



# Repeatability of microenvironment-specific nesting behaviour in a turtle with environmental sex determination

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Evolutionary thermal adaptation is increasingly being elucidated in a variety of systems. However, one of the most striking examples, temperature-dependent sex determination (TSD) in reptiles, has proven stubbornly difficult to decipher. Theoretical models suggest that selection on and heritable variation in thermal sensitivity of embryonic sex determination and maternal behavioural choice of thermal qualities of nest sites control the microevolutionary potential of TSD. To begin addressing this important issue, we conducted a multiyear field study of nesting behaviour in painted turtles (*Chrysemys picta*), which display TSD. We detected a significant field repeatability for overstorey vegetation cover around nests at oviposition, a trait that is correlated inversely with nest temperature during embryonic sex determination and positively with offspring sex ratio (percentage of male). Neither clustered nesting nor individual spatial repeatability in nest site choice caused this striking pattern. Instead, females consistently preferred nest environments with particular quantities of overstorey vegetation cover. These findings confirm a crucial assumption of models concerning the microevolution and adaptive significance of TSD in reptiles. The results also indicate that this system may be able to evolve via long-term maternal nesting behaviour in response to skewed sex ratios caused by environmental perturbations, such as gradual long-term climate change.

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Thermal adaptation is an increasingly hot topic in evolutionary physiology and population biology. Indeed, the adaptive bases of various thermal traits in a variety of systems are regularly being identified (e.g. Johnston & Bennett 1996; Bull et al. 1997; Gilchrist et al. 1997). Either directly or indirectly, this research has important ramifications for biotic responses to climate change for which there is minimal evidence of direct temperature effects (Kareiva et al. 1993; Root & Schneider 1993; but see e.g. Saether et al. 2000). One clear example of direct temperature effects involves the functional covariation between annual fluctuations in ambient temperature and offspring sex ratios in a population of turtles with temperature-dependent sex determination (TSD) (Janzen 1994a). Despite the impact of TSD on this key demographic parameter, the microevolutionary dynamics and potential of TSD have only been clarified in a single species of fish (Conover 1984; Conover & Heins 1987; Conover & Van Voorhees 1990).

Although TSD occurs in a variety of reptilian taxa, its evolutionary significance has puzzled biologists since its discovery 35 years ago (recently reviewed in Shine 1999).

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TSD has originated one or more times in amniote vertebrates (Janzen & Paukstis 1991a, b; unpublished data) and has subsequently differentiated considerably (Bull 1983; Ewert & Nelson 1991; Janzen & Paukstis 1991a; Ewert et al. 1994; Lang & Andrews 1994; Viets et al. 1994; Cree et al. 1995). However, the selective forces involved have not been satisfactorily established empirically in either the macroevolutionary or microevolutionary scenarios (Shine 1999), and only in the latter case have the genetic mechanisms involved even been partially elucidated (see below).

Theoretical models (Bulmer & Bull 1982; Charnov 1982; Bull 1983; Karlin & Lessard 1986) show that, after TSD is established, the microevolution of sex ratio is governed by selection on and heritable variation in two key factors: (1) thermal sensitivity of embryonic sex determination and (2) maternal behavioural choice of thermal qualities of nest sites. Laboratory studies have detected significant heritabilities ( $h^2$ ) for the former trait (Bull et al. 1982; Janzen 1992; Girondot et al. 1994; Rhen & Lang 1998); a single laboratory experiment has examined the quantitative genetic basis of the latter trait in a reptile with TSD (Bull et al. 1988). Although these  $h^2$  values are substantial, the effective heritabilities of these traits are in fact much lower because identical constant

temperatures were used in the experiments instead of a variety of temperatures as found among natural nests, thereby inflating the laboratory estimates of  $h^2$  (e.g. Bull et al. 1982; Janzen 1992). Thus, both sex ratio factors may evolve slowly in response to sex ratio selection in nature. However, neither trait has been evaluated under field conditions.

We conducted a multiyear field study to investigate the repeatability ( $r$ ) of nest site choice behaviour in a population of turtles with TSD (Janzen 1994a, b; Weisrock & Janzen 1999). The repeatability of a trait in microevolutionary analyses is crucial for two reasons: (1) consistent individual differences are required for selection to act on the trait in a meaningful way (e.g. Brodie & Russell 1999) and (2) it places an upper bound on the heritability of the trait (i.e.  $r \geq h^2$ ) and thus on evolutionary responses to selection (Boake 1989; Arnold 1994 but see e.g. Aragaki & Meffert 1998). We focused on assessing the repeatability of overstorey vegetation cover around nests at oviposition for three important reasons: (1) more dramatically than thermal sensitivity of embryonic sex determination, nest site choice behaviour with respect to overstorey vegetation cover may vary geographically (e.g. turtles in southern populations tend to nest in shadier microenvironments than turtles in northern populations; Ewert et al. 1994), suggesting microevolutionary potential, (2) female 'control' of offspring sex ratio is a crucial assumption of recent models concerning the adaptive significance and maintenance of TSD in reptiles (Reinhold 1998; Roosenburg & Niewiarowski 1998), and (3) this trait predicts nest thermal (but not hydric) environments during the period of embryonic sex determination (Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data) as well as offspring sex ratios in environmentally typical years (Janzen 1994b). Consequently, overstorey vegetation cover around nests at oviposition could be a reliable long-term cue by which gravid females 'choose' the thermal characteristics of a nest site to influence their offspring phenotypes, including gender.

## METHODS

### Study Area and Population

We monitored nesting events of individually marked painted turtles (*Chrysemys picta*: Emydidae) from 1995 to 1997 on the south 'beach' (ca. 250 m at the longest point by ca. 75 m at the widest point) of an island in the Mississippi River (see Janzen 1994b). The nesting beach is a level, grassy area, composed mainly of moist loamy soil and interspersed with patches of mature, primarily deciduous, trees; the grass is mowed approximately once weekly throughout the summer by the Army Corps of Engineers. This nesting population is well characterized, having been the focus of sex determination studies since 1988 (Janzen 1994a, b; Weisrock & Janzen 1999; Morjan & Valenzuela in press; C. L. Morjan & F. J. Janzen, unpublished data). Turtles nested primarily in June of each year (474 of 485 nests) and often nested twice within one year (46 of 79 females). We patrolled the nesting area

approximately once hourly between sunrise and sunset. This procedure caused us to miss a number of nesting events that occurred quickly (see below), but minimized our disturbance of females during the nesting process. After each nesting event was completed, we captured the female for identification and immediately recorded the precise location and quantity of overstorey vegetation cover around the nest.

We estimated overstorey vegetation cover with a Model-A convex spherical densiometer (Janzen 1994b; Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data), which is commonly used to assess canopy density (e.g. Lemmon 1956; Rumble 1992). Overstorey vegetation cover on the south and west sides of nests in this population is a strong predictor of nest temperatures during the period of embryonic sex determination (Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data). We did not focus on ground temperature at oviposition because females do not use it as a cue for nest site choice (Morjan & Valenzuela in press). Furthermore, although ground temperature at oviposition is some function of vegetation cover, it is also greatly affected by time of day, cloud cover, temporal proximity of precipitation events, and so forth at the time of a nesting event. Overstorey vegetation cover at a particular location instead is consistent throughout the incubation period at this field site and is uninfluenced by such transient environmental factors (Janzen 1994b). For nesting turtles, overstorey vegetation cover is thus a more reliable cue than ground temperature for predicting relative nest temperature in the future when embryonic sex is being determined.

During the 3 years of this study, we identified 190 different females nesting at the study site. All but 79 of these females (i.e. 111) were documented nesting only once during this period; another 167 nests were laid by females we did not observe. To calculate the repeatability of nest site choice with respect to overstorey vegetation cover, we thus focus on those 79 females for which we documented two or more nesting events between 1995 and 1997. We first obtained the within- and among-female mean squares from a one-way analysis of variance (ANOVA) in JMP version 3.2.1 (SAS Institute 1997) and then used these values and the harmonic mean of the number of nesting events per female to calculate  $r$  properly (see equations 2–5 of Arnold 1994; Lessells & Boag 1987; Boake 1989).

### Spatial Analyses

We evaluated spatial aspects of nest location in three ways. We first quantified overstorey vegetation cover at 5-m intervals throughout the nesting area in a massive grid ( $N=568$  total readings) in June 1998. We make the assumption that these readings provide a reasonable description of available vegetation microenvironments at the field site during the study (*sensu* Janzen 1994b), because essentially all the overstorey vegetation cover was produced by large, slow-growing, mature trees. We subsequently calculated the proportion of readings in each quartile of overstorey vegetation cover (0–25, 26–50,

51–75, 76–100%), did the same for all nests ( $N=480$ ) and for the nests ( $N=207$ ) and nesting preferences ( $N=79$ ) of the focal females, and tested for differences among these data sets (Sokal & Rohlf 1981).

Nests located close together are likely to experience similar amounts of overstory vegetation cover. Therefore, if individual females oviposited in the same location consistently, a repeatability with respect to overstory vegetation cover could arise. Consequently, we also used both geostatistical methods and distance matrix analyses to rigorously assess the level of spatial dependence between nests and overstory vegetation cover. To provide the spatial references for these two approaches, we accurately established  $X/Y$  coordinates for all 207 nests of the 79 focal females and 568 vegetation cover grid points using INTERPNT (Boose et al. 1993). This technique uses triangulation to produce cartesian coordinates for each nest based on distance measurements between nests and to reference points of known coordinates.

We used Cartesian coordinates from INTERPNT to construct separate semivariograms of overstory vegetation cover for the nests and grid points with Kriging Interpolator 3.1 script for ArcView Spatial Analyst (Environmental Systems Research Institute 1996). In these geostatistical analyses, semivariograms model the expected variability in overstory vegetation cover between nests or grid points as a function of distance. The semivariance for points separated by a distance  $h$  is defined as:

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i+h) - z(x_i)]^2$$

where  $N(h)$  is the number of pairs of points separated by  $h$  (Rossi et al. 1992; Aubry & Debouzie 2000). In our case,  $z(x_i)$  is the vegetation cover over nest  $x_i$ , and  $z(x_i+h)$  is the vegetation cover over a nest separated from  $z(x_i)$  by distance  $h$ . The distance between nests or grid points beyond which overstory vegetation cover is no longer autocorrelated occurs when the standardized semivariogram equals one (i.e. when the semivariogram equals the sampling variance) (Rossi et al. 1992). Nests or grid points closer together than the distance at which the standardized semivariogram equals one would be considered spatially dependent with respect to overstory vegetation cover.

To determine the best representation of the data, we compared the semivariograms for nests and grid points to five standard models (circular, exponential, Gaussian, linear with sill, and spherical). We determined the goodness of fit of both semivariograms to the standard models both by visually examining the fit of a semivariogram to the model lines and by Akaike's Information Criterion (AIC) (see Aubry & Debouzie 2000). Specifically,  $AIC = 2 \log_e(\text{likelihood ratio}) + 2(\text{number of parameters in the model})$ ; the model with the smallest AIC value is preferred. The semivariograms for both nests and grid points were best represented by the exponential model. That is, the independence between nests or grid points in terms of overstory vegetation cover increases exponentially with increasing distance. We thus standardized the semi-

variograms by dividing the semivariance by the total variance according to the exponential model (Rossi et al. 1992).

We employed distance matrix methods to evaluate independently the more visually oriented outcome of the geostatistical analyses. Here we ask whether individual females have statistically more similar overstory vegetation cover readings for their nests compared to nests laid by different females while controlling for geographical distance between nest locations. When using these procedures, observable relations are expressed as a distance (or dissimilarity) matrix between all pairs of samples (Sokal et al. 1997). In our case, we computed the geographical distances between nest locations (Euclidean distances from the  $X/Y$  coordinates) and the absolute value of the difference between overstory vegetation cover values for each pair of nests. We also created a design matrix (Sokal et al. 1997) 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. Because overstory vegetation cover was spatially autocorrelated, we performed a three-way Mantel test to calculate the correlation between the design matrix of female identity and the matrix of overstory vegetation cover, holding geographical distance constant (*sensu* Smouse et al. 1986).

## RESULTS

### Repeatability

The 79 focal female turtles averaged (arithmetically) 2.6 documented nests during the 3 years of this study. Of these turtles, 64 nested at least once in each of 2 or more years; the remaining 15 turtles laid multiple nests only within a single year. We included all 79 females in our statistical analyses because there was no evidence of seasonal variation in individual nesting preferences with respect to overstory vegetation cover in this population (C. L. Morjan & F. J. Janzen, unpublished data).

The 79 focal females nested in nearly the full range of possible overstory vegetation cover (0.5–90.6%), and hence in nearly the full range of nest microenvironments, averaging 44.0% overstory vegetation cover on the south and west sides of each nest. Despite this considerable variation, we detected a significant field repeatability of overstory vegetation cover around nests at oviposition (repeatability:  $r=0.18$ ,  $N=79$  females and 207 nests,  $P=0.01$ ). That is, individual females consistently nested in areas with similar amounts of overstory vegetation cover. This result is robust in that even restricting the analysis to the 40 females with three or more nesting events produced a significant repeatability of similar magnitude ( $r=0.21$ ,  $N=40$  females and 129 nests,  $P<0.01$ ).

### Spatial Distribution of Nests

Turtles did not, however, simply all nest in the same location at the field site and thereby produce similar

overstorey vegetation cover values. Although certain regions contained high densities of nests, other localities were also used for nesting (Fig. 1); indeed, lightly and heavily shaded sites are distributed throughout the nesting area, providing an extensive microclimatic array (Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data) within which turtles can choose to nest without having to cluster.

Females instead differed in their individual preferences for overstorey vegetation cover around nests at oviposition despite the intermediate average for overstorey vegetation cover around all nests (Fig. 2). Certain females consistently nested in unshaded sites, others consistently nested in shaded sites, and the majority consistently nested in sites with intermediate shade environments. To illustrate the distribution of these nesting preferences, we compared background levels of available overstorey vegetation cover at the field site quantified by the grid measurements to quantities of overstorey vegetation cover chosen by all females and just by the 79 focal females (Table 1). Note that turtles in this population tended to nest disproportionately in relatively open microsites and clearly avoided the most heavily shaded areas (Table 1).

If individual females simply oviposited repeatedly in the same physical microsite within and across years, the observed repeatability could be caused by the nest sites experiencing similar readings merely because they were laid closer together. However, the significant field repeatability in overstorey vegetation cover around nests at oviposition was not due to individual microspatial philopatry. This result can be illustrated first by examining the semivariograms of nests and grid points with respect to overstorey vegetation cover to determine the range of spatial dependence (i.e. the distance between locations at which the overstorey vegetation cover readings were spatially related) (Fig. 3). For both semivariograms, the range of spatial dependence was ca. 15 m, indicating that overstorey vegetation cover readings were independent for pairs of nests or grid points greater than 15 m apart. In our study, only 21 of 184 pairs of nests by the same female were less than 15 m apart. This result differs substantially from the 184 out of 184 expected if females nested repeatedly in the same 'location' ( $Z$  test:  $Z=16.99$ ,  $N=184$ ,  $P<0.0001$ ). A Mantel test of the relationship between individual females and overstorey vegetation cover holding 'geography' constant independently confirmed this finding (Mantel test:  $r=0.008$ ,  $P=0.0294$ ). Thus, although geographical philopatry within females exists in this population, it occurs at a spatial scale greater than the spatial autocorrelation of overstorey vegetation cover. Microspatial philopatry was therefore absent and cannot account for the repeatability in overstorey vegetation cover around nests that we detected.

## DISCUSSION

For nesting behaviour to influence the microevolution of sex ratios under TSD, females must be able to 'control' the sex ratio of their offspring and this 'control' must be heritable (Bulmer & Bull 1982; Charnov 1982; Bull

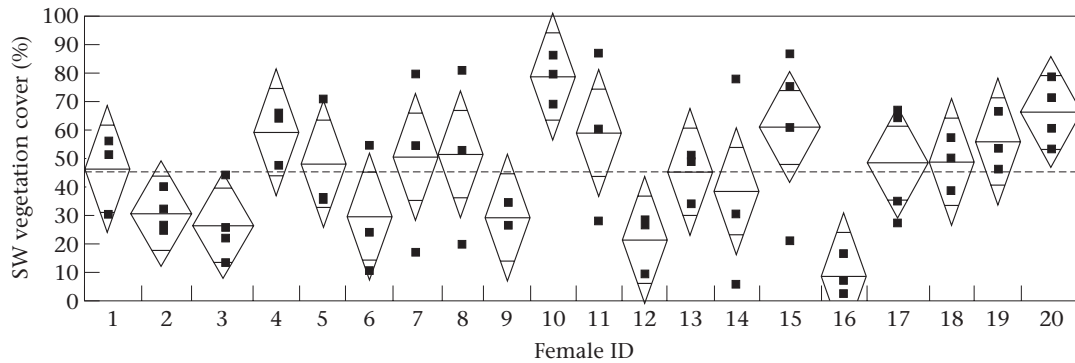
1983; Karlin & Lessard 1986; Carvalho et al. 1998). Our empirical results are roughly in accord with these expectations from theory. That is, individual females in a natural population consistently nested in locations with similar quantities of overstorey vegetation cover that cannot be explained by fidelity to specific microsites. The significant repeatability that we detected suggests that this aspect of nest site choice behaviour may show evolutionary potential. Because overstorey vegetation cover is a reasonable predictor of relative nest thermal environments and offspring sex ratios in this population (Janzen 1994b; Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data), TSD also may be able to evolve adaptively, although probably not rapidly due to annual climatic and other constraints (Janzen 1994a; Carvalho et al. 1998; Valenzuela & Janzen, *in press*), in response to biased sex ratios caused by gradual long-term environmental change.

Climate change may influence the evolution of nest site choice behaviour in species with TSD, but climatic variation may be important as well. When climate varies substantially between nesting seasons at a locality, the relationship between overstorey vegetation cover and nest sex ratio is overridden (Janzen 1994b). In fact Valenzuela & Janzen (*in press*) did not detect a significant within-female repeatability in sex ratio in a 5-year study of the same painted turtle population described herein. This result is not surprising given that in only 1 of the 5 years was air temperature within one standard deviation on either side of the 60-year mean during the sex-determining period. However, concern with short-term, year-to-year climatic variation may not be the relevant perspective with which to view the impact of nest site choice behaviour on sex ratio evolution in this system, given that a typical female painted turtle has a greater than 90% annual survival rate after maturity (Mitchell 1988; Iverson & Smith 1993; but see Frazer et al. 1991). In other words, the pertinent temporal scale for appropriate analysis of sex ratio evolution in taxa with TSD may be a function of maturational longevity in these long-lived species: do females differ in their lifetime offspring sex ratios and, if so, do these differences reflect lifetime variation in nest site choice behaviour among individuals? We anxiously await long-term empirical research that addresses these issues directly.

Nonrandom nesting behaviour in turtles has been documented previously, mainly with respect to macro-environmental parameters like nesting beaches or zones on nesting beaches (reviewed in Janzen 1994b; Roosenburg & Niewiarowski 1998; see also Swingland et al. 1990; Scribner et al. 1993). Although these localities may show different thermal environments and thus influence offspring sex ratios (reviewed in Janzen 1994b; Mrosovsky 1994; Roosenburg & Niewiarowski 1998), actual nest site choice with respect to factors strongly linked to particular thermal microenvironments had not been documented previously in natural populations, and this behaviour seems more likely to exemplify the key maternal behaviour parameter in models describing the microevolution of TSD (Bulmer & Bull 1982; Charnov 1982; Bull 1983; Karlin & Lessard 1986;



**Figure 1.** ArcView diagrams of the main nesting area at the field site indicating (a) overstory vegetation cover in 25% increments (e.g. darkest=76–100% covered) and locations of painted turtle (*Chrysemys picta*) nests in (b) 1995 ( $N=131$ ), (c) 1996 ( $N=153$ ) and (d) 1997 ( $N=196$ ). Five nests in 1997 were laid ca. 10 m north of the northern limit of this area. Note that most regions of the field site contained nests, although some localities were used more heavily than others.



**Figure 2.** Overstory vegetation cover around nests for 20 of the 79 focal painted turtles (*Chrysemys picta*). The dashed line indicates the mean overstory vegetation cover around nests for all 79 females. Each point represents one nest and each diamond represents a statistical summary of overstory vegetation cover around nests for one of the 20 females illustrated. For each diamond, the centre line indicates the mean, the end points indicate 95% confidence intervals for the mean, and the lines flanking the mean indicate overlap marks (i.e. any two means with the same sample size are significantly different if the marks do not overlap) (overlap marks =  $(2^{0.5}/2)$  (length of the confidence interval)). Note the relative consistency of overstory vegetation cover within each female compared with the differences between females.

**Table 1.** Distribution of nesting preferences of painted turtles with respect to available overstory vegetation cover

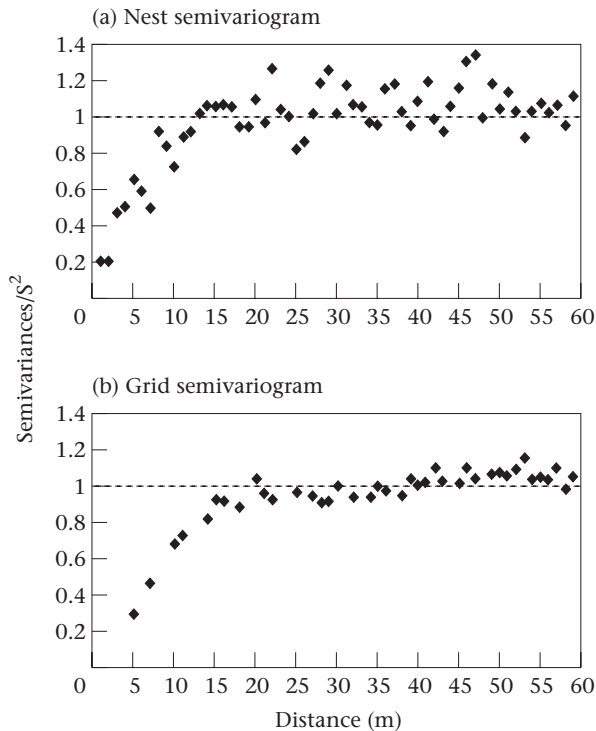
Quartile of vegetation cover*	Transect sites, N (%)	All nests, N (%)	All focal nests, N (%)	Mean focal nests, N (%)
0–25%	89 (15.7)	116 (24.2)	42 (20.3)	8 (10.1)
>25–50%	130 (22.9)	193 (40.2)	82 (39.6)	43 (54.4)
>50–75%	111 (19.5)	125 (26.0)	64 (30.9)	27 (34.2)
>75–100%	238 (41.9)	46 (9.6)	19 (9.2)	1 (1.3)
Total	568	480	207	79

\*Vegetation cover is defined as the average percentage of overstory vegetation cover on the south and west sides of a location of interest (i.e. transect site or nest) as determined by a spherical densiometer (see Methods for more details). The total number of nests here is 480 because data for overstory vegetation cover were not recorded for five nests in 1996. Using the quartile categorization of vegetation cover in the table, turtles nested nonrandomly with respect to this variable regardless of whether all nests ( $G$  test:  $G_3=151.49$ ,  $P<0.0001$ ), all focal nests ( $G_3=86.89$ ,  $P<0.0001$ ), or means for the focal females ( $G_3=81.55$ ,  $P<0.0001$ ) were considered. Nesting preferences overall ( $N=480$ ) did not differ significantly from the subset chosen by the focal females ( $N=207$ ) ( $G_3=2.23$ , NS).

Carvalho et al. 1998). Indeed, previous studies of nest site choice behaviour in species with TSD have been hampered by having to identify how a female chooses a thermally related cue at oviposition when the period of embryonic sex determination occurs weeks subsequent to the nesting event (reviewed in Janzen & Paukstis 1991a). Overstory vegetation cover around nests at oviposition may provide such a predictive mechanism because it covaries with nest thermal environments (Ewert et al. 1994; Weisrock & Janzen 1999; C. L. Morjan & F. J. Janzen, unpublished data) and with offspring sex ratios in typical years (Janzen 1994b).

Of course, vegetation cover might also be linked to factors other than nest temperature during embryonic development that have significant fitness ramifications in turtles, which could influence the evolution of nest site choice behaviour (e.g. Weisrock & Janzen 1999). For our study population we can rule out at least three variables thought to be potentially important in this regard:

(1) substrate moisture, (2) nest predation and (3) hatching success. Increased substrate moisture during incubation is linked in laboratory and field studies to increased body mass of resulting neonatal turtles (reviewed in Packard 1991; see also e.g. Cagle et al. 1993). These larger hatchlings, in turn, have increased survival in the field during early life stages (e.g. Janzen et al. 2000). Analyses of covariance, using mean egg mass of nests at oviposition as the covariate, did not reveal significant relationships between mean offspring mass of nests and their overstory vegetation cover for any of the 3 years of the present study (1995:  $F_{1,41}=0.16$ ,  $P=0.6868$ ; 1996:  $F_{1,24}=1.53$ ,  $P=0.2278$ ; 1997:  $F_{1,147}=1.14$ ,  $P=0.2870$ ; sample sizes are comparatively small for 1995 and 1996 due to extensive nest predation). That is, neither relatively larger nor relatively smaller hatchlings resulted from nests with more or less overstory vegetation cover. The probabilities of nest predation and hatching success are also unrelated to overstory vegetation cover in this



**Figure 3.** Standardized semivariograms for (a) 207 nests of the 79 focal painted turtles (*Chrysemys picta*) and (b) 568 grid readings of background overstorey vegetation cover at the field site (see Materials and Methods for more details). The distance at which the semivariogram value equals the total sample variance (i.e. when the model lines cross the horizontal line) indicates the distance (ca. 15 m in both cases) above which any two physical locations are spatially independent with respect to overstorey vegetation cover. Only 21 of 184 pairs of nests by the same female were less than 15 m apart, suggesting that turtles nest independently of the locations of previous nest sites.

population. Three-way Mantel correlations between predation status (or hatching success) and a design matrix of within-/between-year comparisons, holding vegetation cover constant, detected no repeatability of either fitness measure in particular vegetation microenvironments (Valenzuela & Janzen 1992). In other words, from year to year, nests with particular quantities of overstorey vegetation cover were neither more or less likely to experience characteristic predation or hatching success rates.

The theoretical and biological significance as well as the genetic basis of oviposition site choice behaviour have been documented extensively in various organisms (e.g. Sezer & Butlin 1998). This topic is of particular interest in certain arthropods, where the quality of the oviposition site has substantial sex-specific fitness effects and thereby influences the evolution of sex ratio and sex allocation (e.g. Orzack & Parker 1986; Wrensch & Ebbert 1993; Crozier & Pamilo 1996; Frank 1998). Indeed, the remarkable ability of females to manipulate the sex ratio of their offspring, and the ecological and evolutionary consequences of this behaviour, are receiving increasing attention even in species with genotypic sex-determining mechanisms (e.g. Komdeur et al. 1997; Nager et al. 1999).

Although the sex-specific benefits, if any, of nest site choice behaviour in reptiles with TSD remain unclear, the microenvironment-based nest site fidelity documented in this study could conceivably signify long-term, but probably not short-term (Valenzuela & Janzen, in press), maternal 'control' of offspring sex ratio.

The identification of repeatability in the field of this remarkable behaviour is significant not only because of its demographic consequences, but also because it provides a crucial empirical underpinning for recent maternal effects models concerning the evolutionary maintenance and adaptive significance of TSD in reptiles, both long-standing puzzles in population biology (Shine 1999). In the former case, females are posited to select specific nest thermal environments to influence offspring sex ratio based on sex-specific benefits to the offspring (e.g. egg size effects on hatchling size) derived from maternal condition (Roosenburg & Niewiarowski 1998). In the latter model, nest site philopatry is hypothesized to lead directly to the evolution of TSD because it is inherited maternally and thus male and female fitnesses are affected differentially (Reinhold 1998). Both promising models bring nest site choice behaviour to the heart of the long-standing debate concerning the evolutionary significance of TSD, although both also suffer from a lack of widespread empirical support (C. L. Morjan & F. J. Janzen, unpublished data; Valenzuela & Janzen, in press).

The repeatability that we detected does not mean that females are constrained in their nesting behaviour. This value was not so large as to preclude a reasonable amount of within-female plasticity in nest site choice. For example, females could still alter clutch sex ratios in response to, say, the breeding sex ratio. Even so, there is no evidence for females adjusting offspring sex ratio in relation to breeding sex ratio in this or other systems (e.g. Bensch et al. 1999).

What about the underlying basis of the significant field repeatability? Is it heritable or is it the consequence of some nongenetic effect (e.g. imprinting)? If primiparous turtles nested in a site with a particular overstorey vegetation cover and then simply copied that behaviour in future events, a nongenetically based repeatability with respect to overstorey vegetation cover would arise. Such a scenario, however, seems unlikely to have evolved in this system. Any females nesting (for the first time and then subsequently) in suboptimal sites in terms of overstorey vegetation cover would be at a disadvantage compared with other females, which may explain why essentially no females consistently nested in cold, highly vegetated sites in this northern population (Table 1). In fact, the disadvantages of an imprinting scenario would seemingly select instead for a genetic basis for nest site choice behaviour, as we hypothesize, so that the trait would evolve in response to selection for certain nesting preferences. The 'imprinting' scenario would preclude an evolutionary response to selection and thus would be maladaptive.

Although our results are significant biologically, repeatability probably cannot be equated with heritability in microevolutionary models. Theoretically,  $r \geq h^2$  (Boake

1989; Arnold 1994), and thus presumably  $h^2 \leq 0.18$  in this study. However,  $r$ , and hence inference regarding the maximum value of  $h^2$ , can be underestimated for a variety of reasons (Boake 1989; Aragaki & Meffert 1998). Sampling bias (e.g. the 79 turtles that we evaluated may have been a nonrandom sample of the 190 identified females; but see Table 1) and behavioural interaction (e.g. females may have modified the basis of nest site choice behaviour depending on the frequency or number of mating attempts by males), among other factors, can all cause  $r$  to be underestimated when  $h^2$  is in fact larger (Boake 1989; Aragaki & Meffert 1998). Although breeding designs to estimate  $h^2$  directly are prohibitively difficult to conduct with this system, other methods (e.g. Ritland 1996) may be useful to clarify further the quantitative genetic basis of microenvironment-based nest site choice behaviour.

Our approach allowed a single meaningful variable to be quantified, permitting estimation of a potentially evolutionarily important parameter of nest site choice behaviour under natural conditions. We confirmed the existence and quantified the extent of microenvironment-based nest site choice in a turtle population with TSD, providing important empirical underpinnings for macro- and microevolutionary models of TSD (Bulmer & Bull 1982; Charnov 1982; Bull 1983; Karlin & Lessard 1986; Reinhold 1998; Roosenburg & Niewiarowski 1998; Shine 1999). By nesting non-randomly with respect to available overstory vegetation environments, females may exert a rough measure of long-term control over the sex ratio of their offspring. That this behaviour is repeatable for individual females suggests that, as in fish (Conover 1984; Conover & Heins 1987; Conover & Van Voorhees 1990), TSD in reptiles not only may be adaptive but also may be able to evolve in the long term in response to skewed sex ratios caused by environmental perturbations, such as gradual long-term climate change (Janzen 1994a; Mrosovsky 1994; Rhen & Lang 1998).

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