

Nest-site philopatry and the evolution of temperature-dependent sex determination

Nicole Valenzuela* and Fredric J. Janzen

Department of Zoology and Genetics, Iowa State University, Ames, IA 50011, USA

ABSTRACT

Despite intensive research, there is no clear empirical evidence to explain the evolution and persistence of temperature-dependent sex determination in reptiles. A recent hypothesis presented by Reinhold proposes that natal homing could lead directly to the evolution of temperature-dependent sex determination. According to his hypothesis, daughters are produced in rare high-quality sites (associated with higher survival rates) to which they return and use to nest, thus deriving higher fitness than sons for whom the quality of the natal patch does not affect their reproductive output if they survive to maturity. We performed an initial empirical evaluation of several assumptions and predictions of this hypothesis as applied to painted turtles (*Chrysemys picta*), using data from five consecutive nesting seasons, on a major nesting beach. Females were somewhat philopatric to microgeographic sites and to vegetation cover types within the nesting beach, consistent with one of the assumptions of Reinhold's hypothesis. The variables we examined that influence hatchling fitness (predation, hatching success and sex ratio) were not stable at microgeographic nesting sites or at vegetation cover types. Predation was repeatable within females, whereas hatching success and sex ratio were not. Contrary to Reinhold's hypothesis, females did not nest more frequently in open sites (which tend to produce more females) than in patches with more vegetation (which tend to produce more males). Furthermore, preferred nest sites (as measured by nest density) did not produce predominantly females. However, nests with higher hatching success tended to produce slightly more females (although the magnitude of this effect was very small). Therefore, Reinhold's hypothesis is not applicable to *C. picta* at the level studied – that is, within a nesting beach over a 5 year period – because most of the essential conditions were not met by our data.

Keywords: *Chrysemys picta*, natal homing, nest-site philopatry, Reinhold hypothesis, reptiles, temperature-dependent sex determination.

INTRODUCTION

One of the more intriguing but poorly understood sex-determining mechanisms is temperature-dependent sex determination (TSD), which occurs primarily in reptiles (reviewed in Bull, 1983; Ewert and Nelson, 1991; Janzen and Paukstis, 1991a). Under temperature-dependent sex determination, the sex of individuals is determined permanently

* Author to whom all correspondence should be addressed. e-mail: nvalenzu@iastate.edu
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after conception by temperature, such that some thermal environments produce males and others produce females (Bull, 1983). Several hypotheses have been proposed to explain the evolutionary persistence of temperature-dependent sex determination (Ewert and Nelson, 1991; Burke, 1993; Shine, 1999). The hypothesis most widely studied is based on a model by Charnov and Bull (1977). This model states that temperature has differential sex-specific fitness effects and assumes that the environment consists of nesting patches that differ in thermal conditions unpredictably, such that incubation conditions cannot be controlled by either parents or offspring. Consequently, temperature-dependent sex determination is beneficial because it allows offspring to develop into the sex that will confer the highest possible fitness in a given patch. Studies on reptiles have focused on differential sex-specific fitness effects of embryonic incubation temperature (e.g. post-hatching growth, survival or behaviour); some results are consistent with the model of Charnov and Bull (1977) but are not conclusive (reviewed in Shine, 1999).

Nest-site choice could play an important role in sex-ratio evolution in species with temperature-dependent sex determination (Charnov, 1982; Karlin and Lessard, 1986; Bull *et al.*, 1988). For example, Roosenburg (1996) postulated that a female may choose a warmer nest site if she carries big eggs or a cooler site if her eggs are small, such that larger eggs develop as females and smaller ones as males. Roosenburg (1996) suggested that this scenario provides a sex-specific fitness differential consistent with Charnov and Bull's (1977) model, given the positive relationships between egg size, size at hatching and post-hatching growth. Data from diamondback terrapins support this hypothesis, but those for painted turtles (C.L. Morjan and F.J. Janzen, unpublished data) and for Amazonian giant river turtles (Valenzuela, 2001) do not.

Recently, Reinhold (1998) presented a hypothesis, congruent with the model of Charnov and Bull (1977), of how female natal homing could lead directly to the evolution of temperature-dependent sex determination. According to Reinhold (1998), nesting sites can be thought of as the patches in the model of Charnov and Bull (1977). Moreover, he postulated that high-quality sites are rare, whereas low-quality sites are frequent. Quality is proportional to the survival of hatchlings, and this quality affects the relative fitness of sons and daughters differently (Reinhold, 1998), in the following manner. In species with female natal homing, daughters use the nesting patch of their mothers. Although not stated explicitly by Reinhold, this implies that daughters exhibit a preference for either a particular geographic location or a type of microhabitat similar to that exhibited by their mothers. Therefore, daughters from low-quality sites have lower future reproductive success than daughters from high-quality sites, whereas sons from low-quality sites are assumed not to suffer from this disadvantage. Long-term fluctuations in nest-site quality and imperfect natal homing prevent selection from leading to nesting restricted to high-quality sites (Reinhold, 1998). In the end, temperature-dependent sex determination should be favoured over genotypic sex determination when the sex ratio (proportion of male offspring with TSD phenotype) differs from 0.5 at least at one nesting site type, is ≥ 0.5 at low-quality sites and is ≤ 0.5 at high-quality sites (Reinhold, 1998). This hypothesis has yet to be examined critically, despite its implications for understanding the evolution and persistence of temperature-dependent sex determination in reptiles.

Reinhold (1998) used the term 'nest-site philopatry' and defined it as the mechanism by which 'females come back to the same area where they hatched to lay their own eggs', a mechanism also known as 'natal homing'. Females that show natal homing must also exhibit nest-site philopatry, whereas the converse is not necessary. Therefore, we use the

term ‘natal homing’ explicitly hereafter and restrict the use of ‘nest-site philopatry’ to the return of females to the same geographic location or type of microhabitat irrespective of whether this nest site is their natal site.

Here we present the results of an initial empirical evaluation of Reinhold’s (1998) hypothesis using *Chrysemys picta*, a freshwater turtle that exhibits female nesting-beach philopatry (Schwarzkopf and Brooks, 1987; Lindeman, 1992; Scribner *et al.*, 1993). We evaluated several assumptions and predictions of Reinhold’s (1998) hypothesis at the scale of a major nesting beach over 5 consecutive years by addressing the following:

1. Reinhold’s (1998) hypothesis requires the existence of female nest-site philopatry. We analysed whether *C. picta* females are philopatric to particular geographic sites or to particular types of microhabitat within the nesting beach. We tested for philopatry at a finer scale than the nesting-beach philopatry previously reported (Schwarzkopf and Brooks, 1987; Lindeman, 1992; Scribner *et al.*, 1993) to determine the heterogeneity in nest-site quality within a nesting beach.
2. Reinhold’s (1998) hypothesis requires that offspring of females nesting at high-quality patches breed on average more frequently at high-quality patches than offspring of females breeding at low-quality patches. Consequently, under natal homing, the hypothesis assumes that the conditions which influence hatchling fitness (nest quality) are stable at nesting sites, otherwise the quality of the patch used by the daughters to breed (whether geographic site or microhabitat type) would not match the quality of the patch used by their mother. We explored whether survival rates, sex ratios and incubation conditions were repeatable across years in different nesting locations or types of microhabitat.
3. Reinhold’s (1998) hypothesis states that preferred nest sites are high-quality sites, and that eggs laid in them should benefit from developing into females. At the same time, eggs laid in less-preferred low-quality sites should develop into males, because sons produced at those sites do not suffer from a decrease in reproductive output as their sisters do (Reinhold, 1998). We tested the predictions that (a) nesting density should be negatively correlated with sex ratio (percent males) within the nesting beach, or that (b) nesting females should be less likely to frequent sites with more vegetation cover (which tend to produce more males) than more exposed sites (which tend to produce more females).
4. Reinhold’s (1998) hypothesis predicts that embryonic survival and offspring sex ratio should be correlated. We assessed whether embryonic mortality rates were higher at male-producing sites and lower at female-producing sites.

METHODS

Study site and data collection

The data derive from a natural population of *C. picta* nesting on the southern beach of an island in the upper Mississippi River (Thomson Causeway, Carroll Co., IL) (Janzen, 1994a,b) between 1995 and 1999. The south beach (~250 × ~100 m) is frequented by many females during their June nesting forays (~200 nests per year) (Janzen, 1994b). The method for observing nesting behaviour – mapping and relocating nests, tagging and measuring females, evaluating vegetation cover around nests and sexing offspring – is detailed elsewhere (Janzen, 1994a,b; Weisrock and Janzen, 1999; Janzen and Morjan, 2001).

Data to evaluate Reinhold's (1998) hypothesis were derived from nesting episodes of some females within a single year and the same or other females across several years. Records were taken for 862 nests: female identity was available for 630 of those nests (492 females); 514 nests corresponded to females that nested in multiple years; 263 nests corresponded to females that laid two (112 females) or three (13 females) nests in a single season (Table 1). The following variables were recorded:

- *Sex ratios* produced by each nest were recorded as the proportion of males where sex was determined by macroscopic examination of the gonads (Janzen, 1994a,b). Six hatchlings per nest were examined, such that not all hatchlings were sacrificed (average clutch size = 10.7, range = 5–17, $n = 757$ nests). A sample size of six hatchlings is sufficient to estimate clutch sex ratios with great confidence because most nests are unisexual (Janzen, 1994b). For example, subsampling 6 hatchlings per nest at random from 1989 when 10 hatchlings per nest were examined, indicated that 75% of nests would have received an identical sex ratio designation and 90% would have received a sex ratio designation within 5% of the value based on 10 hatchlings per nest.
- *Predation* was recorded as a binary variable (0 = absent, 1 = present) because all eggs are lost when a nest is found by a predator.
- *Hatching success* was calculated as the proportion of eggs per nest that produced live offspring. Hatching success values of zero were the result of predation as well as other sources of mortality (e.g. infertility, temperature extremes, etc.).
- *Clutch size* was recorded as the number of eggs per nest.
- *Geographic locations* of nests were recorded as x,y coordinates on maps of the beach to the nearest 0.1 m from a common point of origin, using INTERPNT (Boose *et al.*, 1998).
- *Vegetation cover* over nest sites, which consists primarily of mature trees, was assessed using a spherical densiometer (Janzen, 1994b; Weisrock and Janzen, 1999). Percent vegetation cover in the southern and western directions were added and the resulting value was divided by 200 to obtain a combined standardized proportion of vegetation for the two directions. Southern and western values were chosen because the vegetation in these two directions is correlated with sex ratio in this population (Janzen, 1994b).

Table 1. Sample sizes used in the present study

Year	Single-nesting females	Double-nesting females	Triple-nesting females	Total females	Total nests with and without female information
1995	76	8	0	84	130
1996	69	4	1	74	150
1997	72	40	1	113	198
1998	71	30	3	104	178
1999	79	30	8	117	206
All years	367	112	13	492	862

Note: Number of *Chrysemys picta* females arranged by year and by the number of nests laid per reproductive season. Number of single-, double- and triple-nesting females are mutually exclusive.

This vegetation cover influences relative nest temperatures, such that nests with more vegetation cover tend to produce more males, whereas nests in more open sites tend to produce more females.

Statistical methods

Spatial autocorrelation within variables

Adequate tests of Reinhold's (1998) hypothesis require an explicit assessment of spatial structure in the data. To test for spatial structure in each of the variables measured, we subjected the original data to a spatial autocorrelation analysis that tested for positive or negative spatial association of the values with distance. This analysis was done by computing one-dimensional correlograms (see Fig. 1) for each variable using Moran's I and Geary's c measures of self-association at various spatial scales and geographic distances (Sokal and Oden, 1978a,b). Euclidean geographic distance between two nests was based on their x,y coordinates. In this way, we tested if a given variable was autocorrelated and at

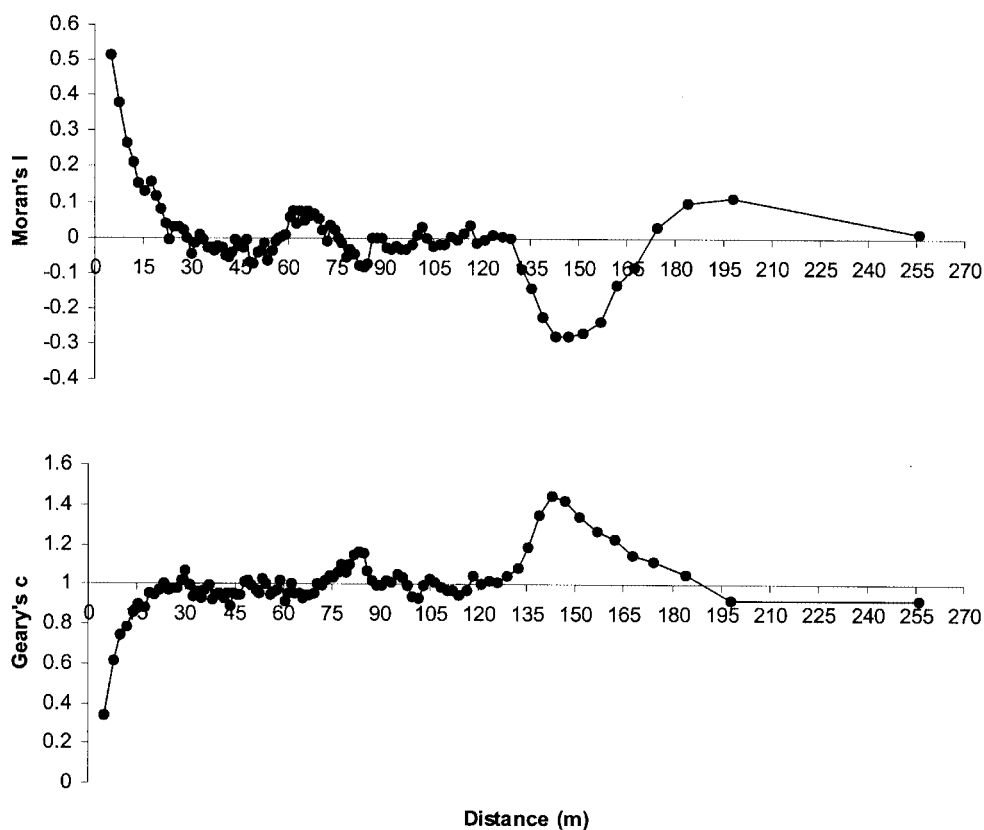


Fig. 1. Spatial correlogram of vegetation cover with geographic distance. Upper and lower panels correspond to Moran's I and Geary's c indices of self-association respectively. Vegetation cover was autocorrelated < 15 m (Geary's c) or < 20 m (Moran's I).

what spatial scale. The overall significance of correlograms was assessed using a Bonferroni test (Oden, 1984).

We used distance matrix methods for data analysis (Sokal *et al.*, 1997). When using these procedures, observable relations are expressed as a distance or dissimilarity matrix between all pairs of samples. In our case, we computed the distances between values of each variable (geographic distances, vegetation cover distances, sex ratio distances, etc.) for every pair of nests and used the resulting distance matrices in the following analyses.

Association between variables (exploratory analyses)

The independence of each pair of distance matrices was evaluated using Mantel tests of association (Mantel, 1967; Sokal, 1979; Smouse and Long, 1992), which can estimate the magnitude of the correlation between sets of variables. A 'three-way' Mantel test (in which the matrix of geographic distances was held constant) was used to calculate correlations when one of the variables was spatially autocorrelated (Smouse *et al.*, 1986). The significance of each Mantel correlation (r_M) was assessed by permutation tests using 1000 iterations, and compared to a Bonferroni-adjusted critical α level. Mantel correlation coefficients are usually smaller than Pearson's moment correlation coefficients.

Specific hypothesis testing (confirmatory analyses)

Hypothesized relationships among pairs of a set of entities can be stated as distance matrices, which are called design matrices in that case (Sokal *et al.*, 1997), by assigning distance values to entries in the design matrix that reflect the expected relationship if the hypothesis were true. Design matrices were subjected to Mantel tests to assess particular hypotheses by estimating the significance of the correlation between the design matrix and the distance matrix that contained the actual values of the variable under scrutiny (correcting for spatial autocorrelation as necessary).

We used the absolute difference between two values as the distance measure for all variables, with the exception of sex ratio, hatching success and vegetation cover, for which chi-square distance was computed because the values were proportions. Spatial and Mantel tests were conducted using PASSAGE (Rosenberg, 2000); all other tests were performed in NTSYSpc (Rohlf, 2000) and JMP (SAS Institute, 1995).

Details of the statistical analysis of each particular hypothesis examined are presented in the Results section.

RESULTS

We used 100 distance classes to test for spatial autocorrelation starting at a small scale (0–5 m). In total, 857 nests were included, resulting in numerous nest pairs per distance class (762 to 3668 pairs per class), which ensured enough statistical power to grant the use of 100 distance classes for all variables. Predation, hatching success, clutch size and sex ratio (percent males) showed no significant spatial autocorrelation. Only vegetation cover exhibited significant non-directional spatial autocorrelation ($P < 0.0001$, $n = 857$) – that is, vegetation cover values at locations less than 15 m apart showed a positive significant association (Fig. 1). Therefore, in every statistical analysis that included vegetation cover, the appropriate correction was made to account for this spatial autocorrelation by using a three-way Mantel test to hold geographic distance constant.

Hypotheses examined

Are females philopatric to particular geographic sites within the beaches?

A Mantel correlation was calculated between the matrix of pairwise geographic distances between nests and a design matrix where cells corresponding to same-female comparisons were given a value of 0 and cells corresponding to between-female comparisons were given a value of 1. If females are philopatric to particular geographic sites within a beach, then the geographic distance between nests within females should be smaller than the distance between nests among females.

Females in this study showed significant philopatry to particular geographic sites, as indicated by the positive correlation between geographic distance among nests and the design matrix of same-/between-female comparisons ($r_M = 0.03$, $P = 0.001$, $n = 630$).

Are females philopatric to particular microhabitat types within the beach?

A Mantel correlation was calculated between the matrix of vegetation cover dissimilarity between nests and the same design matrix of female identity used to test the previous hypothesis. If females are philopatric to particular vegetation types, then the difference between vegetation cover values of nests within females should be smaller than the difference for nests among females.

Females also showed significant philopatry to particular microhabitat types (vegetation cover) within the nesting beach, as indicated by the positive correlation between vegetation cover difference among nests and the matrix of same-/between-female comparisons ($r_M = 0.01$, $P = 0.004$, $n = 630$).

Are nest mortality and sex ratio more similar within than among females?

A Mantel correlation was calculated between the design matrix of female identity and the matrix of predation, hatching success or sex ratio. If sex ratio is more similar within than among females, then the difference between sex-ratio values of nests within females should be smaller than the difference for nests among females. The same would be expected for predation or hatching success.

Nests laid by a given female tended to experience more similar predation (consistently predated or not, depending on the female) than nests laid by different females, as indicated by the positive correlation between predation and female identity ($r_M = 0.02$, $P = 0.001$, $n = 630$). In contrast, nests laid by a given female did not exhibit more similar hatching success, and did not produce more similar sex ratios, than nests laid by different females. Therefore, hatching success and sex ratio were not repeatable within females even though females showed philopatry to geographic location and vegetation cover.

Are hatching rate, predation and sex ratio repeatable across years in different nesting locations?

A three-way Mantel correlation was calculated between the hatching-rate distance matrix and a design matrix where cells corresponding to same-year comparisons were given a value of 0 and cells corresponding to between-year comparisons were given a value of 1, while accounting for geographic location by holding the matrix of geographic distance constant. Similarly, a three-way Mantel correlation was calculated between predation (or sex ratio) and the same design matrix mentioned above, also holding geography constant. If hatching

rates (or predation or sex ratios) are not repeatable across years at particular nesting locations, the difference between values from between-year comparisons should be significantly larger than the difference between values from same-year comparisons.

Hatching success differed significantly among years and, therefore, was non-repeatable in particular geographic locations, as indicated by the positive relationship between hatching success and the design matrix of same-/between-year comparisons when accounting for geographic location ($r_M = 0.04$, $P = 0.006$, $n = 364$). Similarly, predation ($r_M = 0.10$, $P = 0.001$, $n = 857$) and sex ratio ($r_M = 0.03$, $P = 0.001$, $n = 392$) differed significantly among years and, therefore, were also non-repeatable in particular geographic locations.

To allow for the possibility that relative sex ratios (or hatching success) among nests might be repeatable rather than their absolute values, an additional analysis was performed. In this case, rank values were calculated for the sex-ratio data within each year (Sokal and Rohlf, 1995) and a three-way Mantel correlation was calculated between this new rank variable and the same-/between-year design matrix holding geography constant. Rank values were also calculated for hatching success and the three-way Mantel correlation was calculated in the same manner. The results of these two new tests were identical to the results of the original analyses – that is, relative sex ratio ($r_M = 0.11$, $P = 0.001$, $n = 392$) and relative hatching success ($r_M = 0.12$, $P = 0.001$, $n = 364$) differed significantly among years and, therefore, were non-repeatable in particular geographic locations.

Are incubation conditions repeatable in the different types of microhabitat?

An inverse relationship between vegetation cover and nest temperature has already been established for this population and thus it is not considered here (C.L. Morjan and F.J. Janzen, unpublished data). We calculated a three-way Mantel correlation between hatching success (or predation) and the design matrix of same-/between-year comparisons used to evaluate the previous hypothesis, accounting for vegetation cover. If incubation conditions are not repeatable across years at particular types of vegetation cover, the difference between values from between-year comparisons should be significantly larger than the difference between values from same-year comparisons.

Hatching success differed significantly among years and, therefore, was non-repeatable in particular types of vegetation microhabitat, as indicated by the positive relationship between hatching success and the design matrix of same-/between-year comparisons when accounting for vegetation cover ($r_M = 0.04$, $P = 0.006$, $n = 364$). The same result was found for predation ($r_M = 0.10$, $P = 0.001$, $n = 857$).

Are offspring sex ratios produced in different microhabitat types consistent among years?

A three-way Mantel correlation was calculated between sex ratio and the design matrix of same-/between-year comparisons holding vegetation cover constant. If sex ratios are not repeatable across years at particular types of vegetation cover, the difference between values from between-year comparisons should be significantly larger than the difference between values from same-year comparisons. Additionally, we calculated the Mantel correlation between sex ratio and vegetation cover for each year independently.

Sex ratio was significantly different among years and, therefore, was non-repeatable in particular types of microhabitat (vegetation cover), as indicated by the positive relationship between sex ratio and the design matrix of same-/between-year comparisons when accounting for vegetation cover ($r_M = 0.07$, $P = 0.001$, $n = 392$). This finding identifies a possible mechanism for the lack of within-female repeatability for sex ratio reported above.

Furthermore, even though differences in vegetation cover among nests were significantly correlated with differences in sex ratio over all 5 years combined ($r_M = 0.06$, $P = 0.001$, $n = 392$), the relationship was not significant for 1996 and 1997 when analysed independently (Table 2).

Is nesting density correlated with sex ratio, hatching or predation rates?

We divided the nesting area into quadrats of 5×5 m and generated a new matrix of the geographic coordinates corresponding to the centre of each quadrat. We counted the number of nests within each of those quadrats and calculated the average sex ratio produced by those nests. We then generated new distance matrices from these quadrat data (differences in geographic distance, nest density and sex ratio between each pair of quadrats). We calculated the three-way Mantel correlation between density per quadrat (number of nests per quadrat) and average sex ratio (percent males), holding geography constant to account for the fact that many quadrats are contiguous and this geographic non-independence could inflate the correlation estimates. We repeated this procedure for 10×10 m, 15×15 m and 20×20 m quadrats, as well as for average hatching rate and predation (number of nests predated per quadrat). The expectation under Reinhold's (1998) hypothesis is that females are produced preferentially in 'higher-quality patches', which should be frequented more often than 'low-quality' male-producing patches. Consequently, it is expected that more frequented nesting sites should suffer lower mortality rates.

Contrary to expectations, we did not detect a significant relationship between nest density and sex ratio for any of the quadrat sizes examined. Similarly, hatching rate was lower at higher densities for two of the quadrat sizes, as indicated by the significant negative correlation between these two variables in the 10×10 m and 20×20 m quadrats ($r_M = -0.08$, $P = 0.01$, $n = 159$ and $r_M = -0.09$, $P = 0.04$, $n = 56$, respectively). Similarly, predation was higher at higher densities, as indicated by the strong positive correlations between these two variables for all quadrat sizes ($r_M = 0.74, 0.84, 0.88$ and 0.88 respectively; $n = 365, 159, 88$ and 56 respectively; $P = 0.001$ in all cases). Therefore, females were not produced preferentially at more frequented sites and mortality was higher at preferred nesting sites.

Are females less likely to nest at sites with more vegetation cover (which tend to produce more males) than more exposed sites?

We performed the same procedure as in the previous sub-section, but using the matrix of differences in average vegetation cover values between quadrats instead of sex ratios. The

Table 2. Results of the three-way Mantel correlation tests between sex ratio (percent male) and vegetation cover, holding geography constant

Year	<i>P</i> -value	Mantel correlation coefficient	Sample size
1995	0.003	0.18	45
1996	0.79	0.05	70
1997	0.18	0.04	129
1998	0.001	0.14	109
1999	0.004	0.16	39
All years	0.001	0.06	392

expectation under the hypothesis is that patches with a higher density of nesting females should be found in less covered sites, which produce more females (Janzen, 1994b).

Contrary to expectations, females did not nest more often at more exposed nesting sites than at sites with more vegetation cover, as indicated by the lack of a significant correlation between nest density and vegetation cover for all quadrat sizes examined.

Are mortality rates higher at male-producing sites and lower at female-producing sites?

First, we calculated Kendall's coefficient of rank correlation, τ , between hatching success and sex ratio (Sokal and Rohlf, 1995). Second, using data from 5×5 m, 10×10 m, 15×15 m and 20×20 m quadrats, we calculated a three-way Mantel correlation between the matrix of predation (number of nests predated per quadrat) and average sex ratio per quadrat, holding geography constant. The expectation under the hypothesis is that males are produced at lower-quality patches – that is, at patches with lower offspring survival.

Consistent with Reinhold's (1998) hypothesis, nests with lower embryonic mortality produced slightly more females, as indicated by the significant negative correlation between sex ratio (percent males) and hatching success ($\tau = -0.06$, $z = -1.73$, $P = 0.04$, $n = 361$; Fig. 2). The magnitude of this effect, however, was very small and the slope of Kendall's robust line fit was virtually zero. In contrast, mortality due to nest predation was not significantly correlated with sex ratio for any of the quadrat sizes examined.

To test further whether mortality rate was higher at male-producing sites than at female-producing sites, we conducted two additional tests using vegetation cover instead of sex ratio. First, we calculated a three-way Mantel correlation between vegetation cover and hatching success, holding geography constant. Second, we calculated a three-way Mantel test of average vegetation cover and predation (number of nests predated per quadrat) for each of the four quadrat sizes used in previous analyses. The expectation under Reinhold's (1998) hypothesis, given that vegetation cover is positively related to sex ratio (percent males) (Janzen, 1994b), is that nests with more vegetation cover should exhibit lower hatching success or experience higher predation.

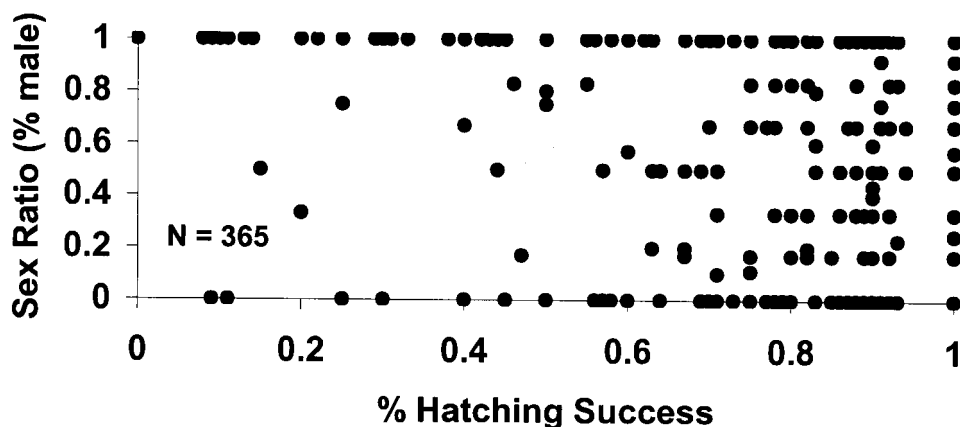


Fig. 2. Scatterplot illustrating the relationship between hatching success and sex ratio in *Chrysemys picta* nests.

Contrary to expectations, mortality was not higher at sites with more vegetation cover compared with more exposed sites, as indicated by the lack of significant correlations in all of the tests.

DISCUSSION

Despite intensive research, no clear empirical evidence explains the evolution and persistence of temperature-dependent sex determination in reptiles. Several proposed hypotheses can be divided into four major categories (reviewed in Ewert and Nelson, 1991; Burke, 1993; Shine, 1999): the 'phylogenetic inertia hypothesis', the 'group-structured adaptation hypothesis', the 'sib-avoidance hypothesis' and the 'differential fitness hypothesis'. Only the 'differential fitness hypothesis' (Charnov and Bull, 1977), which was the first to be suggested, remains a viable candidate to explain the evolution of temperature-dependent sex determination in reptiles (Ewert and Nelson, 1991; Janzen and Paukstis, 1991b; Burke, 1993; Shine, 1999).

Reinhold (1998) proposed that natal homing could lead directly to the evolution of temperature-dependent sex determination by providing sex-specific fitness conditions required by Charnov and Bull's (1977) model. The validity of this and other hypotheses can be tested by studying the fitness consequences of nest-site choices made by females in natural populations (*sensu* Martin, 1998). Support for Reinhold's (1998) hypothesis derives from the sea turtle *Eretmochelys imbricata*, which exhibits natal homing and also shows a positive correlation between offspring survival and the proportion of female offspring in a nest (Swingland *et al.*, 1990; Horrocks and Scott, 1991; Reinhold, 1998). If Reinhold's hypothesis is general, it should hold for many or all species with temperature-dependent sex determination and natal homing. We present an initial empirical evaluation of several assumptions and predictions of Reinhold's hypothesis as applied to the freshwater *C. picta* within a major nesting beach across 5 consecutive years.

Tests of assumptions of Reinhold's (1998) hypothesis

Nest-site philopatry exists

Reinhold's (1998) hypothesis was proposed for species with natal homing and thus requires that females show nest-site philopatry. The scale of female philopatry combined with the ecological factors that influence offspring sex determination and survival must be established to assess the applicability of the hypothesis to a given species. Although *C. picta* females appear philopatric to nesting beaches (Schwarzkopf and Brooks, 1987; Lindeman, 1992; Scribner *et al.*, 1993; F.J. Janzen, personal observation), we explored the existence of nest-site fidelity at a finer spatial scale, and found that females are somewhat philopatric to particular geographic sites within the nesting beach and also to microhabitat vegetation cover. Interestingly, females showed significant philopatry to vegetation cover but not as a result of their philopatry to geographic site, because geography was held constant in the three-way correlation between female identity and vegetation cover. Nest-site philopatry could result from sedentary behaviour, in which females have small home ranges such that they feed and nest in the same area (Bass *et al.*, 1996). However, *C. picta* females feed away from their nesting area, such that the fine-scale philopatry they exhibit is not due to sedentary behaviour. Females that show natal homing must also exhibit nest-site philopatry,

whereas the converse is not necessary. If daughters use the nesting patch of their mothers due to natal homing, then full- and half-sisters born in the same or subsequent years should nest in close proximity or in the same type of microhabitat; thus genetically similar individuals should be clustered in space. A study of natal homing to test for genetic relatedness of nesting females is ongoing. The significant genetic structuring among nesting beaches detected by Scribner *et al.* (1993) suggests that we might detect such fine genetic structuring.

Conditions that influence fitness of hatchlings are stable at nesting sites

For nest-site philopatry to induce sex-specific differential fitness, Reinhold's (1998) hypothesis requires stability of patch quality. This happens because Reinhold's hypothesis states that imperfect natal homing should be rare, such that high-quality patches are used on average more often by the offspring of females nesting at high-quality patches than by offspring of females breeding at low-quality patches. Consequently, because under natal homing daughters use the nesting patch of their mothers, the conditions that influence hatchling fitness must be stable at nesting sites. Otherwise, the quality of the patch used by the offspring to breed (whether geographic site or microhabitat type) would not match the quality of the patch used by their mother. Patch quality is defined in the hypothesis as offspring survival. If incubation conditions reflect patch quality, they should be consistent among years. If patch quality is stable and operates in synergism with nest-site philopatry, then survival rates should be consistent among years. And because temperature-dependent sex determination allows embryos to differentiate into the sex that attains the highest fitness at a given patch quality (*sensu* Charnov and Bull, 1977), then, if patch quality is stable, the offspring sex ratio produced at those patches should be repeatable.

Hatching success, predation and sex ratio at particular geographic locations or at particular types of microhabitat (vegetation cover) were significantly different among years (see also Janzen, 1994a) and, therefore, were non-repeatable across years. Even though differences in vegetation cover among nests were significantly correlated with differences in sex ratio overall (across 5 years) (see also Janzen, 1994b), the relationship was not significant for 1996 and 1997 (two very cold years) when analysed independently. Therefore, sex ratio production at geographic or vegetation patches within the nesting beach is not stable across years, as Reinhold's (1998) hypothesis requires. Moreover, the hatching success and sex ratio of sequential nests laid by given females were not significantly more similar than were those from nests laid by different females. Therefore, hatching success and sex ratio were not repeatable at the individual female level, even though females showed philopatry to geographic location and vegetation cover. In fact, the relationship between vegetation cover and mean-July nest temperatures, the parameter correlated with sex ratio production (Janzen, 1994b), changes among years according to climatic conditions (C.L. Morjan and F.J. Janzen, unpublished data). The repeatability analysis within females included multiple nesting episodes of females within and across years.

On the other hand, nests laid by the same females experienced more similar predation (high or low depending on the female) across years in comparison with nests laid by different females. To explore further whether philopatry to geographic site or microhabitat type could have an advantage in terms of predation (i.e. if philopatry was found only in those females whose nests were not predated), we performed two *a posteriori* tests. First, we created two new design matrices representing two alternative hypotheses. In one matrix, cells corresponding to same-female comparisons and non-predated nests were given a value

of 1 and all other cells were given a value of 0. In the other matrix, cells corresponding to same-female comparisons and predated nests were given a value of 1 and all other cells were given a value of 0. We calculated the three-way Mantel correlation for each of these two matrices and vegetation cover, holding geography constant. The results were not significant in either case, suggesting that the nests of females were not predated (or did not avoid predation) consistently because they were placed in particular types of vegetation cover. However, these two new design matrices contained many more 0's than 1's, which could be responsible for the non-significance of the results. Consequently, we performed a second test after dividing the data set into two subsets, one including all predated nests and the other including all non-predated nests. We then reran the tests of female philopatry to geographic sites and to microhabitat types in each of these subsets, but these tests were also non-significant. Overall, then, we found no evidence of an advantage (or cost) of female philopatry, because philopatry to geographic site or microhabitat type did not result in nest predation avoidance (or risk). The nests of some females were predated consistently over the years, whereas nests of other females survived consistently over the years independently of female philopatry, an observation that may have important implications with respect to the potential biased representation of some female lineages in the population (see also Scribner *et al.*, 1993). At this moment, we cannot provide an explanation for this predation repeatability within females.

Tests of predictions of Reinhold's (1998) hypothesis

Preferred nest sites are high-quality sites and thus produce predominantly females

Reinhold (1998) predicts that high-quality sites (i.e. those with the highest offspring survival) benefit female offspring more than male offspring in the following manner. If females show natal homing, daughters will likely nest at the site of their own nest. Females nesting at high-quality sites will increase the number of prospective offspring by producing predominantly daughters, whereas females nesting at low-quality sites should produce predominantly sons, because the number of prospective offspring produced by males is not affected by the quality of their natal patch (Reinhold, 1998). Although not stated explicitly, Reinhold assumes that males do not exhibit natal homing, otherwise natal sites of males will affect their number of prospective offspring. The conditions of Reinhold's hypothesis are set such that low-quality patches are visited by the few females produced there plus a few others with imperfect natal homing born at high-quality sites. High-quality patches are visited by a large number of females produced in them and by a very few with imperfect natal homing born at low-quality sites. Contrary to this hypothesis, nest density and sex ratio showed no significant relationship for any of the quadrat sizes examined. Moreover, nest density and vegetation cover showed no significant relationship, suggesting that females were not more likely to frequent more exposed sites (which tend to produce more females) than sites with more vegetation. Also contrary to expectations, hatching success declined and predation increased at higher densities, suggesting that mortality was higher at preferred nesting sites. Higher predation at higher nest densities could result from two mechanisms that cannot be distinguished at present. On the one hand, predators may be attracted to nesting sites with higher density. On the other hand, predators may exhibit a random nest-search pattern, but are bound to randomly encounter more nests at high-density sites simply as a function of their abundance. Whatever the cause, this density-

dependent mortality can be an important ecological factor that hinders the applicability of Reinhold's (1998) hypothesis to *C. picta*.

Embryonic survival and offspring sex ratio should be correlated

Patch quality is defined in Reinhold's (1998) hypothesis as offspring survival, and temperature-dependent sex determination allows the production of offspring sex ratios that maximize offspring fitness according to patch quality (Charnov and Bull, 1977). Mortality is then expected to be higher in male-producing patches than in female-producing patches because males are supposedly produced in low-quality sites. For example, nest predation may be non-random with respect to nest temperatures (e.g. Congdon *et al.*, 1983, 1987; Temple, 1987; Janzen, 1994b). This correlation is the most critical test of Reinhold's (1998) hypothesis (K. Reinhold, personal communication). As expected from the hypothesis, we detected a significant and negative correlation between sex ratio (percent males) and hatching success, indicating that nests with lower hatching success produced slightly more males. On the other hand, predation and sex ratio were not significantly correlated. Furthermore, hatching success and predation were not significantly correlated with vegetation cover (the variable that predicts sex ratio), suggesting that, contrary to expectations, microhabitat sites that tend to produce more males (sites with more vegetation) did not exhibit higher quality (lower mortality).

CONCLUSION

We analysed the applicability of Reinhold's (1998) hypothesis to *Chrysemys picta* at the scale of a major nesting beach. His assumption of nest-site philopatry was partially fulfilled in *C. picta* at the level of microgeography and vegetation cover. However, his assumption that conditions related to hatchling fitness (hatching success, predation and sex ratio) were stable at nesting sites (microgeographic sites and vegetation cover type) was not supported. Furthermore, hatching success and sex ratio were not repeatable within females, even though females were philopatric to geographic sites and types of vegetation cover. Only predation was repeatable within females, but independently of female philopatry, such that philopatry was not advantageous or disadvantageous in terms of predation. Reinhold's (1998) predictions that preferred sites were high-quality sites (lower-mortality sites) and produced more females were also not supported. However, higher-quality sites (as measured by hatching success) tended to produce slightly more females, but those sites were not the most frequented by females. Therefore, Reinhold's (1998) hypothesis is not applicable to *C. picta* at the level studied – that is, within a nesting beach – because only a few of the necessary conditions were fulfilled.

Further evaluations, using alternative approaches, of Reinhold's (1998) hypothesis are warranted. For example, the necessary conditions may be fulfilled at a broader spatial scale – namely, when comparing beaches that differ in nesting density. However, at least for sea turtles, a finer geographical scale than between nesting beaches may be the scale relevant to Reinhold's (1998) hypothesis (Julliard, 2000). The use of alternative temporal scales may also provide important insights into this system. Namely, analyses across a multi-year time window were precluded using our current database, as was a more detailed analysis within nesting seasons (although the within-female analyses of repeatability included nesting episodes within and between years). These alternative temporal scales may shed light on perhaps relevant patterns not evident at the scale used in the present study. In particular,

given that it takes several years for an offspring to reproduce, a time-scale corresponding to the generation time may be appropriate (although the existence of overlapping generations also warrants the use of the year-to-year time-scale presented here). Finally, it should be noted that most of the correlations supporting portions of Reinhold's (1998) hypothesis reported in this study, while statistically significant, were weak. Therefore, the biological implications of these results in terms of the applicability of Reinhold's (1998) hypothesis to *C. picta* must be viewed with discretion. For a clear empirical explanation for the persistence of temperature-dependent sex determination in reptilian taxa, further study is required.

ACKNOWLEDGEMENTS

We are especially grateful to K. Reinhold for reviewing the manuscript and providing us with invaluable comments to improve it and to R.B. Huey, L.B. Slobodkin and an anonymous reviewer for their suggestions. We also thank D.C. Adams and M.S. Rosenberg for their advice on some of the statistical analyses. The research was supported in part by NSF grant DEB 962959 to F.J.J., Journal Paper No. J-19393 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3369, and supported by the Hatch Act and State of Iowa Funds.

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