

# A contemporary review of the ecology and evolution of environmental sex determination

**Callan Padron**

Department of Biology  
Lake Forest College  
Lake Forest, Illinois 60045

## Abstract

This article is a review of past and current literature on the study of environmental sex determination. Environmental sex determination (ESD) is a mechanism of sex determination in both plants and animals, which is influenced by the surrounding environmental conditions. These conditions range in a variety of environmental cues that influence this determination to occur. ESD is an alternative to genetic sex determination (GSD) and hermaphroditism, the former of which occurs as the most dominant form of sex determination in both plants and animals. This article aims to compile the evolution of our understanding of this unique method of sex determination, as well as the biological evolution of the method itself. This method has profound effects on the species that utilize it and begs the question of why this method is used over others, namely, GSD. This article will describe some of the main cues involved in ESD and provide examples of species that have evolved this method and how they can be expected to be impacted by a changing environment.

**Key words:** Environmental sex determination, temperature, genetic sex determination, sex allocation theory, sequential hermaphroditism, sex ratios.

Sex determination mechanisms (SDMs) are diverse across all plant and animal life on Earth. One of the lesser studied mechanisms is environmental sex determination (ESD). This method of sex determination operates through the fixed establishment of sex within a period after fertilization by nongenetic cues found in the environment (Janzen, 2006). These cues vary within and between species and are typically influenced by abiotic factors such as physical and chemical conditions. This method of sex determination involves multiple interacting parts and has been attempted to be explained through layers of cues interacting with hormones and genes. This differs from genotypic sex determination (GSD), which uses sex chromosomes and autosomes from the parents to determine sex.

ESD is also typically discussed in the same areas of study that hermaphroditism is, in which sex determination can be influenced by the organism's environment. However, hermaphroditism is important to distinguish as its own sexual system, very different from ESD because sex determination is flexible throughout a hermaphroditic organism's life, and commonly involves sexes besides strict male and female (Warner, 1975). ESD develops only strict males and females, a trait shared with GSD called gonochorism. Considering these differences and similarities, ESD is an incredibly complex sex determination mechanism, sharing traits with the other two mechanisms while simultaneously having unique traits of its own. There are different theories as to why ESD has become the primary form of sex determination over GSD in some species, including the theories that surround the survival of the mechanism itself over time.

The development of ESD as a viable theory has come only recently after decades of pointed observation into the sex determination mechanisms of a multitude of different kinds of plants and animals. The theory of environmental sex determination was first set forth in primary scientific literature in 1966 by Madeleine Charnier, a French zoologist studying the common Agama lizard, *Agama agama* (Charnier, 1966). Her article titled "Action of temperature on the sex ratio in the *Agama agama* (Agamidae, Lacertilia) embryo" presented the first description of environmental sex determination that paved the way for furthering future studies to incorporate the role of estrogen in determining sex, as well as the implications of global warming on reptiles. Charnier's work presented an entirely new area of study for developmental biology that didn't gain much traction until about ten years later. In 1977, James Bull and Eric Charnov published their article titled "When is sex environmentally determined?" (Charnov and Bull, 1977). This article took a more general approach to the topic, positing that

sex determination can occur through a variety of cues, and related this mechanism to principles that define sex ratios.

The purpose of this was to find the underlying reasoning for ESD as a viable and surviving mechanism, using fitness and equilibrium theories to explain the occurrence of ESD. The two foundational theories of natural selection favoring ESD set forth by Charnov and Bull described two possibilities, one of which included the influence on the fitness of offspring as they develop away from their parents, where the environment is patchy and provides differing opportunities for the two different sexes. The second theory describes the possibility that both the offspring and parents have an inability to predict or adapt to the patch type the offspring is born into (Charnov and Bull, 1977). The use of the word "patchy" refers to inconsistencies in the surrounding environment that could produce differential fitness in sexes, producing a difference in advantages in different conditions. Charnov and Bull further discussed potential environmental cues and how they have been observed in recent years relative to the publication of their article. At this time, ESD and sexual hermaphroditism were acknowledged as different but weren't separated in examination. Much of the study of ESD equally involved hermaphroditism, considering social cues producing hermaphroditic organisms to be part of the broader environment.

Charnov and Bull have been cited as two of the foundational researchers of environmental sex determination and provided a more substantial basis for the theory to gain traction in the scientific community for further research. At that time, ESD was primarily studied under the lens of temperature sex determination (TSD) in reptiles, primarily, turtles. Turtles provided a prime subject for ESD studies because of their high susceptibility to temperature during embryonic development, therefore these effects consistently produced tangible evidence for researchers to study the underlying molecular mechanisms of ESD. Many theories underlying ESD have been published in contemporary literature, but some of the most recent focus on the effect of the stress hormone cortisol on gene pathways in ESD organisms (Strakova et al., 2022).

Other sex determination mechanisms have been found to have "master on/off switches" in genes that influence sex to develop as male or female in a fertilized organism. But no clear on-off pattern has shown any indication in any genes for ESD (Crews and Bull, 2009). Regardless of this gap in knowledge, other topics concerning the evolutionary biology of ESD are still being studied. Many studies suggest the presence of aromatase, an enzyme that converts testosterone to estrogen, can play a pivotal role in TSD, as the activity of this enzyme reduces in extreme temperatures. (Godwin et al., 2003). In this study, researchers compiled sex ratio data on a species of fish, *Paralichthys olivaceus*, which exhibits both GSD and ESD. When researchers manipulated temperature, they found that individuals developed as females at approximately 50% at 20°C, and as females at higher and lower temperatures relative to 20°C (Fig 1). Females developed at 100% in gynogenetic, all XX progeny, which implies that XX individuals have TSD, whereas XY individuals have GSD (Godwin et al., 2003). These findings reinforce the hypothesis that estrogens produced by aromatase are the transducing factor for temperature effects on sex determination.

While aromatase does not completely explain the occurrence of ESD in organisms, other supplementary theories involving aromatase have been suggested in the evolution of ESD, such as the occurrence of stress and stress hormones. In Strakova et al. (2020), researchers investigated the possible relationship between stress and sex determination. Specifically, the role of stress-related hormones corticosterone and cortisol. In previous studies conducted on the effects of stress on various species of fish, most results found masculinization of gonads or inhibition of ovarian growth. It was suggested that corticosteroids affect sex determination pathways by "modulating the expression of the follicle-stimulating hormone receptor and aromatase" (Strakova et al., 2020). This could possibly imply a communication between activity in the hypothalamic-pituitary-adrenal axis which regulates cortisol as a response to environmental stressors, and the hypothalamic-pituitary-gonadal axis, which plays a role in reproduction in vertebrates. Furthermore, consideration of different gene pathways such as those including the Jumonji gene KDM6B, which has the ability to silence or alter transcription of the sex-determining gene DMRT1. In studies, knockout of KDM6B has blocked responsiveness of the sex determination pathway to temperature in embryos of certain ESD species. Under stress conditions, KDM6B tends to increase in expression (Strakova et al., 2020). With the potential for this link between the activity of these two axes, combined with interaction with sex-determining genes, there is ample footing

for explaining the development of ESD as it occurred throughout evolutionary time. Looking at ESD through a chronological lens can help to explain why we see ESD occur where it does. ESD is typically associated with reptiles and fish, similarly with hermaphroditism in fish.

Phylogenetically speaking, reptiles and fish represent some of the oldest vertebrate lineages on Earth and still maintain many of the biological and evolutionary traits that first occurred millions of years ago. Based on this scarce occurrence in prevalence, it's commonly hypothesized that ESD remains as a relic and possibly an ancestral mode of sex determination for all amniotes (Miller et al., 2004). A scenario of evolution described in Figure 2 suggests that over geologic time, TSD was once the primary form of sex determination, which was then followed by GSD through a primitive form of sex chromosomes derived through ancestral autosomes. It is possible that GSD became the dominant mechanism over time in most mammals, birds, and other organisms for a variety of reasons, including equilibrium sex ratios following the Fisherian theory of sex ratios (Fisher, 1930.)

Of course, this begs the question of what was ancestral to ESD itself, which is potentially where we return to the similarities between ESD and hermaphroditism. Both mechanisms of sex determination, whether at fertilization or periodically throughout an organism's life, are typically theorized to be influenced by fitness. This is the idea that certain conditions might act as cues to which ESD organisms may respond molecularly, resulting in the fittest population relative to their surroundings; the "optimization" of ESD progeny is based on the greatest value. Strakova et al. (2020) suggest that because of these similarities in producing sex ratios based on their environment, sequential hermaphroditism could have been an ancestral mode preceding ESD, which was selected for over and over again through consistent stressful conditions until it developed into its own gonochoristic mechanism of sex determination at fertilization.

Theories for the maintenance of ESD as a viable SDM vary. A study in 2006 by Janzen et al. suggested multiple different possible theories as to why ESD has remained as successful as it has;

1. **Phylogenetic inertia** – ESD is the ancestral condition in this clade and is simply maintained in extant lineages because it is currently adaptively neutral or nearly so.
2. **Group adaptation** – ESD promotes adaptive control of sex ratio to promote group fitness.
3. **Inbreeding avoidance** – ESD minimizes inbreeding by producing single-sex clutches.
4. **Differential fitness** – ESD provides sex-specific fitness benefits such that some developmental temperatures are better for one sex. (Janzen and Phillips, 2006).

While these four theories are not universally accepted, they do include some of the most highly considered theories as to why ESD still occurs in modern species relative to the dominating success of GSD, especially the theory of differential fitness. It is even possible that multiple theories are correct, acting in cooperation to produce ESD at a frequency higher than that of any one theory alone. This study by Janzen and Phillips also suggests that ESD may have even evolved independently multiple times, further encouraging ESD as a highly effective mechanism of sex determination.

Considering differential fitness as one of the most likely theories underlying ESD, it's important to understand the sex allocation theory, particularly why and how species develop sex ratios relative to their resource base. Sex allocation theory is defined as the distribution of adjustments to male and female offspring in sexual species (Charnov, 1983). In the Fisherian principle of equal sex allocation, when a species produces 1:1 sex ratios of males and females in a given population. This occurs in stable populations where it is assumed that males and females are equally costly to produce by the parents. In a scenario where one is more costly, parents would allocate resources to offspring differentially. Therefore, parents allocate equal investment in the effort of both sexes, to produce a stable ratio of sexes to carry on the population for generations. With this in mind, understanding ESD through the Fisherian principle does not apply in the same way, because the sex ratios of ESD species can be biased based on the conditions producing one sex over the other.

As such, we turn to the Trivers-Willard hypothesis, which deviates from Fisherian sex ratios. This model from 1973 proposed that individuals would skew the sex ratio of males to females as a response to certain

parental conditions. These conditions include food availability, maternal condition/quality, mate attractiveness and quality, and sex change (Trivers and Willard, 1973). While this hypothesis does not directly confront ESD, it does provide a better framework for the functioning of ESD as an SDM capable of producing viable sex ratios without population crash. By predicting more investment in males through parents in good conditions, and greater investment in females in poor conditions (Trivers and Willard, 1973), we can better understand how the surrounding environment directly and indirectly influences populations to produce progeny with the greatest fitness, rather than relying on equal parental investment to produce viable ratios that may or may not thrive in the conditions the species is experiencing. These conditions vary from biotic to abiotic conditions, which can act as "cues" to trigger sex bias in ESD species.

While ESD has been studied primarily in reptiles and fish, especially with attention to temperature sex determination, there are many more species of both plants and animals that exhibit ESD through a variety of environmental cues. Some of these include water availability, pH, CO<sub>2</sub>, parasitism, day length/photoperiod, nutrient availability, and resource availability. It's important to note that these are not all the possible environmental cues that exist, they do however represent some of the most interesting, significant, and susceptible environmental cues across environmental sex determination.

With regards to temperature, the tuatara (*Sphenodon punctatus*), a reptile found only in New Zealand and recognized for its strong lineage to ancestral reptiles, is described as a "living fossil." Most importantly, the tuatara only reproduces through TSD. Members of this species are determined as male in higher temperatures, and with the increasing temperatures consistently occurring through climate change, the tuatara could only produce all male clutches at 100% of nest sites with no variation (Mitchell, 2010). Without any modified nesting behavior or adaptive response of the pivotal temperature in time to change this result, environmental conditions produced by climate change could mean extinction for the tuatara and others like it.

In a study conducted in 2019, Dupoue et al. studied the effects of water sex determination (WSD) as a form of ESD that impacts the sex dynamics and genetic diversity of two species of reptiles, the Asp viper (*V. aspis*) and the European common lizard (*Z. vivipara*), both of which are viviparous species known to display genetic sex determination. This study remarks on the discoveries found in prior research, in which genetic sex determination can be overridden by environmental sex determination, highlighting the fact that sex determination is not strictly a 'one or the other' occurrence, but describes it as a spectrum of the differing influences of both genotypic and environmental factors (Dupoue et al., 2019). Other examples in studies have shown environmental pressures as influences in sex reversal through both biotic and abiotic factors. This study's focus on water could be considered more important than temperature because water is a depreciable resource during gravidity or gestation periods when allocating water among embryos, thus influencing the fitness of offspring. When conducting this study, researchers restricted water mid-pregnancy for the two species, for 29 individuals of *V. aspis* and 420 individuals of *Z. vivipara*, both species found in the study location of Western France. This method was based on the fact that restriction of water is known to dehydrate gravid and gestating females without changing their reproductive investment, any large difference in sex ratios could indicate water sex determination. This study found a male-biased secondary sex ratio in both species following water restriction in mid-pregnancy, and the control group that received water at liberty had a balanced secondary sex ratio. Interestingly, the study also included data on the effects of water restriction at different points in their pregnancy, in which the restriction had less of an effect later in the pregnancy, and more of an effect earlier in pregnancies.

A study in 2002 manipulated CO<sub>2</sub> levels in two species of wild-caught turtle eggs, *Trachemys scripta* and *Graptemys pseudogeographica kohnii*, two species known to exhibit TSD. Clutches of both species were incubated at 28.5°C in boxes aerated with one of four gas mixtures (two in *G. p. kohnii*). Across all eggs, elevated levels of carbon dioxide significantly feminized sex ratios in both species and also reduced hatching success. When eggs were grouped into clutches, all comparisons between 0% and elevated carbon dioxide showed feminization in the portions of the clutches at elevated carbon dioxide, a statistically significant result for *T. scripta* (Ewert et al., 2002). Accumulation of biogenic carbon dioxide from embryonic respiration and other sources is thus likely to affect sex ratios in natural nests under some conditions, perhaps through an effect on some

aspects of embryonic pH.

In cichlid fish, for 3 species of *Pelvicachromis* (including *P. pulcher*) as well as 2 species of the South American dwarf cichlid *Apistogramma* by Rubin (1985) across pH conditions ranging from 5 to 7, under which all species showed male-biased sex ratios at low pH and female-biased ratios at neutral pH. The exact length of the critical period for pH exposure on sex ratio in *P. pulcher* is not known, but treatment for the first 30 days after hatching is effective in influencing sex ratios. In a large comparative study of *Apistogramma* cichlids, Römer and Beisenherz (1996) manipulated both pH and water temperature for over 30 species of *Apistogramma*. Nearly uniformly, these species showed more male-biased sex ratios at lower pH and high temperatures and female-biased sex ratios at neutral pH and lower temperatures. Temperature effects were stronger in some species and pH effects were stronger in others. The only species to show a significant “reversed” temperature effect showed it only at the lowest pH and showed far stronger pH effects in the typical direction than other species. The interspecific differences in the interaction of temperature and pH seen across the species in Römer and Beisenherz’s (1996) study demonstrate that these 2 factors have independent effects at the level of the organism. The variation in the strength of pH and temperature effects may reflect selection for tracking more than one distal variable (Renn and Hurd., 2021).

In 1998, 22 isopod species were studied for their molecular response to parasitism. When exposing these species to parasitic infections of *Wolbachia*, the most positive results in infected species (17 out of 22) were obtained in females only, suggesting that most isopod *Wolbachia* strains cause feminization. Feminization also probably occurs in species where intersex individuals have been detected: *A. vulgare*, *A. album*, *L. oceanica*, and *S. rugicauda*. Furthermore, Vitagliano et al. (1996) showed that sex determination and the female-biased sex ratio in *Asellus aquaticus* are influenced by a cytoplasmic factor, but the two studied populations were not tested for *Wolbachia*. The bacteria detected in this species thus seem probable sex-ratio distorters. Infected males were only detected in five species.

In the crustacean *Gammarus duebeni*, sex is determined by the interaction of a number of environmental, genetic, and parasitic factors, which may, in turn, influence sex ratios and population dynamics. A study by Dunn et al. in 2005 suggests that environmental sex determination (ESD) in *G. duebeni* depends primarily on the interaction of two environmental cues: day length and temperature. Whereas previous work found that male-biased sex ratios were produced under long day conditions and female biases under short days, this study shows that, at the lower temperatures normally experienced by this species at northern latitudes, the reverse pattern can occur. When researchers reared *G. duebeni* broods in four conditions based on temperature and photoperiod duration, initial analysis of the entire data set showed that brood sex ratios differed significantly with day length, temperature, and between the different populations (Dunn et al., 2005). Furthermore, the interaction between photoperiod and temperature significantly affected sex ratios. *G. duebeni* shows between population variation in the mechanism of ESD—both the level of ESD and the cues which affect sex ratios differ in animals from different populations. This study discusses the idea that the use of two environmental cues for sex determination may be adaptive if it allows more precise predictions of growth opportunity before reproduction and so of expected future fitness (Dunn et al., 2005).

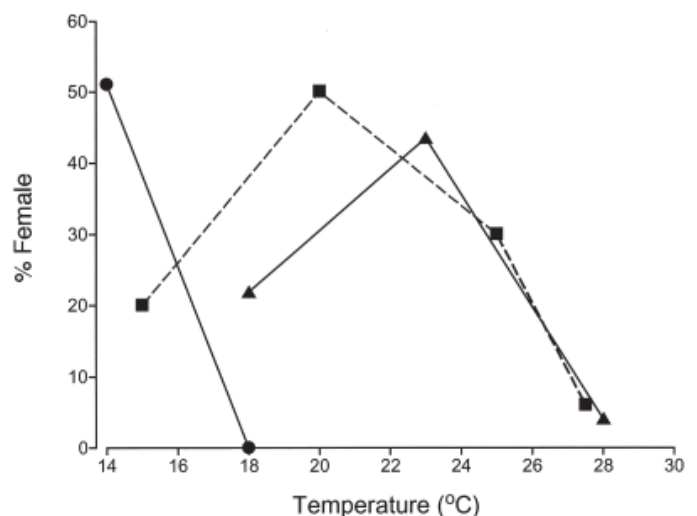
In plants, ESD is also known to occur. A study conducted in 2012 used a species of fern, *Culcita macrocarpa* in varying nutrient conditions to study its effect on sex determination. Initially, most of the gametophytes of *Culcita macrocarpa* were male and subsequently hermaphrodite under different nutrition. The result indicates that its sex determination is protandry. All nutrient conditions were favorable for developing young male gametophytes but only a good environment (high nutrient) was favorable for female gametophyte growth. In all respects, female gametophytes were much larger than the other types of gametophytes. (Ghosh et al., 2012).

Considering the broad and diverse impacts of the environment on sex determination mechanisms, it’s important to consider the consequences that may result from these responses to certain environmental conditions. In certain consistent conditions producing one sex over the other, ESD-specialist species are vulnerable to skewed sex ratios that, if produced for too long, could add stress to a population’s survival and even end in extinction. This is a concern for species such as the tuatara. Additionally, in species that exhibit both GSD and ESD, continual selection for ESD could possibly result in a loss of genetic sex factors that are used in GSD

(Strakova et al., 2020). It’s not necessarily a certainty that GSD would be completely lost within those species, just that it would be reduced to an “inactivated” genetic mode, and could leave species with only ESD at an unfortunate time. Similarly, species with only ESD could experience loss of plasticity, as they become more fixed to specific conditions, their ability to adapt to new conditions could be negatively affected.

Climate change presents an extreme issue to ESD species, specifically in environmental cues most affected by this problem. Temperature, CO<sub>2</sub>, pH, salinity, and water/nutrient availability could prove to be negative cues as conditions in our climate keep shifting towards extremes with less environmental variation. As these conditions remain consistent, species could experience that skewed sex ratio and present a variety of population dynamics issues. Also, the theory of differential fitness may not be the most accurate for all occurrences of ESD, and those sexes produced by those shifts to “one end” of an ESD’s conditional cues may not actually be the most advantageous for that condition. This could produce sex ratios that are maladapted to their environment (Wyneken and Lolavar, 2015).

Environmental sex determination presents many new opportunities for research as a relatively new concept in the scientific community. ESD represents a very broad and diverse mechanism across the plant and animal kingdom, including those that utilize both ESD and GSD or ESD and hermaphroditism. It is encouraged to consider sex determination as a spectrum of mechanisms and sexual systems on multiple axes, and that one species could fall anywhere on that scale, not just at an extreme. There is a lot to still discover about ESD, including the variety of environmental cues and not just how species interact with them.



**Fig. 3.** Flounder sex ratios resulting from development at different temperatures. Depicted are data from barfin flounder (●), Japanese flounder (■), and southern flounder (◆). References are in text. Japanese flounder values are means calculated from Yamamoto (1999).

**Figure 1.** Godwin, J., Luckenbach, J. A., and Borski, R. J. (2003). Ecology meets endocrinology: environmental sex determination in fishes. *Evolution and development* 5(1), 40-49.

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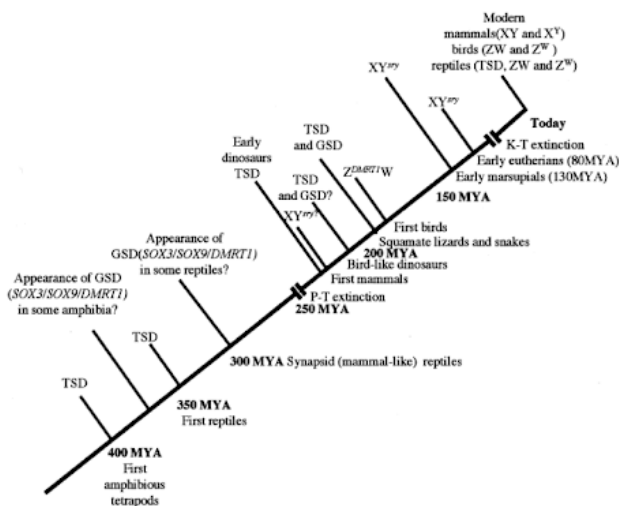
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**Figure 2.** Miller, D., Summers, J., and Silber, S. (2004). Environmental versus genetic sex determination: A possible factor in dinosaur extinction? *Fertility and Sterility*, 81(4), 954-964. <https://doi.org/10.1016/j.fertnstert.2003.09.051>