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Sex-specific survival to maturity and the evolution of environmental sex determination

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Abstract
Four decades ago, it was proposed that environmental sex determination (ESD) evolves when individual fitness depends on the environment in a sex-specific fashion—a form of condition-dependent sex allocation. Many biological processes have been hypothesized to drive this sex asymmetry, yet a general explanation for the evolution of sex-determining mechanisms remains elusive. Here, we develop a mathematical model for a novel hypothesis of the evolution of ESD, and provide a first empirical test using data across turtles. ESD is favored when the sex-determining environment affects annual survival rates equivalently in males and females, and males and females mature at different ages. We compare this hypothesis to alternative hypotheses, and demonstrate how it captures a crucially different process. This maturation process arises naturally from common life histories and applies more broadly to condition-dependent sex allocation. Therefore, it has widespread implications for animal taxa. Across turtle species, ESD is associated with greater sex differences in the age at maturity compared to species without ESD, as predicted by our hypothesis. However, the effect is not statistically significant and will require expanded empirical investigation. Given variation among taxa in sex-specific age at maturity, our survival-to-maturity hypothesis may capture common selective forces on sex-determining mechanisms.

Keywords
Condition-dependent sex allocation, GSD, reptile, sex determination, theoretical model, TSD

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Sex-specific survival to maturity and the evolution of environmental sex determination

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Abstract

Four decades ago, it was proposed that environmental sex determination (ESD) evolves when individual fitness depends on the environment in a sex-specific fashion – a form of condition-dependent sex allocation. Many biological processes have been hypothesized to explain how this sex asymmetry arises, yet a general explanation for the adaptive evolution of sex-determining mechanisms remains elusive. Here, we develop a mathematical model for a novel and general hypothesis of the evolution of ESD, and provide a first empirical test using data across turtles. ESD is favored when the sex-determining environment affects annual survival rates equivalently in males and females, and males and females mature at different ages. We compare this hypothesis to alternative and potentially complementary hypotheses, and demonstrate how it captures a crucially different process. This maturation process arises naturally from common life histories and phenotypic effects and applies more broadly to condition-dependent sex allocation. Therefore, it has widespread implications for animal taxa. Across turtle species, ESD is associated with greater sex differences in the age at maturity compared to species without ESD, as predicted by our hypothesis. However, the effect is not statistically significant and will require expanded empirical investigation to provide a robust test.

Introduction

Across vertebrates, the sex-determining mechanism is an evolutionarily-labile trait, with numerous bidirectional transitions between Genotypic Sex Determination (GSD) and Temperature-dependent Sex Determination (TSD; Bull 1983; Janzen and Paukstis 1991a; Janzen and Krenz 2004; Ezaz et al. 2009; Pen et al. 2010; Holleley et al. 2015). GSD involves activation of the sex-determining pathway by a gene located on sex chromosomes. Under TSD, a form of ESD, the sex-determining pathway is initiated by incubation temperature during embryonic development.

The discovery of TSD in the mid-20th century has compelled numerous hypotheses for its evolution and maintenance (Janzen and Paukstis 1988, 1991a; Shine 1999). The primary adaptive hypothesis (Charnov and Bull 1977) suggests that TSD is a form of condition-dependent sex allocation that is favored over GSD when incubation temperature influences the fitness of individuals in a manner that differs between the sexes (Fig. 1A). Extensive empirical research on TSD has focused on revealing how this sex-specific effect of incubation temperature arises biologically. Hatchling phenotypes may depend on incubation temperature in a different fashion for males and females (i.e., a temperature-by-sex effect on phenotypes relevant for fitness; Shine 1999). Support for this type of effect is taxonomically-scattered and often species-specific (Joanen and McNease 1989; Janzen 1995; Spencer and Janzen 2014). More commonly, temperature influences phenotype similarly for the two sexes (Deeming 2004; Warner and Shine 2005), and this phenotypic effect is assumed to influence fitness differentially for the two sexes (i.e., a phenotype-by-sex effect on fitness; Conover 1984; Warner and Shine 2008; Warner et al. 2009). For

example, TSD may evolve in short-lived species when an effect of incubation temperature on body size impacts first-year reproductive success differently in males and females (Pen et al. 2010; Warner and Shine 2008; Warner et al. 2009). Still, explanations for the occurrence of TSD in any given taxon exhibit an ad hoc flavor.

In addition, adaptive explanations must compete with plausible non-adaptive hypotheses. Recent theoretical and empirical evidence suggests that sex chromosomes may be lost under climatic upheaval, causing a non-adaptive conversion from GSD to TSD (Grossen et al. 2010; Holleley et al. 2015). Similarly, TSD can be maintained in long-lived animals with very low levels of selection under theoretical scenarios (Schwanz and Proulx 2008), raising the possibility that its persistence and taxonomic frequency in many reptile clades is due to nearly-neutral processes (Janzen and Phillips 2006).

While much conceptual focus for the adaptive evolution of TSD has been placed on the importance of temperature for reproductive success as an adult (Conover 1984,Warner et al. 2009), we argue that juvenile survival may be equally or more important in determining lifetime fitness (sensu Sæther et al 2013). Indeed, temperature need only influence survival to maturity differently for males and females to select for biased sex ratios under condition-dependent sex allocation (Schwanz et al. 2006). Here, we present a previously-unappreciated biological process that provides a general explanation for the occurrence (i.e. persistence) of TSD across living organisms. We demonstrate that two straight-forward biological traits – temperature-dependence of annual juvenile survival and sex-differential age at maturity – jointly select for TSD, while either trait in isolation does not. In our novel 'survival to maturity' hypothesis, sex differences in age at maturation can drive the evolution and maintenance of TSD even if incubation temperature affects the

annual survival of juveniles in a similar manner in the two sexes. More broadly, our model can be applied to any condition-dependent sex allocation strategy where an early-life environmental variable other than temperature (e.g. maternal provisioning) impacts annual survival rate post-independence.

Model

We present an optimality model for the evolution of temperature-dependent sex determination with sex-specific ages at maturity and temperature-dependent survival rates using a simple life history. We then demonstrate how these conclusions can be extended to more complex life histories and use this approach to distinguish our present hypothesis from alternative hypotheses presented in the literature.

Simple Life History

Consider an organism that does not reach sexual maturity until many years after hatching. Upon reaching sexual maturity, the animal breeds one time and dies. Eggs are incubated in one of two patch types – a 'cold' patch or a 'hot' patch. Patch temperature influences the survival of a juvenile, such that annual survival of a juvenile from a hot patch ($s\beta$) is higher than the annual survival of a juvenile from a cold patch (s, assuming $\beta > 1$; see Fig. 1B, 'Year 1' line). Oviposition sites are limited and patch frequencies are fixed such that not all females can oviposit in the preferred hot patches. Survival to the age at maturity (S_α, where α is the age at maturity) is the multiplication of each annual survival probability. Thus,

survival to maturity of an individual from a cold patch is $S_{\alpha,c} = s^{\alpha}$, and survival to maturity of an individual from a hot patch is $S_{\alpha,h} = (s\beta)^{\alpha}$.

We model the effect of different ages at maturation on the evolution of TSD using the Shaw-Mohler (1953) equation. We measure the fitness of a mutant mother whose offspring develop with sex ratios of $\hat{r}_c$ (when nesting in cold patches) and $\hat{r}_h$ (hot patches) in a population of wild-type mothers with offspring sex ratios r_c and r_h. If offspring sex is also related to production costs (e.g. sex determination is linked to egg size; Radder et al. 2009), mother-offspring conflict over the sex ratio could arise and alter theoretical predictions. (Kuijper and Pen 2014). However, for this model, we ignore these complications. A mother produces b number of offspring, with P probability of developing in a cold patch, and $1-P$ probability of developing in a hot patch. The mutant mother's fitness (W) is the proportion of her grandchildren in a wild-type population:

$$W = \left[\frac{P\hat{r}_c bs^{\alpha_m} + (1-P)\hat{r}_h bs^{\alpha_m}\beta^{\alpha_m}}{Pr_c bs^{\alpha_m} + (1-P)r_h bs^{\alpha_m}\beta^{\alpha_m}} + \frac{P(1-\hat{r}_c)bs^{\alpha_f} + (1-P)(1-\hat{r}_h)bs^{\alpha_f}\beta^{\alpha_f}}{P(1-r_c)bs^{\alpha_f} + (1-P)(1-r_h)bs^{\alpha_f}\beta^{\alpha_f}} \right]. \quad [1]$$

A wild-type organism has $W = 2$, so the sex ratios r_c and r_h are evolutionarily stable if no mutant values ($\hat{r}_c, \hat{r}_h$) yield $W > 2$. Thus, at the ESS conditions: 1) W is maximized with respect to $\hat{r}_c$ and $\hat{r}_h$, 2) $\hat{r}_c = r_c$ and $\hat{r}_h = r_h$, and 3) $W = 2$. We can find the ESS of eqn [1] by considering the derivatives $dW/d\hat{r}_c$ and $dW/d\hat{r}_h$ and setting $\hat{r}_c = r_c$ and $\hat{r}_h = r_h$:

$$\frac{dW}{d\hat{r}_c} = \frac{P}{Pr_c + (1-P)r_h\beta^{\alpha_m}} + \frac{-P}{P(1-r_c) + (1-P)(1-r_h)\beta^{\alpha_f}} \quad \text{and} \qquad [2.a]$$

$$\frac{dW}{d\hat{r}_h} = \frac{(1-P)\beta^{\alpha_m}}{Pr_c + (1-P)r_h\beta^{\alpha_m}} + \frac{-(1-P)\beta^{\alpha_f}}{P(1-r_c) + (1-P)(1-r_h)\beta^{\alpha_f}}. \qquad [2.b]$$

If we define the population's contribution of males (*M*) and females (*F*) as $M = Pr_c + (1-P)r_h\beta^{\alpha_m}$ and $F = P(1-r_c) + (1-P)(1-r_h)\beta^{\alpha_f}$, we find

$$\frac{dW}{d\hat{r}_c} = \frac{P}{M} - \frac{P}{F} \quad \text{and} \quad \frac{dW}{d\hat{r}_h} = \frac{(1-P)\beta^{\alpha_m}}{M} - \frac{(1-P)\beta^{\alpha_f}}{F}. \qquad [3.a\text{-}b]$$

Analyzing equations 3.a-b, we find that TSD evolves if two conditions are met: 1) age at maturity differs for males and females (e.g., $\alpha_m < \alpha_f$) and 2) an annual survival advantage accrues to individuals developing in one thermal patch over the other (e.g., $\beta > 1$ in Fig. 1B). When these conditions are met, the two derivatives (eqns 3.a-b) cannot simultaneously equal zero. Thus, three TSD outcomes (Table 1; Fig. 2A) are possible based on the frequency of cold and hot patches. When cold patches are rare, cold patches always produce males (r_c=1), while hot patches overproduce females (r_h<1/2) (scenario I). When hot patches are rare, hot patches always produce females (r_h= 0), while cold patches overproduce males (r_c> 1/2) (scenario III). At intermediate frequency of patches, cold

patches produce all males and hot patches produce all females (scenario II). In all cases, cold patches produce more males than warm patches.

The intuitive explanation for this outcome is that the difference in survival to maturity between cold- and hot-incubated individuals is amplified as the age at maturity increases (Fig. 3). This means that, if males and females mature at different ages, temperature exerts a sex-differential effect on juvenile survival (hence fitness). If age at maturity for males (α_m) is earlier than for females (α_f; $\alpha_m < \alpha_f$), then a hot incubation temperature has a stronger benefit for females than for males ($(s\beta)^{\alpha_f} / s^{\alpha_f} > (s\beta)^{\alpha_m} / s^{\alpha_m}$), and individuals are selected to develop as females at hot temperatures and males at cold temperatures (Fig. 2A). Indeed, the survival parameter itself (s) cancels out, so we can say more generally that TSD evolves whenever the survival advantage of patch type differs between the sexes (i.e., when $\beta^{\alpha_f} \neq \beta^{\alpha_m}$). The greater the incubation temperature effect on annual survival (β) and the greater the disparity in age at maturity, the stronger selection for TSD will be.

In contrast, when age at maturity is the same for both sexes ($\alpha_m = \alpha_f$), or if there is no effect of incubation temperature on annual survival ($\beta = 1$), $\beta^{\alpha_m} = \beta^{\alpha_f}$ and the derivatives can simultaneously equal zero. Solving for r_c and r_h we find:

$$r_c = \frac{1}{2} + \frac{1}{2}\frac{(1-P)}{P}\beta^{\alpha_m}(1-2r_h) \qquad [5.a]$$

and

$$r_h = \frac{1}{2} + \frac{1}{2}\frac{P}{(1-P)}\frac{1}{\beta^{\alpha_f}}(1-2r_c)\,. \qquad [5.b]$$

One solution is that, for all proportions of hot and cold patches, 50% males are produced in both hot and cold patches [$r_c = 1/2$ and $r_h = 1/2$] (Fig. 2B). TSD will not be favored in this scenario.

Generalized Life History

Our model can be generalized across more complex life histories and compared to alternative evolutionary models using R_0 as the fitness measure (R_0 = juvenile survival * fecundity * expected adult lifespan, see proofs in Charnov 1997; Schwanz et al. 2006). We modify our notation slightly to accommodate comparison with alternative hypothesis. Each life-history component can differ according to i sex (male, female) and j incubation temperature (hot, cold). If we allow overlapping generations, with adult annual survival $p_{i,j}$ (such that expected adult lifespan is given by $E_{i,j} = 1/(1-p_{i.j})$), and annual fertility, $b_{i,j}$, then the relative fitness advantage of males in the hot patch compared to the cold patch is

$$\frac{R_{0,m,h}}{R_{0,m,c}} = \frac{{s_{m,h}}^{(\alpha_{m,h})} b_{m,h}/(1-p_{m,h})}{{s_{m,c}}^{(\alpha_{m,c})} b_{m,c}/(1-p_{m,c})} \qquad [6.a]$$

Similarly, the relative fitness of females in the hot patch is

$$\frac{R_{0,f,h}}{R_{0,f,c}} = \frac{{s_{f,h}}^{(\alpha_{f,h})} b_{f,h}/(1-p_{f,h})}{{s_{f,c}}^{(\alpha_{f,c})} b_{f,c}/(1-p_{f,c})}$$

[6.b]

These are general equations that can be leveraged to understand the fitness differentials associated with any specific sex × temperature life-history effect, assuming stable age distribution.

Survival to Maturity (SM) hypothesis. Under the assumptions of our survival-to-maturity hypothesis (Table 2), eqns [6.a-b] can be rewritten and simplified as

$$\frac{R_{0,m,h}}{R_{0,m,c}} = \left(\frac{s_h}{s_c}\right)^{\alpha_m}$$

[7.a]

and

$$\frac{R_{0,f,h}}{R_{0,f,c}} = \left(\frac{s_h}{s_c}\right)^{\alpha_f}$$

[7.b]

which is equivalent to saying that the relative fitness advantage of males in the hot patch compared to the cold patch is β^{α_m}, and the relative fitness of females in the hot patch is β^{α_f}.

Thus, examining a generalized life history with R_0 as the fitness measure leads to the same formulation of relative male and female fitness as the simple life history. As proven above, TSD evolves when $\beta^{\alpha_f} \neq \beta^{\alpha_m}$ or when 1) $\frac{s_h}{s_c} \neq 1$ and 2) $\alpha_f \neq \alpha_m$ (assumptions a&b, Table 2). The difference in sex-specific fitness depends entirely on the quantitative effect of incubation temperature on juvenile survival rate (and that this effect persists until both sexes have matured), and the quantitative difference in age at maturity between males and females (Fig. 4A). Specifically, as the benefit of hot incubation temperatures for juvenile survival rate increases (lines in Fig. 4A), it acts synergistically with difference in age at maturity between the sexes (*x*-axis in Fig. 4A) to produce strong selection for TSD. When females mature later than males (right side of Fig. 4A), their fitness benefits from hot incubation temperatures exceed those of males, and selection favors the development of females at hot temperatures and males at cold temperatures. The opposite pattern of TSD is favored when males mature later than females (left side of Fig. 4A).

For the remaining two hypotheses, we posit more broadly that TSD is favored over GSD whenever the relative fitness of males for hot and cold patches does not equal the relative fitness of females from hot and cold patches (assuming stable-age distribution):

$$\frac{R_{0,m,h}}{R_{0,m,c}} \neq \frac{R_{0,f,h}}{R_{0,f,c}}$$

[8]

Temperature-dependent Fertility (TF) Hypothesis. An early hypothesis for the evolution of TSD proposed that the sex × temperature effect on fitness arises when 1) incubation temperature effects post-incubation body size or growth (e.g., via seasonal time of hatching) and 2) body size influences adult fertility more in one sex (e.g., females) compared to the other (Conover 1984). TSD should evolve such that the sex that gains the most in fertility from larger adult body size develops at the incubation temperature associated with greater adult body size (also known as the 'sexual dimorphism hypothesis', Janzen and Paukstis 1991b). These effects explain the occurrence of TSD in silverside fish (Conover 1984), but their general explanatory power in reptile sex-determining mechanisms remains unsupported (Janzen and Paukstis 1991b).

Applying the parameter assumptions for this model (Table 2) to eqns [6a&b] and simplifying leads to:

$$\frac{R_{0,m,h}}{R_{0,m,c}} = \frac{b_{m,h}}{b_{m,c}}$$

[9.a]

and

$$\frac{R_{0,m,h}}{R_{0,m,c}} = \frac{b_{f,h}}{b_{f,c}}$$

[9.b]

A formal proof for a similar condition-dependent sex allocation model can be found in Schwanz et al. (2006). As long as the fertility of one sex is more strongly influenced by incubation temperature than is the fertility of the other sex (assumption a, Table 2), TSD is favored (Fig. 4B, where fitness ratio ≠ 1).For example, when male fertility is not influenced by incubation temperature (Fig. 4B, top line), the strength of selection for TSD increases as the benefit of hot temperatures for female fertility increases (*x*-axis). There is no sex-differential fitness advantage, and no selection for TSD, when incubation temperatures influence fertility the same for the two sexes (Fig. 4B, when fitness ratio = 1 moving down the lines and across the *x*-axis. Note that age at maturity and survival to maturity do not feature in the solution, but that incubation temperature must have a sex-specific influence on fertility regardless of how many years after hatching individuals mature.

Temperature-dependent Maturation (TM) Hypothesis. A recent hypothesis that we will call the 'temperature-dependent maturation' (TM) hypothesis (Warner et al. 2009) shares features with the SM and TF hypotheses yet has distinct selective forces. As in the TF hypothesis, the TM hypothesis proposes that incubation temperature influences post-incubation body size equally for both sexes– warm-incubated offspring hatch early and have a long season to grow whereas cool-incubated offspring hatch late and have little opportunity to grow before winter. Age at maturity also features in the TM hypothesis as an important biological mechanism – size impacts the age at maturity in one sex (females)

more than the other (males). Age at maturity is taken to be the age at first successful breeding, regardless of gonadal development.

There is support for this hypothesis in dragon lizards (Warner and Shine 2008; Warner et al. 2009). Specifically, TSD is more often associated with species where all males are likely to be too small to secure matings at 1 year of age (due to strong, size-based intrasexual competition), and early-hatched females, but not late-hatched females, are large enough to produce eggs at 1 year of age.

The TM hypothesis draws upon models of seasonal sex ratios in birds (Daan et al. 1996; Pen et al. 1999). An important feature of seasonal sex ratio models is whether one assumes that average annual adult survival ($p_{i,j}$) and the resulting adult reproductive lifespan are independent of age at maturity (Pen et al. 1999) or whether delaying age at maturity leads to a decrease in adult reproductive lifespan (Warner et al. 2009). The former is a typical theoretical simplification of invariant adult survival rate. The latter may occur when senescence in viability occurs and there is a finite total lifespan, so that delaying maturity means losing a year of reproduction from a finite number of years. At the extreme (low overlap in generations), seasonal sex ratios can disrupt the stable age distribution required for R_0 to be a viable fitness measure (Werren and Charnov 1978), so a formal model is required to validate the following formulation. We specify age at maturity (and adult lifespan) varying according to sex and incubation temperature (Table 2; eqns [6a&b]):

$$\frac{R_{0,m,h}}{R_{0,m,c}} = s^{(\alpha_{m,h}-\alpha_{m,c})}\left(\frac{(1-p_{m,c})}{(1-p_{m,h})}\right) \quad [10.a]$$

$$\frac{R_{0,f,h}}{R_{0,f,c}} = s^{(\alpha_{f,h}-\alpha_{f,c})}\left(\frac{(1-p_{f,c})}{(1-p_{f,h})}\right) \quad [10.b]$$

where $p_{i,j}$ is a function of $\alpha_{i,j}$. When $p_{i,j}$ is not related to $\alpha_{i,j}$, the $p_{i,j}$ cancel out of both equations and TSD is favored when the differences in age at maturity between incubation temperatures are not equal for the sexes ($\alpha_{f,h} - \alpha_{f,c} \neq \alpha_{m,h} - \alpha_{m,c}$; assumption a, Table 2). For example, if all males mature at the same age (the ratio of fitness of males from hot and cold temperatures is 1), but hot-incubated females mature 1 year earlier than cold-incubated females (the female fitness ratio is $1/s$, a value greater than 1), then females gain more in lifetime fitness from hot incubation compared to cold incubation than do males (Fig. 4C, bottom line). If these are the only effects, than the driving fitness impact of incubation temperature is through its influence on juvenile survival.

If, in addition, maturing earlier (smaller $\alpha_{i,j}$) allows an extra year of reproduction within an individual's lifetime (larger mean $p_{i,j}$), then the impact of incubation temperature on relative fitness is amplified (Fig. 1C, upper lines). As total lifespan becomes short (e.g. 5 years; Fig. 4C, top line), sex-differences in temperature-dependent maturation lead to a strong fitness differential between females and males. In this conceptualization (e.g., Warner et al. 2007), the driving fitness effects include juvenile survival and adult lifespan. Note that survival rates are invariant between the sexes and incubation temperatures, and that it is the *difference* in ages at maturity in each sex that matter (absolute age at maturity matters if $p_{i,j}$ is a function of $\alpha_{i,j}$).

Combined maturity. It is highly plausible that the effect of incubation temperature on size and/or quality simultaneously impacts annual survival rates as well as age- and sex-specific reproductive success, manifest as an earlier age at maturity and higher adult fertility for one or both sexes. These combined effects can be examined numerically using eqns 6a&b. We briefly consider the interactive effects of the two hypotheses related to age at maturity (SM and TM). Specifically, incubation temperature impacts juvenile survival rate, the sexes mature at different mean ages, and incubation temperature influences the age at maturity for females but not males. Eqns 6.a&b are rewritten as:

$$\frac{R_{0,m,h}}{R_{0,m,c}} = \left(\frac{s_h}{s_c}\right)^{\alpha_m} \qquad [11.a]$$

and

$$\frac{R_{0,f,h}}{R_{0,f,c}} = \frac{{s_h}^{(\alpha_{f,h})}}{{s_c}^{(\alpha_{f,c})}} \qquad [11.b]$$

As shown for the SM hypothesis (Fig. 4A), the advantage of hot incubation temperatures for survival rate interacts strongly with the sex difference in age at maturity (Fig. 4D; compare line style groups). If, in addition, hot-incubated females mature earlier than cold-incubated females (lines within line groups) there is an increase in the female:male fitness

differential; however, within-sex difference in maturity interacts very little with between-sex difference in average age at maturity. Interestingly, when mean age at maturity is similar for the two sexes (e.g., *x*-axis = 0), the survival rate advantages and female maturation advantages interact. When hot-incubated females mature earlier than cold-incubated females (e.g., by two years – the top line of each group), increasing the juvenile survival advantage (solid vs. dashed vs. dotted lines) decreases selection for TSD.

Empirical Test of the Survival To Maturity Hypothesis

Testing any of these hypotheses or trying to distinguish among them is exceptionally challenging. Such analyses would best be accomplished by directly testing the assumptions for each hypothesis listed in Table 2. However, while gathering the necessary life-history details for a single species is not trivial, the challenges of gathering such information for multiple species to acquire generality are extraordinary. Because of these limitations, we examine the likelihood of the 'survival to maturity' hypothesis as a general explanation for variation in sex-determining mechanisms (SDMs) at a broad scale, using comparative methods to test whether sex differences in age at maturity in turtle species are associated with SDM.

We compiled data for SDMs, sex-specific ages at maturity, and body sizes for turtles from the primary literature (Table S1). Differences in the age at maturity between males and females may be causally linked to average body size and sexual size dimorphism (Shine 1990), which themselves could provide alternative biological processes selecting for TSD (Lovich et al. 2014). Although direction of size dimorphism was previously shown not associated with SDMs (Janzen and Pauksits 1991b), we used our updated data to examine whether body size variables predict SDMs.

Concomitantly examining these relationships provides an avenue for assessing the general applicability of the three hypotheses. Different ages at maturity for males and females are 1) a necessary driver in the SM hypothesis, 2) not addressed in the TF hypothesis, and 3) an outcome of the TM hypothesis, although reduced if one sex matures across multiple ages. Sex differences in adult body size are 1) a potential outcome of different ages at maturity in the SM hypothesis, 2) a driver in the TF hypothesis, and 3) a potential outcome in the TM hypothesis that decreases in likelihood as variation in age at maturity increases and as adult lifespan increases, overwhelming size differences at maturity. To find support for the SM hypothesis, age at maturity must differ between the sexes more in TSD species than in GSD species. We have no expectation of size differences between the sexes under this hypothesis.

We created data quality filters for the age at maturity data. Because sexual maturity varies among populations of a given species, sex-specific estimates were used only when presented for the same geographic location. When data on both male and female sexual maturity were available for multiple populations, we chose data from the population closest to the center of the species' range.

Phylogenetic generalized least squares (PGLS) models that account for species interdependence were used to determine whether SDMs are associated with: (1) differences in the age at first reproduction between males and females (Diff_maturity); (2) differences in body size between males and females; (i.e. sexual size dimorphism; Diff_size); and/or (3) mean body size inclusive of both females and males (Mean_size). In addition, we tested whether Diff_maturity is predicted by Diff_size and Mean_size.

First, non-phylogenetic GLS models were generated and their residuals were tested for Brownian motion (BM), Ornstein-Uhlenbeck (OU), and white noise models of evolution on pruned ultrametric trees with branch lengths derived from the most recent comprehensive molecular phylogeny of chelonians (Guillon et al. 2012). Akaike information criteria indicated that the OU model of evolution best described GLS covariance structures. Thus, before running PGLS models, α was adjusted accordingly. Analyses were conducted using the Ape, Geiger, nlme, and phytools packages of the R programming language (R Development Core Team 2014). Body size data were referenced from the most comprehensive review (Halámková et al. 2013) of published estimates for female and male turtles (Table S1).

PGLS tests that included Diff_maturity as a response variable were conducted on 28 species for which maturity and phylogenetic information were available (Figure S1). The largest compilation for which both phylogenetic and body size data were available included 55 species (Figure S2).

In all cases where the sexes differed in age at maturity in our dataset, males matured earlier than females. As predicted by the SM hypothesis, the mean difference in age at maturity between males and females was approximately one year greater for species with TSD compared to species with GSD (Fig. S1B). However, this difference was not supported with statistical significance, likely due to the low number of species with GSD (Table 3; Fig. S1). As expected, SDMs did not covary with either sexual size dimorphism or mean body size (Table 3; Fig. S2), although sexual maturity was related to both factors (Table 3).

Discussion

Sex-determining mechanisms have evolved independently numerous times in animals. The most compelling adaptive hypothesis for the evolution of TSD was proposed nearly four decades ago (Charnov and Bull 1977). These authors suggested that TSD is a type of condition-dependent sex allocation that is favored over GSD when fitness depends on incubation temperature in a sex-specific manner. Testing the hypothesis, however, has been hampered by the difficulty of measuring lifetime fitness and the experimental challenge of decoupling temperature and sex.

Empirical support for adaptive hypotheses of TSD has been contentious, being both scattered taxonomically and species-specific (Conover 1984; Janzen 1995; Warner and Shine 2008). Moreover, non-adaptive or nearly-neutral processes have also been invoked to explain the evolution and maintenance of TSD (Janzen and Phillips 2006; Grossen et al. 2010; Holleley et al. 2015). Here, we demonstrate mathematically that a simple biological process related to age at first reproduction provides a broadly inclusive explanation for the adaptive evolution of TSD across all taxa. In this 'survival to maturity' hypothesis (Fig. 3), incubation temperature affects annual juvenile survival. The annual effect is multiplied across successive years of immaturity, so that survival from hatching to a given year depends more strongly on temperature as more years pass. When males and females mature at different ages, the combined impact of incubation temperature on survival to maturity differs between the two sexes, thus establishing a (temperature-dependent) phenotype × sex interaction on fitness. If females mature later than males and annual survival is higher for warmer natural incubation temperatures, then we would expect females to develop at warm

temperatures and males to develop at cold temperatures. Alternatively, if one of those patterns is reversed, we would predict the opposite pattern of TSD.

The 'survival to maturity' hypothesis has great potential to explain the occurrence of TSD across taxa. In reptiles, incubation temperature has diverse and nearly ubiquitous impacts on hatchling phenotypes relevant for survival (e.g., morphology, locomotion, and anti-predator behavior), as well as survival itself (Janzen 1995; Deeming 2004; Warner and Shine 2005). Perhaps counterintuitively, these thermal effects need not differ for males and females for our hypothesis to hold (Rhen and Lang 1995). Crucially, for our hypothesis to be correct, the temperature effect on survival must persist during the ages when one sex has matured, but the other has not. Although this age may be many years after hatching for long-lived species, the assumption is no greater than those made for many alternative hypotheses. For example, the additional two hypotheses contrasted in this paper rely on size differences established by incubation temperature persisting up to and beyond maturity. Such size differences are at least as likely to influence annual survival as they are to influence maturation or fertility. Unfortunately, little is known regarding whether the phenotypic and survival effects persist beyond the limited temporal scale of most experiments (1 month – 1 year). Indeed, post-hatching survival itself is rarely reported (see, e.g., Janzen 1995; Andrews et al. 2000; Booth et al. 2004; Freedberg et al. 2004; Hare et al. 2004), and is a fruitful target for increased empirical research to validate the hypothesis.

If temperature effects on annual survival are near universal for ectothermic animals, then variation in SDMs would be explained largely by the extent of sex differences in age at maturity. GSD would be expected when males and females have similar ages at maturity, while TSD would be expected when males and females have greater differences in ages at

maturity. We provided a first test of this prediction with a comparative analysis across chelonian taxa, and found little support for our hypothesis. Species with TSD had greater differences in age at maturity between males and females than did species with GSD, as predicted, but there are currently too few taxa with GSD and known ages at maturity for both sexes to provide a robust statistical test.

With so few data in the GSD group, the results may also be influenced by a few taxa (Maddison and Fitzjohn 2015). Increasing the number of species for which sex-specific age at maturity is known would greatly improve our ability to test the hypotheses, particularly if non-chelonian (e.g., lizard) taxa could be included in the comparative analysis.

We additionally examined the role of sexual size dimorphism and associated size-related fitness in selecting for TSD (Janzen and Paukstis 1988 and here), but found even less support for these factors being related to SDM. These results also hold when sea turtles are excluded (results not presented).

The distinction between a role for size-related traits or survival to maturity *per se* is important in comparing alternative hypotheses for the adaptive evolution of TSD. We considered two common, traditional hypotheses that are linked critically to differences in body size and its impact on reproductive potential. In the 'temperature-dependent fertility' hypothesis, sex-specific differences in the importance of body size for adult fertility provide the sex-by-temperature fitness effect. In the 'temperature-dependent maturation' hypothesis, body size influences the ability to breed for the first time (i.e., age at maturity) more in one sex than the other. In contrast, the 'survival to maturity' hypothesis does not rely on sexual dimorphism, and any scatter in the link between size and age at maturity is inconsequential. It is difficult, however, to use these data to disprove the 'temperature-

dependent maturation' hypothesis because the variable maturation age in one sex should generate noise in the size and age at maturity data.

Viewing the 'survival to maturity' hypothesis as a novel selective process is not a trivial, theoretical exercise. Its distinctness is of vital importance for empiricists, as demonstrated using a hypothetical empirical study (Fig. 5). Standard avenues of inquiry into the adaptive nature of TSD (e.g., effects of incubation temperature on growth, maturation and fertility) could lead a researcher to erroneously conclude that TSD has no adaptive relevance if negative results arise. Only when the results are considered in the context of our 'survival to maturity' hypothesis is an additional sex-specific effect of incubation temperature apparent (see Fig. 5). While other processes operating in conjunction may be important, our 'survival to maturity' hypothesis provides a sufficient and general explanation for the otherwise persistent conundrum of TSD in long-lived vertebrates.

Moreover, the 'survival to maturity' hypothesis adds a novel dimension and important distinction for empirical research on condition-dependent sex allocation in unisexual taxa with GSD. Our hypothesis would apply when early-life conditions (e.g. maternal condition) influence the post-independence juvenile survival rate differently for males and females and the two sexes mature at different mean ages. The hypothesis contrasts with the common view of the Trivers-Willard hypothesis (Trivers and Willard 1973), which focuses on the sex-specific impact of condition for reproductive success as an adult (but see the broader, 'reproductive value' view, Leimar 1996).

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Figure Legends

Figure 1. Sex-differential fitness effects of incubation temperature select for TSD and arise when incubation temperature impacts annual survival and ages at maturity differs between the sexes. *A*, hypothetical scenario illustrating selection for TSD: Incubation temperature more strongly influences the fitness of females (dotted) than that of males (solid). Note that we could set fitness in a given patch (e.g. cold patch) equal to 1 and compare relative fitness in other patches. In *B*, small differences in annual survival between cold- and hot-incubated individuals are magnified across multiple years. Each line shows survival to the year indicated. If females reach sexual maturity later (Year 6) than males (Year 3), then temperature has a stronger effect on survival to maturity in females compared to in males.

Figure 2. Offspring sex ratios when developing in cold and hot patches with and without TSD. *A*, when the conditions of incubation temperature-dependent annual survival and sex difference in age at maturity are met, TSD is the ESS, here shown as biased primary sex ratios for hot and cold patches. *B*, when age at maturity is equal for males and females, TSD is not favored (both patches produce 50% males).

Figure 3. Schematic of 'survival to maturity' hypothesis for the evolution of Temperature-dependent Sex Determination (TSD). TSD evolves when incubation temperature of embryos impacts annual survival for the rest of the individuals' lives and the two sexes mature at different ages. It is assumed that cold patches are unavoidable. Survival of a clutch of eggs is shown whether incubated in hot or cold patches and whether developing as all males (blue) or all females (red). Annual survival is assessed each year, here shown for

the first 6 years of life. For the sex that matures later (here, females mature at six years), survival to the age at maturity is much higher when incubated at hot temperature than cold temperatures. In contrast, survival to the age at maturity is not as strongly impacted by incubation temperature for the sex that matures earlier (here, males mature at 3 years). The optimal strategy (black boxes) is to develop as a female at warm temperatures to benefit from the stronger survival-to-maturity advantage, and develop as a male at cold temperatures to accept the relatively small cost of survival-to-maturity while benefitting from frequency-dependent selection on sex.

Figure 4. Selection differentials under three hypotheses for the evolution of TSD. Each panel shows the ratio of the temperature advantage for females (eqn. 8, right side) to the temperature advantage for males (eqn. 8, left side) on the *y*-axis (note variation in scale across figure panels). A value of 1 indicates no sex-specific fitness as a function of temperature, thus no selection for TSD. Values greater than 1 indicate selection for females to develop preferentially at warm temperatures, whereas values less than 1 indicate the opposite selection. A) According to the 'survival to maturity' (SM) hypothesis developed in this paper, selection depends on the difference in ages at maturity for males and females (*x*-axis) and the benefit of incubating at warm temperatures for juvenile survival rate (*lines*, top to bottom: 1.2, 1.15, 1.1, 1.05, 1)[$\alpha_m = 4$ for figure]. B) Under the 'temperature-dependent fertility' (TF) hypothesis, selection depends on the benefit in fertility females receive from incubating at warm temperatures (*x*-axis) compared to the corresponding benefit males receive (*lines*, top to bottom: 1, 1.25, 1.5, 1.75, 2). C) Under the 'temperature-dependent maturation' (TM) hypothesis, selection depends on whether

incubation temperature has a greater effect on age at maturation for females (*x*-axis) compared to males ($\alpha_{m,h} - \alpha_{m,c} = 1$), with the additional possibility that total lifespan is finite and is a linear function of age at maturation (*lines*, top to bottom: $E_{i,j} = 5$ yrs, 10 yrs, 20 yrs, infinite)[$s = 0.8$; $\alpha_{f,h} = 1$]. D) The SM and TM hypotheses may simultaneously apply to a population. Increasing the difference in age at maturity between males and females has a strong effect (*x*-axis) when incubation temperature has a large influence on survival rates (*line groups*: s_h/s_c = 1 (solid); 1.1 (dashed); 1.2 (dotted)). The difference in female age at maturity due to incubation temperature (*within group lines*, top to bottom: -2,-1,0) has a fairly constant effect of increasing the female:male fitness ratio [$s_c = 0.8$; $s_h = 0.8, 0.88, 0.96$; $\alpha_m = 3$; $\bar{\alpha}_f = 3,5,7,9$, with $\alpha_{f,h}$ and $\alpha_{f,c}$ distributed evenly around the mean].

Figure 5. The importance of distinguishing the SM hypothesis from the TF and TM hypotheses is demonstrated with hypothetical experimental results. A researcher is studying a species with TSD and wants to test the adaptive significance of this SDM for the species, focusing on sex-differential fitness effects of incubation temperature (Charnov and Bull 1977). For this hypothetical scenario, we imagine the researcher is able to generate males and females across a range of incubation temperatures and starts by measuring body size. If the researcher is not considering the SM hypothesis (conceptual framework in black), then results in line with point (1) or points (2) and (3) would lead to the conclusion that there is no support for adaptive evolution of TSD. However, these results are consistent with the SM hypothesis (gray), and additional data allow further testing the model.

Table 1. Maternal condition and optimal primary sex ratios in a population with discrete generations.

Derivatives		**Parameter Space Regions**	r_c	r_h
I. $\frac{dW}{dr_c} > 0$,	$\frac{dW}{d\hat{r}_h} = 0$	$\frac{P}{1-P} < \beta^{\alpha_m}$	1	$\frac{1}{2} - \frac{1}{2}\frac{P}{(1-P)}\frac{1}{\beta^{\alpha_m}}$ Eqn. [4.a]
II. $\frac{dW}{dr_c} > 0$,	$\frac{dW}{d\hat{r}_h} < 0$	$\beta^{\alpha_m} \le \frac{P}{1-P} \le \beta^{\alpha_f}$	1	0
III. $\frac{dW}{d\hat{r}_h} = 0$	$\frac{dW}{d\hat{r}_h} < 0$	$\beta^{\alpha_f} < \frac{P}{1-P}$	$\frac{1}{2} + \frac{1}{2}\frac{(1-P)}{P}\beta^{\alpha_f}$ Eqn. [4.b]	0

,

Table 2. Comparison of the hypothesis developed in this paper with two popular hypotheses for the adaptive evolution of temperature-dependent sex determination (TSD). The comparison demonstrates how the hypotheses differ in the mechanism by which incubation temperature has a differential effect on male and female relative lifetime fitness.

Hypothesis	Assumptions	Parameters
Survival to Maturity (SM)[1] TSD is favored when annual juvenile survival rate depends on incubation temperature and the sexes mature at different ages	a) Annual juvenile survival differs for T_{inc}, but not sexes;	a) $s_{f,j} = s_{m,j} = s_j$; $s_h \neq s_c$
	b) Age at maturity differs for sex, but not T_{inc};	b) $\alpha_{i,h} = \alpha_{i,c} = \alpha_i$; $\alpha_f \neq \alpha_m$
	c) Fertility and adult lifespan are not influenced by T_{inc} or sex	c) all $p_{i,j} = p$; all $b_{i,j} = b$;
Temperature-dependent Fertility (TF)[2] TSD is favoured when incubation temperature influences fertility differently for males and females	a) Adult fertility may vary by T_{inc} and sex, with T_{inc} effects differing between sexes	a) $\frac{b_{f,h}}{b_{f,c}} \neq \frac{b_{m,h}}{b_{m,c}}$;
	b) Annual juvenile mortality, age at maturity, and adult lifespan are not influenced by T_{inc} or sex	b) all $s_{i,j} = s$; all $\alpha_{i,j} = \alpha$; all $p_{i,j} = p$;
Temperature-dependent Maturity (TM)[3] TSD is favored when incubation temperature influences effective age at maturity differently for males and females	a) Age at maturity may vary by T_{inc} and sex, with T_{inc} effects differing between sexes	a) $\alpha_{f,h} - \alpha_{f,c} \neq \alpha_{m,h} - \alpha_{m,c}$
	b) Total lifespan may be finite such that mean survival rate and adult lifespan are reduced when maturity is delayed	b) $\frac{(1-p_{f,c})}{(1-p_{f,h})} \neq \frac{(1-p_{m,c})}{(1-p_{m,h})}$;
	c) Annual juvenile mortality and adult fertility not influenced by T_{inc} or sex	c) all $s_{i,j} = s$; all $b_{i,j} = b$;

[1]Hypothesis presented herein

[2]Conover 1984, Janzen and Paukstis 1991b

[3]Daan et al. 1996, Pen et al. 1999, Warner et al. 2009, Pen et al. 2010

Table 3. Results of phylogenetic generalized least squares (PGLS) models on Diff_maturity ($N = 28$), Diff_size ($N = 55$), and Mean_size ($N = 55$); * = model residuals with statistically significant ($P < 0.05$) phylogenetic signal (Bloomberg's K).

Model	Source	$_{num}$DF	$_{den}$DF	*F*	*P*
Diff_maturity; (K= 0.057)	Intercept	1	27	66.093	<0.0001
	SDM	1	27	0.877	0.3571
Diff_maturity; ($^{*}K$ = 0.052)	Intercept	1	26	102.53372	<0.0001
	Diff_size	1	26	15.20633	0.0006
	Mean_size	1	26	0.04543	0.8329
Diff_size; ($^{*}K$ = 0.031)	Intercept	1	53	71.266	<0.0001
	SDM	1	53	0.149	0.7009
Mean_size; ($^{*}K$ = 0.026)	Intercept	1	53	72.495	<0.0001
	SDM	1	53	1.483	0.2287

Figure 1

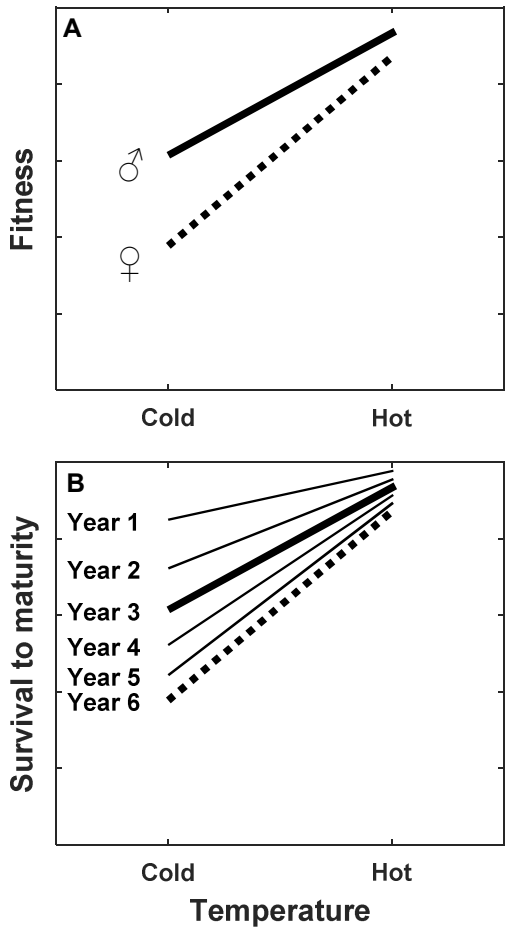

A
Fitness
♂
♀
Cold
Hot
B
Survival to maturity
Year 1
Year 2
Year 3
Year 4
Year 5
Year 6
Cold
Hot
Temperature

Figure 2

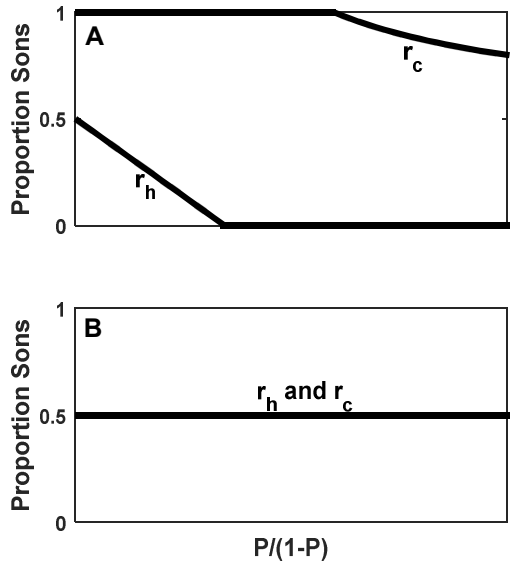

1
A
Proportion Sons
0.5
r_c
r_h
0
1
B
Proportion Sons
r_h and r_c
0.5
0
P/(1-P)

Figure 3.

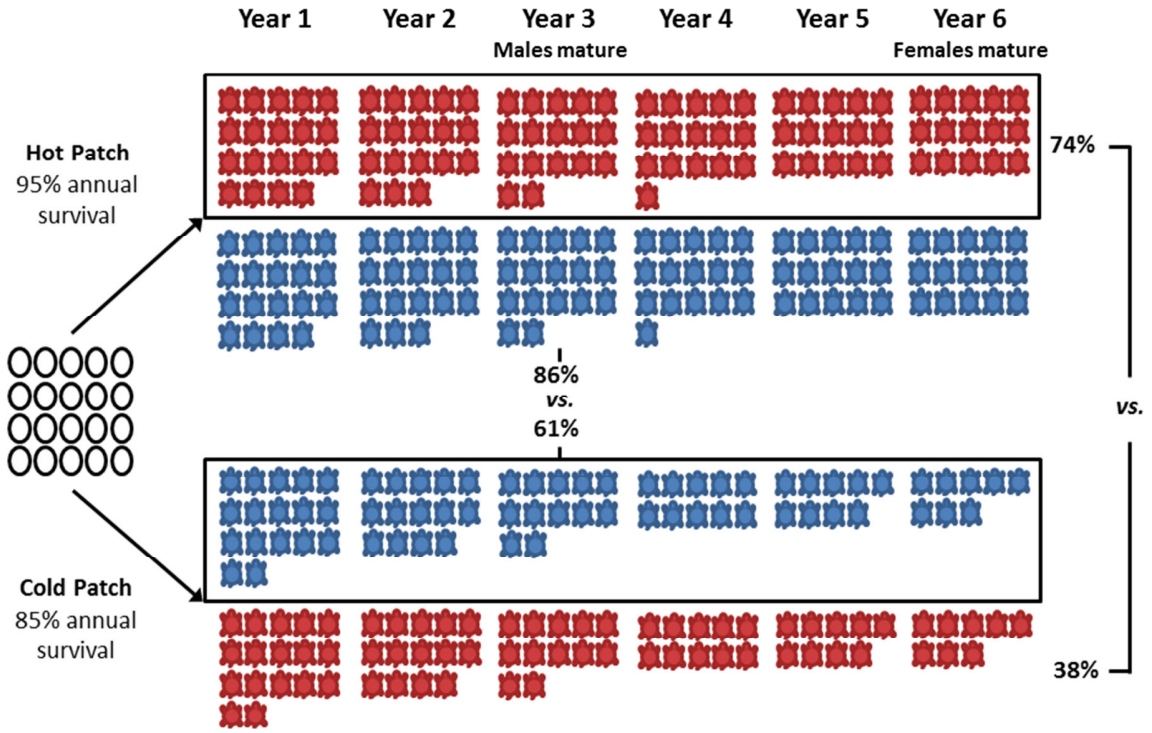

Year 1
Year 2
Year 3
Males mature
Year 4
Year 5
Year 6
Females mature
Hot Patch
95% annual survival
74%
86%
vs.
61%
vs.
Cold Patch
85% annual survival
38%

Figure 4.

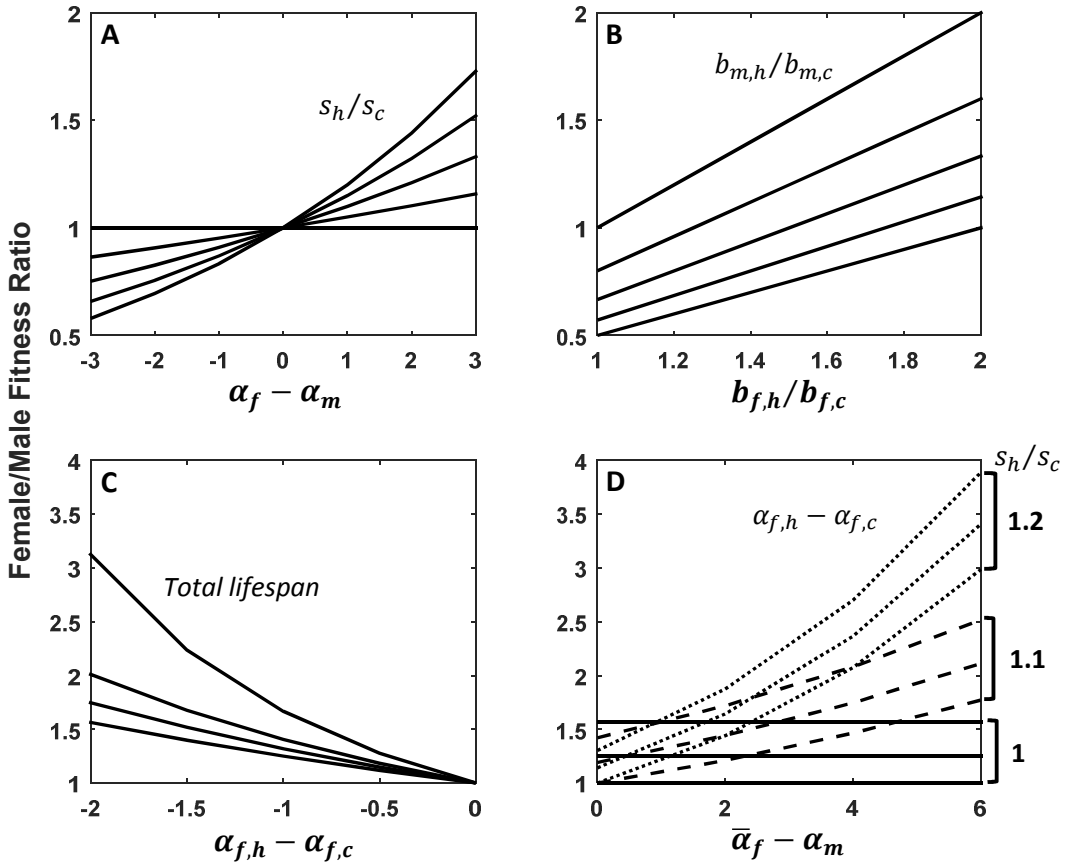
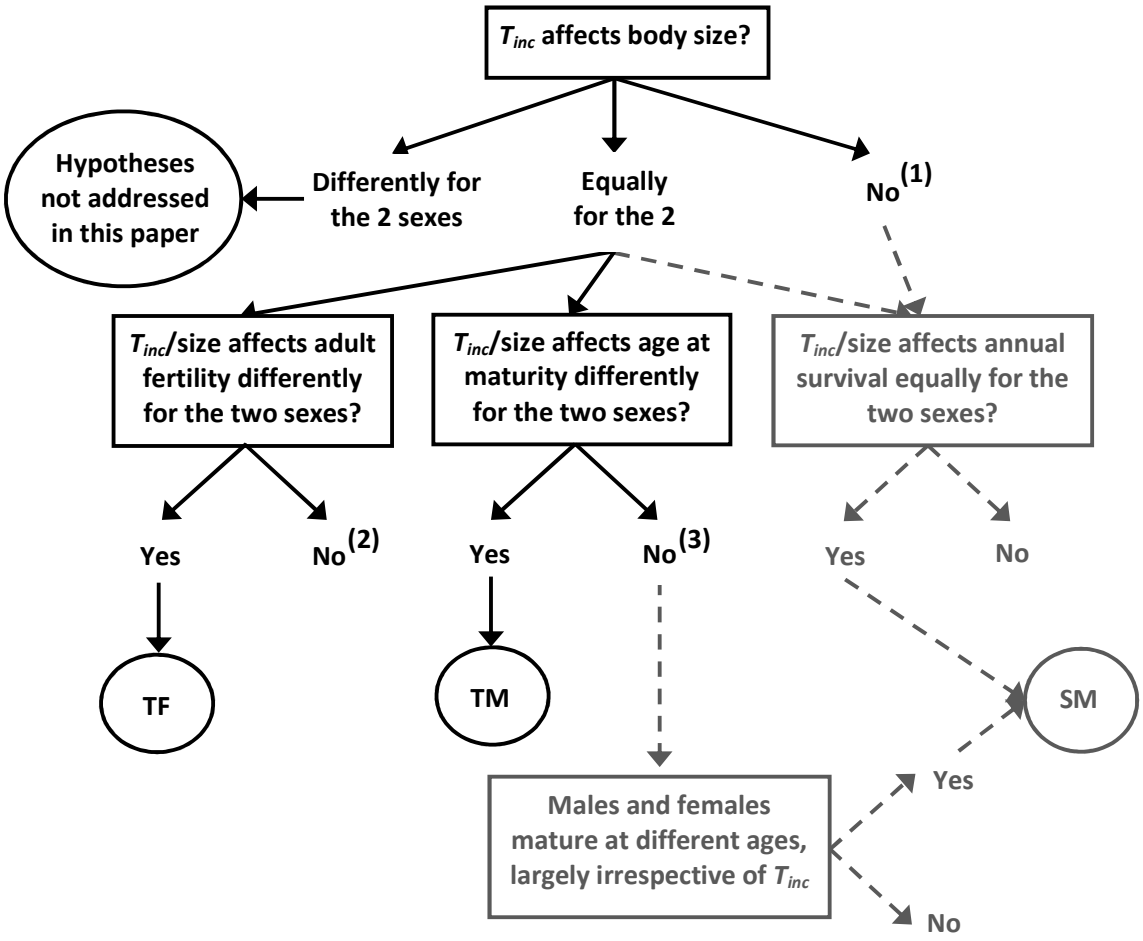

A
s_h/s_c
$\alpha_f - \alpha_m$
B
$b_{m,h}/b_{m,c}$
$b_{f,h}/b_{f,c}$
C
Total lifespan
$\alpha_{f,h} - \alpha_{f,c}$
D
$\alpha_{f,h} - \alpha_{f,c}$
s_h/s_c
1.2
1.1
1
$\bar{\alpha}_f - \alpha_m$
Female/Male Fitness Ratio

Figure 5.


T_{inc} affects body size?
Hypotheses not addressed in this paper
Differently for the 2 sexes
Equally for the 2
No(1)
T_{inc}/size affects adult fertility differently for the two sexes?
T_{inc}/size affects age at maturity differently for the two sexes?
T_{inc}/size affects annual survival equally for the two sexes?
Yes
No(2)
Yes
No(3)
Yes
No
TF
TM
SM
Males and females mature at different ages, largely irrespective of T_{inc}
Yes
No