Climate Change and Temperature-Dependent Sex Determination: Can Individual Plasticity in Nesting Phenology Prevent Extreme Sex Ratios?

Lisa E. Schwanz*
Fredric J. Janzen
Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011

ABSTRACT

Under temperature-dependent sex determination (TSD), temperatures experienced by embryos during development determine the sex of the offspring. Consequently, populations of organisms with TSD have the potential to be strongly impacted by climatic warming that could bias offspring sex ratio, a fundamental demographic parameter involved in population dynamics. Moreover, many taxa with TSD are imperiled, so research on this phenomenon, particularly long-term field study, has assumed great urgency. Recently, turtles with TSD have joined the diverse list of taxa that have demonstrated population-level changes in breeding phenology in response to recent climate change. This raises the possibility that any adverse impacts of climate change on populations may be alleviated by individual plasticity in nesting phenology. Here, we examine data from a long-term study on a population of painted turtles (*Chrysemys picta*) to determine whether changes in phenology are due to individual plasticity and whether individual plasticity in the timing of nesting has the capacity to offset the sex ratio effects of a rise in climatic temperature. We find that individual females show plasticity in the date of first nesting each year, and that this plasticity depends on the climate from the previous winter. First nesting date is not repeatable within individuals, suggesting that it would not respond to selection. Sex ratios of hatchlings within a nest declined nonsignificantly over the nesting season. However, small increases in summer temperature had a much stronger effect on nest sex ratios than did laying nests earlier in the season. For this and other reasons, it seems unlikely that individual plasticity in the timing of nesting will offset the effects of climate change on sex ratios in this population, and we hypothesize that this conclusion applies to other populations with TSD.

* Corresponding author. Present address: Cary Institute of Ecosystem Studies, Millbrook, New York 12545; e-mail: lisa.schwanz@gmail.com.

The impact of recent climate change on the ecology of living organisms is extensive and often indirect (McCarty 2001; Stenseth et al. 2002; Walther et al. 2002; Parmesan 2006). Many recorded changes, including altered phenology, range shifts, and modified trophic interactions, suggest negative outcomes for the persistence of populations. Some changes, however, may serve to rescue populations from extinction by moving them toward conditions that are more typical of historical ecologies, whether through microevolution or individual phenotypic plasticity (Walther et al. 2002; Berteaux et al. 2004; Parmesan 2006). Changes in phenology, for example, have the potential to either realign evolutionary associations between breeding, temperature, and food availability or to create a mismatch among these factors (Stevenson and Bryant 2000; McCarty 2001; Walther et al. 2002; Visser and Both 2005). Phenological changes have been extensive in plants and animals (Beebee 1995; Crick et al. 1997; Forchhammer et al. 1998; McCarty 2001; Walther et al. 2002; Weishampel et al. 2004; Parmesan 2006), and a modest number of studies have shown that changes in breeding phenology in focal populations (mostly birds) are due, at least in part, to individual plasticity in the timing of breeding in response to climatic variation (reviewed in Nussey et al. 2007).

Temperature has a strong impact on physiology, activity, and development of animals. This impact is particularly apparent in species with temperature-dependent sex determination (TSD), wherein the sex of an individual is determined irreversibly by incubation temperatures during embryonic development (Janzen and Paukstis 1991; Mrosovsky and Pieau 1991). As a result, when the link between climate and incubation temperatures is strong, as it is in most species with TSD, climate controls cohort sex ratios (Janzen 1994*a*; Godfrey et al. 1996). Climate extremes produce heavily biased offspring sex ratios (Janzen 1994*a*). Consistent exposure to such extremes can lead to selection to restore the equilibrium sex ratio while maintaining TSD (e.g., selection on the pivotal temperature of sex determination or on nesting behavior), to selection against temperature as the sex-determining mechanism, or to population extinction (Bull and Bulmer 1989; Conover and Van Voorhees 1990; Janzen and Paukstis 1991; Conover et al. 1992; Van Dooren and Leimar 2003).

Changes in breeding phenology in populations with TSD have the potential to rescue populations from adverse consequences of extreme sex ratios (Ewert et al. 2005; Doody et al. 2006). That is, advances in the onset of the nesting season could

move the thermosensitive period of sex determination during embryonic development to an earlier time in the season when thermal conditions are cooler or more representative of conditions historically linked to a period later in the season. Plasticity of breeding initiation in response to local climatic variation may allow populations to adjust to a warming climate and may be adaptive in mitigating the sex ratio effects of historical climatic fluctuations (Houston and McNamara 1992; de Jong 1995). Indeed, in several populations of turtles with TSD, the timing of first breeding has been earlier in recent years, concurrent with climate warming; however, individual phenotypic plasticity has not been investigated thoroughly in these populations (e.g., Weishampel et al. 2004; Pike et al. 2006).

Painted turtles (*Chrysemys picta*) in Illinois have hastened the initiation of breeding over the last 17 yr of study (F. J. Janzen, unpublished data). Between 1990 and 1996, females in this population began nesting, on average, on June 4 (Julian day 155), with the earliest date of May 28. Between 2000 and 2006, nesting initiated around May 26 (Julian day 146), with the earliest date of May 17. Nesting terminates by the end of June, and females sometimes produce a second and third clutch within a season, roughly 2 wk after the previous clutch. Eggs require approximately 3 mo before hatching, with the thermosensitive phase of sex determination for most embryos in this population occurring historically in July (Janzen 1994*a*, 1994*b*). In *C. picta*, warm incubation temperatures during embryonic development lead to female offspring, whereas cool incubation temperatures produce males. Vegetation cover plays an important role in determining nest temperatures and nest sex ratios within a year (Janzen 1994*b*; Weisrock and Janzen 1999), but climate (July air temperature) determines the average nest sex ratio each year and strongly predicts the cohort sex ratio (Schwarzkopf and Brooks 1987; Janzen 1994*a*, 1994*b*; Weisrock and Janzen 1999). Thus, one might expect that climatic warming could eventually lead to an extreme overproduction of female offspring, thereby substantially influencing the ecological and evolutionary dynamics of the population.

In this article, we examine breeding phenology in our population of *C. picta* and its potential to counteract the effects of climatic warming on this population, namely on offspring sex ratio. First, we examine whether individual nesting females demonstrate plasticity in the timing of first nesting in response to local winter and spring climate. This study is one of only a few to utilize extensive longitudinal data from a wild vertebrate population to investigate phenotypic plasticity in breeding, and the first to do so in a reptile (Nussey et al. 2007). Second, we examine whether the timing of first nesting is repeatable within females in order to assess whether this trait has the potential to be heritable and responsive to selection. Third, we evaluate how nest sex ratios correlate with the timing of nesting to determine how changes in timing by a female might affect her nest sex ratio. Finally, we step back and explore the population trends that have been observed during the course of our study to understand how individual nesting relates to population outcome. Namely, what are the ties between winter and summer climate, nesting initiation and median nest date, and lay date and the production of second clutches?

Material and Methods

We monitored a population of painted turtles (*Chrysemys picta*) on the Thomson Causeway Recreation Area (TCRA), a small island in the Mississippi River, near Thomson, Illinois. The turtle population nests primarily in a ~1.5-ha area adjacent to the backwaters of the river. The habitat is a mostly flat, grassy area interspersed with deciduous and coniferous trees (for more description of the study site, see Kolbe and Janzen 2002). The nesting area has been the focus of research on *C. picta* since 1988, with intensive monitoring for nesting activity between mid-May and the end of June since 1990.

During the nesting season, the area was checked hourly for the presence of nesting females from dawn until dusk. Each nest was mapped and the degree of vegetation cover above the nest in the four cardinal directions was recorded with a spherical densiometer. The south + west vegetation cover was used as a summary value of nest vegetation cover (see Janzen 1994*b*; Weisrock and Janzen 1999; Morjan 2003*b* for more details). Since 1995, turtles have been captured after nest completion and individually marked with unique combinations of filings in the marginal scutes.

In September, all nests that were not depredated were excavated to retrieve offspring. Because hatchling painted turtles in our population hibernate in their natal nests over the winter, we can be assured of finding all hatchlings in the nest in September (Janzen 1994*b*). The hatchlings were moved to Iowa State University (Ames), where they remained over the winter. A subset of hatchlings in each nest was euthanized and sexed via visual examination of the gonads, as described by Janzen (1994*b*). Because 66% of nests are typically unisexual (Janzen 1994*b*), sexing only a portion of hatchlings in each nest allows us to reasonably estimate the nest sex ratios (proportion of male individuals).

On the basis of our observations of all nesting events over each season, the first and median nesting dates for each year were determined. In some early years of the study, lay dates were not known for a sufficient number of nests to be able to confidently assign a median nest date. The proportion of nests in a year that were second clutches was calculated from the number of known second clutches and the total number of nests laid. Because knowledge of second nests requires individual identification of females, the proportion of second nests could not be determined before 1995.

Climate data were acquired for Clinton, Iowa (~10 km from the TCRA) from the National Climate Data Center (NCDC; http://www.ncdc.noaa.gov). Heating degree-days (HDD) for the winter before the nesting season (HDD winter) was chosen as the most appropriate climate measure because of its cumulative nature and its temporal correspondence with egg development. Egg follicles develop to roughly 50% of their energy content in the fall before egg laying, and the remaining devel-

opment occurs after females emerge from hibernation (Congdon and Tinkle 1982). Such physiological processes are probably highly thermally dependent in these animals. Monthly HDD values represent the sum of the number of degrees Fahrenheit that each daily mean temperature falls below the base temperature (base temperature = 65°F for NCDC data, or ~18°C). Thus, a higher HDD value indicates that the climate has been colder, requiring greater heating to reach the base temperature. Here, HDD values were summed from monthly values from Clinton for September through April. Mean May air temperature was also explored as a potential predictor of the onset of nesting for the population, but it had no explanatory power. Mean May, June, and July air temperatures were from summary data provided by the NCDC. Monthly air temperatures were available from 1931, whereas winter HDD values could be calculated starting with the year 1952.

To examine plasticity in the timing of first nesting for individual females, the analysis was restricted to only those females that nested in at least 3 yr between 1995 and 2006 (not necessarily in consecutive years). This subset of data contained 1,322 nesting records for 268 females. We used linear mixed models to examine plasticity in first nesting date for individual females (e.g., Nussey et al. 2005b, 2007). A value for HDD winter just before nesting was entered as a fixed effect and year was entered as a random effect to account for variation among years in nesting date that was not due to winter climate. The importance of the random effects of female identity and female × HDD winter for the model were determined using likelihood ratio tests from random regression models, referencing the test statistic to a χ^2 distribution (where degrees of freedom are given by the difference in the number of parameters between the two models). The climate variable (HDD winter) allows us to test for plasticity (i.e., the reaction norm), and female identity and interaction test for differences in height and slope of the reaction norms, respectively, due to differences in individual-level attributes, such as genes or developmental factors.

The same data subset (females nesting at least three times) was used to test for repeatability (r) within females in the timing of first nesting. A one-way ANOVA with female identity as the predictor of first nesting date provided values of variance within and among females. Repeatability in nesting date, which ranges between 0 and 1, was calculated using Equations (2)–(5) in Lessels and Boag (1987).

To examine whether nest sex ratios vary according to nesting date, we examined all nests produced by females of known identity, including nests from females with fewer than three nesting years and nests that were second and third clutches from females within a year. A generalized linear mixed model with binomial error structure and logit link (SAS ver. 9.1, June 2006 release of PROC GLIMMIX) tested for the importance of nesting date (in Julian days; covariate), vegetation cover (covariate), mean July air temperature (covariate), and the interaction between July temperature and nesting date for the response variable, nest sex ratio. Female identity was entered as a random effect in this model to account for repeated measures of females. The data were overdispersed, so we added a multiplicative overdispersion parameter to the model statement.

The overall effects of nesting phenology were examined using linear regressions of median nesting date and proportion of second clutches on first nesting date and year. In addition, the probability of laying a second clutch for each female in a year (1 = second clutch, 0 = no second clutch) was related to lay date of the first nest using a logistic regression, with female identity entered as a random effect. For this analysis, the data set included all females of known identity, regardless of the number of years in which they nested. All statistical analyses were performed with JMP 6, with the exception of the sex ratio model.

Results

Climate and Phenology

Over the course of this study (1990–2006), there was no significant change in mean air temperature for the months of May ($r^2 = 0.00$, $P = 0.84$, $N = 17$), June ($r^2 = 0.01$, $P = 0.66$, $N = 17$), or July ($r^2 = 0.11$, $P = 0.20$, $N = 17$), or for winter HDD ($r^2 = 0.12$, $P = 0.17$, $N = 17$). During our study, the date of first nesting for the population each year was strongly correlated with winter climate, with warmer winters leading to earlier first nesting dates ($r^2 = 0.71$, $P < 0.0001$, $N = 16$; first nest date = 77.55 + 0.012 × HDD winter; Fig. 1).

Plasticity and Individual Nesting Events

Females demonstrated plasticity in their first nesting date in response to winter climate (Tables 1, 2). Following warmer winters (lower HDD winter values), females laid their first nests
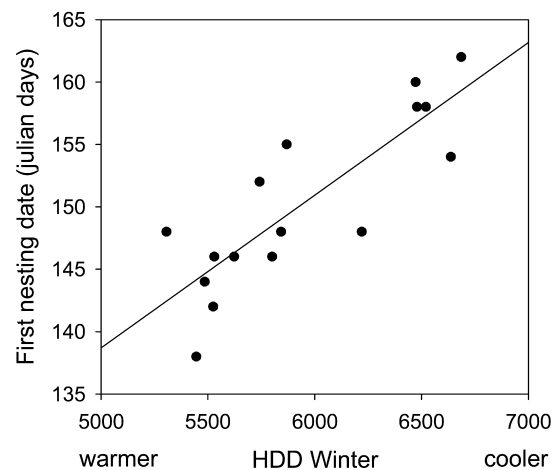

Figure 1. First nesting date (Julian days) of each year for our painted turtle population is earlier after warmer winters (fewer heating degree-days [HDD]; September–April). A Julian day of 150 corresponds to May 30, excluding leap years. HDD winter = HDD for the winter before the nesting season.

Table 1: Significance of adding random effects to linear mixed models of first nesting date for individual females

Random Variables	−2 Log Likelihood	Test	δ df	Likelihood Ratio	P Value
Year	9,340.46				
Year + female (elevation)	9,335.61	1 vs. 2	1	4.85	<.05
Year + female (elevation) + female × HDD winter (slope)	9,326.88	2 vs. 3	2	8.73	<.05

Note. Heating degree-days for the winter before the nesting season (HDD winter) was a fixed effect that was entered into all models. The data include the first nests of females that were known to have nested in at least 3 yr between 1995 and 2006.

earlier in the spring (Fig. 2). In addition, females differed significantly in the elevation and slope of their plastic response (Tables 1, 2). First nesting date was not repeatable within females ($r = 0.03$, $N = 268$).

Nest sex ratios became more female biased as the laying date of a nest occurred later in the season, but this tendency was not significant (Table 3). The interaction between July temperature and laying date was not significant and was subsequently removed from the final model. Vegetation cover had a positive influence on nest sex ratios. In other words, shadier nests produced more male-biased sibships than did less vegetated nests; an increase of 1% in south + west vegetation cover (maximum 200%) leads to a 2% increase in probability of producing sons in a nest. July air temperature had a significant effect on nest sex ratio, with lower nest sex ratio (i.e., more females) predicted when July is warmer (for a given nesting date and vegetation cover). A 1°C rise in July air temperature from the 1931–2006 mean (from 23.9°C to 24.9°C) leads to a ~50% reduction in the sex ratio of a nest.

Population-Level Consequences of Plasticity

Winter climate (HDD winter) did not predict mean air temperatures in May ($r^2 = 0.07$, $P = 0.30$; $N = 17$ for all), June ($r^2 = 0.04$, $P = 0.45$), or July ($r^2 = 0.15$, $P = 0.12$) during our study. Over a greater time frame (1952–2006), warmer winters were correlated with warmer air temperatures in July ($r^2 = 0.14$, $P = 0.005$; $N = 55$ for all) but not in May ($r^2 = 0.04$, $P = 0.13$) or June ($r^2 = 0.02$, $P = 0.26$). During our study, July air temperatures were not predicted by air temperatures in May ($r^2 = 0.00$, $P = 0.97$), when nesting begins, but they did tend to correlate positively with air temperatures in June ($r^2 = 0.21$, $P = 0.07$), which is the month when the majority of nests are laid. A similar pattern was seen with May, June, and July temperatures from 1931 to 2006.

The median nesting date for each year was positively correlated with the first nesting date, but the relationship was allometric ($r^2 = 0.50$, $P = 0.0045$, $N = 14$; median nest date $= 100.14 + 0.432 \times$ first nest date; Fig. 3A). As a result, median nesting date has not occurred earlier over the course of this study (median nest date vs. year; $r^2 = 0.06$, $P = 0.39$, $N = 14$; Fig. 3B).

The production of a subsequent clutch within a year was more likely for females that laid earlier first nests that year (P(first nest date) < 0.0001, P(female) $= 1$, $n_{nests} = 1,822$; Fig. 3C). The proportion of nests that are constructed second in a

season is not significantly predicted by the first nesting date for the population ($r^2 = 0.23$, $P = 0.11$, $N = 12$; note that power in this test [0.19] was quite low), but the frequency of second nests has increased over the course of the study ($r^2 = 0.60$, $P = 0.003$, $N = 12$; Fig. 3D).

Discussion

Few studies to date have used longitudinal data to test phenotypic plasticity in wild vertebrates, and the majority has focused on bird populations (Nussey et al. 2007). Here, we take advantage of an extensive longitudinal data set on breeding in female painted turtles to provide, to the best of our knowledge, the first evidence of within-individual breeding plasticity in wild reptiles. Female painted turtles demonstrated plasticity in the timing of their first nesting event each year. This plasticity was linked to winter climate, with females nesting earlier after warmer winters.

The mechanisms behind this individual plasticity in annual onset of nesting are largely unknown. However, several potential mechanisms exist. First, ambient temperature may directly influence the date on which females can emerge from hibernation and resume egg development, or it may directly influence the rate of egg development during fall and spring through the availability of basking opportunities (Moll 1973; Congdon and Tinkle 1982; Stevenson and Bryant 2000). Second, temperatures may influence the timing of food resources available for female metabolism and egg completion (Walther et al. 2002).

Plasticity in the timing of first nesting clearly contributes to the observed correlations between the first nesting date of the population and winter climate. Microevolution of nesting timing per se is unlikely to have contributed to this population trend because of the lack of repeatability (and, hence, heritability) in nesting timing. Immigration of females from populations with earlier nesting phenology is also a possible contributor to changes in nesting phenology over the course of this study, although it is unlikely given the low variation in nesting dates among *Chrysemys picta* populations across a wide geographic range (Moll 1973; Morjan 2003b; F. J. Janzen, unpublished data).

The phenotypic plasticity observed in female painted turtles in our population seems unlikely to prevent future biased sex ratios caused by climate warming (Janzen 1994a; Morjan 2003a) for several reasons. First, although nests laid earlier in the season produced more males in previous studies on *C. picta*

(Bowden et al. 2000), the seasonal effect on our population was modest compared with the sex effects of climate. If the parameter estimate from our statistical model is accurate, then a nest laid 1 d earlier experiences a 1% increase in sex ratio. If summers warm by 1°C over the next 30 yr (IPCC 2001; Pan et al. 2004), nests laid on a given date will be half as likely to produce sons, and a nest must be laid ∼60 d earlier to offset the effect of climate (63 d for a doubling of the nest sex ratio). Female painted turtles would need to begin nesting in late March to counteract this climate effect. If every month of the winter (September–April) warms by 1°C from the 1931–2006 average, this pattern would lower the HDD winter value from a historical average of 6,000 degree-days to 5,500 degree-days. Such a change of 500 degree-days is predicted to move a female's first nesting date forward by 4 d (on the basis of the predicted reaction norm) and the population's first nesting date forward by 6 d (on the basis of the relationship between the population's first nesting date and the HDD winter value). Clearly, this change in the timing of breeding cannot offset the influence of climate on nest sex ratios.

This prediction, of course, assumes that annual climate profiles remain the same, raised by some constant across the entire year. This pattern may not hold true. Although we found a positive correlation over the past 50 yr between winter and July climates, geographically broader climate patterns over the past 30 yr indicate that the south-central United States has not uniformly experienced increases in summer temperatures to match those observed in the rest of the country and in the winter (IPCC 2001; Pan et al. 2004). Indeed, in Clinton, Iowa, July air temperatures have not changed from 1931 to 2006 (duration of climate data available for Clinton; $r^2 = 0.03$, $P = 0.15$, $N = 76$), whereas winters have warmed significantly since 1952 (when HDD winter values are first available; $r^2 = 0.07$, $P = 0.05$, $N = 55$). This summer "warming hole" is predicted to lead to summer temperatures warming to a lesser extent over the next 30 yr than winter temperatures (Pan et al. 2004). Such asymmetries in climate warming may lead to asynchrony between breeding and resource availability (Stevenson and Bryant 2000; McCarty 2001; Visser and Both 2005; Parmesan 2006). For painted turtles in Illinois, this could mean a mismatch between the timing of the embryonic thermosensitive period of sex determination and thermal conditions. That
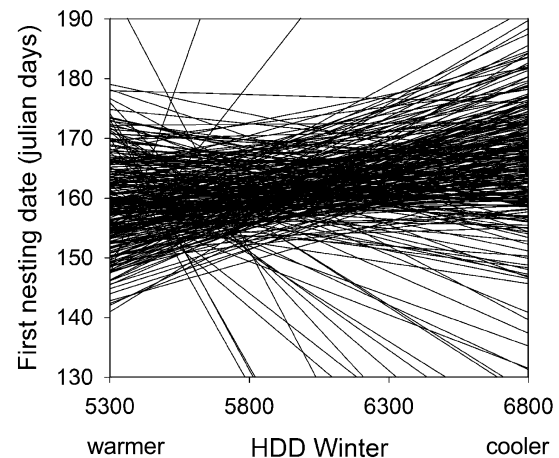

Figure 2. Reaction norms of first nesting date (Julian days) for individual female painted turtles, as a function of winter climate. Most females nested earlier after warmer winters (fewer heating degree-days [HDD]; September–April). Females differed significantly in elevation and slope of the reaction norms. HDD winter = HDD for the winter before the nesting season.

is, nests laid earlier may actually experience cooler incubation temperatures than normal given that mean air temperature for June averages 2°C cooler than for July (1931–2006) and that nest temperatures tend to mirror fluctuations in air temperatures (Weisrock and Janzen 1999). Under this scenario, climate warming could counterintuitively lead to the overproduction of male offspring, again rendering plasticity in nest timing ineffective in offsetting climate effects. Furthermore, if a 1°C rise in summer temperatures reduces a nest's sex ratio by half, HDD winter would need to be reduced to 0–1,000 degree-days to effect a 60-d advance in nesting date and a doubling of nest sex ratio. One thousand heating degree-days would be exceeded if as few as 100 d between September and April had a daily mean air temperature below 55°F, indicating that the warming asymmetry would have to be extreme to offset the sex ratio effects of a modest rise in summer temperatures.

The final reason that individual plasticity is unlikely to counteract the effects of climate warming is that females are more likely to lay a second clutch when their first nest is laid early. As a result, the proportion of nests each year that are second

Table 2: Estimates of fixed and random effects from the final linear mixed model for first nesting date

Effect	Estimate	SE	P Value	Variance Component	SE
Fixed:					
HDD winter	.0079	.002	.0028		
Random:					
Year				9.61	4.60
Female (elevation)				2.21	1.46
Female × HDD winter (slope)				-1.4×10^{-5}	3.83×10^{-6}
Residual				67.09	3.17

Note. HDD winter = heating degree-days for the winter before the nesting season.

Table 3: Results from a generalized linear mixed model of nest sex ratio (proportion male), with binomial error structure and logit link

Predictor	Log Odds Ratio	Numerator df	Denominator df	F Value	P Value
Nesting date	−.011	1	793.7	2.42	.120
Nest vegetation	.021	1	830.9	128.19	<.0001
July temperature	−.697	1	819.4	94.02	<.0001

Note. Female identity was entered as a random effect, and we included a multiplicative overdispersion parameter to account for overdispersion of nest sex ratio. Data include all nests where offspring were sexed that were from females with known identity between 1995 and 2006 (839 nests).

nests has increased and the median date of nesting has not changed significantly. Perhaps as a consequence, July air temperatures have remained important for sexual differentiation in a majority of nests and for cohort sex ratios in this population (F. J. Janzen, unpublished data). If this pattern continues, altered phenology will not "save" the majority of embryos from overly warm Julys because most nests will not shift into cooler, earlier portions of the season.

If plasticity fails to prevent the overproduction of female hatchlings, microevolution may bring the sex ratio back to equilibrium via selection on pivotal temperatures or nest-site selection (Morjan 2003a). For example, the evolution of maternal nest-site selection toward wetter or shadier nests would lead to cooler nests, thus mitigating the effects of warmer air temperatures. The evolution of nesting behavior has clearly been historically important in responding to local climate across geographic ranges in species with TSD (Bull et al. 1982; Morjan 2003b; Ewert et al. 2005; Doody et al. 2006). If climate change is too rapid and extreme, the repeated production of extremely female-biased cohort sex ratios would lead to a deficiency in reproductive males in the population and, potentially, to extinction (Janzen 1994a; Morjan 2003a; L. J. Harmon and F. J. Janzen, unpublished data).

Prospectus for Future Research

Turtles have joined the growing list of taxa exhibiting phenological shifts in response to climate change (McCarty 2001; Walther et al. 2002; Weishampel et al. 2004; Parmesan 2006; Pike et al. 2006; Hawkes et al. 2007; F. J. Janzen, unpublished data). Here we have shown that this change in phenology in one population of painted turtles is the product of labile nesting dates within females. This plasticity as it currently exists does not seem adequate to offset the potentially negative effects of climatic warming on offspring sex ratios. Several future avenues of research may provide further insight into the role of plasticity in mitigating negative impacts of climate change on populations with TSD.

First, additional maternal traits (selection of nest cover and egg hormone deposition) influence nest sex ratios in painted turtles (Janzen 1994b; Weisrock and Janzen 1999; Bowden et al. 2000) and in other species with TSD. These traits should be examined for plasticity in response to climate fluctuations

to determine whether they could contribute to mitigation of climate effects on offspring sex ratios.

Second, the individual variation in the elevation and slope of reaction norms of nesting date (Table 1; Fig. 2) suggest further examination of individual-level differences in plasticity in this system (Nussey et al. 2007). Plasticity in nesting itself may be condition dependent (Nussey et al. 2005a) and sensitive to effects from early life or constraints on adults. External effects on plasticity are an important future avenue of research because of the potential for climate change to alter early life conditions and to impose external constraints. Additionally, plasticity may be heritable and could evolve in response to climate change (de Jong 1995). Estimation of the heritability and fitness consequences of nesting plasticity is not only of interest in basic evolutionary biology but would also allow predictions of how plasticity may evolve in response to climate change.

Third, a mechanistic model of offspring sexual outcome would allow more detailed investigation of the direct and indirect ways climate may impact offspring sex. Such a model should include the effects of nest temperature profiles (from nest cover and air temperature profiles), yolk provisioning, and yolk hormones on offspring developmental rate, sex, and viability (e.g., Georges et al. 2005; Les et al. 2007). When this mechanistic model is informed by climate-induced changes in (1) nesting date, (2) production of second and third clutches (which are depleted in yolk provisions and show altered hormone composition; Bowden et al. 2000, 2004; Harms et al. 2005), and (3) seasonal and daily patterns in climate, we can estimate the outcome for the overall offspring sex ratio. In this study, we have addressed the first two climate impacts. These efforts would advance if we determined the triggers of nesting plasticity, allowing better extrapolation of this response to predicted future climate change.

Climate change poses a serious threat to the persistence of populations of organisms exhibiting environmental sex determination, many of which are already imperiled for other reasons. Clutch sex ratios vary across seasons and oviposition microhabitats in most systems with TSD that have been studied (e.g., Conover and Kynard 1981; Janzen and Paukstis 1991; Godfrey et al. 1996), indicating that adjustment of the sex ratio to climate change is possible. Climate has a strong influence on offspring sex ratios in our study population, leading us to speculate whether our results for painted turtles extend in gen-
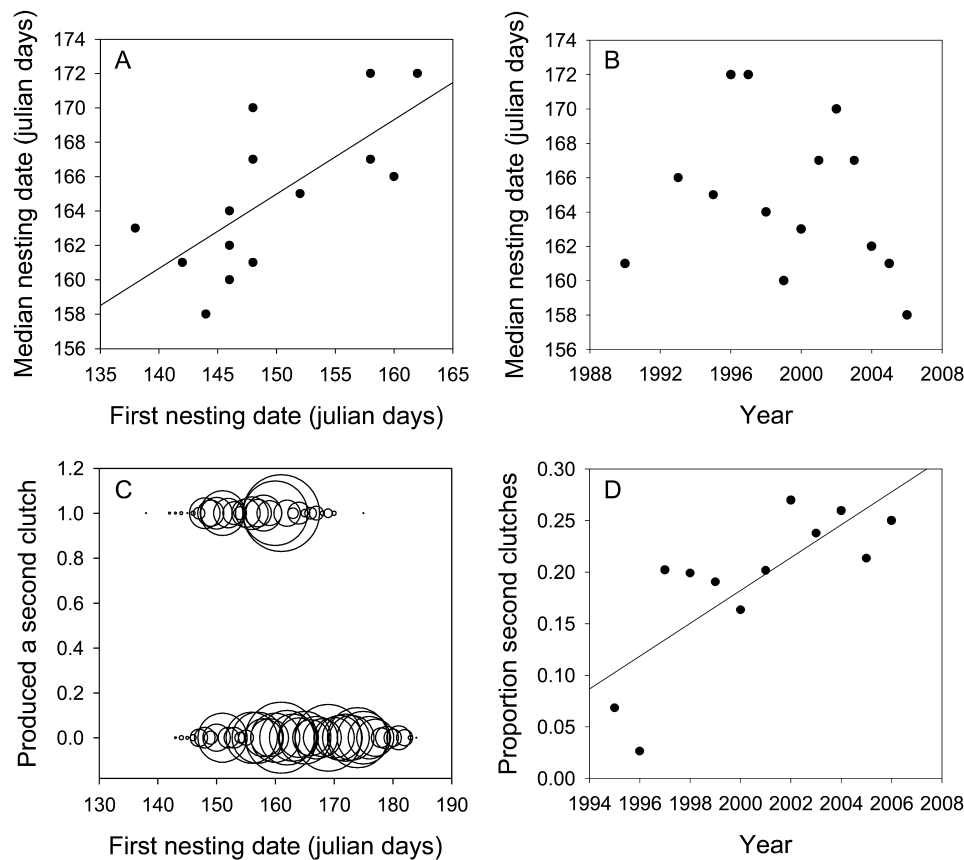
Figure 3. Consequences of phenotypic plasticity in nesting date. In *A*, median nesting date is positively correlated with first nesting date, but in *B*, it has not been made earlier during the course of the study, in contrast with first nesting date. This result is likely explained by *C*, early nesting by a female increasing the chances that she will lay a second clutch in the season at a later date (symbol size indicates counts at the data point). *D*, Proportion of nests laid in a year that are second clutches has increased during this study.

eral to other TSD-exhibiting populations. Specifically, is climate typically such a strong contributor to nest sex ratio that it overwhelms the effects of variation in nest-specific attributes? Clearly, the question of whether population changes will be enough to offset climate change is one of primary interest in all studies of plasticity and climate change and phenological responses to climate (Ewert et al. 2005; Visser and Both 2005; Doody et al. 2006). The addition of more population-focused studies will determine whether any generalities can be made. In particular, the results of this study underscore the applied importance, as well as the basic importance, of understanding the ecology and evolution of phenotypic plasticity (particularly labile traits). Such studies can be accomplished only through examination of extensive longitudinal data. The value of long-term data for retrospective and predictive analyses is immeasurable, and our reliance on such data for studies of the biotic impact of climate change will surely increase in the future.

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