

Heritable Variation for Sex Ratio Under Environmental Sex Determination in the Common Snapping Turtle (*Chelydra serpentina*)

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ABSTRACT

The magnitude of quantitative genetic variation for primary sex ratio was measured in families extracted from a natural population of the common snapping turtle (*Chelydra serpentina*), which possesses temperature-dependent sex determination (TSD). Eggs were incubated at three temperatures that produced mixed sex ratios. This experimental design provided estimates of the heritability of sex ratio in multiple environments and a test of the hypothesis that genotype \times environment (G \times E) interactions may be maintaining genetic variation for sex ratio in this population of *C. serpentina*. Substantial quantitative genetic variation for primary sex ratio was detected in all experimental treatments. These results in conjunction with the occurrence of TSD in this species provide support for three critical assumptions of Fisher's theory for the microevolution of sex ratio. There were statistically significant effects of family and incubation temperature on sex ratio, but no significant interaction was observed. Estimates of the genetic correlations of sex ratio across environments were highly positive and essentially indistinguishable from +1. These latter two findings suggest that G \times E interaction is not the mechanism maintaining genetic variation for sex ratio in this system. Finally, although substantial heritable variation exists for primary sex ratio of *C. serpentina* under constant temperatures, estimates of the effective heritability of primary sex ratio in nature are approximately an order of magnitude smaller. Small effective heritability and a long generation time in *C. serpentina* imply that evolution of sex ratios would be slow even in response to strong selection by, among other potential agents, any rapid and/or substantial shifts in local temperatures, including those produced by changes in the global climate.

THE evolution of sex ratio has intrigued biologists for many decades (*e.g.*, DARWIN 1871), and has been an especially popular subject among theoretical geneticists in recent years (HAMILTON 1967; TRIVERS and WILLARD 1973; MAYNARD SMITH 1978; CHARNOV 1982; BULL 1983; KARLIN and LESSARD 1986). The theoretical basis for much of the current work was developed by FISHER (1930), who derived a simple model showing that the population sex ratio ought to evolve toward unity. This result has been used to explain the 1:1 sex ratios typically observed in natural populations of a wide variety of non-haplo-diploid organisms (reviewed in BULL and CHARNOV 1988). In fact, sex ratios other than 1:1 in populations of such species are commonly thought to be temporary excursions from unity, in the absence of complications such as local mate competition. This application of sex ratio theory has only recently been questioned despite the general lack of evidence for several assumptions of the theory concerning the presence and nature of genetic variation for sex ratio (BULL and CHARNOV 1988).

Models derived from FISHER's theory for the gradual adaptive evolution of sex ratio typically assume that genetic variation (1) exists for the primary sex

ratio, (2) is caused by genes of modest effects and (3) is exposed to Mendelian segregation. Unfortunately, evaluation of the quantitative genetic basis for sex ratio has been hindered by the inability to observe the primary sex ratio in many species or to rule out major-gene and sex-linked factors that might control the primary sex ratio (KING 1918; WEIR 1962; BAR-ANAN and ROBERTSON 1975; CONOVER and HEINS 1987; NAYLOR, ADAMS and GREENWOOD 1988; M. ANTOLIN, personal communication). Consequently, reports of quantitative genetic variation for primary sex ratio are uncommon (BULL, VOGT and BULMER 1982; PARKER and ORZACK 1985; ORZACK 1986, 1990; ORZACK and PARKER 1986, 1990; SULLIVAN and SCHULTZ 1986; ORZACK, PARKER and GLADSTONE 1991). In particular, species that reproduce viviparously, which can obscure the primary sex ratio due to differential mortality *in utero*, or that possess sex chromosomes pose considerable difficulties in determining a quantitative genetic basis for the primary sex ratio. In contrast, a number of species of reptiles exhibit temperature-dependent sex determination (TSD), in which sex of the offspring developing in the eggs is determined after conception by temperatures experienced in the nest during incubation (reviewed in JAN-

ZEN and PAUKSTIS 1991). Low incubation temperatures typically produce male offspring in turtles and female offspring in lizards and crocodylians, whereas the converse is true at high incubation temperatures (see BULL 1980). However, both sexes are produced over a short span of temperatures centered at about 28° (*i.e.*, approximately the threshold temperature of sex determination), which may be subject to genetic control since it differs both within and among species (PAUKSTIS and JANZEN 1990; JANZEN and PAUKSTIS 1991). As a result, species with TSD permit estimation of quantitative genetic variation in primary sex ratio that can be separated experimentally from effects of sex-biased mortality, major-gene factors, or sex-linked genes. Such findings would be of fundamental theoretical and evolutionary importance, because the lack of genetic variation would restrict evolution of sex ratio even under the strongest selection pressure.

To assess the existence and extent of quantitative genetic variation in sex ratio, I investigated the response of hatchling sex ratio to incubation temperature among families of the common snapping turtle (*Chelydra serpentina*), a species with TSD (reviewed in JANZEN and PAUKSTIS 1991). I used an experimental design in which families were crossed with three incubation temperatures to determine if genotype × environment (G × E) interactions might be the mechanism by which genetic variation in sex ratio is maintained in this system.

MATERIALS AND METHODS

Experimental procedures: Eggs of common snapping turtles (*Chelydra serpentina*) were collected from 15 recently-laid nests on National Wildlife Refuge land, Whiteside Co., Illinois, from 4 to 8 June 1990. Upon removal from nests, eggs were numbered and then stored in Styrofoam chests housed in a cool cellar prior to transport to the University of Chicago on 8 June.

In the laboratory, eggs were cleaned and then were weighed to the nearest 0.01 g. Two eggs from each clutch were randomly assigned to each of 16 plastic shoeboxes containing moistened vermiculite (−150 kPa) (JANZEN *et al.* 1990). The 30 eggs in each box were randomly assigned a position within a 5 × 6 matrix. The substrate of each container was rehydrated once weekly to provide a relatively constant hydric environment. Maintenance of a constant hydric environment precludes the effects of variation in water potential on sex determination (*e.g.*, GUTZKE and PAUKSTIS 1983; PAUKSTIS, GUTZKE and PACKARD 1984).

Four boxes were assigned to each of two incubators, one calibrated to 27.5° and the other to 28.5°. The remaining eight boxes were placed in an incubator set at 28.0°, which was expected to be the threshold temperature of sex determination for this population (*i.e.*, the temperature at which the sex ratio is 1:1) (F. J. JANZEN, unpublished data). This incubator arrangement provides an estimate of the reaction norm of sex ratio, that is, the clutch × temperature interaction that results in a differential phenotypic expression of sex ratio (DE JONG 1990). Boxes were rotated daily within the incubators to mitigate the potential influence of undetected thermal gradients on sexual differentiation of devel-

oping embryos (see BULL, VOGT and BULMER 1982).

Eggs hatched in approximately 2 months. Over 95% of all eggs hatched (458 out of 480). Turtles were weighed upon hatching and then were raised individually in plastic cups containing about 2 cm tap water at 24° under the natural photoperiod. Hatchlings were not fed, but subsisted on residual yolk resorbed internally prior to hatching. This procedure ensures that the gonads are well formed and unambiguous. After approximately 100 days, turtles were killed with an overdose of anesthetic (0.8 ml of 1:1 distilled H₂O:Nembutal) injected into the pericardial cavity. All specimens are preserved and are available for inspection.

Sex of the hatchlings was determined by macroscopic examination of the gonads (YNTEMA 1976; JANZEN *et al.* 1990). The sex of 40 randomly chosen individuals was confirmed by histological preparation of the gonads. In the statistical analyses of heritability of sex ratio, males were assigned a value of 1, females were assigned a value of 0, and the one "intersex" was given a value of 1/2.

Statistical procedures: The possible influence of clutch and treatment on hatching success were analyzed with a two-way analysis of variance, using arcsine square-root transformations of the percentages of eggs hatching as the dependent variable (SOKAL and ROHLF 1981). The potential effects of nesting date and differential mortality on sex ratio were evaluated with separate Pearson product-moment correlation analyses. The likelihood that sex-specific mortality influenced sex ratio also was determined using a heterogeneity G-test (SOKAL and ROHLF 1981).

Heritability of sex ratio was calculated for each temperature treatment separately and for the combined data, using the methods of BULL, VOGT and BULMER (1982) for a threshold trait with an underlying quantitative genetic basis. Since this method is somewhat difficult to reconstruct from the original account, I present a brief description as follows. First, the between-clutch sum of squares is

$$S = \sum_{i=1}^f (p_i - \bar{p})^2, \quad (1)$$

for f families of size n , where \bar{p} = the treatment sex ratio (*i.e.*, proportion of males). Consequently, the expected value of the mean square is

$$E\{S/(f-1)\} = \{(n-1)\sigma_g^2 + \sigma_B^2\}/n, \quad (2)$$

where σ_g^2 = the between-clutch variance in sex ratio and σ_B^2 = the phenotypic variance of sex = $p(1-p)$. The between-clutch variance in sex ratio (σ_g^2) and the within-clutch correlation of sex (ρ_B), respectively, are estimated as

$$\sigma_g^2 = Sn/\{(f-1)(n-1)\} - \sigma_B^2/(n-1), \quad (3)$$

$$\rho_B = \sigma_g^2/\sigma_B^2. \quad (4)$$

The within-clutch correlation of the continuous variable underlying sex, that is, the tetrachoric condition of sex (ρ_X), is calculated as

$$\rho_X = \sin\{(\pi/2)\rho_B\}, \quad \text{if } p = 1/2. \quad (5)$$

For $p \neq 1/2$, a table of appropriate transformations from Appendix I of BULL, VOGT and BULMER (1982) was used. Consequently, the heritability of sex (h_X^2) is $2\rho_X$ for full-sib families and $4\rho_X$ for half-sib families. This approximation assumes additive genetic variance, no maternal effects, and a constant thermal environment (BULL, VOGT and BULMER 1982). Confidence intervals on the estimates of heritability were calculated by dividing S by the lower and upper 2.5% of the χ^2 distribution with 14 d.f., because the ratio of S to the theoretical variance is distributed as χ^2 . These two values

TABLE 1

Analysis of hatching success of eggs of common snapping turtles (*C. serpentina*) incubated at three constant temperatures

Source of variation	d.f.	Sum of squares	Mean square	F value	P value
Clutch	14	0.866	0.062	3.966	0.0009
Temperature	2	0.087	0.044	2.805	0.0776
Error	28	0.437	0.016		

The dependent variable is the arcsine square-root transformation of the percentage of eggs hatching from each clutch in each treatment.

were substituted separately into Equation 2 with the results being substituted sequentially into Equations 3–5. Doubling the results of the final substitutions into Equation 5 yielded the 95% confidence intervals for h^2 .

Sex ratios also were analyzed as reaction norms. An analysis of variance of sex using clutch, temperature, and a clutch \times temperature term, was computed to test for heterogeneity of slopes. A significant interaction term would indicate a genotype-environment interaction for sex ratio (FALCONER 1989), implying that phenotypic expression of the genotype is not static and instead depends on the environment it experiences.

Finally, potential genotype-environment interactions for sex ratio were evaluated with an alternative approach. Genetic correlations of sex ratio across environments were calculated using method 1 of VIA (1984). In this procedure, the correlation of family means is used to approximate the standard full-sib genetic correlation

$$r_m = \text{cov}_{m(XY)} / \{(\text{var}_{m(X)})(\text{var}_{m(Y)})\}^{1/2}, \quad (6)$$

where $\text{cov}_{m(XY)}$ = the covariance of family mean sex ratio between two environments (X and Y), and $\text{var}_{m(X)}$ and $\text{var}_{m(Y)}$ = the variances of family mean sex ratio for each of two environments. Although the correlation of family means contains an unestimable portion of the within-family error variance or covariance (VIA 1984), the correlation approaches the true genetic correlation as family size increases. The number of offspring from each family in each treatment in this experiment (8 or 16) is probably moderate in size.

To derive more rigorous estimates, confidence limits and modified genetic correlations were subsequently generated from the original data using Tukey's jackknife method (SOKAL and ROHLF 1981). A z -transformation was not employed however because it mathematically excludes confidence intervals greater than one (S. H. ORZACK and F. J. JANZEN, in preparation) and because none of the untransformed pseudovalues (ϕ of SOKAL and ROHLF 1981) was significantly skewed or kurtotic ($t < 0.50$ and $P > 0.50$ in all six instances). In any case, genetic correlations significantly less than one imply the existence of $G \times E$ interactions (YAMADA 1962).

RESULTS

Preliminary analyses: Hatching success of eggs of common snapping turtles (*C. serpentina*) differed significantly among clutches, but was unaffected by incubation temperature (Table 1). Nonetheless, only three of 15 clutches lost more than two of the initial 32 eggs during incubation. Neither hatching success nor date of nesting, however, was significantly correlated with the sex ratio of hatchling turtles ($r_{\text{hatch}} =$

TABLE 2

Heritability of sex ratio in common snapping turtles (*C. serpentina*)

Incubation temperature	Heritability (h^2)	95% confidence interval
27.5°	0.60	0.1008–1
28.0°	0.76	0.3099–1
28.5°	0.34	0–1
Combined	0.56	0.2569–1

These values were derived using methods presented in BULL, VOGT and BULMER (1982) for estimating the heritability of a threshold character. This approach assumes all genetic variance is additive, families consist of full-sibs, no maternal effects exist, and incubation temperatures were constant (see MATERIALS AND METHODS for details).

0.050, $n = 15$, $P = 0.859$ and $r_{\text{nest}} = 0.154$, $n = 15$, $P = 0.583$). To examine further the possibility that sex-specific survivorship of embryos may have biased the results of this experiment, the among-family heterogeneity in sex ratio was calculated both before and after accounting for embryonic mortality (heterogeneity G -test from SOKAL and ROHLF 1981). In the latter instance, the sex of unhatched eggs was assigned so as to shift a given family's sex ratio closer to the mean sex ratio for the treatment. The among-family heterogeneity in sex ratio was greater than expected in both cases ($G_{H(\text{ORIGINAL})} = 348$, $G_{H(\text{MODIFIED})} = 100$, 12 d.f., $P < 0.001$). Observed differences in sex ratio among families consequently cannot be attributed to differential mortality of eggs, nor can they be derived from effects of ambient conditions prior to placement of eggs in constant temperature environments in the laboratory, because sex in this species is unaffected by temperature until at least 12 days after oviposition (YNTEMA 1979).

Heritability estimates: Three of the four full-sib estimates of the heritability of sex ratio were statistically different from zero (Table 2). Estimates were strongly positive in all four cases, indicating substantial quantitative genetic variation for sex ratio. These estimates of heritability were not detectably different however, supporting results generated by the analysis of reaction norms of sex ratio (see below).

Analyses of genotype-environment interactions: Gonadal sex of hatchling turtles was significantly influenced by clutch and by incubation temperature, but not by the interaction of those two variables (Table 3). Certain families, in other words, tended to produce more males than others (*e.g.*, one clutch produced 0% males in all three treatments) (Figure 1). As expected under temperature-dependent sex determination, more males hatched from eggs incubated at the coolest temperature than were produced at the intermediate temperature, which, in turn, produced more males than the warmest incubation temperature. However, the *pattern* of intraclutch sex ratio did not vary significantly across temperature treat-

TABLE 3

Analysis of variance for sex ratio of hatchlings of common snapping turtles (*C. serpentina*) from eggs incubated at three constant temperatures

Source of variation	d.f.	Sum of squares	Mean square	F value	P value
Clutch	14	11.907	0.850	6.418	<0.0001
Temperature	2	7.508	3.754	28.327	<0.0001
Clutch × temperature	28	4.827	0.172	1.301	0.1429
Error	413	54.731	0.133		

The dependent variable is the sex of the hatchlings (1 = male, 0 = female, 1/2 = "intersex"). The model explained approximately 1/5 of the variance in sex among families and incubation temperatures ($r^2 = 0.324$).

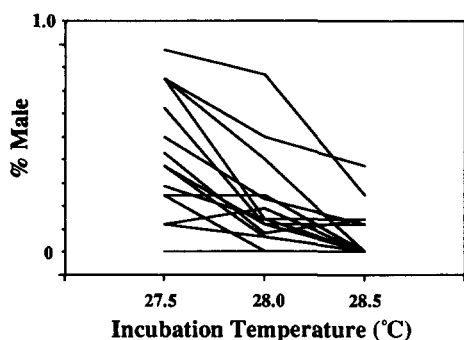


FIGURE 1.—Variation in primary sex ratios among families of common snapping turtles (*C. serpentina*) as a function of the temperature at which eggs were incubated. Each line represents one of 15 families. Differences in elevation of these lines indicate genetic variation, whereas non-parallelism of the lines indicates genotype-environment interactions.

ments (Table 3, Figure 1), suggesting that the genetic factors underlying sex determination in these turtles act similarly within families. In short, the phenotypic response across environments generally could be predicted from information for a single clutch.

Analysis of genetic correlations of sex ratio across incubation temperatures detected large, positive values (Table 4). These positive genetic correlations suggest that sex ratio in each environment is controlled by a majority of the same genetic factors. The results of this analysis imply the absence of genotype-environment interactions, similar to the findings with the analysis of variance approach (Table 3), because confidence limits on all three genetic correlations essentially include +1 (Table 4) (YAMADA 1962).

DISCUSSION

R. A. FISHER's (1930) model for the microevolution of sex ratio seemingly is supported by the strong evidence of balanced sex ratios in many natural populations of non-haplo-diploid organisms (BULL and CHARNOV 1988), although these 1:1 sex ratios also could be explained simply by Mendelian segregation of chromosomes (e.g. WILLIAMS 1979; TORO and CHARLESWORTH 1982). Much of the previous research

TABLE 4

Genetic correlations of sex ratio across incubation temperatures in common snapping turtles (*C. serpentina*)

Variables	Correlation (r_m)	95% confidence interval	Genetic variance (%)
27.5° × 28.0°	0.73	0.47–0.99	53
27.5° × 28.5°	0.52	0.07–0.96	27
28.0° × 28.5°	0.67	0.37–0.97	45

Genetic correlations, based on the same 15 families in each treatment, were derived using method 1 (family mean correlations) presented in VIA (1984). The correlations and confidence intervals presented in this table were calculated using Tukey's jackknife method (SOKAL and ROHLF 1981) without the z-transformation on the original genetic correlations (see MATERIALS AND METHODS for details). Values in the last column indicate the proportion of the genetic variance of each correlation referable to the same genes or to linkage disequilibrium or pleiotropy.

in this area has demonstrated a genetic basis for sex ratio, but has not shown, as required by adaptive sex ratio theory (see BULL, VOGT and BULMER 1982), that this genetic variation (1) exists for the primary sex ratio, (2) is due to genes with small effects, and (3) is subject to Mendelian segregation (KING 1918; WEIR 1962; BAR-ANAN and ROBERTSON 1975; CONOVER and HEINS 1987; NAYLOR, ADAMS and GREENWOOD 1988; M. ANTOLIN, personal communication). If, however, it were true that sex ratio was not under genetic determination, then sex ratio could not evolve.

Estimation of heritability of sex ratio: My results suggest that considerable quantitative genetic variation for sex ratio exists in a reptile with TSD, the common snapping turtle (*C. serpentina*). An increase in the number of families used in this experiment would have improved the precision of the estimates of heritability and probably would have permitted the statistical bounding of all estimates from zero. Consequently, these results and those discussed below for genotype-environment interactions and genetic correlations should be interpreted cautiously. Nonetheless, three of the four values for heritability were significantly greater than zero (Table 2). This variation in sex ratio exhibited among families cannot be attributed to differential hatching success, to sex-biased mortality, or to the date on which a nest was laid (Table 1; see RESULTS). Additionally, randomization of the incubation substrate among families eliminated substrate moisture as a possible factor influencing sexual differentiation in this study (GUTZKE and PAUKSTIS 1983; PAUKSTIS, GUTZKE and PACKARD 1984) and thereby potentially prevented artificial inflation of the among-family variance for sex ratio.

The methodology employed in this experiment for estimating heritability involves several key assumptions that may affect interpretation of the results (see BULL, VOGT and BULMER 1982). This approach assumes that all genetic variance detected for sex ratio

is due to additive effects without influence by epistasis, dominance, maternal effects, major genes, or sex-linked genes. The effects of these factors on the heritability estimates can be addressed indirectly. Epistasis, which arises from interactions between genes at different loci, contributes only a small amount to the covariance among relatives and probably exhibits negligible influence in nature (see FALCONER 1989), although it is necessary for sex determination in *Drosophila* (e.g., ERICKSON and CLINE 1991). Directional dominance, which results from interactions among alleles at a single locus, may inflate estimates of heritability based on full-sib analyses (FALCONER 1989). However, multiple paternity is known to occur in at least one species of turtle (HARRY and BRISCOE 1988) and sperm storage is thought to be ubiquitous in turtles (GIST and JONES 1989). Sperm storage may lead to multiple paternity which, in turn, results in half-sib families. Consequently, the values for heritability of sex ratio calculated herein would be underestimated if the clutches of snapping turtle eggs consisted of half-sib families. On the other hand, the presence of nongenetic maternal effects on sex ratio would inflate the estimates of heritability and, hence, would tend to cancel out the influence of multiple paternity in this experiment. Finally, the presence of TSD in this species implies an absence of major gene effects on sex ratio and precludes the control of sex ratio by sex chromosomes or by sex-linked genes. Consequently, I infer a significant quantitative genetic basis for primary sex ratio in *C. serpentina*, which provides empirical support for several major assumptions of FISHER's theory for the gradual evolution of sex ratio.

Genotype-environment interaction and maintenance of genetic variation for sex ratio: Genotype-environment interactions may greatly affect phenotypic evolution, evolution of genetic variances and covariances, and the extent of genetic variation for the interacting traits (VIA and LANDE 1985, 1987; GILLESPIE and TURELLI 1989). Indeed, maintenance of quantitative genetic variation for a trait may be supported through environmental heterogeneity (LEVENE 1953). Selection for a particular phenotype under one set of environmental conditions concomitantly would favor, via correlated response to selection, the retention of traits whose character states under different environmental conditions may be selected against. Graphical evaluation of the reaction norm of sex ratio in this experiment indicated that families tended to maintain their "rank" for sex ratio across treatments, although a considerable crossing of lines is evident (Figure 1). Nonetheless, the magnitude of the $G \times E$ interaction for sex ratio calculated using an analysis of variance was small and statistically non-significant (Table 3). It is not impossible though that

inclusion of more than 15 families in this experiment may have produced a significant genotype-environment interaction. Lack of a significant $G \times E$ interaction nevertheless suggests that effects of the genotype and the environment on sex ratio are independent and, assuming a constant environmental variance, that estimation of the genetic variation in one environment should provide a representative value.

Estimation of genetic correlations for a given trait across environments provides an additional method for assessing the existence of genotype-environment interactions (YAMADA 1962; VIA 1984). Low genetic correlations imply that different genes control phenotypic expression of a given trait in various environments. On the other hand, traits that exhibit substantial positive or negative genetic correlations are expected to have a similar genetic basis (VIA 1984). The positive genetic correlations for sex ratio across incubation treatments detected in this study (Table 4) thereby suggest that similar genetic factors underly sexual differentiation in each environment. Although these values are not exceedingly large, the specific method used in this experiment (method 1 of VIA 1984) may underestimate the actual magnitude of a genetic correlation when family sizes are small (e.g., see Table 1 in VIA 1984), because the numerator of the correlation may include both sampling error and genetic covariance whereas the denominator may possess unabstracted within-family variance. In any respect, all three genetic correlations are essentially indistinguishable from one, indicating the absence of genotype-environment interactions (YAMADA 1962), similar to the results obtained from the analysis of variance discussed above.

Even though substantial quantitative genetic variation for sex ratio exists in this population of common snapping turtles, the mechanism by which this variation is maintained is unclear. There are, in theory, several mechanisms other than $G \times E$ interactions by which genetic variation for fitness components may be maintained, including (1) disruptive selection, (2) fluctuating environments, (3) frequency-dependent selection, (4) heterozygote advantage, (5) migration, (6) mutation, and (7) negative genetic correlations between traits closely linked to fitness (reviewed in MOUSSEAU and ROFF 1987). Although empirical support for the prevalence of these mechanisms in natural populations is somewhat sparse and controversial (CHARLESWORTH 1987; FALCONER 1989), there is reason or evidence, which is beyond the scope of this paper to detail, to suspect that one or more of these mechanisms may be plausibly operating in this system. Furthermore, snapping turtles exhibit a lower threshold temperature of sex determination ($\approx 21^\circ$) in addition to the upper threshold temperature ($\approx 28^\circ$) investigated herein (see reviews in PAUKSTIS and JAN-

ZEN 1990; JANZEN and PAUKSTIS 1991). Consequently, evaluation of the heritability of sex ratio at the lower threshold temperature and its potential genetic correlation with sex ratio from the higher threshold temperature could provide useful insight into the evolution of sex ratio in these species.

Evolutionary significance of the results: The estimates of heritability for sex ratio calculated in this experiment are quite large for a trait presumably related closely to fitness (Table 2) (MOUSSEAU and ROFF 1987). Nonetheless, these values are comparable to the only other estimate of the heritability of sex ratio in an organism with environmental sex determination [$h^2 = 0.82$ for the Ouachita map turtle (*Graptemys ouachitensis*)] (BULL, VOGT and BULMER 1982). These authors show, however, that the effective heritability of sex ratio in *G. ouachitensis* is actually much lower, because evolution of sex ratio in species with TSD is affected by variance in nest temperatures. Assuming no covariance between nest temperature and genotype,

$$\text{Effective } h_x^2 = (h_x^2)(\sigma_x^2)/(\sigma_x^2 + \sigma_T^2), \quad (7)$$

where σ_x^2 = the phenotypic variance of the embryonic sex trait and σ_T^2 = the phenotypic variance of nest temperatures. Using approximate values of σ_x^2 (≈ 0.09) and σ_T^2 (≈ 1) for turtles, then the relative variance of $X \approx 0.083$ (BULL, VOGT and BULMER 1982). As a consequence, the effective values for the estimates of heritability of sex ratio presented in Table 2 are reduced to 0.05, 0.06, 0.03 and 0.05, respectively. In other words, the reality of substantial variation in temperatures of turtle nests in nature mitigates the potential influence of genetic factors of the embryo on sex determination. On the other hand, these same results can be employed to show that maternal choice of nest location may have a considerably greater effect on the evolution of sex ratios (BULL, VOGT and BULMER 1982). The only experiment to address the genetic basis of maternal nest choice in an organism with TSD, however, calculated a rather low maximum estimate of heritability ($h^2 < 0.20$) for the leopard gecko (*Eublepharis macularius*) (BULL, GUTZKE and BULMER 1988). The effective heritabilities of sex ratio and of nest choice imply that the evolution of sex ratio in organisms with TSD may be constrained despite substantial estimates of heritability calculated under controlled conditions.

These findings have critical implications for the persistence of populations of species with TSD in view of rapidly changing habitats and shifts in environmental conditions (JANZEN and PAUKSTIS 1991). Rapid degradation or human development of land that creates open, homogeneous nesting areas, in addition to the predicted 3–5° average increase in global temperature within the next 100 years (see for example COHN

1989), may dramatically skew sex ratios for populations of many species with TSD. The results of this study and others (BULL, VOGT and BULMER 1982; BULL, GUTZKE and BULMER 1988) imply that the evolution of sex ratios by altering either the threshold temperature of sex determination or the maternal choice of nest site would be extremely limited. Instead, species with TSD may have to change their sex-determining mechanism, alter their geographic ranges, or dig deeper nests in order to avoid extinction of many populations.

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