the elevation and slope of the reaction norm for nesting date (reviewed by Brommer 2013), then counter-gradient selection might result in a shallower reaction norm slope in ON compared to MI (Jinks and Connolly 1975, Gavrilets and Scheiner 1993, Schlichting and Pigliucci 1993, Springate et al. 2011); in other words, reaction norm slope might evolve primarily as a correlated response to selection on reaction norm elevation (Fig. 2d). To complement the population-level analysis, we used random regression procedures to investigate whether among-individual variation in nest date plasticity is less prevalent in the relatively cold ON environment than in the relatively warm MI environment, as convergence of individual reaction norms is one possible outcome of persistent directional selection on reaction

norm slope and elevation (Fisher 1930, Springate et al. 2011).

MATERIALS AND METHODS

Study system

Snapping turtles and painted turtles are excellent systems to study phenotypic plasticity of reproductive traits. Their long reproductive lives, ~35 and 40 yr, respectively (Congdon et al. 2003, 2008), ensure that individuals are subjected to substantial environmental variation during their lifetime, thereby exposing most of the reaction norm to selection and facilitating the evolution of adaptive plasticity (de Jong 2005). In the present study, turtles at the ON locale are exposed to lower temperatures, which results in a later start to the activity season. For example, the average initiation of nesting is 12.4 ± 6.6 d later for snapping turtles (mean ± SD; range 2–23 d), and 13.1 ± 3.9 d later (range 5–18 d) for painted turtles at the ON locale compared to the MI locale (R. J. Brooks and J. D. Congdon, *unpublished data*). At both sites, female painted turtles typically produce 1 or 2 clutches each spring (Congdon and Tinkle 1982; Samson 2003, McGuire et al. 2011) with nests of second clutches constructed 14–21 d after the first clutch. Snapping turtles produce a maximum

of 1 clutch each spring (Congdon et al. 2008) and typically initiate nesting 5–7 d after painted turtles begin nesting. Ultimately, the length of time in which temperatures are high enough to support embryonic development is shorter in ON than MI, and in general, painted turtle second clutches and snapping turtle clutches have relatively less time to complete development than the first clutch of painted turtles at both locales.

In natural nests of both species of turtles, embryos require between 60 and 90 d to complete development (Congdon et al. 1987, Cagle et al. 1993). At both locations, hatching must occur before winter, as embryos of both species that remain in eggs do not survive the winter (Obbard and Brooks 1981, Packard and Packard 2001). Painted turtle hatchlings that emerge from the egg have the ability to tolerate sub-freezing temperatures in the nest (Packard and Packard 2001, Costanzo et al. 2008) and, in both populations, the majority of hatchlings over-winter in the nest and emerge in the spring (Breitenbach et al. 1984, Nagle et al. 2000, Riley et al. 2014). Snapping turtle hatchlings cannot tolerate freezing, and fall emer-gence of hatchlings from the nest cavity is essential, as hatchlings overwinter in wetlands (Obbard and Brooks 1981, Costanzo et al. 1995). Embryonic survivorship is more stochastic in ON than in MI (Brooks et al. 1988) and variation in embryonic survival is strongly related to annual variation in summer temperature in ON, but not in MI (Appendix S1: Fig. S1). Nesting relatively early at a given temperature (i.e., a low reaction norm elevation), and/or exhibiting a relatively steeper norm of reaction of nest timing to temperature (Fig. 2) may therefore com-prise adaptation of nest timing to time constraints, and adaptation is most likely to occur in traits where the time constraint is strongest, i.e., in snapping turtle clutches, and in painted turtle second clutches.

Long-term monitoring

Nesting data for painted turtles and snapping turtles were collected as part of two long-term mark–recapture studies (Table 1), spanning 41 yr in Algonquin Park, in Ontario, Canada (ON, 45°34′40″ N, 78°30′49″ W; Fig. 1) and 43 yr on the University of Michigan E. S. George

Reserve (ESGR), Michigan, USA (MI, 42°27′46″ N, −83°57′50″ W; Fig. 1). Most turtles in both populations have been given unique markings so that individuals can be identified (Table 1).

In MI, the nesting ecology of painted turtles and snapping turtles was studied each year from 1976 to 2007. Painted turtles were studied in East Marsh at the MI site, which is approximately 4 ha in size and predominantly <1.5 m deep. Snapping turtles were studied in East Marsh and a nearby (<2 km west) 7.3 ha wetland complex con-sisting of a swamp, marsh, and pond, all with water depths less than 2 m. East Marsh was encircled by a drift fence from 1983 to 2006 that was monitored at approxi-mately 20 minute intervals during all days of all nesting seasons to capture females exiting to nest. Female painted turtles were identified, and their reproductive condition determined by palpation and x-radiography; an average of 23% (min–max, 6–40%) of reproductive females pro-duced a second clutch within a season (McGuire et al. 2011). Female snapping turtles were captured at the East Marsh fence, and during frequent searches of nesting areas adjacent to both aquatic areas on the MI site.

In ON, two populations of snapping turtles were moni-tored. The Lake Sasajewun (ON-Lake) population was monitored from 1972 to 2013 and the Arowhon Ponds population (ON-Pond) was monitored from 1989 to 2013. The ON-Lake population inhabited a network of rivers, lakes, and wetlands, but the majority of the females nested on a 100-m section of land beside a dam on the south shore of Lake Sasajewun. During the nesting season, the dam was patrolled every 30 minutes from approximately 06:00 to 23:59. The ON-Pond population of snapping turtles was about 25 km west of ON-Lake, and the turtles inhabited two ponds that were 500 m apart, predominantly 1.5 m in depth, between 0.9 and 1.8 ha in size. Females in ON-Pond nested on an abandoned railway embankment that bisects the ponds. During the reproductive season, the embankment was patrolled every 30–40 min from approx-imately 06:00 to 23:59. A large population of painted turtles also inhabited the same ponds and nested on the same embankment, and they were monitored from 1990 to 2012 along with the snapping turtles (for more details on nesting patrols see Rollinson et al. [2012]).

TABLE 1. Summary data for analysis of individual plasticity in phenological timing for painted turtles and snapping turtles.

Species	Population	N observations	N females	Median N observations per female†	N years	Incubation temperature (°C)‡
Painted turtle	ON	596	199	2 (1–11)	23 (1990–12)	17.83 (1.12)
Painted turtle	MI	493	157	2 (1–18)	28 (1980–07)	21.28 (1.10)
Snapping turtle	ON-Pond	259	46	5 (1–15)	25 (1989–13)	18.19 (0.92)
Snapping turtle	ON-Lake	893	106	7 (1–33)	41 (1972–13)	18.19 (0.92)
Snapping turtle	MI	1124	199	3 (1–25)	32 (1976–07)	21.47 (1.072)

Note: ON, Ontario, Canada; MI, Michigan, USA; N, number of.
† Range given in parentheses.
‡ Mean air temperature of the 90-d period after the first nest of the season for N years; we tested the assumption that air tem-peratures from weather stations were correlated with nest temperatures from wild nests in both populations (Appendix S1: Fig. S2) SD is given in parentheses.

Determining the temperature periods that predict nesting

Daily minimum and maximum air temperatures were obtained from weather stations situated <50 km from the respective study locations (Table 1). Mean daily temperature was calculated as the average of the daily minimum and maximum. We used a sliding window approach (e.g., Brommer et al. 2008, Porlier et al. 2012) to identify the period that best predicted the onset of the nesting season (first nest date) for each population. For the first nest of painted turtles and snapping turtles, we estimated the correlation between first nest date for each year and the mean temperature for all 10–30 d periods beginning on 1 January of each year and terminating the day before the earliest nest ever observed in a given population. The time period with the highest adjusted r^2 was chosen as the temperature period that best predicted the onset of nesting and used in further analyses.

Accurately determining the temperature period that predicts nesting requires accurate estimates of the first nest date for each population. However, sampling efficiency probably increased with time in each study population (Appendix S1: Fig. S2). Given that the vast majority of female painted turtles and snapping turtles in each population typically produce at least one clutch per year (Congdon et al. 2003, Samson 2003), we lowered the potential bias imposed by low annual sample sizes by excluding data from years in which the number of nests observed was <60% of the maximum number of nests observed in any year (60% roughly corresponds to 1 standard deviation in the data set).

We used a sliding window approach to identify the temperature period that best predicts the length of the interval between the first and second clutch for painted turtles (the within-season "inter-nesting interval" in days). We tested the correlation between length of the mean annual inter-nesting interval and the mean temperature of all 10–30 d periods, beginning on the first nest date of each year. We selected the period with the highest adjusted r^2 value for further analyses. We used data only from years in which at least 10 pairs of first and second clutches were collected, to reduce errors in the estimate of the population-mean inter-nesting interval associated with small sample sizes.

Population-mean response to temperature

We tested whether plasticity of nest timing (i.e., first nest date of each year for both species, inter-nesting interval for painted turtles only) with respect to temperature differed between populations using ANCOVA. For each population pair, population was included as a fixed effect, and temperature was included as a covariate, using the temperature estimated from sliding window analyses for each population. If the Population × Temperature interaction term was not statistically significant ($\alpha = 0.05$), we removed it and re-ran the model with main effects only. For all analyses, we only used nesting data from same years that were used to estimate the temperature window.

Population-level analyses are based on the assumption that mean air temperatures map similarly onto mean female body temperature in all populations within each species. The assumption seems reasonable as populations of painted turtles and snapping turtles in ON-Pond and MI inhabit small ponds that are similar in perimeter and depth (see above). Nevertheless, we explicitly assessed the influence of habitat variation on the population-level response to temperature to clarify whether any population-level differences detected among locales can be attributed to habitat differences. To do so, we assessed phenotypic plasticity in different habitats in ON, specifically by comparing snapping turtles in ON-Pond to those in ON-Lake. Given that the ON-Pond and ON-Lake snapping turtle populations are not genetically differentiated at neutral loci (Davy 2013), any difference in plasticity can likely be attributed to differences in the thermal characteristics of their respective habitats. Further, we compared snapping turtles in MI to snapping turtles in ON-Pond and in ON-Lake, to help clarify whether phenotypic differences observed between the two locales are due to a bias that we imposed by using mean air temperatures (rather than mean body temperatures) in our analysis. In all cases we use $\alpha = 0.05$.

Individual plasticity

Females in both populations of painted turtles typically produced one or two clutches per year, with the second nest constructed approximately two to three weeks after the first (Table 2). Occasionally, the first nest of a female was missed by surveyors and second clutches may have been misidentified as first clutches. For analyses of individual plasticity, we eliminated the possibility of clutch misidentification by including nesting events only from female painted turtles for which both first and second clutches were observed. For snapping turtles, all nesting data were included because this species produces only one clutch per year at both locations.

We assessed among-individual variation in phenotypic plasticity (the interaction of individual and environment; I × E) using random regression models (Nussey et al. 2007, Martin et al. 2011). First nest date (nest date), and inter-nesting interval were fit as a continuous function of the year-specific air temperature of the temperature period (see *Determining the temperature periods that predict nesting*). Phenological traits were centered on the mean at the population level across all years, and temperature was centered on the mean at the individual level, such that temperature values for each turtle represent the range of environments it experienced, thereby allowing plasticity to be assessed (Charmantier and Gienapp 2014). For nest date analyses, both the relative age of each turtle with respect to its first observed year of nesting

TABLE 2. Results of sliding window analysis identifying time periods (Calendar Date 1 is 1 January) for which mean daily air temperature (°C) was most highly correlated with the date of first nest (nest date) and the inter-nesting interval (interval).

Species	Population	N years†	First day/interval‡,§	Window start/duration¶	Temperature (°C)#	r§
Nest date						
Painted turtle	ON	23 (17)	155.1 ± 7.0	124 (29)	11.81 ± 1.69	−0.88
Painted turtle	MI	30 (26)	141.6 ± 8.2	125 (27)	15.91 ± 2.11	−0.80
Snapping turtle	ON-Pond	25 (13)	160.1 ± 7.4	123 (30)	11.88 ± 1.60	−0.89
Snapping turtle	ON-Lake	42 (38)	161.4 ± 6.5	131 (24)	12.78 ± 1.98	−0.74
Snapping turtle	MI	32 (26)	146.4 ± 7.0	121 (30)	15.3 ± 2.0	−0.76
Interval						
Painted turtle	ON	23 (15)	16.68 ± 3.16	30	17.30 ± 1.36	−0.75
Painted turtle	MI	28 (20)	19.76 ± 4.64	29	19.40 ± 1.30	−0.87

† Value in parentheses is the number of years used in the sliding window analysis.
‡ Mean first day/interval ± SD.
§ Values based only on years used in sliding window analysis.
¶ Window start and duration for nest date; window start for interval.
# Values based on entire population-level data set (mean ± SD).

(NAge) and the year-specific temperatures that were defined using our sliding window analysis (temperature) were modeled as fixed effects. While keeping the fixed effects unchanged, we extended the random effects structure hierarchically to test patterns of variation in individual phenotypic plasticity using Proc Mixed (SAS v 9.4; SAS Institute, Cary, North Carolina, USA). We allowed residual variance to vary with temperature, thereby providing a conservative test for individual plasticity (Brommer 2013). Ultimately, we tested whether among-individual variation exists in average values of nest date (I, individual intercepts/elevations for nest date), whether individuals vary in their response to temperature (I × E, individual slopes and intercepts/elevations for nest date), and we tested the strength of the phenotypic covariance between slopes and intercepts. The statistical significance of random effects was assessed using a likelihood ratio test after fitting models with REML (Pinheiro and Bates 2000). We successively compared the following models:

Model 1: Nest date = NAge + Temperature,
Model 2: Nest date = NAge + Temperature, random = Year
Model 3: Nest date = NAge + Temperature, random = Year + Individual Identity (intercept)
Model 4: Nest date = NAge + Temperature, random = Year + Individual Identity (intercept) + Individual Identity × Temperature (slope)

The same procedures were followed to model individual variation in the inter-nesting interval, although NAge was not included in the models as it was never an important predictor of inter-nesting interval (data not shown). Individual-level analyses were not performed for ON-Lake snapping turtles because differences among the diverse habitat types inhabited by turtles at this site (ponds, lakes, and streams) render interpretation of I × E difficult.

RESULTS

Determining the temperature periods that predict nesting

For all species and populations, the best temperature window (i.e., the one most highly correlated with first nest date) began in early May and lasted about four weeks (Table 2). The temperature period was remarkably consistent within species and between populations, facilitating a biologically meaningful comparison of plasticity within species (Nussey et al. 2007). In all cases, we found a strong negative correlation between temperature and first nest date (r between −0.74 and −0.83), indicating that female turtles of both species nest earlier in years with warmer springs (Fig. 3). Temperature was also negatively related to the length of the inter-nesting interval in both painted turtle populations (Table 2, Fig. 3).

Population-mean response to temperature

Painted turtles in MI experienced warmer temperatures than turtles in ON (Fig. 3). After accounting for differences in temperature, painted turtles in ON and MI did not differ in first nest date (Appendix S1: Table S1), and the slope of the relationship between nest date and temperature was strikingly similar for both populations (Fig. 3). Yet, a different pattern was observed for inter-nesting intervals: after accounting for differences in temperature between sites, significantly shorter inter-nesting intervals were observed in ON compared to MI painted turtles (Fig. 3). The slope of the relationship between inter-nesting interval and temperature was less negative in ON than in MI painted turtles, but the difference was not significant ($P = 0.087$) (Fig. 3; Appendix S1: Table S1).

After accounting for temperature differences between locales, both the ON-Pond and ON-Lake snapping turtle populations exhibited a significantly later mean nest date
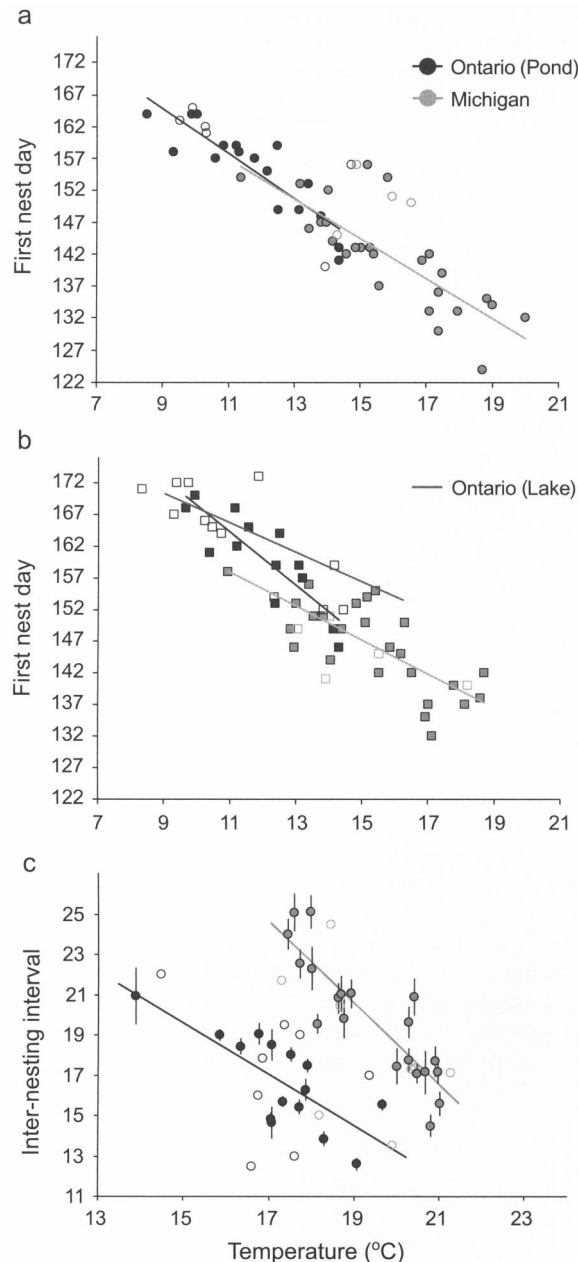
FIG. 3. Phenological traits as a function of the temperature of the window that best predicts the onset of (a and b) nesting season or (c) the inter-nesting interval. Open symbols are from years with small sample sizes, and these years were not used to determine the temperature window for a given population; these points were also not included in statistical analyses. Appendix: Table S1 has statistical significance values for population-level comparisons. (a) First nest date for painted turtles and (b) snapping turtles as a function of temperature. Note that data points from Ontario lake have been omitted for clarity in panel b. (c) The relationship between inter-nesting interval and temperature for painted turtles. Values are mean ± SE. [Color figure can be viewed at wileyonlinelibrary.com]

than the MI snapping turtles (Fig. 3). However, the slope of the relationship between nest date and temperature did not differ in any pairwise test between ON-Lake,

ON-Pond, and MI snapping turtles (Fig. 3; Appendix S1: Table S1). Finally, the relationship between nest date and temperature was significantly shallower for the ON-Lake snapping turtles compared to ON-Pond turtles (Fig. 3; Appendix S1: Table S1).

Individual plasticity

In all populations of painted turtles and snapping turtles, significant variation in nest date was associated with individual identity (I). Within both painted turtle populations, no among-individual variation in plasticity (I × E) was observed (Table 3; Appendix S1: Table S2), indicating that the slope of the relationship between temperature and nest date was similar among individuals within each population (Fig. 4). Snapping turtles in MI did not exhibit significant variation in I × E for nest date (Table 3; Appendix S1: Table S3), but significant variation in I × E was observed among snapping turtles in ON-Pond. The ON-Pond snapping turtles exhibited greater among-individual variation in nest timing in warm springs (Fig. 4), and the intercept and slope of nest date were strongly correlated ($r = 0.998$). Finally, in both painted turtle populations, significant variation in the length of inter-nesting intervals was associated with individual identity (I), but among-individual variation in plasticity (I × E) was not significant in either population (Table 3; Appendix S1: Table S4).

We note that there were differences in the sampling design of the two long-term studies, such that there was a greater chance of accurately quantifying nest date of individuals in the MI population, compared to the ON populations (Appendix S1). We performed simulations to estimate how our ability to detect significant variation in I and I × E is affected by uncertainty in individual nest date. We found that sampling uncertainty, which is probably greater in ON, is likely to have decreased the amount of variation attributable to I, such that variation in I is likely underestimated in our plasticity analysis of ON populations. However, our simulations also showed that a failure to detect significant I × E in all MI models cannot be attributed to sampling design, as increasing the uncertainty in our MI datasets did not increase the likelihood of detecting significant I × E (Appendix S1: Fig. S3).

DISCUSSION

We used a conceptual model (Fig. 2) to explore phenotypic plasticity in populations of two turtle species across a large spatial scale, following a comparative mensurative approach (Hulbert 1984). Given that the short activity season reduces embryonic survival in northern environments (Appendix S1: Fig. S1), we expected that northern populations in Ontario (ON) might exhibit reaction norms that are lower in elevation (i.e., earlier temperature-corrected mean phenological traits) than more southern populations in Michigan (MI; Fig. 2b). We also expect that reaction norm slope for nest timing

TABLE 3.   Statistical significance of adding random terms in mixed-effects models that tested the relationship between nest date and temperature, and for painted turtles only, the relationship between inter-nesting interval and temperature.

Species, trait, population, and parameters	Log-likelihood	Model comparison	df	χ^2	Pseudo r^2
Painted turtle					
Nest date					
ON					
A,T	−1,788.6				
A,T, Year	−1,528.1	1 vs. 2	1	521.1**	
A,T, Year, I	−1,471.5	2 vs. 3	1	113.1**	0.464
A,T, Year, I, I × T	−1,470.0	3 vs. 4	2	3.1	0.0525
MI					
A,T	−1,547.1				
A,T, Year	−1,471.6	1 vs. 2	1	151.0**	
A,T, Year, I	−1,430.7	2 vs. 3	1	81.8**	0.419
A,T, Year, I, I × T	−1,430.0	3 vs. 4	2	1.5	0.00
Snapping turtle					
Nest date					
ON-Pond					
A,T	−795.2				
A,T, Year	−712.6	1 vs. 2	1	165.1**	
A,T, Year, I	−687.8	2 vs. 3	1	49.7**	0.385
A,T, Year, I, I × T	−683.6	3 vs. 4	2	8.3*,†	0.0522
MI					
A,T	−3,564.2				
A,T, Year	−3,313.3	1 vs. 2	1	501.9**	
A,T, Year, I	−3,166.5	2 vs. 3	1	293.6**	0.401
A,T, Year, I, I × T	−3,165.4	3 vs. 4	2	2.1	0.00
Painted turtle					
Interval					
ON					
T	−1,484.0				
T, Year	−1,385.3	1 vs. 2	1	197.3**	
T, Year, I	−1,374.3	2 vs. 3	1	22.1**	0.190
T, Year, I, I × T	−1,374.2	3 vs. 4	2	0.2	0.00
MI					
T	−1,388.0				
T, Year	−1,372.1	1 vs. 2	1	31.9**	
T, Year, I	−1,367.8	2 vs. 3	1	8.5**	0.120
T, Year, I, I × T	−1,367.8	3 vs. 4	2	0.00	0.00

Notes: Temperature (T) and N-Age (A) were modeled as fixed effects; year and individual (I) were modeled as random effects. $*P < 0.05$; $**P < 0.01$.
† Correlation between random slope and intercept = 0.998.

might be steeper in ON compared to MI (Fig. 2c). Of the three comparisons made, only the mean length of the inter-nesting interval was in the predicted direction, as the interval was shorter in ON than in MI (Fig. 3c), reflecting a lower reaction norm elevation in ON. In contrast, mean first nesting date for snapping turtles was *later* in ON than in MI (Fig. 3b), reflecting a relatively high reaction norm elevation in ON; this finding does not support any of our predictions (Fig. 2). Perhaps most interesting was that population-level patterns in reaction norm slope were broadly similar within each species we examined (Fig. 3). Finally, we also found that the only population to exhibit among-individual variation (I × E) in phenotypic plasticity was the ON snapping turtles

(Table 3, Fig. 4). Overall, our results suggest that time constraints have had an effect on the evolution of reaction norm elevation in painted turtles, particularly with respect to inter-nesting intervals, but in snapping turtles there was no straightforward association between patterns of nesting phenology and time constraints.

Reaction norm elevation (i.e., population-mean trait values) differed between painted turtle populations in a manner that is consistent with time constraints on nest timing. Weak time constraints on first clutches of painted turtles may explain why reaction norm slope for first nest date was similar in the ON and MI populations, and why reaction norm elevation is similar in both populations after accounting for differences in the thermal environment
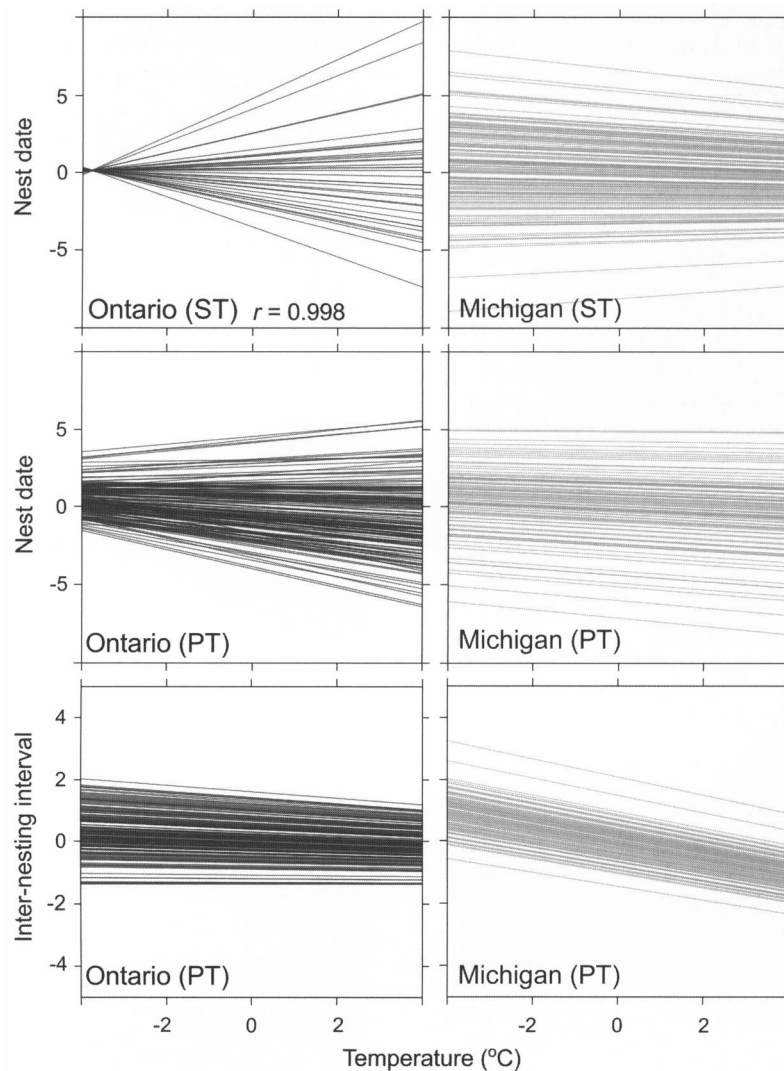
FIG. 4. Fitted values for linear models estimating the interaction of individual identity and environment (I × E) as a function of temperature for phenological traits: nest date for snapping turtles (ST) and painted turtles (PT), and inter-nesting interval for painted turtles. The effect of year (all models) and age of turtles was held constant, illustrating patterns of I × E for all individuals with more than one observation. Phenological traits are centered on the population mean, and temperature is centered with respect to the temperatures experienced by individual turtles. [Color figure can be viewed at wileyonlinelibrary.com]

(Fig. 3a). Embryos of first nests may have a higher overall probability of survival than second nests, given that embryos in first nests have much more time (~14–21 d) to develop and hatch than embryos in second nests (Table 2). In fact, a stronger time constraint on second clutches is clearly reflected in the relatively low reaction norm elevation for inter-nesting interval among ON painted turtles (Fig. 3c). Although we did not measure selection, we assume that the inter-nesting interval is under relatively strong and negative directional selection in ON (Griffith and Watson 2005, Refsnider and Janzen 2012), and that the difference in reaction norm elevation we observed reflects long-term response to selection.

Reaction norm slope of the inter-nesting interval was shallower in ON compared to MI, although the difference

was not statistically significant (Appendix S1: Table S1). Nevertheless, it is interesting to note that the direction of this potential difference is in accordance with Jinks-Connolly rule (Jinks and Connolly 1975). Specifically, when selection on reaction norm elevation is in the direction opposite to that of environmental influence (e.g., selection for early nesting in cold environments), an evolutionary decrease in reaction norm slope can occur, provided that the reaction norm elevation and slope are genetically correlated (Fig. 2d). It is not known whether a genetic correlation exists in the populations we studied, but analysis of I × E in snapping turtles (see below) revealed a strong phenotypic correlation between elevation and slope (i.e., $r = 0.998$), suggesting that a genetic correlation may also exist (see also Brommer 2013).

Further research is required to determine if the (nonsignificant) decrease in reaction norm slope that was observed in ON painted turtles can be explained by strong counter-gradient selection coupled with genetic constraints.

We expected that time constraints on nest timing would also be relatively strong for northern snapping turtles (e.g., Appendix S1: Fig. S1). This expectation arises because hatchlings must emerge from nests and seek aquatic refugia before winter (Packard and Packard 2001, Ultsch et al. 2007), and snapping turtles also exhibit a longer average incubation duration and later first nest timing than painted turtles (Ernst and Lovich 2009). Yet, after accounting for temperature differences between locales, we found that ON snapping turtles had a higher reaction norm elevation (i.e., initiated nesting relatively later) than MI snapping turtles, even though reaction norm slope was similar between locales (Fig. 3; Appendix S1: Table S1). It is difficult to explain why reaction norm elevation was higher in ON snapping turtles (e.g., Fig. 2), and it seems unlikely that this finding can be attributed to any obvious source of bias associated with habitat type. For instance, we observed that the slope of the relationship between nest date and temperature in ON-Lake snapping turtles was shallower than in the ON-Pond population; this probably reflects more gradual heating of lakes relative to shallow ponds, rather than a true difference in reaction norm slope. Yet, neither ON-lake nor ON-Pond differed from MI in reaction norm slope, and both ON populations exhibited higher reaction norm elevation than the MI population (Appendix: Table S1). Further, temperature windows were very similar for ON and MI (Table 2), such that population comparisons are biologically meaningful (Nussey et al. 2007, Husby et al. 2010).

The apparent mismatch between phenotype and environment in ON snapping turtles may, in part, reflect maladaptation to northern environments. Both a close examination of our results and a comparison of the natural history of painted turtles and snapping turtles lends some credence to the maladaptation hypothesis. It is likely that northern populations of painted turtles and snapping turtles have inhabited cool, thermally challenging environments for only a short time since the end of the Wisconsinan glaciation (Fig. 1a). It was not until approximately 10,000 years before present (ybp) that rapid warming and the retreat of the Laurentide ice sheet allowed reptiles to recolonize the Great Lakes region (Anderson and Lewis 1992, Holman 1992). Paleontological evidence suggests that painted turtles were established in Michigan by 5,000 ybp (Holman 1992). Considering that maximum longevities of painted turtles and snapping turtles are at least 40 and 60 yr respectively, and that both species have long cohort generation times (painted turtles, ~16 yr; snapping turtles, ~25 yr in MI, ~31 yr in ON), relatively few generations have passed since turtles colonized Ontario (Galbraith and Brooks 1987; Galbraith et al. 1989, Congdon et al. 1994, 2003,

2008, Armstrong and Brooks 2014). Based on a conservative assumption that recolonization of northern Ontario occurred around 5000 ybp, then only about 300 generations of painted turtles and about 160 generations of snapping turtles have occurred. Maladaptation to the thermally challenging environment in the north might therefore be more likely in snapping turtles, compared to painted turtles.

Evidence from the present and other studies suggests that painted turtles may be better adapted to colonizing northern environments. For instance, painted turtles feature a greater range of physiological adaptations to cool environments, such as anoxia tolerance in adults and freeze tolerance in hatchlings (St. Clair and Gregory 1990, Packard and Packard 2001, Costanzo et al. 2008), with the result that painted turtles range further north than snapping turtles (Ernst and Lovich 2009). In the present study, the only evidence of phenological adaptation to the north comes from painted turtles, where the ON population exhibited shorter inter-nesting intervals than in MI (Fig. 3). It is also interesting that ON snapping turtles exhibited a greater propensity for significant I × E in first nest date than did ON painted turtles (Fig. 3, Table 3). If I × E in northern snapping turtles also indicates underlying G × E (e.g., Husby et al. 2010), then our findings suggest reaction norms might be optimized in MI but not ON snapping turtle, despite stronger selection for early nest timing in the ON population (Appendix S1: Fig. S1; see also Charmantier et al. 2008, Springate et al. 2011, Porlier et al. 2012).

Although we find the maladaptation hypothesis attractive, we acknowledge there are traits other than phenological timing upon which selection can act when time constraints exist. For example, the development rate of embryos is known to vary along latitudinal clines (Lonsdale and Levinton 1985, Conover and Schultz 1995, Ewert et al. 2005), where turtle embryos from colder geographic regions often develop more rapidly than embryos from warmer regions when reared in a common environment (Ewert et al. 2005, Zhao et al. 2015). Thus, a higher reaction norm elevation for snapping turtle nest timing might be balanced by rapid embryonic development in the north, and rate of embryonic development will ultimately affect the strength of selection for early reproduction. Future work would certainly benefit by simultaneously considering nesting phenology and rates of embryonic development.

In summary, we found evidence of phenological adaptation to time constraints in painted turtles but not snapping turtles. Most importantly, adaptation occurred by virtue of changes in reaction norm elevation, whereas none of the population-trait comparisons revealed differences in reaction norm slope. The similarities in reaction norm slopes might be related to historically recent recolonization of northern environments following the Wisconsonian glaciation, coupled with both the long generation time of turtles and perhaps a complex genetic architecture that underlies plasticity. Last, we also provide

some evidence that a population of snapping turtles near their northern range limit might be phenologically maladapted to the environment, as revealed by pronounced among-individual variation in plasticity and, at the population level, a relatively high reaction norm elevation. More broadly, the present study emphasizes that time constraints imposed by seasonality can play a role in the evolution of reaction norm elevation for phenological timing, but other factors may ultimately influence reaction norm slope, especially among long-lived reptiles that inhabit postglacial environments.

LITERATURE CITED

Altwegg, R. 2002. Predator-induced life-history plasticity under time constraints in pool frogs. Ecology 83:2542–2551.

Anderson, T. W., and F. F. M. Lewis. 1992. Climatic influences of deglacial drainage changes in southern Canada at 10 to 8 ka suggested by pollen evidence. Géographie Physique et Quaternaire 46:255–272.

Armstrong, D. P., and R. J. Brooks. 2014. Estimating ages of turtles from growth data. Chelonian Conservation and Biology 13:9–15.

Bobyn, M. L., and R. J. Brooks. 1994a. Incubation conditions as potential factors limiting the northern distribution of snapping turtles, Chelydra serpentina. Canadian Journal of Zoology 72:28–37.

Bobyn, M. L., and R. J. Brooks. 1994b. Interclutch and interpopulation variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (Chelydra serpentina). Journal of Zoology 233:233–257.

Breitenbach, G. L., J. D. Congdon, and R. C. van Loben Sels. 1984. Winter temperatures of Chrysemys picta nests in Michigan: effects on hatchling survival. Herpetologica 40:76–81.

Brommer, K. E. 2013. Phenotypic plasticity of labile traits in the wild. Current Zoology 59:485–505.

Brommer, J. E., K. Rattiste, and A. J. Wilson. 2008. Exploring plasticity in the wild: laying date–temperature reaction norms in the common gull Larus canus. Proceedings of the Royal Society B 275:687–693.

Brooks, R. J., D. A. Galbraith, E. G. Nancekivell, and C. A. Bishop. 1988. Developing management guidelines for snapping turtles. Pages 174–179 in R. C. Szaro, K. E. Severson, and D. R. Patton, editors. Symposium on management of amphibians, reptiles, and small mammals in North America. USDA Forest Service General Technical Report. USDA Forest Service, Fort Collins, Colorado, USA.

Cagle, K. D., G. C. Packard, K. Miller, and M. J. Packard. 1993. Effects of the microclimate in natural nests on development of embryonic painted turtles, Chrysemys picta. Functional Ecology 7:653–660.

Charmantier, A., and P. Gienapp. 2014. Climate change and timing of avian breeding and migration: evolutionary versus plastic changes. Evolutionary Applications 7:15–28.

Charmantier, A., R. McCleery, and L. Cole. 2008. Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science 320:800–803.

Congdon, J. D., and D. W. Tinkle. 1982. Reproductive energetics of the Painted Turtle (Chrysemys picta). Herpetologica 38:228–237.

Congdon, J. D., D. W. Tinkle, G. L. Breitenbach, and R. C. van Loben Sels. 1983. Nesting ecology and hatching success in the Blanding's turtle Emydoidea blandingi. Herpetologica 39:417–429.

Congdon, J. D., G. L. Breitenbach, R. C. van Loben Sels, and D. W. Tinkle. 1987. Reproduction and nesting ecology of snapping turtles (Chelydra serpentina) in southeastern Michigan. Herpetologica 43:39–54.

Congdon, J. D., A. E. Dunham, and R. C. van Loben Sels. 1994. Demographics of common snapping turtles (Chelydra serpentina): Implications for conservation and management of long-lived organisms. American Zoologist 34:397–408.

Congdon, J. D., R. D. Nagle, O. M. Kinney, R. C. van Loben Sels, T. Quinter, and D. W. Tinkle. 2003. Testing hypotheses of aging in long-lived painted turtles (Chrysemys picta). Experimental Gerontology 38:765–772.

Congdon, J. D., J. L. Greene, and R. J. Brooks. 2008. Reproductive and nesting ecology of female snapping turtles. Pages 123–134 in A. C. Steyermark, M. S. Finkler, and R. J. Brooks, editors. Biology of the snapping turtle (Chelydra serpentina). Johns Hopkins University Press, Baltimore, Maryland, USA.

Conover, D., and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology and Evolution 10:248–252.

Costanzo, J. P., J. B. Iverson, M. F. Wright, and R. E. Lee. 1995. Cold hardiness and overwintering strategies of hatchlings in an assemblage of northern turtles. Ecology 76:1772–1785.

Costanzo, J. P., R. E. Lee, and G. R. Ultsch. 2008. Physiological ecology of overwintering in hatchling turtles. Journal of Experimental Zoology 309A:297–379.

Czesak, M. E., C. W. Fox, and J. B. Wolf. 2006. Experimental evolution of phenotypic plasticity: How predictive are cross-environment genetic correlations? American Naturalist 168:323–335.

Davy, C. M. 2013. Conservation genetics of freshwater turtles. Dissertation. University of Toronto, Toronto, Ontario, Canada.

de Jong, G. 2005. Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. New Phytologist 166:101–118.

Ejsmond, M. J., O. Varpe, M. Czarnoleski, and J. Kozlowski. 2015. Seasonality in offspring value and trade-offs with

growth explain capital breeding. The American Naturalist 186:E111–E125.

Ernst, C. H., and J. E. Lovich. 2009. Turtles of the United States and Canada. Johns Hopkins University Press, Baltimore, Maryland, USA.

Ewert, M. A. 1985. Embryology of turtles. Pages 75–267 *in* A. C. Gans, editor. Biology of the Reptilia development. John Wiley & Sons, New York, New York, USA.

Ewert, M. A., J. W. Lang, and C. E. Nelson. 2005. Geographic variation in the pattern of temperature-dependent sex determination in the American snapping turtle (*Chelydra serpentina*). Journal of Zoology 265:81–95.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford, UK.

Galbraith, D. A., and R. J. Brooks. 1987. Survivorship of adult females in a northern population of common snapping turtles, *Chelydra serpentina*. Canadian Journal of Zoology 65:1581–1586.

Galbraith, D. A., R. J. Brooks, and M. E. Obbard. 1989. The influence of growth rate on age and body size at maturity in female snapping turtles (*Chelydra serpentina*). Copeia 1989:896–904.

Gavrilets, S., and S. M. Scheiner. 1993. The genetics of phenotypic of reaction norm shape V. Evolution of reaction norm shape. Journal of Evolutionary Biology 48:31–48.

Ghalambor, C. K., J. K. McKay, S. P. Carrol, and D. N. Reznick. 2007. Adaptive versus non adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21:394–407.

Griffith, T. M., and M. A. Watson. 2005. Stress avoidance in a common annual: Reproductive timing is important for local adaptation and geographic distribution. Journal of Evolutionary Biology 18:1601–1612.

Holman, J. A. 1992. Late Quaternary herpetofauna of the Central Great Lakes region, USA: Zoogeographical and paleoecological implications. Quaternary Science Reviews 11:345–351.

Hurlbert, S. H. 1984. Pseudoreplication and the design on ecological field experiments. Ecological Monographs 54:187–211.

Husby, A., D. H. Nussey, M. E. Visser, A. J. Wilson, B. C. Sheldon, and L. E. B. Kruuk. 2010. Contrasting patterns of phenotypic plasticity in reproductive traits in two great tit (*Parus major*) populations. Evolution 64:2221–2237.

Jinks, J. L., and V. Connolly. 1975. Determination of the environmental sensitivity of selection lines by the selection environment. Heredity 34:401–406.

Levins, R. 1968. Evolution in changing environments: some theoretical explorations. Princeton University Press, Princeton, New Jersey, USA.

Lonsdale, D. J., and J. S. Levinton. 1985. Latitudinal differentiation in copepod growth: an adaptation to temperature. Ecology 66:1397–1407.

Lourdais, O., R. Shine, X. Bonnet, M. Guillon, and G. Naulleau. 2004. Climate affects embryonic development in a viviparous snake, *Vipera aspis*. Oikos 104:551–560.

Lovich, J., M. Agha, M. Meulblok, K. Meyer, J. Ennen, C. Loughran, S. Madrak, and C. Bjurlin. 2012. Climatic variation affects clutch phenology in Agassiz's desert tortoise *Gopherus agassizii*. Endangered Species Research 19:63–74.

Martin, J. G. A., D. H. Nussey, A. J. Wilson, and D. Réale. 2011. Measuring individual differences in reaction norms in field and experimental studies: a power analysis of random regression models. Methods in Ecology and Evolution 2:362–374.

McGuire, J. M., J. D. Congdon, K. T. Scribner, and J. D. Capps. 2011. Variation in female reproductive quality and reproductive success of male painted turtles (*Chrysemys*

picta marginata). Canadian Journal of Zoology 89:1136–1145.

Menzel, A., N. Estrella, and P. Fabian. 2001. Spatial and temporal variability of the phenological seasons in Germany from 1951 to 1996. Global Change Biology 7:657–666.

Nagle, R. D., O. M. Kinney, J. D. Congdon, and C. W. Beck. 2000. Winter survivorship of hatchling painted turtles (*Chrysemys picta*) in Michigan. Canadian Journal of Zoology 78:226–233.

Nussey, D. H., E. Postma, P. Gienapp, and M. E. Visser. 2005. Selection on heritable phenotypic plasticity in a wild bird population. Science 310:304–306.

Nussey, D. H., A. J. Wilson, and J. E. Brommer. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology 20:831–844.

Obbard, M. E., and R. J. Brooks. 1981. Fate of overwintered clutches of the common snapping turtle (*Chelydra serpentina*) in Algonquin Park, Ontario. Canadian Field-Naturalist 95:350–351.

Packard, G. C., and M. J. Packard. 2001. The overwintering strategy of hatchling painted turtles, or how to survive in the cold without freezing. BioScience 51:199–207.

Parker, A. L., and R. M. Andrews. 2007. Incubation temperature and phenotypic traits of *Sceloporus undulatus*: Implications for the northern limits of distribution. Oecologia 151:218–231.

Pelletier, F., D. Réale, D. Garant, D. W. Coltman, and M. Festa-Bianchet. 2007. Selection on heritable seasonal phenotypic plasticity of body mass. Evolution 61:1969–1979.

Pigliucci, M. 2001. Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Maryland, USA.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: Where are we going now? Trends in Ecology and Evolution 20:481–486.

Pigliucci, M. 2010. Genotype-phenotype mapping and the end of the "genes as blueprint" metaphor. Philosophical Transactions of the Royal Society B 365:557–566.

Pinheiro, J., and D. Bates. 2000. Mixed effect models in S and S-Plus. Springer Verlag, New York, New York, USA.

Porlier, M., A. Charmantier, P. Bourgault, P. Perret, J. Blondel, and D. Garant. 2012. Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within-population comparisons. Journal of Animal Ecology 81:1041–1051.

Refsnider, J. M., and F. J. Janzen. 2012. Behavioural plasticity may compensate for climate change in a long-lived reptile with temperature-dependent sex determination. Biological Conservation 152:90–95.

Riley, J. L., G. J. Tattersall, and J. D. Litzgus. 2014. Potential sources of intra-population variation in painted turtle (*Chrysemys picta*) hatchling overwintering strategy. Journal of Experimental Biology 217:4174–4183.

Roff, D. A. 1997. Evolutionary quantitative genetics. Chapman & Hall, New York, New York, USA.

Rollinson, N., R. G. Farmer, and R. J. Brooks. 2012. Widespread reproductive variation in North American turtles: temperature, egg size and optimality. Zoology 115:160–169.

Rowe, L., and D. Ludwig. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. Ecology 72:413–427.

Rowe, L., D. Ludwig, and D. Schluter. 1994. Time, condition, and the seasonal decline of avian clutch size. American Naturalist 143:698–722.

Samson, J. 2003. The life history strategy of a northern population of midland painted turtles, *Chrysemys picta*

marginata. MSc. thesis, University of Guelph, Guelph, Ontario

Scheiner, S. 1993. Genetics and evolution of phenotypic plasticity. Annual Review of Ecology, Evolution, and Systematics 24:35–68.

Scheiner, S. M., and R. F. Lyman. 1991. The genetics of phenotypic plasticity. II. Response to selection. Journal of Evolutionary Biology 4:23–50.

Schlichting, C., and M. Pigliucci. 1993. Control of phenotypic plasticity via regulatory genes. American Naturalist 142: 366–370.

Schmalhausen, I. I. 1949. Factors of evolution: the theory of stabilizing selection. Blakiston, Oxford, UK.

Springate, D. A., N. Scarcelli, J. Rowntree, and P. X. Kover. 2011. Correlated response in plasticity to selection for early flowering in *Arabidopsis thaliana*. Journal of Evolutionary Biology 24:2280–2288.

St. Clair, R. C., and P. T. Gregory. 1990. Factors affecting the northern range limit of painted turtles (*Chrysemys picta*): winter acidosis or freezing? Copeia 1990:1083–1089.

Stearns, S. 1989. The evolutionary significance of phenotypic plasticity. BioScience 39:436–445.

Stearns, S., and J. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40:893–913.

Telemeco, R. S., M. J. Elphick, and R. Shine. 2009. Nesting lizards (*Bassiana duperreyi*) compensate partly, but not completely, for climate change. Ecology 90:17–22.

Ultsch, G. R., M. Draud, and B. Wicklow. 2007. Post-emergence movements and overwintering of snapping turtle, *Chelydra sperentina*, hatchlings in New York and New Hampshire. Canadian Field-Naturalist 121:178–181.

Yamahira, K., and D. Conover. 2002. Intra-vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology 83:1252–1262.

Zhao, B. O., Y. E. Chen, H. Lu, Z. Zeng, and W. Du. 2015. Latitudinal differences in temperature effects on the embryonic development and hatchling phenotypes of the Asian yellow pond turtle, *Mauremys mutica*. Biological Journal of the Linnean Society 114:35–43.

Supporting Information