Maternal Condition and Nest Site Choice: An Alternative for the Maintenance of Environmental Sex Determination?  

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SYNOPSIS. Egg size and nest site selection are two potential effects that can have a persistent influence on the phenotype of offspring. In this paper, I develop the maternal condition-dependent choice hypothesis for the maintenance of environmental sex determination. The hypothesis stipulates three conditions: 1) there must be variation in the maternal effect, 2) the variation in the maternal effect must influence fitness of the offspring differently between the sexes, and 3) female reproductive behavior is determined by her condition or how her condition will influence her offspring’s fitness. Females with the ability to recognize environments that have a higher probability of producing the sex that would benefit the most from maternal condition will have an advantage. Using egg size as a maternal effect, I test this hypothesis in the diamondback terrapin, an emydid turtle with temperature-dependent sex determination. Terrapins have large variation in egg size among clutches and little variation within clutches. Egg mass is the primary determinant of hatching mass and can result in as much as a three year difference in reaching minimum size of first reproduction in females, but may not affect age or size of first reproduction in males. Finally, terrapins select open nesting sites with warmer incubation conditions and place larger eggs there. Females place smaller eggs in cooler sites. Terrapin reproduction is consistent with the prediction of the maternal condition-dependent nest site choice hypothesis. The model and supporting data demonstrate how maternal effects can be an important factor to consider in studies of environmental sex determination.

INTRODUCTION

Studies of life histories elucidate factors that affect survivorship and reproduction within and among populations of organisms. Genetic, environmental, and maternal effects are all recognized as important factors contributing to phenotypes of organisms; however, until recently, much of the work on life history evolution has ignored maternal effects. Maternal effects can be physiological (energetic or endocrinological), genetic (e.g., mtDNA) and behavioral.

In particular, oviparous organisms can provide energetic maternal effects by varying the amount of resources available in the egg. Similarly, these organisms can have behavioral maternal effects by selecting the environment where their eggs incubate. The energetic component of eggs produced by organisms that abandon their young after parturition has been identified as parental care (Congdon and Gibbons, 1985, 1990; Congdon, 1989). Furthermore, this parental investment has been divided into two components, 1) the energy necessary to produce a complete embryo (PIE) and 2) additional energy that can be used after emergence from the egg (parental care, PIC [Congdon, 1989; Congdon and Gibbons, 1990]). PIE is the minimum investment per egg required for successful embryogenesis and PIC is supplemental provisioning for the neonate. Both PIE and PIC are maternal effects that can produce fitness differences among offspring. Experimental work manipulating energy levels in eggs has demonstrated that offspring size is tightly coupled to egg size, but also that egg size contributes to performance and growth (Sinnerup and McEdward, 1988; Sinnerup and Huey, 1990; Sinnerup and Huey, 1990; Sinnerup and Licht, 1991a, b). Sprint speed responds allometrically to changes in egg size; however, stamina and growth rate exhibit fixed differences independent of egg size among lizards (Sinnerup and McEdward, 1988; Sinnerup and Huey, 1990). These elegant experiments demonstrated the importance of egg size to various life history traits, but more importantly, they demonstrated that egg size can change as a function of selection on covarying traits, i.e., body size. Furthermore, hatchinglings engineered to extreme large or small body sizes had lower survivorship, suggesting that selection might optimize egg size through offspring survival (Sinnerup et al., 1988), instead of a tradeoff between number and quality of offspring. These findings are particularly interesting because a general assumption of models of egg size evolution is that larger size is optimal for the offspring (Smith and Fretwell, 1974; Travis 1974; Brockelman, 1975).

The work of Sinnerup and colleagues has demonstrated that maternal effects can be targeted by selection directly, or indirectly through covarying traits. Choice of parturition or oviposition site by the female can be a behavioral maternal effect. Environmental conditions during development can determine embryo survivorship (Crump, 1991; Horrocks and Scott, 1991; Janzen, 1993a; Burger, 1993; Spotila et al., 1984; Reiter and Shreeve, 1996), size (reviewed in Packard and Packard, 1988), performance (Janzen, 1993a, b), growth (Laws and Joanen, 1982; Joanen et al., 1987; Brooks et al., 1991; McKnight and Gutzke, 1991; Bobyn and Brooks, 1994), behavior (Burger, 1989, 1990, 1991) and sex (reviewed in Bull, 1980, 1983; Korpe-lainen, 1981; Kooiker, 1983; Kooiker and Peters, 1981; Janzen and Paukstis, 1991a). Thus, the choice of oviposition site may affect fitness through a variety of different traits. Furthermore, some of these traits may covary in manners that make it difficult to tease apart the actual target of selection when these traits are evaluated independently. Unfortunately, most laboratory incubation studies can evaluate only a few of the many variables that influence offspring phenotype or fitness. Additionally, laboratory studies rarely mimic the fluctuations in environmental conditions that occur in the wild.

One possibility that has been rarely considered in life history studies is an interaction among maternal effects on offspring phenotypes. For example, males or females in a population might be influenced by a female’s physiological condition affecting the quality of her eggs. If egg quality interacts with the incubation environment to determine offspring quality, then selection would favor females that choose oviposition sites that enhance offspring fitness. One case where such an interaction might exist is in organisms that have environmental sex determination (ESD).

ESD occurs in crocodilians, many turtles, some lizards, fishes, and some invertebrates (reviewed in Bull, 1980, 1983; Korpelainen, 1990; Ewert and Nelson, 1991; Janzen and Paukstis, 1991a). In organisms with ESD the sex of the developing embryo is determined primarily by the environmental conditions during early development, in contrast to genotypic sex determination (GSD) in which the sex of the offspring is determined by sex chromosomes (reviewed in Bull, 1983; Ewert and Nelson, 1991; Janzen and Paukstis, 1991a). As a result, organisms with ESD frequently produce clutches that have skewed sex ratios (Bull, 1985) while organisms with GSD usually produce sex ratios close to 1:1. Because of the potential to deviate considerably from a 1:1 sex ratio in ESD species, it has been difficult to establish the selective advantage of ESD over GSD (Bull and Charnov, 1988).

Four explanations have been suggested for the wide spread occurrence of ESD (reviewed in Ewert and Nelson, 1991; Janzen and Paukstis, 1991a; Burke, 1993; Ewert et al., 1994). First, it has been suggested that ESD is shared among many taxa through common descent; however, there is debate
concerning whether it is a derived or ancestral trait for the major taxonomic groups in which it occurs (Ewert and Nelson, 1991; Janzen and Paukstis, 1991b; Ewert et al., 1994). Second, ESD has been suggested as a mechanism to prevent inbreeding in situations where the probability of sib-matings is high. By producing individuals of all the same sex, within family matings would be prevented (Ewert and Nelson, 1991). Third, the "groupl-stratification adaptation" of the ratio hypothesis suggests ESD provides a mechanism to maintain female-biased sex ratios (Ewert and Nelson, 1991). For species with small, localized populations with a high probability of extinction, female-biased sex ratios can be maintained by group selection by providing large numbers of females to maintain and found populations. Burke (1993) has questioned whether the latter two explanations identify ESD or sex ratio as the trait under selection. Fourth, the most studied hypothesis is the temperature-dependent differential fitness hypothesis for the evolution of ESD (the Charnov-Bull model [Charnov and Bull, 1977, described below]). One derivation of the Charnov-Bull hypothesis is the sexual-size dimorphism hypothesis (Ewert and Nelson, 1991; Ewert et al., 1994) which predicts that the larger sex might have physiological advantages (e.g., faster growth) when raised at warmer temperatures. Data supporting a selective advantage for ESD in vertebrates is limited and a general understanding of ESD in reptiles has yet to emerge. Adaptive explanations for ESD have been supported in turtles (Janzen, 1995), lizards (Gutzke and Crews, 1988), alligators (Joanen et al., 1987), and fish (Conover, 1984). Janzen (1995) found that snapping turtles incubated at temperatures that produced mixed sex ratios had lower survivorship than hatchlings incubated at temperatures that produced only males or females. Gutzke and Crews (1988) showed that female leopard lizards incubated at warm temperatures were more aggressive, did not respond to courtship behavior and did not reproduce. Females from cool temperatures and males from warm temperatures did participate in reproductive activity. Joanen and colleagues (1987) found that alligators incubated at extreme temperatures had slower growth rates than individuals that were raised at intermediate temperatures. Perhaps the best evidence for the adaptive-significance of ESD has been found in the Atlantic silverside, Menidia menidia (Conover, 1984; Conover and Heins, 1987). Southern populations of this fish have ESD (cooler temperatures produce females) and there is a fecundity advantage to larger females. Females produced earlier in the year have a longer growing season and thus attain larger body size. Males do not have a size-dependent fecundity advantage, but do benefit from larger body size through increased survivorship. ESD is adaptive in silversides because it increases the proportion of females produced early that benefit from a longer growing season and thus have a fecundity advantage. In more northern populations ESD is replaced by GSD, the growing season becomes shorter, and the adaptive advantage of ESD is diminished (Conover and Heins, 1987). Interestingly, the alligator and the silverside study both have associated ESD with aspects of growth.

One mechanism that has been suggested for the maintenance of ESD is maternal nest site choice and the effect a female's decision may have on the sex ratio of her clutch (Bull, 1983). Females may assess the current sex ratio and choose specific sites that would maximize the probability of her offspring becoming the less common sex. Because the ability to assess the current sex ratio and predict future sex ratio is unlikely, particularly for organisms with long generation times such as turtles, nest site choice can be a mechanism to maintain ESD has received little attention. Perhaps, a more proximate basis for nest site choice could result if offspring fitness was the result of an interaction between egg size and sex, thereby providing an advantage for ESD. For example, females with the ability to assess variation in P/E and PIC could place their eggs in microhabitats with a greater probability of becoming a particular sex.

The maternal condition-dependent choice hypothesis

The Charnov-Bull (1977) hypothesis states that four conditions are necessary for the evolution and maintenance of ESD: 1) the environment is patchy with respect to factors that determine sex, 2) the fitness of the offspring depends on the sex they become in a particular patch (i.e., females from a male patch will have lower fitness than females from a female patch), 3) neither the mother nor the offspring can select the patch in which sex is determined, and 4) mating is random among individuals from different patches. Thus, if the incubation environment differentially affects the fitness of the males and females, then selection will favor a sex determination mechanism that produces offspring with higher fitness with respect to the local environment during development (Charnov and Bull, 1977).

A special case of the Charnov-Bull hypothesis can be developed that relaxes condition three of patch choice, by incorporating a generalized case of the Trivers-Willard hypothesis (Trivers and Willard, 1973; Bull, 1983; Charnov, 1983). The Trivers-Willard hypothesis suggests that offspring body size is affected by maternal sex; larger, more robust male offspring are produced than female offspring. The model was developed to explain slightly male-biased sex ratios in polygamous mammals, in which larger males were more successful at obtaining matings than smaller males. Charnov (1983) suggested that the model also could apply to species in which females have a size advantage. Fitness of female offspring may be affected more through size or age at maturity than the fitness of male offspring, suggesting that selection could favor sex ratio bias of larger offspring toward females. In general, larger offspring should be biased to the sex that may incur the greatest fitness advantage as a larger or earlier maturing adult.

The maternal condition-dependent choice (MCDC) hypothesis suggests that ESD is a mechanism to bias offspring sex ratio through maternal choice of nest site or incubation position. Three conditions must be satisfied to support the MCDC hypothesis. First, there must be variation in a maternal effect (e.g., propagule or offspring size) within a population. Second, the maternal effect must interact with sex to affect the fitness of one sex more than the fitness of the opposite sex (sensu condition 2. Charnov and Bull, 1977). Third, a female's behavior must be influenced by her condition (physiological state) either directly or through the condition of her eggs. If these three conditions are met, then females with the ability to recognize nest sites with a greater probability of producing the sex that would benefit the most from the maternal effect would have an advantage. When met, these conditions, in addition to conditions one and four of the Charnov-Bull hypothesis, would provide a selective advantage to nest site choice based on maternal condition as a mechanism for the maintenance of ESD.

I test the MCDC hypothesis in the diamondback terrapin, Malaclemys terrapin, using egg size as the maternal effect. The predictions were: 1) egg size variation should be present in the population, 2) egg size should affect the life history and thus fitness of terrapins, 3) the fitness advantage due to egg size should differ between the sexes, 4) nest site choice should occur among the four sites based on climatic differences, and 5) females should place eggs in habitats where they would develop into the sex that would incur the greatest advantage caused by the variation in egg size.

METHODS

Since 1987 I have been conducting a detailed study of the population biology and reproductive ecology of the diamondback terrapin, Malaclemys terrapin, in the Patuxent River, a tributary of Chesapeake Bay. The diamondback terrapin is an excellent species in which to study reproduction and nest site choice for several reasons. First, terrapins nest on small isolated beaches where nesting densities can be as high as 225 nests/0.2 hectare and females are easy to locate while nesting (Roosenburg, 1994). Terrapins are iteroparous, nesting annually and as many as three times in a nesting season (Roosenburg, 1991). They are nest site philopatric (nesting on the same beach within and among years) so data of multiple nests can be obtained for many females. Additionally, terrapins are sexually dimor-
phic; males mature at approximately 300 g between 4 and 7 years of age and females mature at 1100 g between 8 and 13 years of age (Roosenburg 1991). Finally, terrapins have pattern la ESD (Ewert et al., 1994); warmer incubation temperatures produce females while cooler incubation conditions produce males (Jeyasuria et al., 1994; Roosenburg and Kelley, 1996).

I synthesized demographic and experimental data collected from 1987-1992, some of which are published elsewhere (Jeyasuria et al., 1994; Roosenburg, 1990, 1991, 1992; Roosenburg and Kelley, 1996). More detailed descriptions of the methods can be found in those papers. First, the demography described the natural history and individually-based variation in life history traits. Standard mark-recapture techniques assessed the relationship between age and size of terrapins and investigated traits such as age and size of first reproduction and growth rates. Additional information on the reproductive biology (egg size, clutch size, and microhabitat location of the nests) of female terrapins was obtained by continuous observation of nesting beaches throughout the nesting season. Finally, every nest discovered on nesting beaches was given a microhabitat classification with respect to the exposure to direct solar radiation and the amount of vegetation in a 0.25 m^2 area around the nest. Random transsects of the nesting beaches were used to generate expected frequencies of nest sites available and were compared to nest sites chosen by females using a x^2 analysis. Two nesting beaches, Marsh Point Beach and Burton's Beach were monitored; 95 to 99% of the nests laid on these beaches were identified. Details of the nesting areas used and terrapin nesting behavior can be found in Roosenburg (1992, 1994).

An experiment investigating growth rates of terrapins (Roosenburg and Kelley, 1996) brought terrapins from the Patuxent population into the laboratory and incubated them at a high and low temperatures. For the following 3 years hatchlings were raised in captivity. During years 1 and 2 hatchlings were held in 1 m diameter baby pools. Individuals in the pools were randomized after each cleaning (usually once a week). During the last year of the study, terrapins were held in a 1.2 x 2.4 x 0.3 m tank. Feeding of hatchlings began after all individuals hatched. Turtles were fed ad libitum with food remaining in the tanks after each feeding.

RESULTS AND DISCUSSION

Egg size variation

Egg size in the diamondback terrapin varies little within a clutch and considerably among clutches (Fig. 1). Mean egg size among clutches ranges from 8 to 12 g, a 50% range from minimum to maximum mean egg size. In repeated nestings, an individual female can vary egg size over the same range that is observed in the population (Fig. 2).

Several factors may contribute to egg size variation in turtles. First, egg size may be a function of body size, larger females producing larger eggs. This has been demonstrated for painted turtles, Chrysemys picta, and chicken turtles, Deirochelys reticularia, where the shape and thus, egg size appear to be controlled by the width of the pelvic opening (Crombill and Gibbons, 1987). Second, egg size may vary inversely with clutch size as predicted by optimal egg size theory (Smith and Fretwell, 1974; Brodkelman, 1975; Sinervo and Licht, 1991a). Replicate clutches by individual female terrapins vary considerably in egg size, suggesting that neither body size or clutch size-egg size tradeoff are major determinants of egg size in terrapins (Fig. 2). Although, the causal agents of egg size have not been identified (Roosenburg and Dunham, in preparation), there is significant egg size variation within females and it is equivalent to the egg size variation among females in the population. The variation in terrapin egg size meets the first condition of the MCDC hypothesis that egg size must vary.

Effect of egg size on fitness

Egg mass is the primary determinant of hatching mass in terrapins (Least Squares Regression, r^2 = 0.97 for males, r^2 = 0.96 for females, Roosenburg and Kelley, 1996). Additionally, the relationship between egg mass and hatching mass does not differ between the sexes (ANOVA, F(1,42) > 0,14, Roosenburg and Kelley, 1996). Packard et al. (1995) and Cagle et al. (1993) suggested that egg size is the primary determinant of hatching size in field experiments. Other studies have demonstrated that the incubation environment, particularly soil moisture, can affect hatching size (Brooks et al., 1991; Bobyn and Brooks, 1994; reviewed in Packard and Packard, 1988) and performance (Miller et al., 1987). Brooks et al. (1991) showed in snapping turtles that differences in hatching mass attributable to soil moisture were not detectable after 7 months, although Bobyn and Brooks (1994) were able to detect moisture effects up to 10 months later. Larger hatchling turtles have increased survivorship (Swingle and Coe, 1979; Janzen, 1993a, b) and performance (Miller et al., 1987), however to support the MCDC hypothesis it is necessary to demonstrate that the fitness effects attributable to maternal or offspring condition differ between the sexes of offspring. For terrapins, egg size must have a greater impact on the fitness of one sex compared to the other sex. Demographic and experimental data of age and size were used to address these issues.

Terrapin females are larger and mature later than males in the Patuxent population (Roosenburg, 1991). Females vary from 7 to 13 years in reaching minimum size for reproduction (1,100 g, Fig. 3). Many factors are likely to affect growth and age or size
at maturity in turtles, including environmental variation in resources, microclimatology of the local habitats, and the presence of conspecifics in areas where growth occurs (Avery et al., 1993; McKnight and Gutzke, 1993). Maternal effects also can contribute to growth and age and size at maturity. Terrapins raised in captivity for three years had considerable differences in size that were, in part, attributable to variation in egg size (Fig. 4; Roosenburg and Kelley, 1996). Importantly, the egg size effect was significant for females ($P < 0.01$, $r^2 = 0.59$, Fig. 4), but not for males ($P > 0.05$). Variation in size of three-year-old lab-reared individuals (Fig. 3) when compared to three-year-old wild individuals (Fig. 4) suggests that females from larger eggs may reach minimum size at first reproduction 2–3 yr sooner than individuals from smaller eggs. Thus the maternal effect of egg size can have a large influence on fitness for females. The males, however, were approaching size at maturity at three years of age and a similar maternal effect was not present. This supports conditions 2 and 3 of the MCDC hypothesis that maternal or offspring condition affects fitness and that the effect is greater in one sex than the other.

Growth has been studied in other reptiles with ESD. Data for snapping turtles are consistent with conditions 2 and 3 of the MCDC hypothesis, for example, differences between clutches and egg mass affect hatching size and size at 7 months and beyond (Brooks et al., 1991; McKnight and Gutzke, 1993; Bobyn and Brooks, 1994). Growth rates in snapping turtles were greatest among male producing temperatures in all cases. This is consistent with the MCDC because males are the larger sex in this species (Gibbons and Lovich, 1990). Studies of the desert tortoise also found that males had greater mass gain than females during their first 40 days, however mass gain in both males and females was greater in individuals incubated closer to the pivotal temperature (Spotila et al., 1994). Once again, males are larger in desert tortoises (Gibbons and Lovich, 1990). Growth rates of alligators appear to be affected by incubation temperature independent of sex, such that intermediate incubation temperatures result in faster growth rates (Joanen et al., 1987).

Female nest site selection

The MCDC hypothesis predicts that females use nest site choice to adjust the sex ratio of her offspring so that the fitness gain attributable to maternal condition occurs in the sex that can better exploit this advantage. Evidence to support this condition would include the following: 1) oviposition sites chosen are different from what is available on nesting areas, 2) microclimatic conditions among nest sites chosen span the range of temperatures necessary to produce both males and females, 3) nest sites chosen are associated with the sex most likely to benefit from the maternal effect. In the case of terrapins, larger eggs should be placed in warmer habitats and smaller eggs in cooler habitats.

Terrapins prefer to nest in sunny habitats. The nesting sites chosen are significantly different from those available for the 9 microhabitat classifications (1989, $x^2 = 384.47$, df = 8, $P > 0.001$; 1991 $x^2 = 284.53$, df = 8, $P > 0.001$, Fig. 5). Nesting activity on both beaches occurs primarily in the sun-open and sun-edge habitats; less nesting occurs in the sun-vegetation areas. The predominant microhabitats on Marsh Point Beach are sun-vegetation, and semi-shade-vegetation (Roosenburg, 1992). The locations where terrapins nest suggest that females can discriminate among nesting sites. Nest site use was similar on Burton’s Beach (Roosenburg, 1992) and other beaches throughout the Patuxent River (personal observation).

Distinct microclimatic conditions are associated with the different microhabitats. Sun nests are warmer than semi-shade nests which are warmer than shade nests (Jeyasurya et al., 1994). Similarly open nests are warmer than edge nests which are warmer than vegetation nests (Jeyasurya et al., 1994). Soil temperature profiles indicate that 1) there is considerable daily variation in nest temperatures and 2) increasing vegetation decreases both the mean temperature and the amplitude of the diel temperature fluctuation (Fig. 6). Unfortunately, the incubation temperatures that produce males or females in fluctuating environments are not well understood; however it is likely that temperatures in open and edge habitats
Confounding effects may be more important for females than males in polygamous systems. Similarly, for species with ESD in which the males are the larger sex, females might bias the effect of age at first reproduction for females. Finally, female terrapins place smaller eggs in cooler (males producing) sites as is predicted by the MCDC hypothesis for this species, contrary to a previous suggestion that maternal provisioning is not likely to differ between the sexes in reptiles with ESD (Bull and Carlsson, 1988). Additional data is needed to test the MCDC in terrapins and other species with ESD. This study is unlikely to explain the origin of ESD in organisms such as reptiles, where sex is determined by components of the abiotic environment. However, it may be that the interaction of maternal effects in species with ESD prevents the evolution of genotypic sex determination.

CONCLUSIONS AND PROSPECTS

The maternal condition-dependent nest site choice hypothesis has been developed and supported with data from the diamondback terrapin. Other reptiles with ESD may provide additional corroborative evidence. Turtle species in which egg size is correlated with body size may represent a simpler situation in which nest site choice could evolve. For example, females reproducing at small sizes at earlier ages should select nest sites that would benefit smaller individuals and later in life, after continued growth, select sites that would give an advantage to the larger individuals. It has been suggested that the direction of ESD and the direction of sexual dimorphism may be correlated, with warmer temperatures producing the larger sex (Ewart and Nelson, 1991; Ewart et al., 1994), however see Janzen and Paukstis (1994) for a different perspective. The MCDC presents an alternative connection between sexual dimorphism and ESD, however sexual dimorphism is not required for the hypothesis to hold. If maternal condition affects growth and timing of reproduction, then mothers could produce the sex most likely to benefit from earlier reproduction. One possibility is that an earlier age of first reproduction may be more important for females than males in polygamous systems. Similarly, for species with ESD in which the males are the larger sex, females might bias the effect of age at first reproduction for females. Finally, female terrapins place smaller eggs in cooler (males producing) sites as is predicted by the MCDC hypothesis for this species, contrary to a previous suggestion that maternal provisioning is not likely to differ between the sexes in reptiles with ESD (Bull and Carlsson, 1988). Additional data is needed to test the MCDC in terrapins and other species with ESD. This study is unlikely to explain the origin of ESD in organisms such as reptiles, where sex is determined by components of the abiotic environment. However, it may be that the interaction of maternal effects in species with ESD prevents the evolution of genotypic sex determination.

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