

ADAPTIVE VALUE OF SEX DETERMINATION MODE AND HATCHLING SEX RATIO BIAS IN REPTILES.—Ewert and Nelson (1991) presented important new data on the distribution of temperature-dependent sex determination (TSD) and genetic sex determination (GSD) modes among turtle taxa. In TSD species, egg incubation temperature influences the sex of hatchlings, whereas offspring sex of GSD species is not influenced by incubation temperature.

Ewert and Nelson discussed four alternative adaptive explanations for the existence of TSD in turtles and other reptiles. They also presented evidence apparently contrary to each of these explanations and were obviously unsatisfied with any of them as comprehensive explanatory scenarios for the observed patterns. However, in their concluding remarks, they described the two lesser-known models as "additional models worthy of consideration" and say that all four "may be important" (1991:63). Here I suggest that most previous studies have lacked relevant comparison with sister taxa. I further consider the four models, compare them to available information on population genetics and natural history of relevant species, and suggest directions for further investigation.

First, it is necessary to point out that in the search for explanations for the origin of TSD, limiting research to the examination of species with TSD is unlikely to be profitable. Phylogenetic analysis of the distribution of TSD in reptilian taxa does not clearly show whether TSD is the primitive state in turtles, but the pattern in lizards (Janzen and Paukstis, 1991a) suggests that GSD is primitive for Sauria. Whatever selective advantage TSD provided when it evolved may have been dependent on conditions that no longer exist, and demonstration of current selective advantages can only suggest why it persists, not why it evolved. Thus, current selective mechanisms that may maintain

TSD are the focus of the four explanations discussed by Ewert and Nelson (1991).

It must further be noted that two of these explanations, "group-structured adaptation in sex ratios" and "sib-avoidance," are attempts to explain offspring sex ratio bias, not TSD specifically. Ewert and Nelson show that in 14 of 16 reported populations of TSD reptiles, offspring sex ratios were female biased (they state that "some" of these estimates may be free of sampling error) and propose their two hypotheses as adaptive explanations. Unfortunately, they do not present similar sex ratio data from GSD species for comparison. Thus, it is not possible to determine from their presentation whether female-biased sex ratios are unique to TSD species. Nevertheless, if biased sex ratios are adaptive, then TSD would be just one of several possible mechanisms that vertebrates use to accomplish this result (see Frank, 1990, for review of these mechanisms, and Frank and Swingland, 1988, and Charnov and Bull, 1989, for other explanatory hypotheses for offspring sex ratio bias). The other two explanations, "phylogenetic inertia" and "temperature-dependent differential fitness," are specific to TSD.

Group-structured adaptation.—Ewert and Nelson suggested that the observed hatchling sex ratio patterns may be a by-product of selection for sex ratio biases in small breeding kin groups (Hamilton, 1967). In such systems, a small number of kin groups are restricted to breeding among themselves, and the usual Fisherian argument for 1:1 sex ratio allocation is violated, typically when competition between brothers for mates becomes severe. As the number of kin groups increases, the optimal sex ratio quickly reaches 1:1. Thus, this type of breeding system is highly vulnerable to invasion from outside and must be isolated to persist. For example, a male from another population that entered the breeding population of such a group could experience very high reproductive success at the expense of the other males of the group. Ewert and Nelson implicitly suggested that at least some TSD reptiles are regularly restricted to breeding in small groups.

For these species, the number of kin groups must be small, and either there must be few attempts at invasion from other groups or individuals must be good at identifying outsiders and excluding matings with them. If the number of kin groups is small, then inbreeding is common. Inbreeding's normal genetic result, a deficiency of heterozygotes, would be expected, especially in comparison to GSD species (if this were a TSD-specific phenomenon). Unfortu-

TABLE 1. REPORTED MULTILOCUS HETEROZYGOSITY (H) FOR VARIOUS TSD REPTILE SPECIES.*

Species	Mean H	Range	#Loci	n	Populations
CHELONIA					
<i>Trachemys scripta</i> ^b	0.127	0.105–0.190	19	535	16
<i>Emys orbicularis</i> ^c	0.036		14	1	1
<i>Caretta caretta</i> ^d	0.034	0.019–0.062	13	106	4
<i>Chelonia mydas</i> ^d	0.119	0.103–0.135	13	21	2
<i>Kinosternon flavescens</i> ^e	0.146	*	13	34	*
<i>K. scorpioides</i> ^e	0.107	*	13	20	*
<i>K. acutum</i> ^e	0.076		13	1	1
<i>K. leucostomum</i> ^e	0.134	*	13	19	*
<i>Sternotherus carinatus</i> ^e	0.076	*	13	12	*
<i>S. minor</i> ^e	0.125	*	13	20	*
<i>S. odoratus</i> ^f	0.124	0.080–0.138	25	41	7
<i>Rhinoclemmys areolata</i> ^g	0.000		19	1	1
<i>R. pulcherrima incisa</i> ^g	0.106	*	19	8	*
<i>R. p. manni</i> ^g	0.004	*	19	13	*
<i>Mauremys mutica</i> ^c	0.036		14	1	1
<i>Melanochelys trijuga</i> ^c	0.080	*	14	2	*
<i>Chinemys reevesi</i> ^c	0.035	*	14	2	*
<i>Testudo h. hermanni</i> ^h	0.023	*	16	20	1
<i>T. h. boettgeri</i> ^h	0.036	*	16	1	1
<i>T. graeca</i> ^h	0.000	*	16	4	1
CROCODYLIA					
<i>Alligator mississippiensis</i> ⁱ	0.022	0.014–0.033	17	78	3
LEPIDOSAURIA					
<i>Agama stellio</i> ^j	0.065	0.043–0.087	25	242	9
GSD or sex determination mode unknown ^k	0.081	0.00–0.714	82 species		

* = Not reported or not determinable from data presented.

References: ^aEwert and Nelson, 1991; ^bJanzen and Paukstis, 1991a, 1991b; ^cPackard and Packard, 1988; ^dSmith and Scribner, 1990; ^eSites et al., 1984; ^fSmith et al., 1977; ^gSeidel et al., 1986; ^hSeidel et al., 1981; ⁱSites et al., 1981; ^jSqalli and Blanc, 1990; ^kAdams et al., 1980; ^lNevo, 1981; ^mfrom data reported in Nevo et al., 1984.

nately, relevant published data are scarce, especially for comparison with taxonomically and/or ecologically similar pairs of GSD and TSD species. This is due in part to the fact that all crocodylians have TSD, most lizards apparently have GSD, and there are few genetic data for the relatively few turtles with GSD.

For a preliminary analysis, I collected estimates of heterozygosity (H) for 23 TSD species (Table 1) including 21 turtles, one crocodylian, and one lizard. Values are well within the reported range (see Table 1) for 82 reptile species that either have GSD or for which the sex determination mode is unknown (H values from Nevo et al., 1984). Although summary statistics may not be appropriate given the taxonomic unevenness and potential for important methodological differences between investigators, clearly there is considerable overlap among H values for the two groups. This summary strongly suggests that there is no genetic evi-

dence that inbreeding is more common in TSD species.

If the group structure hypothesis is correct, then it might also be expected that female-oriented offspring sex ratio biases would be negatively correlated with H values. In other words, extreme female biases would be expected in species with high levels of inbreeding (and therefore low H values). Three of the species in Table 1 are also listed in Ewert and Nelson's table of hatchling sex ratios. Of the three, the species with the highest percentage of female offspring (*Alligator mississippiensis*) also has the lowest H value, well below the average for a large sample of reptiles (Nevo et al., 1984). This result is consistent with the group structure hypothesis. However, *Chelonia mydas* has an intermediate offspring sex ratio but a relatively high H value. Finally, *Caretta caretta* has a nearly 1:1 sex ratio but an H value that is both intermediate among these three TSD species and less than half the

Nevo et al. (1984) average. Here again, it appears there is little genetic support for a correlation between offspring sex ratio and indications of high levels of inbreeding.

Breeding groups of freshwater turtles might consist of all the turtles in a network of ponds, or only of those in a single pond, or several groups might exist within one pond. Ewert and Nelson did not explicitly describe the size of the breeding groups required by their hypothesis, although their reference to the data of Scribner et al. (1986) suggests pond-level groups, rather than separate groups within a pond, or individuals dispersed over multiple ponds.

Indications of group structure level may also be found by evidence of migration and entry into new breeding populations. Because the group-structured population explanation requires the occurrence of little or no immigration from other groups, it is similar to the population genetic "island model" for defining populations. Evidence of migration between ponds, for example, would imply that the breeding group must be more inclusive and therefore on a higher level, encompassing the metapopulation within which migration occurs. Migration estimates are only available for a few TSD species. For example, Smith and Scribner (1990) showed that migration rates of the turtle *Trachemys scripta* (a TSD species) between breeding populations in different ponds in South Carolina were between 1.25 and 5 individuals per generation, relatively high for vertebrates. This movement is strongly male biased (Gibbons, 1986); thus it presents a strong threat to female-biased groups. Given this level of immigration, it seems unlikely that pond populations could persist as isolated breeding units. It seems even more unlikely that multiple ponds, such as the 16 sampled by Smith and Scribner (1990) form a large, rarely invaded unit.

If the typical breeding unit is not at the pond level or higher, it may be composed of multiple groups within each pond. Such groups could not be geographically isolated, as those in different ponds could be. Instead, an effective group recognition system, perhaps using behavioral or olfactory clues, would be required to avoid matings with members of other groups. I am unaware of convincing evidence that such a system, or any other type of kin recognition, exists in TSD reptiles, unlike that which may occur in tadpoles (Blaustein and O'Hara, 1986). Based on the above arguments, it does not appear that group structuring is likely to be an important influence on offspring sex ratio modification in reptiles. Proponents of the group-structure explanation must provide data show-

ing that small, exclusive breeding groups exist in TSD species, particularly in contrast to closely related GSD species. Such evidence might be behavioral or genetic. Population genetic data could be analyzed using techniques such as those of Weir and Cockerham (1984) or Long et al. (1986) to determine whether such structuring exists, and if so, the level at which it occurs.

Sib-avoidance.—Ewert and Nelson pointed out that some reptile species with TSD have been reported to commonly lay unisexual clutches. They presented data for eight TSD species where the proportion of unisexual clutches varied from 29% to 86%. Obviously, offspring of unisexual clutches cannot interbreed. Ewert and Nelson (following Thompson, 1983) suggested that TSD may be a mechanism for reducing inbreeding. This may be advantageous where both the cost and potential for full-sib matings is high. Ewert and Nelson postulated that for bisexual clutches the potential for inbreeding may be high when clutch survival tended to be all or none, and a surviving clutch might become a substantial part of the future local breeding population.

Ewert and Nelson recognized some difficulties with this scenario, and these bear further discussion. This explanation is unsatisfying largely because other types of kin matings are not reduced. It appears that female turtles may store sperm for an extended period (Gist and Jones, 1989), so offspring from subsequent clutches in the same year or in following years may also be full sibs. Unless these different clutches were all of the same sex, full-sib inbreeding might still occur. Production of single-sex clutches also does not reduce breeding between half-sibs, parent and offspring, or other types of inbreeding, unless females consistently choose nest sites that produce offspring of the same sex in each reproductive episode. Also, given that dispersal into other breeding populations is apparently common, it appears unlikely that such a specialized mechanism is necessary to reduce inbreeding. Finally, there are no data suggesting that inbreeding should be a particular problem for the species that have TSD, especially relative to the species that have GSD.

If TSD was useful in reducing inbreeding, then inbreeding would be more common in GSD species than in TSD species, the reverse of the prediction made under the group-structured explanation. In this case TSD species would have higher H values than GSD species. It is again relevant that H values for TSD species are quite similar to those for GSD species (Table 1). Sup-

port for the sib-avoidance explanation must include a demonstration that inbreeding is reduced through TSD, that TSD species are more likely to have unisex clutches, or even that inbreeding is sufficiently deleterious to TSD species that this special mechanism evolved to reduce it.

Phylogenetic inertia.—Because of the continuing investigations into sex determination mode in turtles, data are now available for at least one species in each extant turtle family (Ewert and Nelson, 1991; references in Janzen and Paukstis, 1991a, 1991b). Unfortunately, the pattern within most families is almost entirely unknown, and it is clear that species-level investigations can be profitable (see below). The current data are insufficient to determine definitively which of the two modes is ancestral. Bull (1980, 1983) discussed the use of evidence from heteromorphic sex chromosomes to determine the primitive state in turtles, but results of this noncladistic approach appear inconclusive. Both modes are present in the most primitive families of turtles (using the phylogeny of Gaffney and Meylan, 1988, and Gaffney et al., 1991). Using cladistic procedures with amphibians and mammals as multiple outgroups for turtles, there is no evidence conflicting with the hypothesis that GSD is primitive for turtles. Heteromorphic sex chromosomes are ubiquitous in mammals and are probably homologous within the class (Graves, 1991), although the same is not true for amphibians (Schmid et al., 1991).

Ewert and Nelson suggested that phylogenetic inertia is an unlikely explanation for the observed sex determination pattern in chelonians because of TSD/GSD plasticity in this highly conservative class. This plasticity takes two forms: First, two patterns of TSD have been observed; thus there is important variation even among TSD species (Ewert and Nelson, 1991). Second, if TSD is primitive for turtles, GSD has evolved independently at least four times (see Janzen and Paukstis, 1991a); if GSD is primitive, TSD has evolved at least nine times. Quantification of the impact of phylogenetic inertia will require not only robust cladograms but also data on sex determination mode for many more species. Too little is known of the pattern within families to assess the importance of phylogenetic inertia on a finer scale at this time.

Temperature-dependent differential fitness.—Charnov and Bull's (1977) hypothesis is perhaps the most widely accepted model for the adaptive significance of TSD but suffers from a lack of clearly supportive data. The research needed

to test this hypothesis is logistically difficult; Ewert and Nelson (1991) and Janzen and Paukstis (1991b) summarized the available inferential support. One important question that Charnov and Bull did not consider in developing their model is the evolution of GSD from TSD. However, Bull and Bulmer (1989) suggested that TSD species might be more vulnerable than GSD species to climatic changes, resulting in severe populationwide sex ratio biases. Under climatic variation, their analysis showed much stronger selection against TSD in short- than in long-lived species. Some empirical support for this prediction was presented by Janzen and Paukstis (1991b). Thus, researchers interested in testing this explanation may be more successful if they emphasize short-lived species.

As a test of temperature-dependent differential fitness, eggs from both species might be incubated under a range of temperature and moisture conditions and the resulting hatchlings subjected to a variety of performance tests. Although probably all fitness tests are subject to criticism, a battery of locomotor and reproductive performance tests could be used to compare "fitness." Similar field tests might be more informative but would be much more difficult. Charnov and Bull (1977) predicted that, for TSD species, the majority sex at each incubation temperature should have higher fitness. GSD species would not be predicted to show such differences.

Where some answers might be found.—Investigations of polytypic genera are possible in both lizards and turtles. For example, Ewert and Nelson reported that, in the turtle genus *Clemmys*, *C. guttata* has TSD and *C. insculpta* has GSD. TSD appears to be primitive for the Family Emydidae, based on the phylogeny of Gaffney and Meylan (1988) and Gaffney et al. (1991) and the sex determination pattern reported by Ewert and Nelson (1991) and Janzen and Paukstis (1991a, 1991b). Thus the condition in *C. insculpta* is clearly derived. Comparison of the life history, ecology, and population genetics of *C. insculpta* with its sister taxa may be productive, although the available data do not strongly indicate why GSD might have evolved in *C. insculpta*.

Similarly, TSD is known in only a few lizard families (Janzen and Paukstis, 1991b), and this pattern seems to be the result of independent evolutionary events. Both modes are found within the lizard genus *Gecko* (references in Ewert and Nelson, 1991). These taxa are particularly appealing as experimental subjects to test theories of the evolution of sex-determination

mode because they tend to be short lived (Bull and Bulmer, 1989).

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LITERATURE CITED

- ADAMS, S. E., M. H. SMITH, AND R. BACCUS. 1980. Biochemical variation in the American alligator. *Herpetologica* 36:289–296.
- BLAUSTEIN, A. R., AND R. K. O'HARA. 1986. Kin recognition in tadpoles. *Sci. Amer.* 254:108–116.
- BULL, J. J. 1980. Sex determination in reptiles. *Quart. Rev. Biol.* 55:3–21.
- . 1983. Evolution of sex determining mechanisms. Benjamin/Cummings, Menlo Park, California.
- , AND M. G. BULMER. 1989. Longevity enhances selection of environmental sex determination. *Heredity* 63:315–320.
- CHARNOV, E. L., AND J. J. BULL. 1977. When is sex environmentally determined? *Nature* 266:828–830.
- , AND ———. 1989. The primary sex ratio under environmental sex determination. *J. Theor. Biol.* 139:431–436.
- EWERT, M. A., AND C. E. NELSON. 1991. Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia* 1991:50–69.
- FRANK, S. A. 1990. Sex allocation theory for birds and mammals. *Ann. Rev. Ecol. Syst.* 21:13–55.
- , AND I. R. SWINGLAND. 1988. Sex ratio under conditional sex expression. *J. Theor. Biol.* 135:415–418.
- GAFFNEY, E. S., AND P. A. MEYLAN. 1988. A phylogeny of turtles, p. 157–219. *In: The phylogeny and classification of tetrapods, Vol. 1.* M. J. Benton (ed.). Clarendon Press, Oxford, England.
- , AND A. R. WYSS. 1991. A computer assisted analysis of the relationships of the higher categories of turtles. *Cladistics* 7:313–335.
- GIBBONS, J. W. 1986. Movement patterns among turtle populations: applicability to management of the desert tortoise. *Herpetologica* 42:104–113.
- GIST, D. H., AND J. M. JONES. 1989. Sperm storage within the oviduct of turtles. *J. Morph.* 199:379–384.
- GRAVES, J. A. M. 1991. Mammalian genome evolution—new clues from comparisons of eutherians, marsupials, and monotremes. *Comp. Biochem. Phys. (A)*. 99:5–11.
- HAMILTON, W. D. 1967. Extraordinary sex ratios. *Science* 156:477–488.
- JANZEN, F. J., AND G. L. PAUKSTIS. 1991a. A preliminary test of the adaptive significance of environmental sex determination in reptiles. *Evolution* 45:435–440.
- , AND ———. 1991b. Environmental sex determination in reptiles: ecology, evolution and experimental design. *Quart. Rev. Biol.* 66:149–179.
- LONG, J. C., N. M. NAIDU, H. W. MOHRENWEISER, H. GERSHOWITZ, P. L. JOHNSON, J. W. WOOD, AND P. E. SMOUSE. 1986. Genetic characterization of Gainj and Kalam speaking people of Papua New Guinea. *Amer. J. Phys. Anthro.* 70:75–96.
- NEVO, E. 1981. Genetic variation and climatic selection in the lizard *Agama stellio* in Israel and Sinai. *Theor. Appl. Gene.* 60:369–380.
- , A. BEILES, AND R. BEN-SHLOMO. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. *Lec. Notes Biomath.* 53:13–213.
- PACKARD, G. C., AND M. J. PACKARD. 1988. The physiological ecology of reptilian eggs and embryos, p. 523–605. *In: Biology of the Reptilia, Vol. 16.* C. Gans and R. B. Huey (eds.). Alan R. Liss, New York, New York.
- SCHMID, M., I. NANDA, C. STEINLEIN, K. KAUSCH, J. T. EPPLEN, AND T. HAAF. 1991. Sex-determining mechanisms and sex chromosomes in Amphibia, p. 393–432. *In: Amphibian cytogenetics and evolution.* D. M. Green and S. K. Sessions (eds.). Academic Press, Inc. New York, New York.
- SCRIBNER, K. T., J. E. EVANS, S. J. MORREALE, M. H. SMITH, AND J. W. GIBBONS. 1986. Genetic divergence among populations of the yellow-bellied slider turtle (*Pseudemys scripta*) separated by aquatic and terrestrial habitats. *Copeia* 1986:691–700.
- SEIDEL, M. E., J. B. IVERSON, AND M. D. ADKINS. 1986. Biochemical comparisons and phylogenetic relationships in the Family Kinosternidae (Testudines). *Copeia* 1986:285–294.
- , S. L. REYNOLDS, AND R. V. LUCCHINO. 1981. Phylogenetic relationships among musk turtles (Genus *Sternotherus*) and genetic variation in *Sternotherus odoratus*. *Herpetologica* 37:161–165.
- SITES, J. W., JR., J. W. BICKHAM, B. A. PYTEL, I. F. GREENBAUM, AND B. A. BATES. 1984. Biochemical characters and the reconstruction of turtle phylogenies: relationships among Batagurine genera. *Syst. Zool.* 33:137–158.
- , I. F. GREENBAUM, AND J. W. BICKHAM. 1981. Biochemical systematics of Neotropical turtles of the genus *Rhinoclemmys* (Emydidae: Batagurinae). *Herpetologica* 37:235–264.
- SMITH, M. H., AND K. T. SCRIBNER. 1990. Population genetics of the slider turtle, p. 74–81. *In: Life history and ecology of the slider turtle.* J. W. Gibbons (ed.). Smithsonian Institution Press, Washington, D.C.
- , H. O. HILLESTAD, M. N. MANLOVE, D. O. STRANEY, AND J. M. DEAN. 1977. Management implications of genetic variability in loggerhead and green sea turtles, p. 302–312. *In: XIIIth Congress of Game Biologists.* J. J. Peterle (ed.). 11–15 March 1977. Wildlife Management Institute, Washington, D.C.
- SQALLI-HOUSSAINI, H., AND C. P. BLANC. 1990. Genetic variability of four species of the genus *Testudo* (Linnaeus, 1758). *J. Herpetol. Assoc. Africa.* 37:1–12.
- THOMPSON, M. B. 1983. The physiology and ecology of the eggs of the pleurodiran tortoise *Emydura macquarii* (Gray), 1831. Unpubl. Ph.D. diss., Univ. of Adelaide. Adelaide, Australia.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating F statistics for the analysis of population structure. *Evolution* 38:1358-1370.

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