A shifting switch: How plasticity shapes the evolution of environmental sex determination

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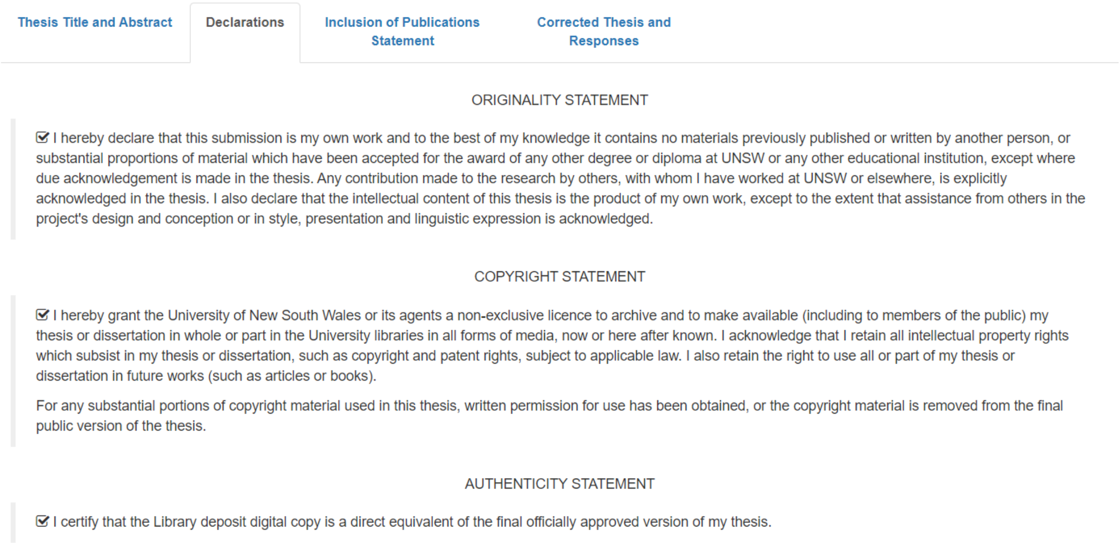

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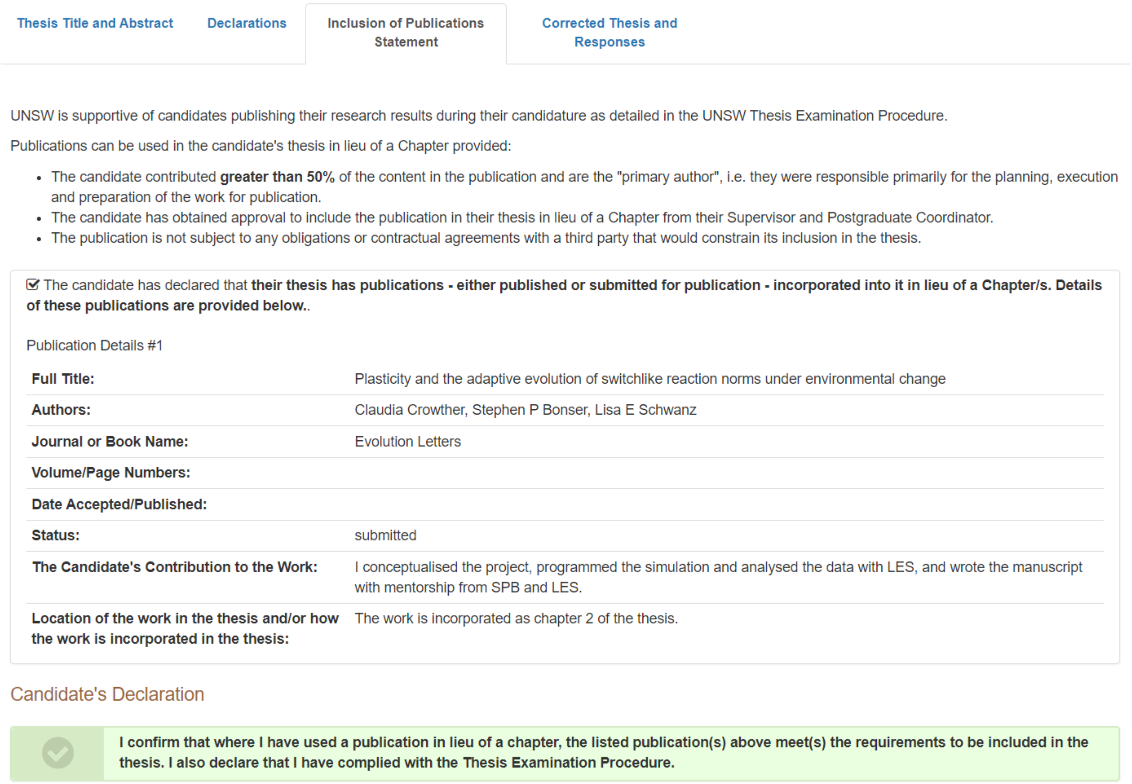

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Publication Details #1

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Publications, Presentations, and Awards

Publications

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UNSW Biological Earth & Environmental Sciences Outstanding Ecology & Evolution Presentation 2019 – Runner-up

“At the present time there is hardly any question in biology of more importance than this of the nature and cause of variability.”

Charles Darwin

‘Studies in the Theory of Descent’

“Life in plastic, it's fantastic.”

Aqua

‘I’m a Barbie Girl’

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Abstract

Phenotypic plasticity, the variation of gene expression in response to the environment, is a universal characteristic of living organisms. Because natural selection acts on phenotypes, plastic trait expression must influence evolution, yet the nature of this influence is not well understood. This thesis explores the role of phenotypic plasticity in the evolution of environmental sex determination (ESD), a widespread polyphenism with significant consequences for individual fitness and population survival. In particular, I focus on species with temperature-dependent sex determination (TSD), a form of ESD. I use simulation modelling and demographic data from a wild population with TSD, to investigate the role of plasticity in the adaptation of TSD species to novel environments. In Chapter Two, I examine the influence of plasticity on the evolution of discrete traits, such as sex in species with TSD. I simulate how plasticity in the slope and inflection point of the TSD reaction norm affects the evolution of the pivotal temperature of sex determination (T_{piv}). I find that the influence of plasticity on T_{piv} evolution depends on whether plasticity increases or decreases the strength of sex ratio selection. In Chapter Three, I assess T_{piv} plasticity in a wild population of turtles with TSD. I investigate whether fluctuating nest temperatures can account for previously observed patterns of plasticity in T_{piv}, and if plastic responses covary with maternal traits. I detect T_{piv} plasticity, but no evidence of adaptive variation in T_{piv} or maternal influence on sex ratios. In Chapter 4, I use simulation modelling to examine the coevolution of two traits that respond to sex ratio selection in TSD species: T_{piv}, and maternal nesting behaviour (*Nb*). I find that T_{piv} has a greater capacity to adapt to climatic warming than *Nb*, but that this relationship is strongly influenced by plastic responses to the environment, such as temperature-dependent survival and seasonal variation in *Nb*. Overall, my thesis demonstrates that plasticity can influence selection on genotypes, exposing traits to stronger selective pressures or shielding genes from selection. I highlight that the influence of plasticity on selection can create a trade-off between adaptive plasticity and trait innovation. Species with ESD navigate this trade-off with adaptation in a diverse range of traits, both fixed and plastic, in response to the strength of selection for the rare sex.

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Chapter One

General Introduction

Claudia Crowther

Plasticity and its Effect on Evolution

Phenotypes are a product of genes and the *environment*. Organisms cannot avoid the influence of their surroundings interceding in the expression of their genomes, to potentially deleterious effect (Ghalambor et al. 2007). Yet phenotypic plasticity may also allow organisms to adjust, throughout their lives, the fate handed to them by genetics (Pfennig 2021). Regardless of adaptive value, the alteration of genotypes by plastic responses to the environment must greatly influence evolution, as changes in phenotype lead to differences in fitness and thus, differences in surviving genotypes. Because of this, understanding the sources of variable responses to the environment, and their specific effect on evolution, is of great importance to the field of evolutionary biology.

Adaptive phenotypic plasticity has been proposed as a mechanism by which organisms may survive environmental change, either within the species' existing range, or during the colonisation of a new environment (Agrawal 2001; Lande 2009; Chevin et al. 2010; Scheiner et al. 2017; Kelly 2019). New phenotypes generated by responses to novel conditions could be adaptive and allow the persistence of a species in an environment where the original phenotype could not survive (Paenke et al. 2007; Scheiner et al. 2017). Behavioural plasticity allows for flexible interactions with the environment and changes in behavioural responses within an organism's lifetime can promote species survival (Mery and Burns 2010). Plastic responses to novel environments may ultimately lead to speciation, if variation in trait expression creates reproductive separation between populations of the same species responding to different environments (Endler 1995). In contrast, plasticity may also shield poorly adapted genotypes from selection, preventing adaptive evolution. The potential of plasticity to limit evolutionary change was first observed in behavioural traits and termed the

“Bogert effect” for Charles Bogert, the initial proponent of the theory (Bogert 1949, 1959; Muñoz 2021). Bogert’s observations were later formalised by Huey et al. (2003) who described the phenomenon as “behavioural inertia”. Inertia occurs when plasticity produces a highly fit phenotype, such that no additional selection acts on the plastic trait, thus preventing trait evolution (Huey et al. 2003; Ghalambor et al. 2007; Snell-Rood et al. 2018).

While individual plasticity has long been researched with respect to environmental change, plasticity across generations has recently emerged as equally important. Transgenerational plasticity, a type of parental effect, can have a large impact on offspring phenotype and has been shown to play a critical role in a population's response to change (Agrawal et al. 1999; Schwanz et al. 2010). Examples of adaptive parental effects are well documented. Parents may induce early development of environment-specific traits in offspring (e.g., plant anti-predator defences), choose oviposition sites that promote their offspring’s survival, or provide care, in the form of nutrition or protection, that maximises their offspring’s fitness (Agrawal et al. 1999; Refsnider and Janzen 2010; Wong et al. 2013; but see Uller et al. 2013). One of the most striking examples of individual plasticity and parental effects colliding to determine a population's response to change occurs in species with environmental sex determination, where sexual development (male or female) depends on the developmental environment and parents are responsible for selecting that environment (Charnov and Bull 1977). In this thesis, I will explore how interactions between developmental plasticity and parental effects shape the course of evolution in species with environmental sex determination.

Offspring Sex Ratios and the Environment

Sex is a fundamental trait, with significant consequences for individual fitness, population dynamics, and evolution. Population persistence in sexually-reproducing species requires a mixed-sex ratio; female-biased sex ratios promote population growth, yet strongly female-dominated demography is rare in natural populations (Bull and Charnov 1988; Girondot et al. 2004; Schwanz and Georges 2021). The persistence of near-equilibrium sex ratios, despite their negative effect on population growth, can be explained by the impact of population sex ratio on individual fitness. The theory of sex ratio selection, commonly attributed to Ronald Fisher, states that because individuals of the rare sex contribute more genetic material to the next generation, selection should favour the production of the rarer sex (Fisher 1930; Edwards 1998; Bull and Charnov 1988). Thus, when species encounter conditions that produce biased sex ratios, negative frequency dependent selection acts on processes that govern sex allocation, favouring genotypes that produce the rare sex, consequently returning the population to a stable sex ratio (Bulmer and Bull 1982; Bull and Charnov 1988; Schwanz and Georges 2021).

With this evolutionary perspective, it is easy to assume that the processes that determine sex (male or female) would be highly conserved and fixed. Indeed, as humans, we are familiar with the idea of genotypic sex determination (GSD). Our sex-determination process is initiated by sex chromosomes, and it is easy to assume that the X and Y chromosome have the last word on sexual development. But nothing could be further from the truth. Across kingdom *Animalia*, a huge diversity of sex-determining mechanisms can be observed. From sequentially hermaphroditic species that decide sexual phenotype based on interactions with conspecifics, to species where sexual development is hijacked by intracellular parasites, to species where abiotic conditions determine their path of sexual development (Korpelainen 1990; Beukeboom and Perrin 2014; Capel 2017; Picard et al. 2021). It is the last category with which this thesis is concerned. In species with environmental sex determination (ESD), sex is determined by the abiotic conditions experienced during development (Charnov and Bull 1977). In ESD species, genes activated early in the process of sexual development are sensitive to an environmental factor, such as pH, nutrient availability, or, most commonly, temperature (Box 1, Fig.1) (Beukeboom and Perrin 2014). Exposure to the specific factor influences the developmental pathway of undifferentiated gonads into ovaries or testes, eventually canalising an individual’s sex as female or male (Beukeboom and Perrin 2014). In this thesis, I primarily refer to temperature-dependent sex determination (Box 1), however many of my findings can be generalised to species with other forms of ESD.

The Compelling Biology of Environmental Sex Determination

ESD is a fascinating case study for an examination of the role of plasticity in adaptation and evolution for several reasons, not least because of the importance of the plastic phenotype in question. When sexual phenotype is at the whim of the elements this presents unique challenges for population persistence. Individuals cannot rely on the even division of meiosis to produce a stable sex ratio, ensuring they have at least a chance of securing a mate of the opposite sex. Environmental variation can cause imbalances in sex ratios, and continued, directional environmental change can lead to population collapse (Bulmer and Bull 1982; Janzen 1994; Hawkes et al. 2007; Chu et al. 2008; Mitchell et al. 2008; Tucker et al. 2008; Refsnider and Janzen 2016; Bokony et al. 2019). Yet despite this precarity, ESD species persist. Understanding how is both an intriguing challenge for evolutionary biology, and vital to the conservation of ESD species.

Box 1. Temperature-Dependent Sex Determination

Temperature-dependent sex determination (TSD) is a form of ESD, where the temperature experienced during development determines if an individual will become male or female (Fig. 1). TSD is common among reptiles, fish, and invertebrates, and a related process, temperature-dependent sex-reversal has been observed in amphibians, reptiles, and fish (Valenzuela and Lance 2004; Capel 2017; Picard et al. 2021).

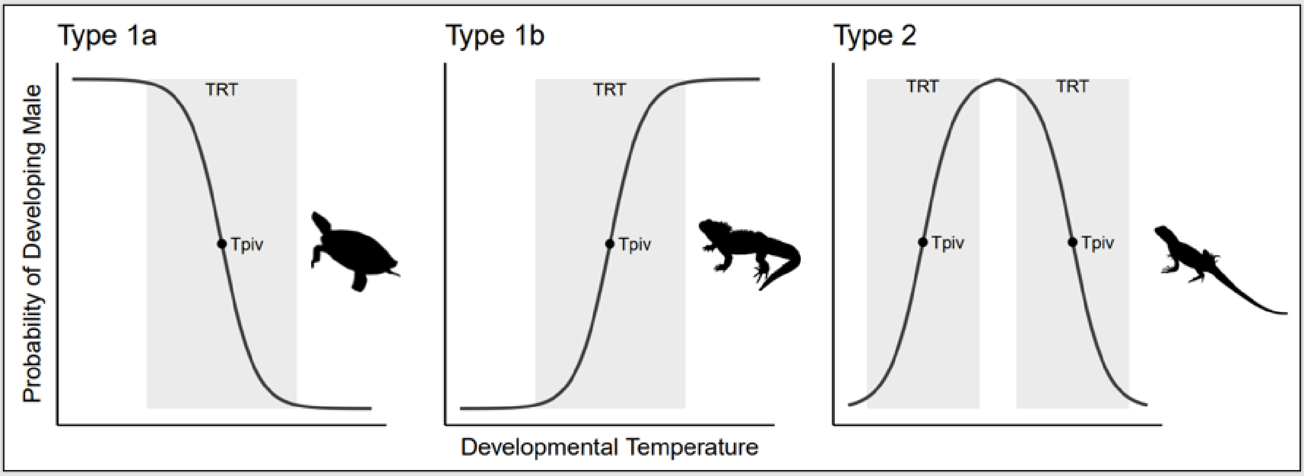

Figure 1. Reaction norms for different forms of TSD (the term ‘reaction norm’ refers to the relationship between phenotype and environment in a plastic trait). In type 1 TSD, there is one switch point between the sexes. In type 1a, males are produced at cold temperatures and females are produced at hot temperatures (e.g., most turtles). In type 1b, the reverse is true (e.g., tuatara and some squamates). In Type 2 TSD, there are two switch points of sexual development, one sex is produced at moderate temperatures and the other sex is produced at extreme temperatures (e.g., most squamates) (Mitchell and Janzen 2010). In TSD the switch point of sexual development is called the pivotal temperature (T_{piv}, the black dots on the graphs) and the slope of the reaction norm is called the transitional range of temperatures (*TRT*, grey boxes on the graphs). Silhouette sources: (O’Meara 2022; Traver 2022; Scroggie 2022).

The second and related reason that ESD is so compelling is that strong frequency dependent-selection on sexual phenotype creates dramatic evolutionary responses to environmental change. An ancient phenomenon, ESD has persisted though times of great climatic upheaval in many taxonomic groups, likely due to the significant capacity for reaction norm adaptation evident in some species (Conover et al. 1992; Janzen and Phillips 2006; Organ and Janes 2008). Conversely, when adaptation of ESD is not possible, frequency-dependent selection can drive changes in sex determining mechanisms (Janzen and Krenz 2004; Capel 2017; Picard et al. 2021). For example, in reptiles, many transitions from TSD to GSD have occurred. In rare occasions such species may revert to TSD, if conditions favourable to ESD emerge (Janzen and Krenz 2004; Gamble et al. 2015). What may be the beginning of this process is currently being observed in the Australian Central Bearded Dragon (*Pogona vitticeps*) (Holleley et al. 2015; Schwanz et al. 2020). Outside of reptiles, ESD has evolved from GSD in several instances, particularly in fish and invertebrates, where switches between sex determining mechanisms, including evolution of polygenic control, are commonplace (Korpelainen 1990; Conover 2004; Capel 2017; Picard et al. 2021). How plasticity influences the varied and occasionally counter-intuitive responses of ESD to selection is an important, unanswered question.

A third reason to explore how plasticity in ESD impacts evolution is that ESD is a threshold trait, a specific form of plasticity where environmental variation causes switches between discrete phenotypes of a trait. Discrete plasticity is common in nature and includes physiological and behavioural phenotypes such as digit number variation, aquatic vs. terrestrial leaf morphology, variant colour morphs, alternate male mating types, eusocial castes, divorce vs. fidelity, and migration vs. non-migration (Wright 1934; Ostrowski et al. 2000; Wells and Pigliucci 2000; Suzuki and Nijhout 2006;

Kamakura 2011; Germain et al. 2018; Debes et al. 2020). Despite their importance, plasticity in discrete traits is less well studied than in continuous traits. Furthermore, the specific expression of plasticity in discrete traits may provide an important opportunity to better understand how plasticity influences evolution. The reaction norm (Box 1, Fig. 1) of a discrete trait has two possible targets of plastic expression: the inflection point, the level of environmental condition at which each phenotype is equally likely; and the slope, or range of environmental conditions where either phenotype could be expressed. The inflection point may shift plastically, changing the level of environmental condition that produces an even chance of developing as either phenotype and a 1:1 ratio of phenotypes within a population. In addition, the slope may become shallower or steeper, changing the sensitivity of the trait to the environment. These forms of plasticity could affect evolution differently, and can both be observed in species with discrete traits, such as ESD, providing an invaluable opportunity to compare their influence on evolution.

A final reason for the value of ESD as a model plastic trait is the critical role that parental effects play in the development of offspring sex. Sexual phenotype in ESD species is decided early in development, as a result, parents can exert control over the developmental environment to influence offspring phenotype (Refsnider and Janzen 2010). In this way, sexual phenotype is influenced not just by the physiological plasticity of embryos to environmental cues, but by the behavioural plasticity of adults attempting to maximise their own fitness. All of these plastic traits are potential targets of selection, as they have the capacity to influence an individuals sex, yet how they interact with sex ratio selection and ultimately influence the evolution of ESD species is not well understood.

Plastic Responses to Sex-Ratio Selection in TSD species

In order to understand how plasticity influences the way ESD species respond to selection, we must identify plastic variation in traits that influence sexual development. Here I focus on examples from species with TSD, however many of these traits have corollaries in other forms of ESD (Korpelainen 1990; Picard et al. 2021). In TSD species, the two forms of reaction norm plasticity (defined in the 'threshold trait' paragraph above) that influence sexual phenotype are the pivotal temperature ($T_{piv},$ inflection point of the reaction norm) and the transitional range of temperatures (*TRT*, slope of the reaction norm) (Fig. 1). Parental behaviour in the form of oviposition site choice also influences offspring sex ratios. A great deal of phenotypic variability can be observed in these traits, some of which may be plastic.

Variation in T_{piv} and *TRT* has been recorded in TSD populations. In some cases, this variation appears to convey adaptive benefits, with differences occurring between populations and species experiencing different climates (Ewert et al. 2005; Refsnider et al. 2014, but see Carter et al. 2019). Variation in embryonic sensitivity to temperature may be a result of plasticity in parental physiology. In oviparous species, maternal allocation of nutrition or exogenous sex hormones to eggs may influence gonadal commitment in embryos (Bowden et al. 2000; Bowden et al. 2001; Elf 2003; Bowden et al. 2004; Warner et al. 2007; Radder et al. 2009). Other aspects of maternal physiology, such as age, appear to covary with offspring sex (Bowden et al. 2004). However, whether these factors can respond to environmental variation in a way that balances sex ratios is unknown.

Variation in oviposition site choice has been observed in TSD species (Doody 2006; Refsnider and Janzen 2010; Delaney et al. 2020). Parental choice of nesting phenology, nest depth, shade cover, substrate type, and moisture content influence the thermal conditions of the nest, and therefore offspring sex (Morjan 2003; Ewert et al. 2005; Doody et al. 2006; Doody 2009; Telemeco et al. 2009; Mitchell and Janzen 2010; Refsnider and Janzen 2012; Somaweera and Shine 2013; Mitchell et al. 2013; Refsnider et al. 2014). In some cases, this variation has an adaptive effect on sex ratios (Morjan 2003; Doody et al. 2006; Mitchell et al. 2013).

Species, populations, and individuals with ESD vary greatly in traits that influence sex determination, and while some of these differences likely result from genotypic change, others are probably due to plastic expression (Morjan 2003). This raises the question, if species respond to novel environments by producing new plastic phenotypes, how will this affect their evolution? Furthermore, if many traits respond simultaneously to a new environment, how will plasticity in one trait affect the evolution of other traits? And how do environmental variables and other selective pressures change the relationship between plasticity and evolution? Even though new plastic phenotypes can appear within the course of a single generation, their effects on evolution may not be apparent for hundreds or thousands of generations. Answering these questions requires an examination of environmental sex determination on an evolutionary timescale.

The Challenges of Studying Evolution

Evolution is a slow process. This presents a challenge to researchers, as we may not possess the resources or lifespan to observe large evolutionary shifts at the pace they naturally occur. We may perform experimental manipulations on populations, if we are lucky enough to have a study system with a short lifespan and large reproductive capacity. However, for many species, lifespan and logistics prohibit experimental evolution. To draw conclusions, evolutionary biologists must employ alternative techniques that allow us to make predictions about past and future evolutionary change from limited data. In this thesis, I use data from a long-term study of a wild species with ESD and computer simulated populations to explore how plasticity in these populations may affect their evolution.

Long term observations of populations have played a key role in evolutionary biology research (Reinke et al. 2019). Perhaps most famously, the effort of Peter and Rosemary Grant to study beak evolution in Galápagos finches over a period of 30 years, has provided invaluable data on species radiations (Grant 1981). The Lenski laboratory's demonstration of adaptive evolution in *E. coli*, and David Reznick's study of life-history evolution in guppies also made significant contributions to the field (Lenski et al. 1991; Reznick et al. 1990). Because long-term population studies have a greater chance to capture the response of a population to environmental variation and observe naturally rare occurrences, they are likely to produce novel findings (Reinke et al. 2019). However, they also require a significant commitment of time and resources, and dedication on the part of investigators. As such it is important to use data from these studies to its full potential. This thesis will draw on reproductive data from a wild population of a freshwater turtle with environmental sex determination, *Chrysemys picta*, collected over a period of 14 years. This data will allow me to examine plasticity in the responses of a species with TSD to environmental change over a long timescale.

An alternative approach to studying evolution in action is to examine it theoretically. Theoretical investigations have revealed important insights into key questions in evolutionary biology, such as: How do interactions between multiple species affect their evolution? How does local adaptation occur? What governs plant investment in growth vs. defence? And what drives the evolution of mating preferences (Kirkpatrick and Ryan 1991; Herms and Mattson 1992; Vermeij 1994; Kawecki and Ebert 2004).

Theoretical approaches are useful because they allow biologists to test the validity of ideas about evolution by reducing the complexity of nature, allowing them to focus on their variables of interest (Kokko 2007). Theoretical models can help decide if an idea is worth testing empirically or generalise ideas from empirical work to make broader conclusions (Kokko 2007; Krakauer et al. 2011). Models generally fall into two categories: analytical models, which are useful because they provide mathematically definite answers to some types of questions; and simulation models, which are useful because they can account for stochastic variables (Kokko 2007). In this thesis, I will use simulation modelling to test predictions about the evolution of ESD. This approach will allow me to include environmental fluctuation and genetic variation in my assessment of the interactions between plasticity and evolution.

Thesis Outline

Phenotypic plasticity is ubiquitous in living organisms and contributes to biological variation that is acted on by natural selection (Sultan 2000; Agrawal 2001; Nijhout 2003). However, we do not fully understand how plasticity influences evolution. If a species possesses traits that significantly determine an individual's fitness and those traits are influenced by the environment, plasticity may have huge consequences for the evolution of that species. This may be the case in species with environmental sex determination. This thesis seeks to explore the role of plasticity in the adaption of ESD species to novel environments. Chapter 2 introduces the idea of plasticity in embryonic sensitivity to temperature and tests how this plasticity influences the evolution of ESD. Chapter 3 tests for the presence of TSD plasticity in a natural population of turtles and investigates maternal physiological factors that could be responsible for plasticity in embryonic responses to temperature. Chapter 4 broadens this investigation to include parental behaviour as a trait co-evolving with physiological factors (T_{piv}) and investigates how plasticity and external selective pressures influence this co-evolutionary relationship.

In Chapter 2, I examine the influence of plasticity on the evolution of discrete traits, using ESD as a model form of plasticity. I develop an individual-based simulation model to examine evolutionary responses to environmental change over a long timescale. Previous examinations of the influence of plasticity on continuous traits have produced variable results, with some investigations showing that plasticity promoted evolution, and others finding that it hindered adaptation. Discrete traits provide a unique opportunity to address this problem, as they contain two different forms of plasticity, the slope and inflection point of the reaction norm. These forms of plasticity have different effects on fitness and testing their influence on evolution will allow us to understand if it is the effect on fitness that determines how plasticity influences evolution.

In Chapter 3, I examine plasticity in sex determination in a wild population of *C. picta*, a freshwater turtle with TSD. The results of Chapter 2 suggest that plasticity in the T_{piv} and slope (or transitional range of temperatures) should influence the evolution of species with temperature-dependent sex determination. I aim to assess *C. picta* for the presence of variable expression of these traits and determine if this plasticity may reduce sex ratio bias. First, I examine a previously identified relationship between climate and plasticity in the TSD reaction norm, to determine if fluctuating nest temperatures influence these findings. I then examine relevant maternal traits to determine if any of these traits co-vary with offspring sex ratios.

In Chapter 4, I broaden my focus on plasticity in ESD to include the influence of parental behaviour on sex determination. I examine the co-evolutionary relationship between two potential responses to sex ratio selection in ESD species: pivotal temperature and nesting behaviour. Using an individual-based model I first assess the adaptive capacity of T_{piv} and Nb in solitary evolution. I then assess how the adaptive capacity of these traits changes when both evolve simultaneously. Finally, I investigate the impact of temperature-dependent survival and behavioural plasticity on the evolution of pivotal temperature and nesting behaviour.

As a whole, this thesis aims to reveal how plasticity in environmental sex determination influences the evolution of this biology and integrate these findings into our current understanding of sex ratio selection on species with ESD. The following Chapters provide detail on how plasticity influences the response of sex-determining traits to selection and demonstrate how the fitness consequences of plasticity drive adaptation. Additionally, this research shows that seemingly unrelated factors such as maternal traits or selection for offspring survival can influence sex ratios, and therefore the evolution of ESD. Overall, these results highlight how frequency-dependent selection underpins every aspect of sex ratio evolution. There exists a multitude of avenues by which species with environmental sex determination may adapt to conditions that affect sex ratios, and the role of plasticity in this adaptive response can be anticipated by its interaction with selection for the rare sex.

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Chapter Two

Plasticity and the adaptive evolution of switchlike reaction norms under environmental change

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Publication Statement/Author Contributions

CC, LES, and SPB conceptualised the project, LES and CC programmed the simulation, CC and LES analysed the data, CC wrote the manuscript with mentoring from LES and SPB. This manuscript was submitted for publication and is under review at *Evolution Letters*.

Abstract

Phenotypic plasticity is often posited as an avenue for adaptation to environmental change, whereby environmental influences on phenotypes could shift trait expression toward new optimal values. Conversely, plastic trait expression may inhibit adaptation to environmental change by reducing selective pressure on ill-adapted traits. Here we examine plasticity in a trait with great ecological and evolutionary significance: sexual phenotype in species with environmental sex determination (ESD). ESD is a discrete trait where development switches between male and female at an environmental threshold (the inflection point). The inflection point is a key trait for adaptive responses to a changing environment and should evolve toward the new optimum in order to maintain evolutionarily stable sex ratios. We used an individual-based theoretical model to investigate how two forms of plasticity in the ESD reaction norm – a shifting inflection point and the slope of the reaction norm – influence the evolution of the inflection point. Three levels of each type of plasticity were initiated in simulated populations exposed to two levels of climate warming. We observed the evolution of the reaction norm over a long timescale to ascertain the potential for plasticity to promote novel phenotypes. We found that steeper reaction norm slopes (higher plasticity) promoted evolution toward new optimal phenotypes (higher inflection points). In contrast increased plasticity (shift) in the inflection point hindered adaptive evolution of the inflection point. Additionally, populations in moderate warming scenarios showed greater adaptive evolution of the inflection point compared with extreme warming scenarios, suggesting that the proximity of existing phenotypes to new optimal phenotypes influences evolutionary outcomes. Our results demonstrate that different forms of phenotypic plasticity have crucially different effects on adaptive evolution. Plasticity that prevented sex ratio bias hindered the evolution of the inflection

point, while plasticity that exacerbated sex ratio bias promoted adaptation to environmental change.

Introduction

Phenotypic plasticity is found in most taxa and can play a critical role in responding to new and variable environments (Sultan 2000; Agrawal 2001; Nijhout 2003). However, it remains unclear whether plasticity facilitates or hinders evolutionary responses to environmental change (Ghalambor et al. 2007; Connover et al. 2009; Hendry 2016). Plasticity that increases an individual's fitness across environments (adaptive plasticity) will promote survival after environmental change and perhaps migration to new environments, thereby facilitating evolution (Agrawal 2001; Lande 2009; Chevin et al. 2010; Scheiner et al. 2017; Kelly 2019). However, selective pressure on plastic traits may be reduced if the plastic phenotype is close to the new optimal trait value, which could hinder evolution toward new optimal phenotypes (Huey et al. 2003; Ghalambor et al. 2007; Snell-Rood 2018). This phenomenon was first observed in response to behavioural plasticity and is known in those cases as the Bogert effect or behavioural inertia, however this effect is not limited to behavioural traits (Ghalambor et al. 2007; Muñoz 2021). Reviews of the topic highlight conflicting conclusions, however it is likely that the impact of plasticity on evolution depends on the particular type of plasticity and its fitness consequences (De Jong 2005; Crispo 2007; Ghalambor et al. 2007; Paenke et al. 2007; Connover et al. 2009; Diamond and Martin 2016; Hendry 2016; Snell-Rood et al. 2018). The impact of plasticity on evolution has largely been explored in continuous traits. In contrast, we know almost nothing about how plasticity in discrete traits impacts evolutionary responses to environmental change.

Plastic traits that are discrete or discontinuous in distribution are common in nature, including sex (male/female), alternate male morphologies, colour morphs, variant leaf morphology, number of digits on a limb, eusocial castes, migration vs. residency, or divorce vs. mate fidelity (Wright 1934; Charnov and Bull 1977; Moczek and Emlen

1999; Ostrowski et al. 2000; Wells and Pigliucci 2000; Suzuki and Nijhout 2006; Kamakura 2011; Chevin and Lande 2013; Germain et al. 2018; Debes et al. 2020). These traits and their plasticity often have a significant influence on individual fitness and population survival (Charnov and Bull 1977; Germain et al. 2018; Debes et al. 2020). Plasticity in a discrete trait is typically described by a switchlike reaction norm, with two extreme trait states, infrequent expression of intermediate traits, and an inflection point (threshold environmental value) where trait expression switches from one extreme to the other. Similar to plasticity in continuous traits, discrete plasticity may be shaped by selection to maximize fitness in a variable environment (Schwanz and Proulx 2008; Schwanz et al. 2010; Chevin and Lande 2013). Furthermore, empirical and theoretical investigations have established that the slope and the inflection point of switchlike reaction norms are likely targets of evolutionary responses to novel environmental change (Hulin et al. 2009; Mitchell and Janzen 2010; Chevin and Lande 2013). Therefore, plasticity in the reaction norms of discrete traits could have a significant impact on their evolution.

Discrete traits provide an excellent opportunity to explore how plasticity influences evolution because they inherently contain two major forms of plasticity to compare within the same selective environment (Fig. 1). First, the slope of the reaction norm determines how sensitive a trait is to its environment (i.e., the range of phenotypes that can be produced in a particular environment) (Fig. 1a, b). A trait with a steep slope is more responsive to the environment and therefore more plastic, while the reverse is true of traits with shallow slopes. Second, the inflection point itself can be plastic by shifting towards optimal values as the environmental mean changes (Fig. 1c, d). That is, the whole reaction norm shifts sideways along the environmental axis if the current mean environment across individuals is likely to deviate from the long-term environmental mean. Shift plasticity allows the production of a rare phenotype under unusual environmental conditions, and thus is particularly useful for traits under negative frequency-dependent selection. In actuality, shift plasticity is similar to plasticity in a continuous trait, since the inflection point is continuously distributed (i.e. a reaction norm of inflection point versus environment, Fig. 1d), however the phenotypic outcome is discrete. A trait with an inflection point that can shift to match the new environment is highly plastic, whereas a trait with an inflection point that is constant regardless of the environment is not plastic. These forms of plasticity are likely to have different effects on the evolution of the inflection point, as high shift plasticity moves phenotypes closer to their optimum value, while high slope plasticity may exacerbate maladaptive phenotypes. Despite the importance of switchlike reaction norms in nature, and their potential as a model system for investigating the evolutionary impact of different forms of plasticity, there has been limited research investigating how plasticity in discrete traits affects trait evolution in response to environmental change (Suzuki and Nijhout 2006; Chevin and Lande 2013).

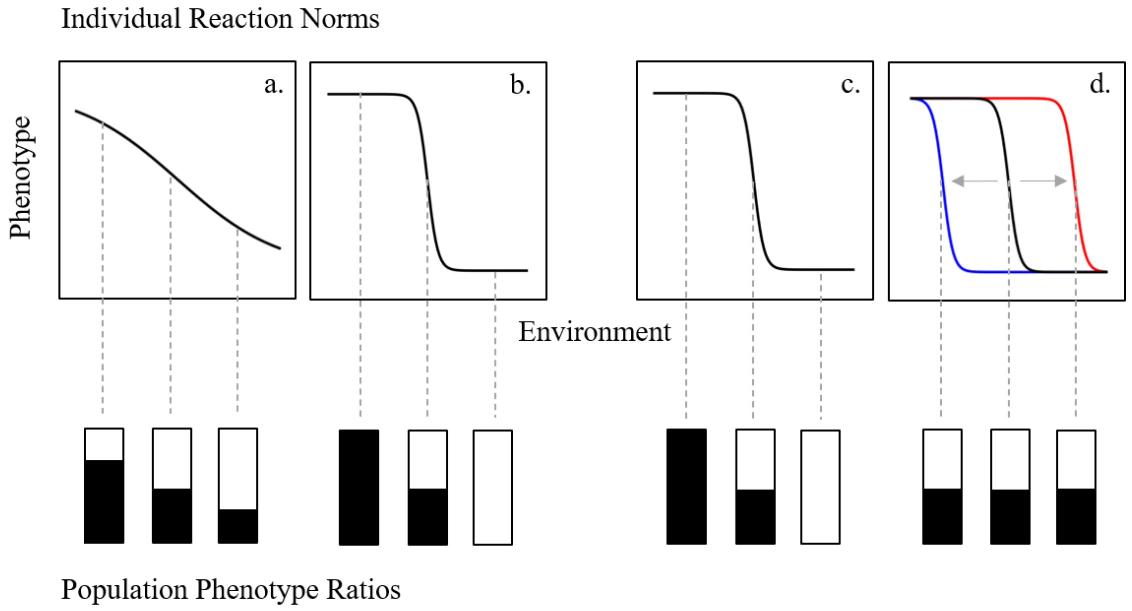

Figure 1. Two forms of plasticity are possible in the reaction norm of a discrete trait, plasticity in the slope (a & b), and plasticity in the inflection point between discrete phenotypes, termed ‘shift’ plasticity in our model (c & d). On an individual level, variation in the reaction norm of a discrete trait may be challenging to detect, as this variation does not always result in a switch in phenotype. However, variation can be observed in population level phenotype ratios. (a) a shallow slope is less plastic and produces a mixed ratio of phenotypes under most environmental conditions. (b) a steep slope is highly plastic. Extreme environments produce individuals of the same phenotype, and moderate environments produce mixed phenotypes. (c) In a reaction norm with low shift plasticity, the inflection point does not respond to environmental conditions, phenotypic ratios depend on the slope of the reaction norm. (d) In a reaction norm with high shift plasticity, the inflection point is responsive to environmental conditions. The blue and red curves represent reaction norm shift in extreme conditions. High shift plasticity produces a mixed phenotypic ratio across most environments.

We aim to investigate how plasticity in a fundamental, discrete trait – the sex expressed by an individual – impacts evolutionary responses to changing environments. Where sexual development depends on the environment (environmental sex determination; ESD), directional change in the average environment often leads to biased sex ratios and strong frequency-dependent selection to equilibrate the sex ratio (Charnov and Bull 1977; Bull 1981; Schwanz and Georges 2021). The response to this selection may include evolution of the inflection point of sexual phenotype to match the new environmental mean, and likely depends on the slope and shifts in the inflection point of ESD reaction norms. Here, we focus on ESD based on developmental temperatures (temperature-dependent sex determination; TSD), as understanding how plasticity will affect the evolution of thermally-sensitive traits is particularly important under current climatic warming. However, our study is applicable to any form of ESD or environmentally-determined threshold trait. Using an individual-based simulation model, we ask how the two kinds of plasticity within switchlike reaction norms affect the evolution of the inflection point (here, the pivotal temperature). We hypothesize that frequency dependant selection for the rare sex directs evolution of the pivotal temperature, such that plasticity that leads to biased sex ratios will promote evolution of the pivotal temperature. This hypothesis aligns with theoretical and empirical evidence that evolution in plastic traits is driven by the specific fitness outcome of plasticity (Ghalambor et al. 2007; Connover et al. 2009; Hendry 2016). Specifically, we predict that high slope plasticity will exacerbate sex ratio biases and will therefore promote adaptive evolution of the pivotal temperature. In contrast, we expect high plasticity in the pivotal temperature (shift) will ameliorate sex ratio biases and will therefore impede adaptive evolution of the pivotal temperature.

Methods

Overview of Plasticity Scenarios

We designed an individual-based simulation model to examine how the pivotal temperature (T_{piv}) of sexual development evolves in response to climatic warming in the presence of two different kinds of plasticity in the TSD reaction norm. First, we investigated the effect of plasticity in the slope of the switchlike reaction norm (‘slope’) (Fig. 2a). Second, we investigated the effect of pivotal temperature plasticity in response to annual climatic fluctuations (‘shift’) (Fig. 2b).

In order to examine how plasticity impacts the evolutionary response of T_{piv} under a directionally changing climate, we varied the level of plasticity in slope or shift across plasticity scenarios (Fig. 2). High plasticity in the reaction norm slope arises from highly negative values of this parameter (see equation 1 below), wherein a very steep and negative slope means that sexual phenotype is highly dependent on the environment. As a result, there are fewer temperatures that could produce either a male or a female hatchling. Low plasticity in the slope arises from slightly negative values, meaning that the relationship between sex and environment is shallower and there is a wider range of temperatures that have a chance to produce a male or female hatchling. Thus, high plasticity in the reaction norm slope *increases* sex ratio biases in an atypical or changing climate (Fig. 1a, b, Fig. 2a). Shift plasticity modifies an individuals' genetic T_{piv} in response to annual climate fluctuations (see equation 1). High plasticity in shift arises from high values in this parameter, meaning the phenotypic T_{piv} tracks more closely to annual mean nest temperatures (i.e. warmer T_{piv} in warmer years). Low plasticity arises from low values of shift, where the same T_{piv} is expressed regardless of environmental temperature. Thus, high plasticity in shift *reduces* sex ratio biases in an atypical or changing climate (Fig. 1c, d, Fig. 2b). The potential values for these variables are presented in Table 1. In 'slope scenarios', both the T_{piv} and slope traits could evolve via mutation, while starting shift was at zero (highest potential for sex ratio bias), with no mutation (Fig. 3a). In 'shift scenarios', both T_{piv} and shift could evolve via mutation, while the initial slope was set at the steepest level (highest potential for sex ratio bias), with no mutation during the simulation (Fig. 3b). The plasticity scenario (shift/slope) and the level of plasticity (low, medium, high) were chosen randomly at the start of each replicate simulation, with approx. 25 replicates of each scenario (sample sizes are presented in Table S1, Appendix A).We explored the impact of the two types of plasticity on T_{piv} evolution across three levels of mean climate and two levels of climate variability. Including a range of mean climates and climatic variability is important because we expected that the relationship between plasticity and evolution would depend on the selection pressure invoked by climatic differences and the consistency of that pressure across years. Our simulation model contained three global temperature scenarios (T_{glob}): a base climate scenario of 28°C, a moderate warming scenario of 30°C, and an extreme warming scenario of 32°C. There were also two climate variability scenarios, determined by the standard deviation of climate between years (SDbw): high variability (1.5°C) and low variability (0.75°C), selected based on natural nest temperatures in wild reptile species (Schwanz et al. 2020). Global temperature and climatic variability were chosen randomly from these options at the start of each simulation replicate.

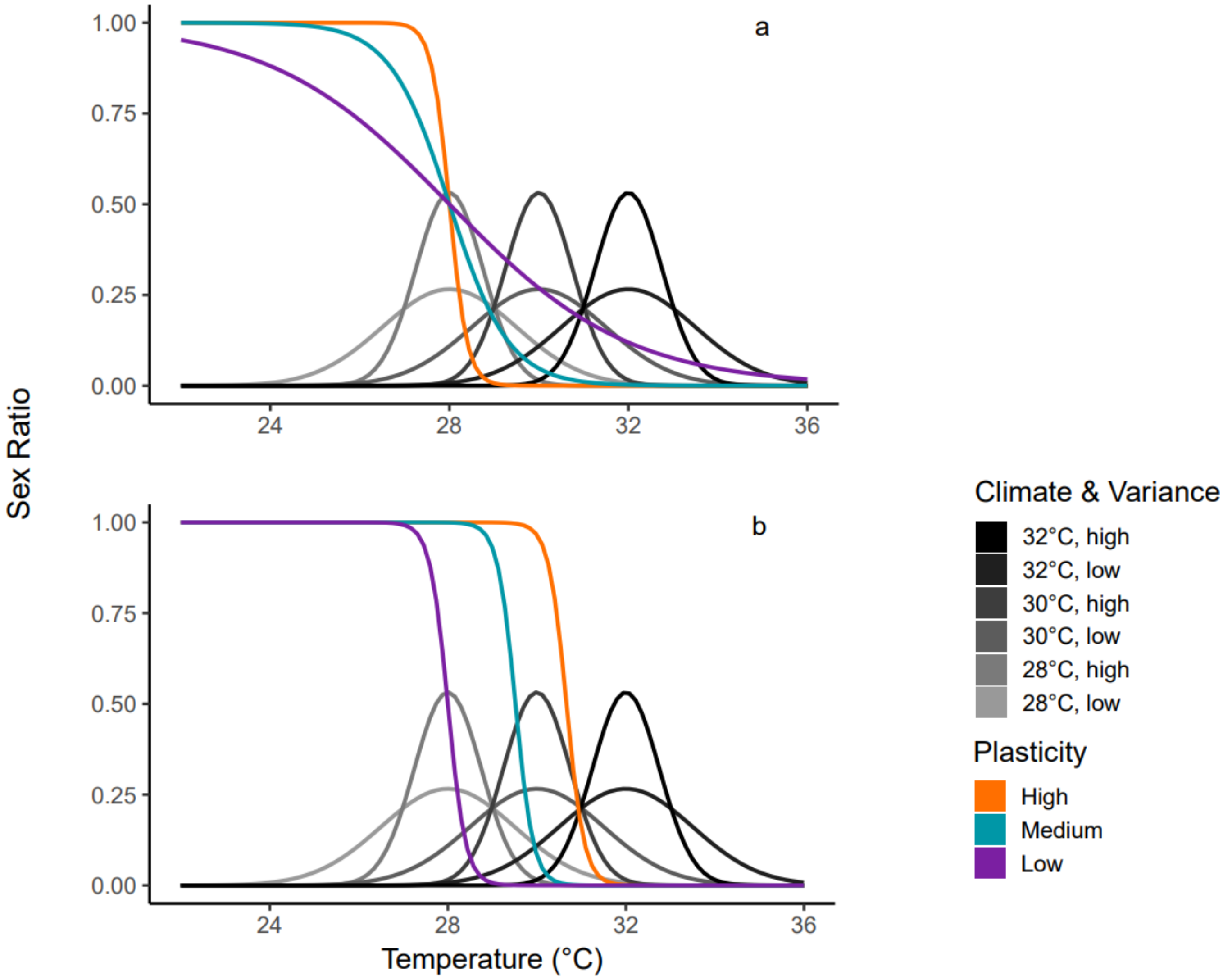

Figure 2. Shapes of simulated reaction norms at different levels of slope and shift plasticity. (a) low, moderate, and high values of reaction norm slope plasticity. (b) Reaction norms produced by low, moderate, and high shift plasticity, with a T_{piv} of 28°C in a 32°C climate. Values for plasticity parameters are presented in Table 1. In both panels, the grey curves represent distributions of nest temperatures at 28°C, 30°C, and 32°C with different levels of variability (high = 1.5°C, low = 0.75°C).

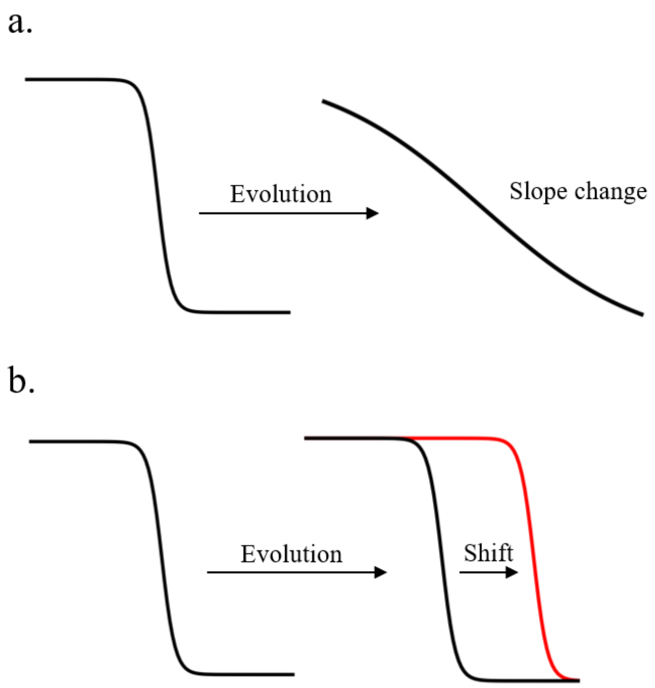

Figure 3. The evolution of traits in slope and shift scenarios. (a) In slope scenarios the slope of the reaction norm could evolve to become more or less steep but shift plasticity could not evolve. (b) In shift scenarios shift plasticity could evolve, i.e. T_{piv} could evolve to be more or less responsive to annual climate, but the slope of the reaction norm could not change. The base value T_{piv} could evolve in all scenarios.

Temperature-dependent sex determination

The simulation used a diploid genetic system, which prevents the rapid spread of extreme phenotypes and reflects the biology of species with TSD. Individuals in the population had three genes (T_{piv}, slope, and shift), with two alleles at each locus. Phenotypes were expressed as the mean of both alleles. We modelled TSD as a logistic curve (Fig. 2) with males developing at low temperatures and females developing at high temperatures, as in type 1a TSD (Valenzuela 2004). We used a modified version of Schwanz and Proulx's (2008) equation for sex determination that includes shift:

$$r = \frac{1}{1+e^{-\left(td-\left(T_{piv}+shift(T_{ann}-T_{base})\right)\right)slope}} \qquad (1)$$

Where *r* is the probability of being male, *td* is the developmental temperature, T_{piv} is the pivotal temperature, *slope* is the slope of the logistic curve (slope plasticity, Fig. 2a.), *shift* is the pivotal temperature shift (shift plasticity, Fig. 2b), T_{ann} is the annual climate, and T_{base} is the baseline climate (28°C).Individual sex was determined stochastically by comparing *r* to a random number chosen from a uniform distribution between 0 and 1. Individuals with higher values of *r* were assigned male and lower values of *r* were assigned female.

Population cycle

At the start of each replicate simulation, populations were seeded with 500 individuals that varied in developmental temperature and genetics. For these individuals, we assumed the mean developmental temperature was 28°C regardless of which climate scenario was chosen. This was done to prevent the immediate formation of a population with no males in warm climate scenarios, and accurately represents an established population that subsequently experiences climatic warming. Each individual was assigned alleles at the three TSD genes based on the chosen scenario. Alleles were drawn from a normal distribution with a mean of the chosen scenario parameter value (slope and shift) and a standard deviation of approximately 1% of the potential evolutionary range (Table 1). We assumed populations were initially adapted to the base climate scenario and assigned mean population T_{piv} equal to 28°C, with a standard deviation that placed extreme genotypic values near the moderate warming scenario (30°C).

Each year (iteration) the annual mean temperature (T_{ann}) was drawn from a normal distribution with T_{glob} as the mean and SDbw as the standard deviation. When females reproduced, each nest temperature was randomly chosen from a normal distribution with a mean equal to the annual temperature and a standard deviation of SD_{wi} (1.2°C), which reflects average among-nest variation in wild reptile nests (Schwanz et al. 2020). These nest temperatures became the developmental temperature (*td*) of the offspring from that nest. It is important to note that our simulation does not model plasticity in maternal nesting behaviour.

Mating occurred in each iteration. Male mates were chosen randomly with replacement for each adult female. Clutch size (CS) was exponentially related to maternal developmental temperature in order to establish sex-differential fitness as a function of temperature and, therefore, selection for TSD. Because climatic warming and frequency-dependent selection on sex can lead to selection for the loss of TSD, we wanted to promote the maintenance of TSD via selection. Clutch size was determined by the following equation:

$$\mathrm{CS} = (F_{scale}(td)^{Phi})(\frac{OSR}{OSR+M_{lim}}) \qquad (2)$$

The fecundity scaler (F_{scale}) was multiplied by the female incubation temperature (*td*) to the power of *Phi*, so that the clutch size ranged from 10-25 over temperatures 22°C - 35°C. In our simulation, female fertility was also limited by the number of males present in the population. We used the male limitation equation from Rankin and Kokko (2007). The *OSR* is the operational sex ratio and M_{lim} represents the impact that male population proportion has on clutch size. We chose $M_{lim} = 0.01$ to ensure males only limited female fecundity when the proportion of males was below 10%. This value prevents the unrealistic continuation of a population with very low males, and the rapid evolution of a population by the spread of genes from a few unusual males.

New offspring received one allele from each parent for their T_{piv}, slope, and shift, chosen randomly from each parent's allelic complement. Offspring had a 2% chance to become a mutant. Mutants experienced mutations on all alleles at each locus that was assigned to mutate based on the scenario. Mutant allelic values were chosen randomly from a normal distribution with a mean equal to the original allelic value and a standard deviation of 2% of the biologically realistic range for the trait (Table 1).

Adult mortality occurred at a fixed rate (10%) after breeding. Juvenile mortality was density-dependent, given by the following equation:

$$Mort = 1 - e^{(-const(n_{Adults}))} \qquad (3)$$

Where *Mort* is the probability if mortality, *const* is a density-dependence constant with the value 0.01, and n_{Adults} is the number of adults alive in the iteration. Surviving juveniles were recruited to the adult population at the end of each iteration. That is, animals matured at 1 year of age.

Model Validation and Sensitivity Analysis

Across our scenarios, we hypothesized that we would have simultaneous selection for higher T_{piv} (driven by warmer climates), higher slope plasticity (driven by sex-differential fertility/fecundity), and higher shift plasticity (driven by annual fluctuations in climate and sex ratios; e.g. Schwanz and Proulx 2008; Schwanz et al. 2010). Because our scenarios examined concomitant evolution of T_{piv} and plasticity (shift/slope), we briefly examined the evolution of each trait independently (without mutation in the other traits) to validate our assumptions (see Appendix A). We confirmed that the pivotal temperature evolved to near-perfectly match the warmed climatic mean (30°C and 32°C; slope fixed at ‘high’, shift fixed at ‘low’). We validated that a sex-differential link between fitness and temperature drives TSD towards appreciably negative slopes under the base climate (shift fixed at ‘low’). Finally, we found that there was a threshold starting shift at ~0.4 that facilitated the evolution of very high shift values (>0.8).

Additionally, we performed analyses to assess sensitivity of the simulation results to mutation range and adult mortality rate (see Appendix A).

The simulation was conducted in MATLAB 2021a (MATLAB 2021) with the Statistics and Machine Learning Toolbox add-on. Graphs were constructed in RStudio using the Tidyverse and Patchwork packages (Wickham et al. 2019; Pederson 2020; R Core Team 2021).

Table 1: Description of simulation parameters. Parameters that varied across simulation scenarios are bolded.

Parameter	Description	Value(s)
Climate		
$\mathbf{T_{glob}}$	**The average global temperature of a scenario.**	**28°C, 30°C, 32°C**
$\mathbf{SD_{bw}}$	**The between-year standard deviation in annual mean temperatures.**	**0.75°C, 1.5°C**
T_{base}	The climate that individuals are initially adapted to (mean $T_{piv} = T_{glob}$).	28°C
T_{ann}	The annual temperature, chosen from a normal distribution with T_{glob} as the mean. and SD_{bw} as the standard deviation.	Generated randomly each year in the simulation
SD_{wi}	The within-year standard deviation in developmental temperatures.	1.2
td	Developmental temperature of a simulated nest, chosen from a normal distribution with T_{ann} as the mean and SD_{wi} as the standard deviation.	Generated randomly each year in the simulation
Genetics		
Slope	**The slope of the switchlike reaction norm.**	**-0.5, -1.5, -5**
Slope SD	Standard deviation of initial population variation in slope.	0.05
Shift	**The ability of an individual to express a phenotypic $\mathbf{T_{piv}}$ other than its genetic $\mathbf{T_{piv}}$, influenced by the deviation of $\mathbf{T_{ann}}$ from $\mathbf{T_{base}}$.**	**0, 0.38, 0.6666**
T_{piv}-shift SD	Standard deviation of initial population variation in shift.	0.01
T_{piv}	The initial temperature with an equal chance of producing a male or female hatchling.	28°C
T_{piv} SD	The standard deviation of initial population variation in T_{piv}.	0.5

Mutation rate	The percentage of hatchlings that receive mutations.	2%
Mutation range (Mutvar_array)	The standard deviation of mutation possible for T_{piv}, shift and slope.	0.08, 0.1, 0.02
Demographics		
Ni	The initial population size of each replicate simulation.	500
T	The maximum number of iterations (years) in each simulation.	50000
Mortality (Ad_mort)	The proportion of adults that leave the population each year.	0.1
Phi	A constant in the female fertility equation.	2
F_{scale}	Scale for female fecundity.	0.02
const	Density dependant constant for 1st-year survival.	0.01
M_{lim}	Expression of male limitation on female fertility.	0.01.

Results

Persistence

The majority of replicates survived for the full duration of the simulation (Fig. 4). Only 4.6% of populations became extinct before reaching 50000 iterations. The majority of replicates resulting in extinction came from 32°C low climate variability scenarios with starting slope = -5 and shift = 0. These are the parameter combinations that cause the greatest sex ratio biases. Higher shift appears to have a buffering effect on sex ratios in these scenarios, as runs where shift started at moderate or high values did not go extinct and runs in which shift started at 0 but was able to evolve had a slightly higher chance of survival compared to when it could not evolve (Fig. 4).

In some populations where the slope was allowed to evolve, the slope became so shallow as to constitute a loss of TSD (defined as slope >= -0.1) (Fig. 4). This occurred primarily in 32°C scenarios with moderate or shallow slopes.

Changes in Pivotal Temperature Over Time

When plasticity in the slope of the TSD reaction norm was allowed to evolve there was an interaction between climate and plasticity that affected the speed of pivotal temperature evolution. In 30°C scenarios, high and moderate plasticity runs (steeper slopes) reached a mean T_{piv} of 30°C (Fig. 5b, orange and blue) much quicker than the low plasticity (purple). In contrast, in 32°C scenarios the few high plasticity runs that survived reached a mean pivotal temperature of 32°C after substantial delay (Fig. 5d, orange), while moderate and low slope plasticity experienced little evolution of the pivotal temperature at all (Fig. 5d, blue and purple).

Shift plasticity had a less pronounced effect on the speed of T_{piv} evolution, with the results being somewhat reversed from slope plasticity (Fig. 5f, h). Across both climate scenarios, it was the low plasticity scenarios (purple) where T_{piv} continued to increase for a longer period of time than in the moderate or high plasticity scenarios (blue and orange), with the latter scenarios plateauing at around the same time but at lower values of T_{piv} (Fig. 5f, h). T_{piv} evolution followed nearly-identical patterns under high climate variability (Appendix A, Fig. S6).

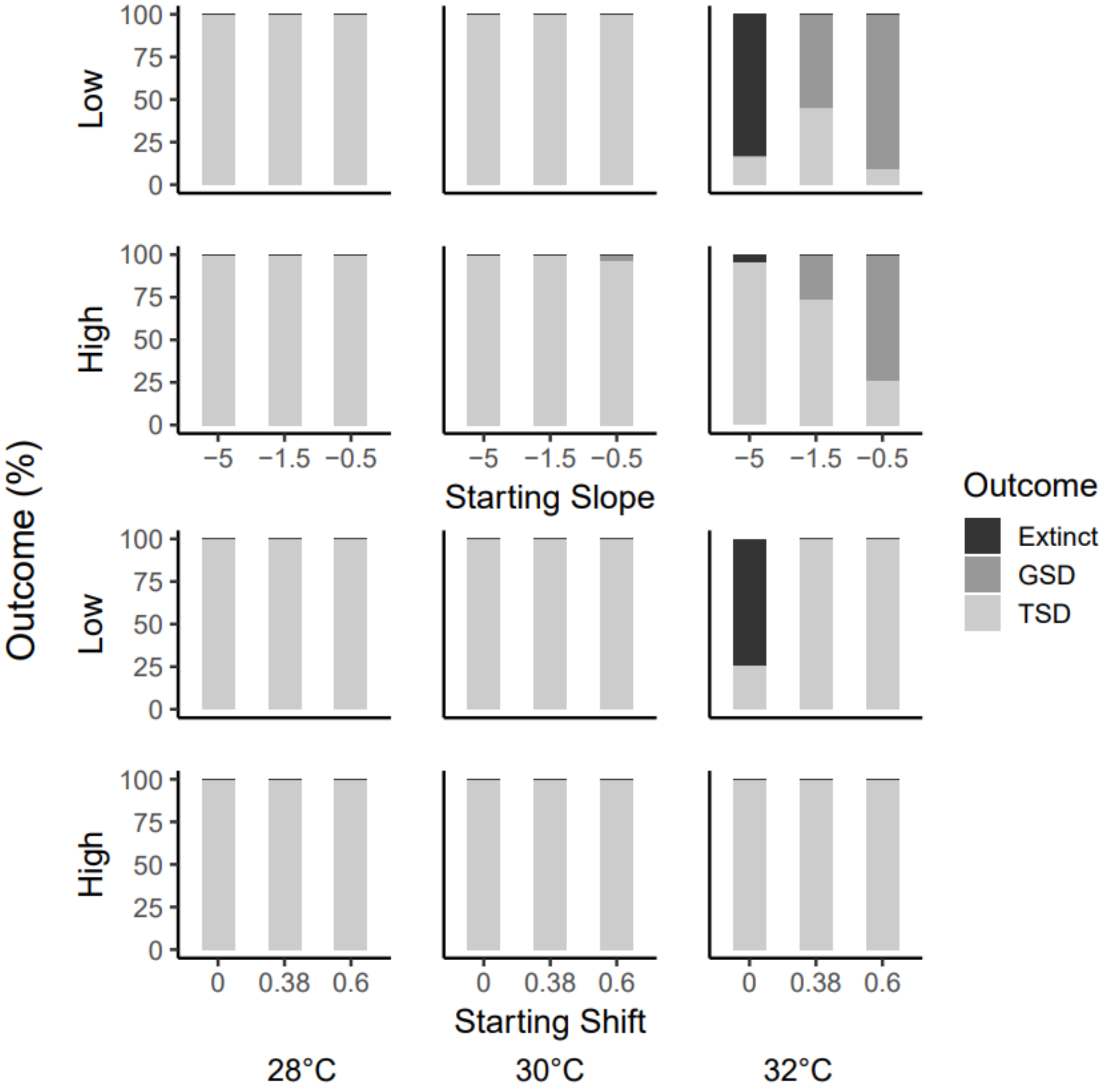

Figure 4. Outcome for replicates in all scenarios, including Extinct, GSD and TSD. Climate variability is on the y-axis (high = 1.5°C, low = 0.75°C). Extinct populations became 100% female before 50000 generations (the length of the simulation). A reaction norm slope more positive than -0.1 was classified as a switch to genetic sex determination (GSD). Sample sizes for each scenario are presented in Table S1.

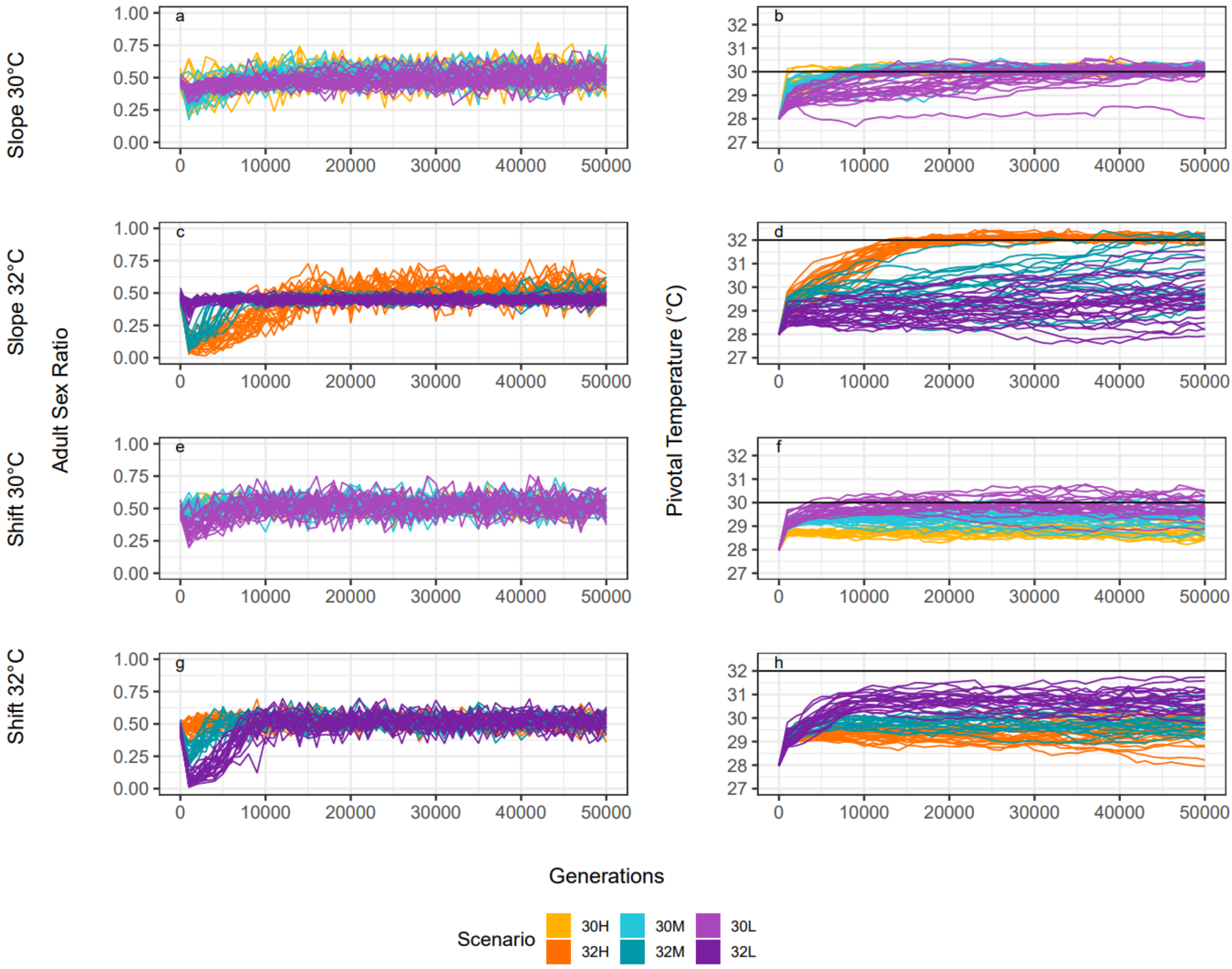

Figure 5. The adult sex ratios and mean pivotal temperatures (T_{piv}) for populations in replicate simulations over time, at different levels of climate and plasticity. Only results from warmed climate scenarios are presented as there was little change in T_{piv} or adult sex ratio in baseline 28°C climate populations. A ratio of 1 is 100% male, a ratio of 0 is 100% female. Levels of plasticity in the scenario key are: H= High, M= Moderate, L= Low. Results shown are from low climate variability treatments.

Changes in Sex Ratios Over Time

The impacts of climate and plasticity on adult sex ratios mirrored the patterns observed for T_{piv} evolution. In all 30°C slope plasticity scenarios, sex ratios were initially female-biased, but quickly increased to parity (Fig. 5a). In contrast, in the 32°C scenarios, moderate and high plasticity led to strongly female-skewed sex ratios initially, with a substantial delay to reach parity, particularly in the high plasticity runs where a 50:50 sex ratio appears coincident with T_{piv} reaching 32°C (Fig. 5c).

A similar pattern occurred when shift plasticity was allowed to evolve, reversed with respect to plasticity level (Fig. 5e, g). In 30°C scenarios sex ratios quickly reached a stable trajectory between 40-60% male. In the 32°C scenarios, low plasticity populations were strongly female skewed for a greater period of time than moderate or high plasticity scenarios. Across all scenarios, higher climate variability increased fluctuations in sex ratios on a per-cohort basis, though the directional patterns through time remained similar (Appendix A, Fig. S6e, g).

Impact of Slope Plasticity on Final T_{piv}

Under baseline (28°C) and mild warming (30°C) scenarios, the three levels of slope all produced final mean T_{piv} roughly equalling the global climate (Fig. 6a, b). In these scenarios, the slope generally stayed steep (for high, orange) or became steeper (for moderate, blue, and low, purple). The exception is for some 30°C, low plasticity replicates, where the slope became shallower and the T_{piv} did not reach 30°C. In contrast, in the 32°C scenarios high slope plasticity promoted T_{piv} evolution (Fig. 6a, b). Replicates with high plasticity (steep slopes) evolved pivotal temperatures closer to 32°C (if they did not go extinct) compared with the replicates with shallower starting slopes where the slope simply evolved to be very shallow (Fig 6b, c, orange vs. purple and blue). This pattern was also the same in the high variability climates, where most of the high slope replicates persisted. With high climate variability scenarios, however, a greater proportion of the moderate slope replicates retained ESD-like slopes and had final pivotal temperatures near 32°C (Appendix A, Fig. S7).

Impact of Shift Plasticity on Final T_{piv}

In contrast to the positive relationship between slope plasticity and T_{piv} evolution, we found a negative relationship between the level of shift plasticity and evolution of the pivotal temperature. Across both warmed climate scenarios (30°C or 32°C), increased shift plasticity reduced the final extent of T_{piv} evolution (Fig. 6d, e).

Additionally, global climate had an effect on T_{piv} evolution. As is to be expected, replicates evolved warmer pivotal temperatures in warmer global climates. In the 30°C and 32°C scenarios, lower plasticity runs showed greater increases in pivotal temperature during the course of the simulation (Fig. 6d). Climate variability appears to have a negligible impact on T_{piv} evolution when interacting with shift plasticity.

Sensitivity to Mutation and Adult Mortality

A higher mutation rate was associated with increased T_{piv} evolution in low plasticity scenarios for both slope and shift plasticity (see Appendix A, Fig. S4). Increased mortality rate (shorter lifespans) increased T_{piv} evolution for the low plasticity (shallow) slope scenario, with T_{piv} reaching the climatic mean and ESD being maintained. In contrast, increased mortality decreased T_{piv} evolution for low plasticity shift scenario, as high shift values evolved, precluding the need for T_{piv} evolution.

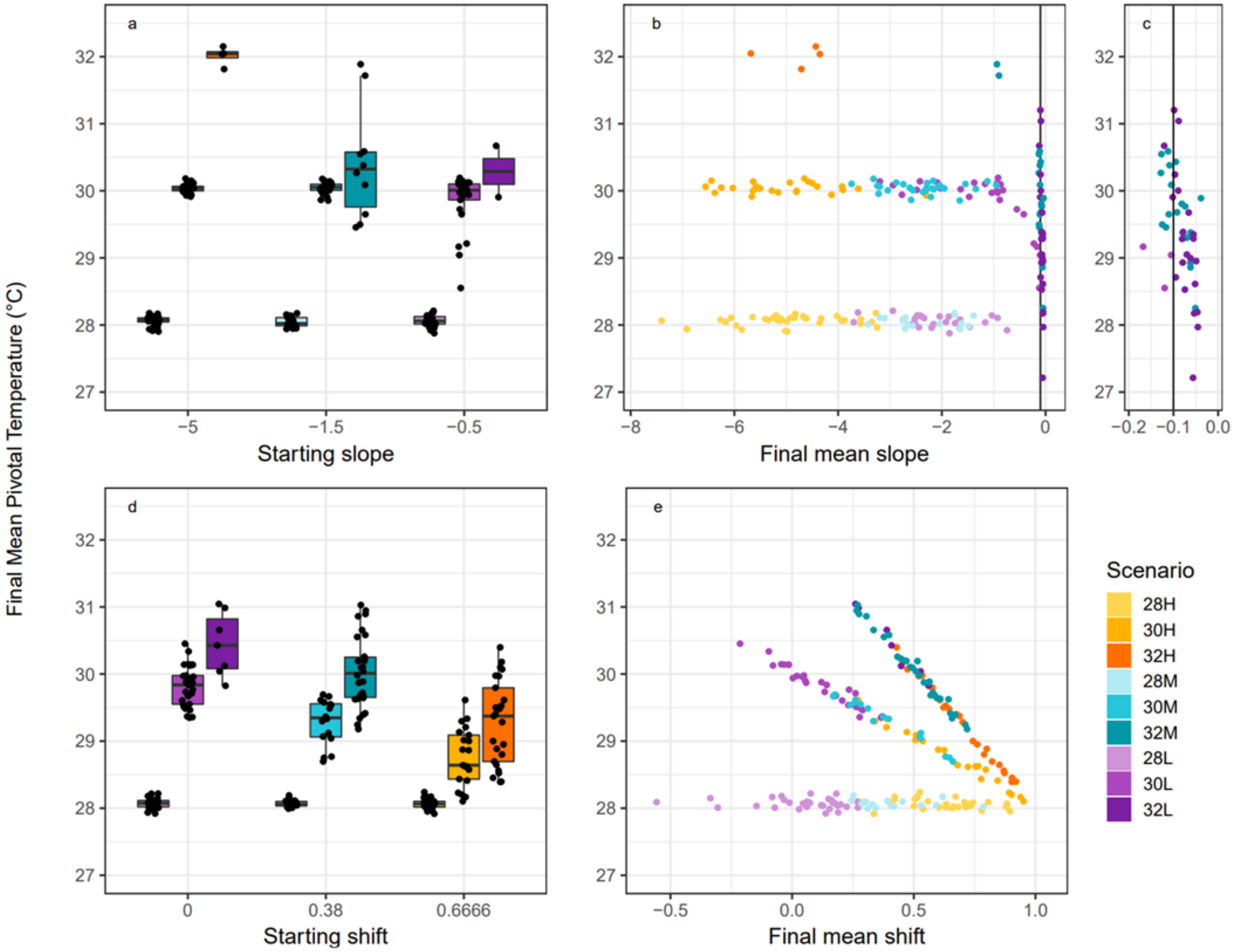

Figure 6. The final mean pivotal temperature of replicate simulations over starting and final mean plasticity levels (mean of the last 1000 generations). Levels of plasticity in the scenario key are: H= High, M= Moderate, L= Low. Results shown are from low climate variability treatments. (a) Final mean pivotal temperatures for the three levels of starting slope plasticity in all climate scenarios. Only replicates that maintained ESD are included in the boxplots. (b) Final mean pivotal temperatures and final mean slopes of replicates in slope plasticity scenarios, the black line indicates the boundary between ESD and a GSD-like slope. (c) An expansion of (b), centered around the black line. It shows the scenarios that lost ESD (slope > -0.1) on the right side of the black line. (d) Final mean pivotal temperatures for the three levels of starting shift plasticity in all climate scenarios. (e) Final mean pivotal temperatures and final mean shifts of replicates in shift plasticity scenarios.

Discussion

We aimed to determine how the evolution of pivotal temperatures (T_{piv}) in species with ESD is affected by two forms of plasticity in the ESD reaction norm: the slope and a shift in the pivotal temperature. We found that the level of plasticity in the slope and shift have opposite effects on the evolution of T_{piv}, but that these opposing effects are similarly related to the fitness consequences associated with frequency-dependent selection on sex. Steep slopes, which indicate a highly plastic relationship between sex (male/female) and the environment, promoted the evolution of T_{piv} toward the new mean temperature of the environment (Fig. 6a, b). In contrast, high plasticity in the pivotal temperature itself (shift) hindered T_{piv} evolution (Fig. 6d, e). The simultaneous evolution of plasticity and T_{piv} often exacerbated the impact of initial slope and shift values on the evolution of T_{piv}, as the evolution of shallow slopes and high shifts made T_{piv} evolution redundant. Additionally, it was easier for T_{piv} to reach the new mean temperature in the moderately warmed environment, as starting populations often already contained some alleles that were suited to this environment. Adaptation to the extreme warming scenario was a much more difficult prospect under both forms of plasticity. Our investigation demonstrates that adaptive plasticity in discrete traits can hinder evolution in novel environments, as has been observed in continuous traits, and that when selection on a discrete trait is frequency dependant, population trait proportions will drive interactions between plasticity and evolution (Ghalambor et al. 2007).

The evolution of the pivotal temperature proceeded in vastly different ways depending on the form of phenotypic plasticity examined. These differing effects appear to be determined by how the plasticity affects sex ratios and the resulting strength of frequency-dependent selection. Populations with high plasticity in the slope of the ESD reaction norm produce sex ratios that are more biased under warm climates than those produced by populations with shallow slopes (Fig. 2a), as they have a stronger relationship between sex and temperature. Analogously, fixed reaction norms that do not shift in response to climate (low shift plasticity) produce more biased sex ratios compared to reaction norms with high shift plasticity (Fig. 2b). Because there is strong negative frequency-dependant selection operating on sex ratios, alleles for producing the rare sex at the new mean temperature of the environment are highly favoured in these biased populations (Charnov and Bull 1977, Bull 1981; Schwanz and Georges 2021; Chevin et al. 2021). Thus, adaptive evolution of the pivotal temperature is promoted in populations with high slope and low shift plasticity.

When sex ratio biases were ameliorated by high shift plasticity, selection on T_{piv} was reduced. It has been argued that adaptive plasticity that shifts phenotypes close to the new optimal phenotype, while maintaining selective pressure for evolution toward the optimal phenotype, can promote adaptive evolution (Ghalambor 2007). Our shift plasticity results suggest that in the case of switchlike reaction norms under frequency-dependent selection, even moderate adaptive plasticity in the inflection point of a reaction norm reduces selection on the pivotal temperature and can hinder long term evolution toward the new optimum phenotype. Our finding is supported by significant evidence that adaptive plasticity prevents selection on genotypes (Sultan 1987; Sultan 1995; Sultan 2000; Huey and Kingsolver 1993; Huey et al. 2003; Ghalambor 2007).

While steep slope and low shift promoted the evolution of T_{piv}, these scenarios also experienced negative consequences under climate warming. In particular, populations in these scenarios experienced a high rate of extinction (especially when individual lifespan was shortened, see Appendix A). The observed high T_{piv} evolution was partly a result of population-level selective survival. Across wild reptile populations with ESD, steeper reaction norms are associated with more single-sex nests than shallow reaction norms and have previously been predicted to make populations vulnerable to extinction under extreme environmental change (Hulin et al. 2009; Mitchell and Janzen 2010). Thus, populations with steep slopes and no shifting of the pivotal temperature must have substantial evolvability of T_{piv} to avoid rapid extinction. Indeed, higher mutation rates of all traits reduced extinctions (see Appendix A). It is important to note that most of the steep slope populations required a longer time for T_{piv} to plateau and equilibrate sex ratios than the comparable scenarios where the T_{piv} plateaued at a lower value (Fig. 5c, orange vs. purple and blue). If populations with heavily female-biased sex ratios are demographically vulnerable, this may constitute a substantial additional risk.

The coevolution of plasticity in our model highlighted that there are several means by which populations can regain evolutionarily stable sex ratios under climate warming. Shallow slopes and high shifts represented alternative solutions to the problem of biased sex ratios. Thus, as discussed above, scenarios starting with these levels of plasticity had reduced T_{piv} evolution, and evolution of plasticity during the simulation often moved in these directions to further reduce the need for T_{piv} evolution. For shift, there was a negative linear relationship between final T_{piv} and final shift values - where shift increased from starting levels, T_{piv} evolved to a lesser extent. For slope plasticity, the trade-off between plasticity and T_{piv} evolution was non-linear. Specifically, T_{piv} evolved to the new climatic mean equally for all final slopes up until a shallow threshold of ~-0.1 when T_{piv} evolution seemed to drift, having lost its relevance in very shallow (even GSD) reaction norms (Fig. 6b, c). For organisms with shallow starting slopes in extreme environments, relaxing the relationship between sex and temperature and effectively losing ESD was more evolutionarily accessible than evolving a higher T_{piv}. In extreme warming scenarios, we saw a nearly bimodal distribution in final mean slope, suggesting that intermediate slopes are unstable. Our observation of the loss of ESD supports research suggesting that switches between sex determining systems are likely to occur as the climate changes (Grossen et al. 2011). However, the capacity for T_{piv} or plasticity to respond to sex ratio biases will depend on their relative evolvability. We attempted to minimize any parameter bias by making all evolving traits mutate with a range of 1% of the anticipated adaptive response. It is notable that an increase in mutational capacity of all traits promoted the evolution of the T_{piv} in both low slope plasticity and low shift plasticity. These results suggest that evolution of the pivotal temperature in response to climate change will be more sensitive to its own heritability than to the heritability of the slope and shift plasticity.

If sex ratio biases are resolved via evolution of higher shift or shallower slopes, the loss of these mechanisms would be selected against by frequency-dependant selection on sex, as only individuals with high shift or shallow slopes can produce the rare sex in warmer climates. Relying on these plastic mechanisms to avoid biased sex ratios could put populations at a disadvantage compared to those with adapted pivotal temperatures. Plasticity in the pivotal temperature (high shift) may involve costs (for example, investing in yolk hormones) that would remain mandatory for populations where adaptive evolution of the pivotal temperature was prevented (Auld et al. 2010; Snell-Rood et al. 2010; Murren et al. 2015; Bowden and Paitz 2021). In addition, maintaining shallow slopes prevents individuals from enhancing their fitness by developing into the sex favoured by the sex-differential fitness, which may ultimately reduce overall population fitness (Charnov and Bull 1977).

The possibility of adapting to a new environment depends significantly on the distance between the possible phenotypes present in a population and the new optimal phenotype. Accordingly, in our study, climate scenario stood out as having a significant effect on pivotal temperature evolution. This is in part because climate is the selective pressure that drives T_{piv} evolution, but it also reflects the influence of starting phenotypic variation on evolution. Initial genetic variation seeded in all populations included extreme T_{piv} alleles near 30°C. Thus, adapting to 30°C required modest mutational change and the proliferation of extreme alleles already in the population. In contrast, entirely new mutations were required to adapt to 32°C. As noted above, increasing the mutation rate of T_{piv} promoted adaptation to extreme environments. Our results agree with previous theoretical predictions that reaction norms will maintain sub-optimal values if the genetic variation necessary to evolve the reaction norm is not present (Gomulkiewicz and Kirkpatrick 1992; Huey and Kingsolver 1993).

Climate variability had unexpected effects on the evolution of the pivotal temperature. In the first five hundred generations, climate variability significantly affected population persistence, with most population extinctions occurring in low climate variability scenarios (Fig. 4). Population extinction under low climate variability is contrary to a wealth of literature suggesting that highly variable climates are more likely to result in extinctions (McLaughlin et al. 2002; Boyce 2006; Mustin et al. 2013; Vincenzi 2014). However, our finding is likely to be specific to phenotypes subject to negative frequency-dependent selection, as climates with high variability have occasional extreme years that allow development of the rare sex (males), thereby enabling reproduction. On an evolutionary timescale however, climate variability did not significantly interact with plasticity to influence pivotal temperature evolution (see Appendix A). This lack of interaction is surprising considering that plasticity is expected to be an adaptive evolutionary response to environmental variability (Gomulkiewicz and Kirkpatrick 1992; Schlicting and Pigliucci 1998; reviewed in Murren et al. 2015). As such, we might expect the evolution of greater shift or shallower slopes in response to selection to reduce sex ratio fluctuations in high variability climates, and correspondingly different co-evolutionary paths between the inflection point and plasticity. The lack of interaction is most likely explained by specifying populations with substantial overlapping generations (average adults lifespan = 10 years), as mixing of cohorts across fluctuating climates ameliorates adults sex ratio biases, and thus reduces selection on plasticity (Bull and Bulmer 1989; van Dooren and Leimar 2003; Schwanz and Proulx 2008; Schwanz et al. 2010). This conclusion is supported by the dramatic increase in shift plasticity (and corresponding decrease in T_{piv} evolution) that we observed for populations of short-lived animals exposed to warm, low variability climates (see Appendix A).

We have shown that plastic responses of species with ESD can greatly affect the evolutionary trajectory of ESD reaction norms and that these effects depend considerably on the type of plasticity in operation. Additionally, the degree of warming and available phenotypic variation are likely to impact long-term evolutionary responses to climate change. Extinction or transition to genetic sex determination are possibilities for ESD species experiencing increased temperatures, and the form of reaction norm plasticity is likely to influence these outcomes. Surviving ESD populations will experience a genetic bottleneck as the ESD reaction norm adapts to warming temperatures, resulting from the disproportionate fitness of a small number of males. This could have significant consequences for future population survival. Despite these potential negative outcomes, multiple pathways of plasticity and pivotal temperature evolution lead to population survival, even in the most extreme warming scenarios, suggesting that for species with ESD and other discrete polyphenisms, persistence is possible.

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Chapter Three

Plasticity in environmental sex determination in response to climate and maternal traits

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Author Contributions

CC and LES conceived the idea for the project. FJJ collected the data for the project. LH calculated the nest CTEs, and CC analysed the data, and wrote the manuscript with mentoring from LES, LH, and FJJ.

Abstract

In order to maintain stable sex ratios, and avoid population collapse, species with environmental sex determination (ESD) must respond to environmental fluctuations, both natural and anthropogenic. Frequency-dependent selection for the rare sex should favour adaptive plastic responses that balance sex ratios, however the presence and mechanisms of this plasticity are not well established. Evidence from species with temperature-dependent sex determination (TSD) suggests that maternal physiological traits can adaptively influence offspring sex. In this chapter, we investigate evidence for inter-annual variation in the pivotal temperature (T_{piv}) and transitional range of temperatures (*TRT*) in a wild population of *Chrysemys picta*, a freshwater turtle with TSD. Additionally, we test for correlation between clutch sex ratios and two potential mechanisms of maternal control over offspring sex: egg size and maternal age. We observed significant inter-annual covariation between T_{piv} and mean nest temperatures, in line with previous work. However, we did not find evidence of adaptive plasticity in T_{piv} or *TRT*, when we accounted for fluctuating incubation temperature. Similarly, variation in clutch sex ratios was not significantly related to egg size or maternal age, though there was a nonsignificant trend for older females to produce female-biased clutches. The differing results from two measures of nest temperature may be explained by the feminising effects of fluctuating nest temperatures, or maternal behavioural responses to climate variability. The lack of evidence for adaptive plasticity in *C. picta* may be a cause for concern under current rapid climate change.

Introduction

When the sex of offspring depends on environmental factors (environmental sex determination, ESD), populations can experience significant variation in sex ratios in response to fluctuations in their environment (Bulmer and Bull 1982; Janzen 1994a; Refsnider and Janzen 2016; Bokony et al. 2019). In addition to these natural fluctuations, there is evidence that some species with ESD are experiencing persistent biased sex ratios as a result of climate change and that these biases will be exacerbated with future warming (Hawkes et al. 2007; Chu et al. 2008; Mitchell et al. 2008; Tucker et al. 2008; Jensen et al. 2018). Biased sex ratios could lead to population extinctions, either by directly reducing recruitment of new individuals or by loss of genetic diversity as individuals of the common sex are unable to contribute genes to the next generation (Mitchell and Janzen 2010; Schwanz and Georges 2021). However, sex ratios are under strong frequency-dependent selection, as production of the rare sex is highly advantageous. As a result, species with ESD are predicted to respond to environmental fluctuation in ways that push sex ratios towards an evolutionarily stable value (Bulmer and Bull 1982; Bull and Charnov 1988; Conover and Van Voorhees 1990; Butka and Freedberg 2019). Thus, past selection arising from historical climatic fluctuations may have favoured traits that help to balance sex ratios in response to climatic change (Mitchell and Janzen 2010).

One under-appreciated means by which sex ratios can remain unbiased is via plasticity in the relationship between sex allocation and the environment. Specifically, theory predicts that offspring sex should be determined based on the relative environment they will experience (i.e. the sex ratio anticipated from the mean and variance of expected environments), rather than an absolute environmental value (Charnov and Bull 1977; Charnov 1982; Schwanz et al. 2010a). In effect, there should be plasticity in the ‘pivotal environment' where a 1:1 sex ratio is produced. This principle has been observed in species with a variety of different sex-determining mechanisms. Simultaneous and sequential hermaphroditic species may allocate to eggs vs. sperm (a form of sex allocation akin to sex ratios) based on their relative size compared to breeding partners, rather than at a set body size (Charnov et al. 1978; Angeloni and Bradbury 1999). In addition, offspring sex ratios have been linked to relative maternal condition in species with genotypic sex determination (Trivers and Willard 1973; Cameron and Linklater 2007). Relative sex allocation has also been observed in short-lived, semelparous species with ESD. In the parasitic nematode, *Romanomermis nielseni*, offspring sex is determined by the relative size of the host, where the minimum host size required to produce a female increases in populations where larger hosts are available (Blackmore and Charnov 1989).

For iteroparous species, selective pressure on sex ratios may be reduced: as long as the environment fluctuates among cohorts, cohort interbreeding reduces the impact of biased sex ratios in a single generation on the total breeding sex ratio (Bull and Bulmer 1989; Schwanz and Proulx 2008; see also van Dooren and Leimar 2003). Despite this reduced selective pressure, theoretical models of long-lived species still suggest that we should observe plasticity in sex allocation relative to the environment (Schwanz et al. 2010a). If this were to occur in a long-lived species with ESD, we would expect the ESD reaction norm (the curve relating sex to environment) to shift each breeding season in response to changes in the mean environment (e.g. in a hot vs. cold year).

The most likely pathway to enable plasticity in sex determination is via nongenetic maternal effects that translate information about the relative environment into offspring sensitivity to the developmental environment. However, maternal allocation of offspring sex in response to relative conditions is not possible unless there are maternal physiological or behavioural adaptions for influencing offspring sex. There is a significant body of research concerning maternal influences on sex determination in reptiles with temperature-dependent sex determination (TSD, Fig. 1). While nesting behaviour (variation in nest timing, depth, soil moisture, and vegetation cover) greatly impacts nest temperatures, thus playing an important role in moderating the sex ratios of reptiles with TSD, it largely changes the temperatures experienced by the embryos, not the sensitivity of the embryos to those temperatures (i.e. the pivotal temperature and the transitional range of temperature) (Janzen and Morjan 2001; Doody et al. 2004; Doody et al. 2006; Warner and Shine 2008; Telemeco et al. 2009; Refsnider and Janzen 2010; Schwanz et al. 2010b; Mitchell et al. 2013; Refsnider et al. 2014). Instead, there is a growing body of research that suggests multiple secondary factors (in addition to the primary factor of temperature) are involved in sex determination, and that some of these factors may be under maternal control (Bowden et al. 2000; Bowden et al. 2001; Elf 2003; Bowden et al. 2004).

Maternal egg allocation can be influenced by demography and the environment in ways that could translate climatic variation into plastic temperature sensitivity in sex ratio (Fig. 1). Maternal diet and egg size can influence sex determination in lizards (Warner et al. 2007) (Radder et al. 2009). In addition, maternally-derived yolk hormones can affect offspring sex in species with TSD (Bowden et al. 2001; Elf 2003, Radder 2007; Warner et al. 2017). In painted turtles, older females produce larger eggs with lower concentrations of yolk testosterone (Bowden et al. 2004). Moreover, a female’s second clutch in a year has higher progesterone and estradiol concentrations than her first clutch (Bowden et al. 2001). This is supported by additional research that suggests that resource allocation to eggs differs between old and young females, and between first and second annual clutches (Harms et al. 2005). Additionally, environmental conditions may influence reproductive strategies in an age-dependent manner. Warmer winters can lead females to nest earlier and increase their chances of producing an additional clutch, with older females more likely to produce such clutches (Bowden et al. 2001; Schwanz and Janzen 2008; Tucker et al. 2008). Thus, many lines of evidence suggest that climate-induced changes in egg provisioning or relative contributions of young and old females to the total egg pool could impact the average sex ratio that emerges from a given nest temperature. However, the majority of this research has been conducted under laboratory conditions, and it is not known whether the effects of maternal age or egg size are detectable under the environmental and demographic complications present in wild populations. Investigating maternal influences on offspring sex may be the key to understanding if and how populations with ESD can respond to environmental fluctuations to maintain stable sex ratios in the short-term. Additionally, ascertaining the prevalence parental effects on offspring sex is the first step to understanding how these effects influence the evolution of TSD species.

The aim of this study is to examine plasticity in sex determination in a wild, long-lived species with TSD. Specifically, we examine the clutch sex ratios of a freshwater turtle, the painted turtle (*Chrysemys picta*). In *C. picta* the TSD reaction norm (the curve that relates the clutch sex ratio to incubation temperature) varies with annual mean July temperature, suggesting that offspring sex is allocated based on relative environmental conditions (Schwanz et al. 2010a). We revisit these findings, using an alternate measure of nest temperature. Additionally, we aim to determine if maternal age or egg size are potential covariates of variation in clutch sex ratios. We focused our investigation on two different scales, population-wide variation in the TSD reaction norm on an annual basis and nest-level variation in sex ratio outcomes based on maternal traits. we predict that:

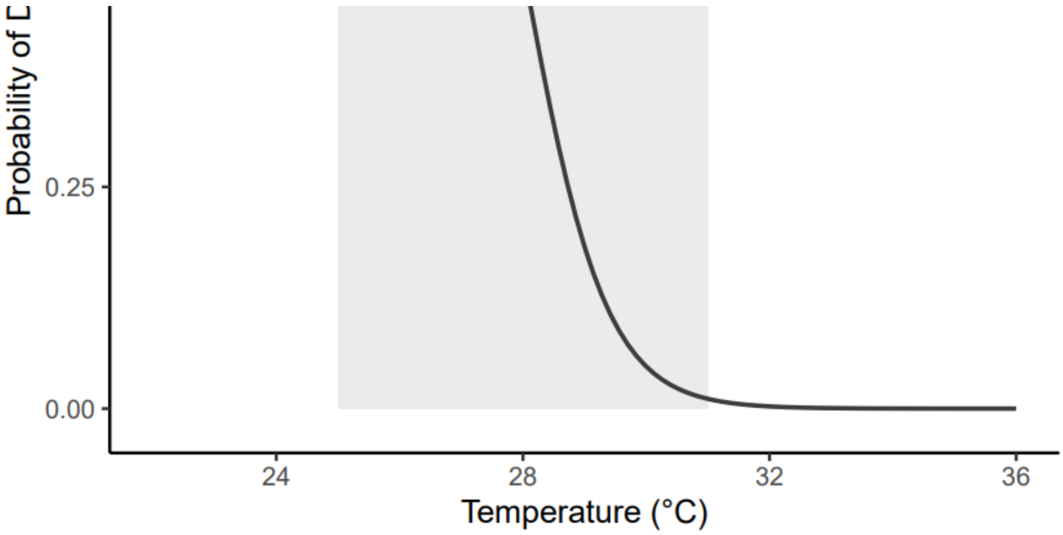
1. The TSD reaction norm of *C. picta* will vary with annual climate. Specifically, the pivotal temperature and transitional range of temperatures will be positively correlated with annual temperature.
2. Clutch sex ratios (proportion of sons) will decline with larger maternal body size and larger average egg mass (Fig. 2).

Methods

Study Species

Chrysemys picta is a freshwater turtle native to North America. *C. picta* displays Type 1a TSD (Fig. 1), where males develop from cold temperatures and females develop from hot temperatures (Carter et al. 2019). We examined nest temperatures and sex ratios in a population of *C. picta* nesting on the Thomson Causeway, in the Mississippi River, Illinois from 1995 – 2017 (Schwanz et al. 2009 metadata; Schwanz et al. 2010b; Murphy et al. 2020). At this site, females lay eggs from late May to early July, and the period of embryonic development associated with sex determination typically occurs in July. After hatching, juveniles remain in their nests throughout the northern hemisphere winter (Schwanz et al. 2010b; Janzen 1994b). We used two parameters to describe the TSD reaction norm: the pivotal temperature (T_{piv}) and the transitional range of temperatures (*TRT*) (Fig. 1).

Figure 1. The relationship between sex and temperature in Type 1a. TSD, where males develop at cold temperatures and females develop at hot temperatures. The pivotal temperature (T_{piv}), marked on this graph by the black point, is the temperature that is estimated to produce a 1:1 ratio of males to females. The transitional range of temperatures (*TRT*), the grey box on this graph, is the range of nest temperatures that is estimated to produce a mixed sex ratio.



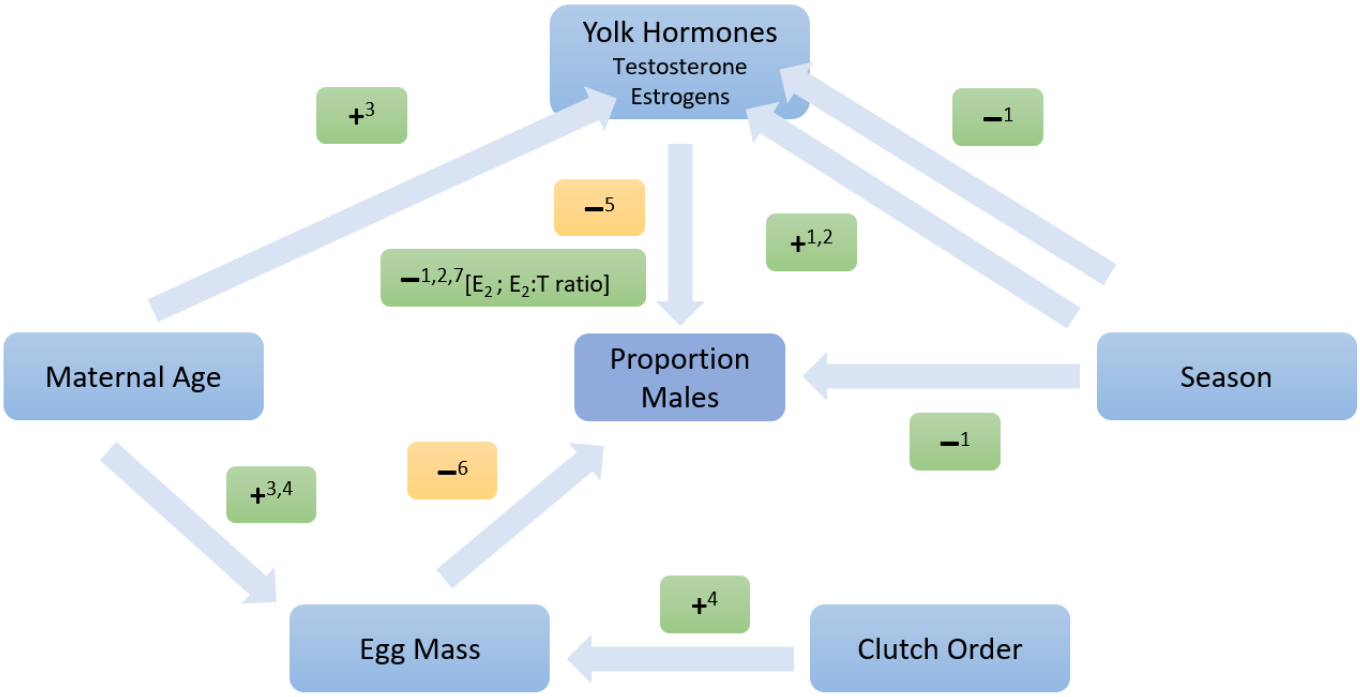


Figure 2. A summary of research on the relationship between maternal age, egg mass, clutch order, seasonality, yolk hormones, and sex ratio in *C. picta*. Green citations concern *C. picta* directly, while the yellow reference concerns other TSD reptiles. Arrows indicate the direction of effect, +/- signs indicate a positive or negative effect. "E_2" refers to maternally derived yolk estrogen, "T" refers to maternally derived yolk testosterone. Refs: 1) Bowden et al. 2000, 2) Bowden et al. 2001, 3) Bowden et al. 2004, 4) Harms et al. 2005, 5) Radder 2007, 6) Radder et al. 2009, 7) Warner et al. 2017.

Data Collection

Nests were identified by monitoring the site hourly for nesting females, from late May to early July. On completion of nesting, females were captured, their plastron length was measured, and they were given unique marks on their marginal scutes (see Morjan and Janzen 2003). Eggs were excavated from the nest and weighed, before being returned to their original nest (Morjan and Janzen 2003). Nest vegetation cover was measured to the South and West using a reflective spherical densiometer (see Janzen

1994a). A subset of nests was selected for temperature monitoring, and data loggers were placed inside the nest. From 1995-2002 HOBO data loggers were placed in nest at the end of June (Morjan and Janzen 2003). From 2003-2017 ibutton data loggers were used, and they were placed in the nest at the time of laying (Schwanz et al. 2010b). After hatching, nests were excavated and an average of 4.5 hatchlings from each nest (range:1-13) were randomly selected for sex determination, these individuals were euthanized, and sex was determined by examination of the gonads (see Janzen 1994a). This sex ratio was then extrapolated to the rest of the clutch.

Calculation and analysis of population-level TSD parameters

First, we investigated whether the TSD reaction norm, as estimated by the annual population-level T_{piv} and TRT, was influenced by annual climate. For each year with sufficient data, we characterized the TSD reaction norm at a population level, including only nests where both nest temperatures and sex ratio were recorded. From the logger data, we calculated two measures of nest temperature relevant for sex determination: 1) the mean temperature in the month of July (when most embryos are in the temperature-sensitive phase); and 2) the constant-temperature equivalent (CTE), which incorporates both mean and variance in nest temperature (Georges 1989; Georges et al. 2004), calculated for 21-40 days after nest-laying. Temperature values > 40°C and < 0°C were removed as they are out of the range of reasonable temperatures for the study site in the summer. Of the 20 years of data available, we excluded 8 years with fewer than 5 nests with both temperature and sex ratio data and 2 years where nests with temperature data produced all males (see Tables S1 & S2. in Appendix B). The remaining 11 years had 9-73 nests with Mean July Nest Temperature values and 5-73 nests with Nest CTE values.

We used the ‘embryogrowth v. 8.0’ package in R to calculate TSD parameters each year using first Mean July Nest Temperature and then Nest CTE (Girondot, 2020). The package is specifically designed for analyses involving the sexual development and growth of embryos. we used the function *tsd* with the logistic model to estimate a logistic equation for the TSD reaction norm, the T_{piv}, the *TRT*, and 95% confidence intervals for these parameters. The logistic model was selected as it assumes a symmetrical distribution of hatchling sex around the pivotal temperature which has been determined to accurately reflect sex determination patterns in *C. picta* (Carter et al. 2019). In the estimation using Nest CTE, two years (1996 and 2009) produced unusually broad confidence intervals around the estimated T_{piv}, so were excluded from the analyses with climate below (see Table S2 in Appendix B).

We wanted to know if the relationship between sex ratio and nest temperature (T_{piv} and TRT) is related to the anticipated cohort sex ratio (e.g., is the temperature threshold for producing females lower in cooler years, when a male biased sex ratio is likely). We used two measures of annual climate that could influence the cohort sex ratio: July air temperature and July nest temperature. we used the latter to accommodate variation in maternal nesting behaviour as a function of climate (Schwanz and Janzen 2008; Refsnider and Janzen 2010). If T_{piv} or *TRT* are plastic in response to changes in nest temperature distributions each year in such a way that ameliorates sex ratio biases, we predicted that the annual T_{piv} and/or *TRT* would be positively related to the annual temperature.

Annual July Air Temperature data for Clinton, Iowa (~10 km from the Thomson Causeway) was acquired from the National Climate Data Center (NCDC; http://www.ncdc.noaa.gov). To calculate the average nest temperature in a year, we included temperature estimates for all nests laid in a year in order to avoid any bias in nests chosen for temperature records. To include nests without direct temperature recordings, we estimated their nest temperatures using vegetation cover over the nest, which has been shown to accurately predict mean July nest temperatures (Weisrock and Janzen 1999; Schwanz et al. 2010b). We collected all the temperature data for nests with July nest temp and CTEs. For July temperature data, each year (n = 11) ranged between 6-73 nests, with a total of 229 nests. For CTE data, each year (n = 9) ranged between 5-73 nests, with a total of 189 nests. For each year, we ran linear regressions of nest temperature vs. vegetation cover over the South and West sectors of the nest (SWveg, Appendix B, Tables S3, S4). We used the estimated slope and intercept from each regression to calculate July nest temp and CTE for each nest that had data for vegetation cover but lacked temperature data (n = 2334 nests). Subsequently, we combined known and estimated nest temperatures to calculate the annual average July Nest Temperature and the annual average Nest CTE for all nests.

To test our main prediction that the TSD reaction norm is positively related to climate, we ran separate linear regressions with the two reaction norm estimates (*TRT* and T_{piv}) as the response variables and mean July Air Temperatures or mean nest temperatures (Mean July Nest Temperature or Nest CTE) as the predictor variable (using function *lm* from base R). We used the T_{piv} and *TRT* derived from the mean July nest temperature as the response variable in the air temperature analysis.

Analysis of nest level variation in sex ratio response to temperature

Examining plasticity in the TSD reaction norm at a population level provides an overview of patterns of sex determination in a species, however it erases the detail of patterns at the nest level and does not inform us of the influence of maternal traits on sex determination. Therefore, we looked next at how much individual nest sex ratios varied after accounting for nest temperature, and what may explain this variation.

We focused on two environmental variables and two maternal traits that may influence sex ratios: nesting year, air temperature, average egg mass, and maternal plastron length. We examined year and air temperature because we wanted to know if nesting mothers respond to annual conditions in ways that influence sex ratios. Similarly, egg mass has been shown to be related to factors influencing sexual development and is a measure of maternal investment in offspring (See Fig. 2). Plastron length is a proxy for maternal age in *C. picta*, which may impact sexual development in this species (Wilbur 1975; Zweifel 1989; Hoekstra et al. 2018).

Because we were not restricted to using years with mixed sex nests, our sample size for these analyses was larger than those in the previous section. Our models include data from 12 years with a range of 5-72 nests per year and a total of 245 nests.

We used the R package glmmTMB v.1.0.2.1 to run binomial generalized linear models examining relationships between nest sex ratio, nest temperature (CTE) and our variables of interest (Magnusson et al. 2020). We first examined whether nest sex ratio varied among years (fixed effect) after accounting for Nest CTE. We then examined if nest sex ratios were related to mean July Air Temperature or its interaction with Nest CTE. Finally, in two separate models, we examined if the maternal traits (plastron length or average egg mass) influenced nest sex ratios, alone or in interaction with Nest CTE. We included year and maternal ID as random effects in the models, except in the model testing the effect of year, where year was a fixed effect. Because clutch sizes vary, the models were weighted with the number of live known-sex hatchlings from a nest. Intersex hatchlings were excluded because they were not included in the sex ratio.

We used R package DHARMa and the simulateResiduals() function to assess the fit of the binomial models (Hartig 2020). There were significant deviations in the residual plots, so we elected to use a beta binomial model which better fit our data. The results of the glmm suggested that our fixed effects required re-scaling. The scale() function from base R produced a model with ill-fitting residuals, so we scaled nest temperature, egg mass and plastron length with a second order polynomial term. To obtain p-values for effect of egg mass and any interactions, we conducted parametric boot strapping for each of the models.

Results

Population level

We found a positive relationship between the annual average of the July Nest Temperature of all nests and the annual T_{piv} (Table 1, Fig. 3). Years with warmer nest temperatures also had warmer pivotal temperatures. This relationship was not statistically significant when we used CTE as a measure of nest temperature (Table 1, Fig. 3). We found no substantive relationship between annual average nest temperatures and the width of the TRT when using either measure of nest temperature (Table 1, Fig. 3).

Nest level

Nest sex ratios did not vary overall among years once accounting for variation in nest CTE ($P = 0.366$). Similarly, once the impact of nest CTE was incorporated, we found no effect of July Air Temperature on clutch sex ratios ($P = 0.637$) and no interaction between July air temperature and nest CTE ($P = 0.772$).

Maternal body size appeared to have a negative influence on the proportion of males in a nest ($P = 0.078$), but there was a negligible interaction between nest temperature and maternal body size ($P = 0.121$). There was also no relationship between average egg mass and nest sex ratio (P = 0.733) and no interacting effect of nest temperature and egg mass on nest sex ratio (P = 0.395).

Table 1. Summary of results from linear regressions comparing two different measures of annual nest temperature with annual T_{piv} and annual TRT.

TSD parameter	**Temp measure**	**SE**	**DF**	**F-statistic**	**R^2**	**P-value**
T_{piv}	Annual average July Nest Temperature[1]	0.10	9	6.63	0.36	**0.030**
	Annual average Nest CTE[2]	0.14	7	2.10	0.12	0.191
	July Air Temperature[1]	3.46	9	3.39	0.19	0.099
TRT	Annual average July[1] Nest Temperature	0.67	9	0.13	-0.10	0.527
	Annual average Nest CTE[2]	1.22	7	0.03	-0.14	0.869
	July Air Temperature[1]	0.85	9	0.42	-0.06	0.532

[1] Annual T_{piv} and TRT estimated using Mean July Nest Temperatures

[2] Annual T_{piv} and TRT estimated using Nest CTE

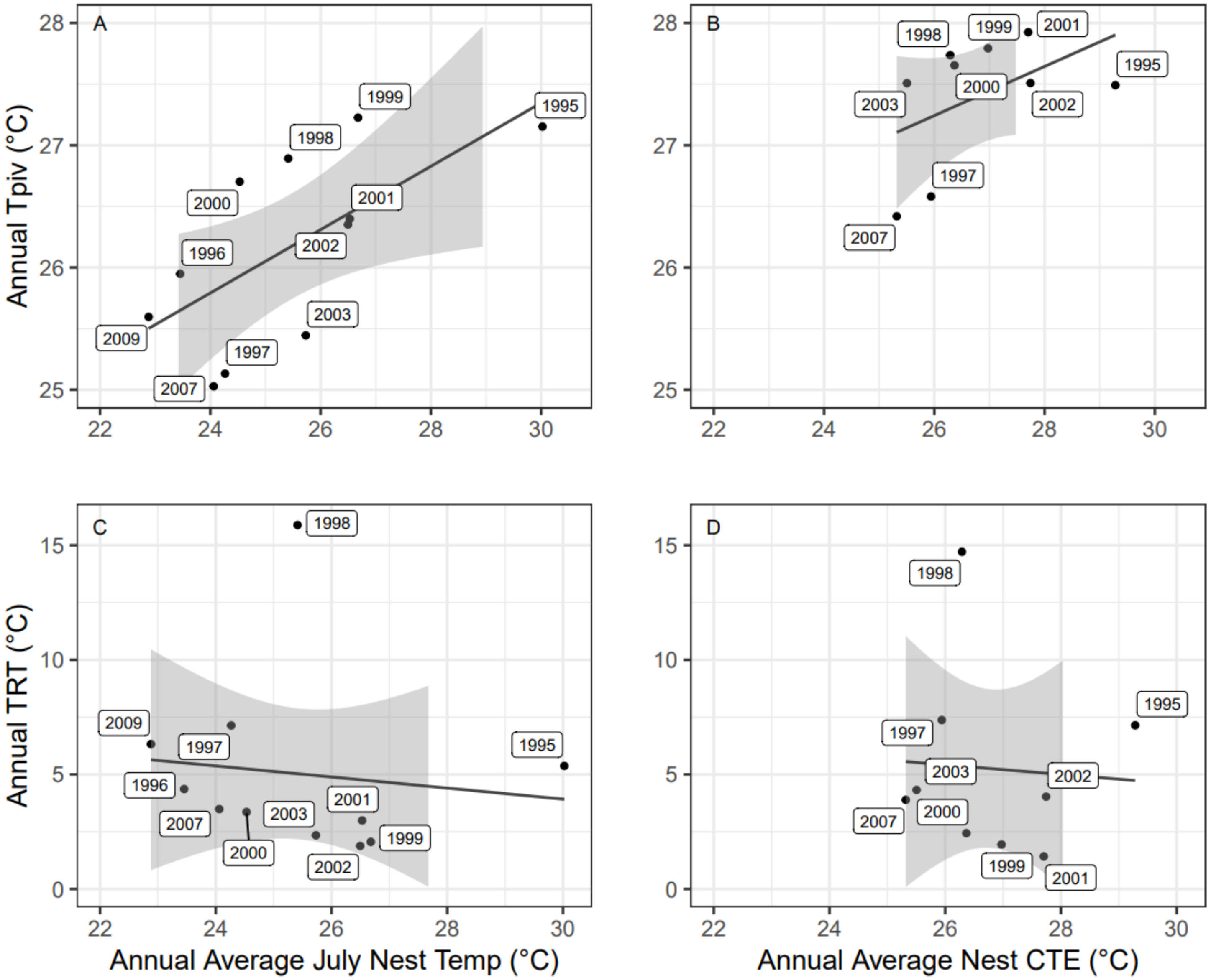


Figure 3. T_{piv} is positively correlated with nest temperature when Annual average July Nest Temp is the temperature measure (1.A), but not when Annual average nest CTE is used (1.B). The TRT is not related to nest temperature when either method of calculating Annual Average Temperature is used (1.C & 1.D).

Discussion

In our population of a long-lived turtle with TSD *(Chrysemys picta)*, the temperature that produces a 1:1 sex ratio (T_{piv}) across the population varies among years and is positively correlated with the annual average July Nest Temperature, a measure of the annual climate and anticipated cohort sex ratio (here and Schwanz et al. 2010a). This plasticity in the pivotal temperature suggests that *C. picta* is responding to inter-annual temperature variation in ways that balance sex ratios. However, we found the relationship was not statistically significant when we used the nest Constant Temperature Equivalent (CTE) as our measure of nest temperature instead of mean July Temperature.

There are two biological explanations for the differences in our findings when using Nest CTE and Mean July Nest Temperature. One explanation lies in the fact that the Mean July Nest Temperature was calculated from the temperature data collected in the month of July, while the Nest CTE was calculated during the time period when *C. picta* embryos' sex are sensitive to temperature (21- 40 days), which may include days in June and August. Thus, Nest CTE incorporates differences in the timing of nesting that occurs among years in response to climate (Schwanz and Janzen 2008, Telemeco et al. 2009; Janzen et al. 2018). This distinction is important: a nest laid late (e.g., end of June) in a cold year would have a Mean July Nest Temperature that underestimates the temperatures actually experienced by an embryo during the temperature-sensitive phase (e.g., August; captured in the Nest CTE). Thus, in these cold years, nests with daughters would have colder Mean July Nest Temperatures than Nest CTEs, leading to lower estimates of pivotal temperatures. This difference in calculation is reflected in the reduced variation in the annual nest temperature estimates (compare Fig. 3A,C to Fig. 3B,D). An alternate explanation is that our results indicate significant fluctuations in nest temperatures during the study period. As CTE accounts for changes in developmental rate that occur with temperature fluctuations (Georges et al. 1989; Georges et al. 2004), greater fluctuations in temperature would lead to more significant differences between the Nest CTE and Mean July Nest Temperature.

Alternatively, the pattern may still exist when CTE are used, but our analysis lacks the power to detect it. The slope of the relationship using CTE is very similar to that using mean July Nest Temperatures. Unfortunately, our CTE analysis had a smaller sample size resulting from the removal of years with unusually broad confidence intervals around the T_{piv}. These were the two coldest female-producing years in the dataset (1998 and 2009), and the reduced number of females made it difficult to estimate a T_{piv}. The loss of these two datapoints, in addition to the compression of nest temperature variation that arises when using CTE, means that the regression relationship is not as strong. It is possible that with a larger sample size there would be a detectable relationship between annual climate and T_{piv} using CTE as the temperature measure. Regardless, the difficulties of estimating T_{piv} in cold years owing to lack of females highlights the limitations of any sex ratio plasticity to compensate for climate fluctuations in this population of painted turtles, at least in the colder ranges of the environmental variation.

While annual differences in developmental temperatures may not predict plasticity in the T_{piv}, there is still surprising variation in T_{piv} on a yearly basis that is of interest. This finding supports the idea of multifactorial sex determination and reflects laboratory and field studies in several reptile species, including painted turtles, that point to a complex inter-relatedness of yolk hormones, maternal age, egg mass, season, clutch order (first or second) and clutch sex ratio (Fig. 2). The variation that we observed in annual average pivotal temperatures may result from multiple factors that influence sex ratios in addition to temperature.

We investigated two aspects of this relationship, maternal body size (as a proxy for age) and egg mass. Based on previous research that established a relationship between maternal age, egg size, and yolk hormones, we predicted that clutches with larger eggs and clutches laid by larger (older) mothers would produce more female hatchlings at a given temperature (Bowden et al. 2000, Bowden et al. 2001, Bowden et al. 2004, Harms et al. 2005). This observation would have suggested that annual variation in the average pivotal temperature of *C. picta*, was a result of demographic fluctuations in the nesting population. However, we did not find strong evidence to support this conclusion. We may have been unable to detect age effects due to lack of data on maternal nesting experience. We used plastron length as a proxy for maternal age as there is a relationship between body size and age in this species. However, this relationship plateaus over time, so plastron length is less indicative of the age of older turtles (Wilbur 1975; Zweifel 1989; Hoekstra et al. 2018). Additionally, *C. picta* body size is influenced by the environmental conditions experienced by an individual. Turtles in good conditions will grow faster than those in bad conditions, so plastron length should not be considered an absolute indicator of age. However, it may be that the influence of maternal age and egg mass on *C. picta* sex ratios is obscured by environmental variation when these factors are studied in a natural setting. Our study does not imply that maternal size or egg allocation are unimportant for clutch or population sex ratios, only that their influences on temperature sensitivity are hard to detect in a wild population.

These results leave the variation in that we observed in T_{piv} unexplained. Several other factors may influence sex determination in *C. picta* and explain this annual variation. In particular, the variation could be related to maternal plasticity in response to winter climate. Females have an increased likelihood of laying a second clutch after warmer winters (Schwanz and Janzen 2008), and second clutches have higher concentrations of oestradiol, which favour female development (Bowden et al. 2001). This combination could yield yearly changes in T_{piv} in *C. picta*.

Additionally, annual variation in nesting behaviours may contribute to plasticity T_{piv}. There is a significant body of evidence outlining the impact of nesting behaviour on the sex ratios of reptiles with TSD (Doody et al. 2004, Doody et al. 2006, Warner & Shine 2008, Warner et al. 2008, Telemeco et al. 2009, Refsnider and Janzen 2010; Mitchell et al. 2013, Refsnider 2014). Many of the nesting behaviours investigated, such as selection of vegetation cover, nest depth, and nest timing affect offspring sex by changing the temperature of the nest. Our study controls for this direct impact of nesting behaviour on nest temperatures. However, nesting behaviour may influence sex ratios by means other than temperature, in particular, the soil particle size and moisture of a nest may influence clutch sex ratio independent of temperature (Paukstis et al. 1983; Mitchell and Janzen 2019, but see also Packard et al. 1987). This variation could help explain the observed fluctuations in annual pivotal temperature.

Plasticity in sex determination that compensates for the impact of climatic variation on sex ratios appears to be limited, or at least difficult to detect, in *C. picta.* While our study demonstrates that pivotal temperatures in this species are variable, that does not necessarily translate to an adaptive benefit or amelioration of sex ratio biases. Long-lived species like *C. picta* may not require adaptations that stabilize sex ratios in a naturally-fluctuating climate. That is, biased sex ratios produced by particularly hot or cold years may not greatly impact population demographics as offspring from these years can interbreed. Theoretical models show that the impact of fluctuating cohort sex ratios on sex-ratio selection and adaptive plasticity is reduced in species with overlapping generations (Schwanz et al. 2010b). This reduction occurs with only a few cohorts of overlap (3 cohorts; Schwanz et al. 2010b), making it relevant to a wide range of life histories. Crucially, this lack of plasticity for dealing with fluctuating temperatures will be problematic for ESD species experiencing rapid, directional climate change. The impacts of biased sex ratios induced by increasing temperatures (or other directional changes in the environment) on population persistence will be species-specific, and depend on whether males or females are over-produced (Mitchell and Janzen 2010). However, extreme warming will ultimately cause changes in population demographics that may lead to species decline (Boyle et al. 2016).

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Chapter Four

Tipping the TSD balance: What factors affect the coevolution of pivotal temperature and maternal behaviour in novel environments?

Claudia Crowther and Lisa E Schwanz

Author Contributions

CC and LES conceptualised the project, CC and LES programmed the simulation, CC analysed the data and wrote the manuscript with mentoring from LES.

Abstract

Species adaptation to environmental change involves co-occurring responses from many traits. Trait adaptation is influenced by changes in other traits and phenotypic plasticity induced by the new environment. In species with temperature-dependent sex determination (TSD), the pivotal temperature (T_{piv}) and maternal nesting behaviour (*Nb*) have been identified as traits with the potential to evolve in response to rising temperatures that destabilise sex ratios. However, the impact of plasticity on the coevolution of these traits has not been explored. Here we use individual-based simulation modelling to ascertain the relative adaptive capacity of T_{piv} and *Nb* and determine how temperature-dependent embryonic survival and plasticity in maternal nesting behaviour influence the coevolution of these two traits. We found that T_{piv} evolved to ameliorate sex ratio bias more readily than *Nb*, though *Nb* played an important role in adaptation to extreme environments. Selection favoured increased evolution of *Nb* when embryonic survival depended on nest temperature. In contrast, the presence of behavioural plasticity to annual climate fluctuation reduced the adaptive capacity of *Nb*. Our research demonstrates the complexity of the interactions that occur when species adapt to new environments and highlights the importance of plastic responses in shaping the course of evolution.

Introduction

When species encounter new environmental conditions, multiple traits respond simultaneously to selection pressures from the new environment (Endler 1995; Ghalambor et al. 2003; Laland et al. 2017). These trait responses influence each other, in ways that can be co-operative or antagonistic (Ghalambor et al. 2003; Laland et al. 2017; Hague et al. 2018). At the same time, novel environments generate new plastic responses that also influence selection on and interactions between traits (Schlichting 2008). Therefore, adaptation to the new environment will be more complex than predicted by examinations of single traits. In order to understand how species adapt to environmental change, we must explore the coevolutionary relationships between traits, including traits that exhibit plasticity. The adaptation of species with environmental sex determination (ESD) to changing environments provides an ideal system with which to examine trait coevolution, as sex in ESD species is the product of multiple, environmentally-sensitive trait interactions, including physiological and behavioural factors (Bulmer and Bull 1982).

Temperature-dependent sex determination (TSD, Fig. 1), where sex is determined during embryogenesis by developmental temperature, is a common form of ESD, found in reptiles, fish, and invertebrates (Conover 1984; Naylor et al. 1988; Blackmore and Charnov 1989; Valenzuela and Lance 2004; Warner and Shine 2008a; Pen et al. 2010; Beukeboom and Perrin 2014; Capel 2017; Picard et al. 2021). Species with TSD experience variation in developmental temperatures, both geographically (between populations) and temporally (climatic changes across time), which can lead to biases in population sex ratios (Bull et al. 1982; Janzen 1994; Hays et al. 2003; Morjan 2003a; Janzen and Phillips 2006; Organ and Janes 2008; Schwanz et al. 2010; Carter et al. 2019). Theory predicts that negative frequency dependent selection (FDS) for the rarer sex should act on traits that influence offspring sex, such as the pivotal temperature (T_{piv}) and maternal nesting behaviour (*Nb*), to balance sex ratios (Fisher 1930; Bulmer and Bull 1982; Bull and Charnov 1988; Morjan 2003a, 2003b; Mitchell and Janzen 2010; McGaugh and Janzen 2011; McGaugh et al. 2011; Mitchell et al. 2013; Schwanz and Georges 2021). It is likely that T_{piv} and *Nb* coevolve in response to environmental variation that destabilises sex ratios, and that plastic responses induced by novel environments influence their coevolution. Quantifying this relationship will further our understanding of how TSD species adapt to environmental change (Janzen 1994; Morjan 2003b; Mitchell and Janzen 2010; McGaugh and Janzen 2011).

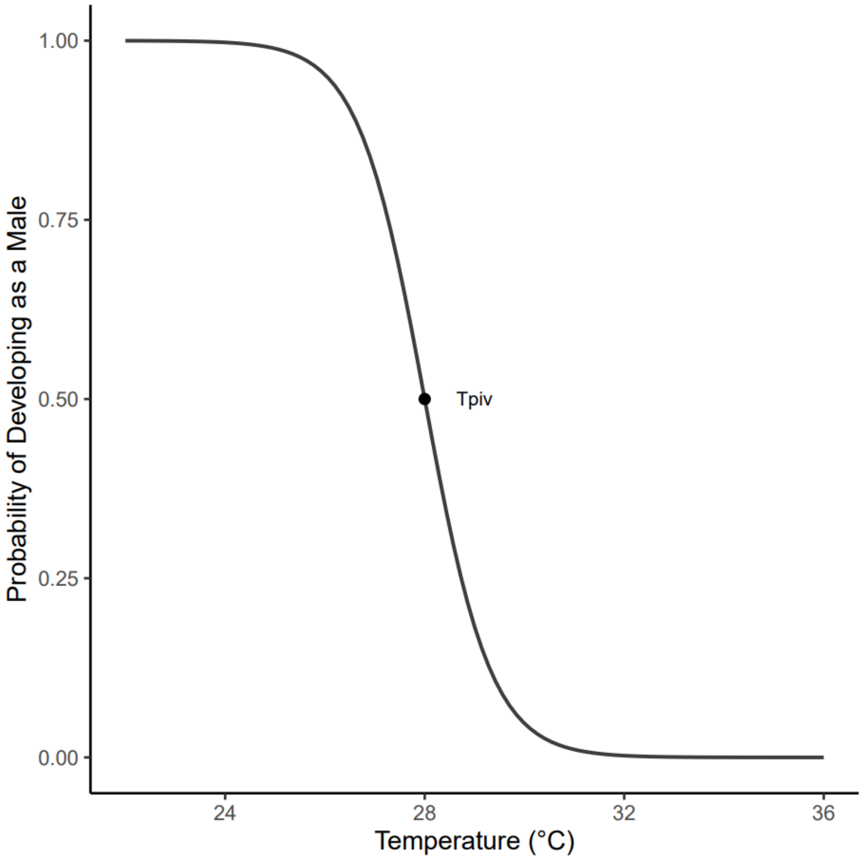

Figure 1. Diagram showing the relationship between temperature and sex determination in Type 1a. TSD. The probability of male development decreases as temperature increases. The pivotal temperature (T_{piv}), at which male and female development are equally likely, is marked with a black point.

When TSD populations experience climatic change, T_{piv} and *Nb* could work in additive ways to prevent sex ratio bias, and it is not immediately obvious which trait would prove more important to sex ratio evolution. Between-population variation in T_{piv} and *Nb* suggests that both traits have the capacity to adapt to local environmental factors (Morjan 2003a; Ewert et al. 2005; Refsnider and Janzen 2012; Refsnider et al. 2014; Carter et al. 2019). Heritability affects the response of T_{piv} and *Nb* to selection and may determine the relative adaptive capacity of these traits. Heritability estimates for *Nb* and T_{piv} vary. Initial investigations found that, while significant genetic variation in T_{piv} existed in several TSD species, the effective heritability of T_{piv} was low, as it was thought that wild populations lacked the range of nest temperatures necessary for T_{piv} genotypes to display variation (Bull et al. 1982; Janzen 1992; but see Rhen and Lang 1998). Recent revaluations of these findings suggest that constant incubation temperature experiments under-estimate T_{piv} heritability, and that the fluctuating nest temperatures of wild populations allow for the phenotypic expression of genetic variation in T_{piv} (McGaugh et al. 2010; McGaugh et al. 2011; McGaugh and Janzen 2011). In comparison, effective heritability estimates of nesting behaviours, such as onset of nesting and nest vegetation cover are low, and dependent on environmental factors (McGaugh et al. 2010; McGaugh and Janzen 2011). These heritability estimates, in conjunction with evidence from simulation modelling, suggest that T_{piv} has a greater capacity to respond to sex ratio bias caused by environmental shift (Morjan 2003b, McGaugh and Janzen 2011).

In previous analyses, one crucial aspect has been overlooked: the presence of temperature-dependent survival may increase selective pressure on *Nb*, tipping the balance in favour of nesting behaviour evolution. In reptiles, hatching success declines rapidly at very hot and cold incubation conditions (Warner and Shine 2009; Noble et al. 2018; Noble et al. 2021). Maternal nesting behaviour can influence developmental temperatures to promote offspring survival, and as a result, nesting behaviour may be under strong viability selection, in addition to sex ratio selection (Schwarzkopf and Brooks 1987; Weisrock and Janzen 1999; Leslie and Spotila 2001; Ewert et al. 2005; Refsnider and Janzen 2010; but see Mitchell et al. 2013). In contrast, changes in pivotal temperature cannot promote offspring survival, even if they reduce the chance of producing the common sex in the new environment. Previous studies have compared sex ratio selection and embryonic survival as drivers of nesting behaviour evolution, but no study has investigated the influence of temperature-dependent survival on the coevolutionary relationship between T_{piv} and *Nb* (Mitchell et al. 2013).

Another factor with potential to influence the evolution of T_{piv} and *Nb* is within-female plasticity in nesting behaviour. Despite substantial repeatability of nesting behaviour within females, considerable nesting plasticity has also been observed (Janzen and Morjan 2001; Morjan 2003a; Ewert et al. 2005; Schwanz and Janzen 2008; Warner and Shine 2008b; Doody 2009; Telemeco et al. 2009; McGaugh et al. 2010; Mitchell and Janzen 2010; Refsnider and Janzen 2012; Mitchell et al. 2013; Somaweera and Shine 2013; Delaney et al. 2020; Heredero Saura et al. 2022). Nesting females in species with TSD have shown plasticity in nest timing, frequency, depth, vegetation cover, distance from water, and substrate type, which can influence nest temperature, and by extension, offspring sex ratios (Tucker et al. 2008; Telemeco et al. 2009; Mitchell and Janzen 2010; Refsnider and Janzen 2012; Somaweera and Shine 2013). If adaptive plasticity in nesting behaviour prevents extreme nest temperatures in fluctuating climates, then plastic nesting behaviour could ameliorate sex ratio biases caused by new environmental conditions (Refsnider and Janzen 2012). In Chapter 2, we demonstrated that plasticity that reduces sex ratio bias can weaken selective pressure on targets of sex ratio selection, a process originally known as the Bogert effect, whereby plasticity limits trait evolution (Bogert 1949; 1959; Muñoz 2021). Therefore, nesting plasticity that stabilises sex ratios may reduce the adaptive capacity of females' fixed nesting preferences.

We aim to compare two potential pathways of adaptation for species with temperature dependant sex determination – evolution of T_{piv} and evolution of Nb – to determine which is most likely to buffer sex ratios of these species against climatic warming. Additionally, we aim to investigate how temperature-dependant survival of offspring and plasticity in nesting behaviour influence the co-evolution of T_{piv} and Nb. When T_{piv} and Nb coevolve under climate warming, we predict that:

1. The relative contribution of T_{piv} evolution to adaptation to changing climates will be greater than that of Nb.
2. The relative adaptive potential of T_{piv} and Nb will be influenced by the relative heritability of these two traits.
3. The presence of temperature dependant survival will promote the evolution of cooler Nb, and accordingly reduce selection for T_{piv} evolution.
4. *E*volution of T_{piv} and Nb will be hindered by plasticity in nesting behaviour, as the ability to respond to annual temperature fluctuations will reduce selective pressure on both traits.

Methods

Model overview

We designed an individual-based simulation model to investigate the co-evolutionary relationship between pivotal temperature (T_{piv}) and nesting behaviour (*Nb*) in species with temperature-dependant sex determination. We first tested the fundamental evolutionary potential of T_{piv} and *Nb* to respond to temperature change, both in solitary evolution experiments and when both traits evolved simultaneously. Next, we examined how this co-evolutionary relationship was affected by the introduction of temperature-dependant survival. Finally, we tested how the presence of behavioural plasticity in response to annual climate fluctuations would affect the evolution of T_{piv} and *Nb*. In all trials, we examined the effect of varying the relative heritability of T_{piv} and *Nb*. Model parameters and values are described in Table 1, and scenario sample sizes are listed in Appendix C.

Climate

All populations were initiated with traits adapted to a base climate scenario with a global temperature of 28°C (T_{base}, see ‘Genetics and Heritability’ below). Populations were exposed to one of three climate scenarios: a control scenario of with a global temperature of 28°C, a moderate warming scenario with a global temperature of 30°C, and an extreme warming scenario with a global temperature of 32°C. Global temperature was selected randomly at the start of each simulation replicate and populations in warming scenarios experienced an immediate, rather than gradual, increase in temperature. Interannual variability in annual mean temperature (SD_{bw}), was 1.25°C. This reflects the variation observed in wild reptile nest temperatures (Schwanz et al. 2020).

Evolution

In the model, maternal nesting behaviour (*Nb*) alleles combined with annual climate (T_{ann}) to determine offspring developmental temperature (*td*), and the offspring's T_{piv} alleles determined the likelihood of becoming a male at that developmental temperature (see 'Temperature-dependent Sex Determination' below for details). Tracking the evolutionary dynamics of T_{piv} and *Nb*, in solitary or coevolution, was the main focus of the simulation. On initiation of the simulation, one or both traits were selected to evolve, populations were seeded with initial variation in those trait(s), and were subject to mutation each generation. The relative heritability of T_{piv} and *Nb* was manipulated by the addition of environmental noise to phenotypic expression of one trait (see 'Genetics and Heritability' below). In scenarios were either T_{piv} or *Nb* did not evolve, the trait was invariant at initiation of the simulation and had no capacity to change during the simulation, and was not subject to variation in heritability. Temperature-dependent embryonic survival and climate-related nesting plasticity were manipulated in coevolution scenarios, with simulated populations being subjected to either none, one, or both of the experimental treatments (see Tables S1 & S2 in Appendix C for sample sizes).

Table 1: Names, values, and descriptions of simulation parameters. Parameters that varied across iterations are shown in bold.

Parameter	Description	Value(s)
Individual traits that can evolve		
T_{piv}	The temperature with an equal chance of producing a male or female hatchling.	Initial: 28°C ± T_{piv} SD
Nb	The nest temperature change caused by maternal nesting behaviour.	Initial: 0 ± NbSD
Other parameters related to individual traits		
T_{piv} SD	The standard deviation of starting variation in T_{piv}.	0.5
Nb SD	The standard deviation of starting variation in *Nb*.	
Mutation rate	The percentage of offspring with allele mutations.	2%
Mutation range (Mutvar_array)	The standard deviation of mutation values for T_{piv} and *Nb*.	0.08
$\boldsymbol{H_{var}}$	**The standard deviation in the realized $\boldsymbol{T_{piv}}$ and *Nb*, given an individual's bi-allelic mean trait value.**	**0, 0.1, 0.2, 0.3, 0.4, 0.5**
Slope	The slope of the TSD reaction norm.	-1.5
td	The developmental temperature of a clutch.	determined by annual temperature (Tann), maternal nesting preference (*Nb*) and relNb
relNb	**The capacity for the population to express *Nb* other than genetic *Nb*, in response to the deviation of $\boldsymbol{T_{ann}}$ from $\boldsymbol{T_{base}}$.**	**0, 0.3, 0.6**

General model parameters		
T_{glob}	**The mean global temperature of a scenario.**	**28°C, 30°C, 32°C**
SD_{bw}	The standard deviation of variation in annual mean temperatures.	1.25°C
T_{base}	The climate that the initial population is adapted to (mean $T_{piv} = T_{glob}$).	28°C
T_{ann}	The annual temperature, chosen from a normal distribution of T_{glob} ±SD_{bw}.	Generated randomly each year in the simulation
Ni	The starting population size of each simulation replicate.	500
T	The maximum number of iterations (years) in each replicate simulation.	10000
σ	**A survival probability constant that determines the degree of influence that temperature has on survival. Lower values of σ produce steeper temperature-dependant survival curves.**	**3, 1e10**
Mortality (Ad_mort)	The proportion of adults that are removed from the population each year.	0.1
Phi	A constant for female fertility.	2
F_{scale}	Scale for female fecundity.	0.02
const	Density dependant constant for juvenile survival.	0.01
M_{lim}	The degree of male limitation on female fertility.	0.01.

Genetics and Heritability

Individuals in the population had two genes (T_{piv}, and *Nb*), with two alleles at each locus. To manipulate heritability of T_{piv} and *Nb*, phenotypes were randomly selected from a normal distribution with the average of both alleles as the mean, and the heritability variation constant, H_{var}, as the standard deviation. In the solitary evolution scenarios, we used six values of H_{var}. We reduced this to three values in the coevolution scenarios to simplify results from the multifactorial experiment (see Table. 1 for H_{var}

values). However, we conducted a sensitivity analysis of the effect of varying heritability on the coevolution of T_{piv} and Nb with a wider range of H_{var} values (See 'Effect of H_{var} on coevolution' Appendix C).

Populations were seeded with alleles such that the mean T_{piv} was 28°C (SD = 0.5) and the mean Nb was 0°C (SD = 0.5). Nb was expressed as the difference between the nest temperature (td) and the annual temperature (T_{ann}) due to nesting behaviour, e.g., a mother with an Nb of -1°C will choose a nest site 1°C cooler than the average temperature of the year.

Nesting Plasticity

In addition to genetic nesting preference (Nb), individual maternal nesting behaviour was also influenced by nesting plasticity ($relNb$). $relNb$ represented the response of an individual's nesting behaviour to annual climate variability (see 'Developmental Temperature' below). While it is highly likely that plasticity varies among females, and is capable of evolution, we parametrised $relNb$ as a constant for simplicity in understanding the other focal variables of this study (Schwanz and Janzen 2008).

Developmental Temperature

The developmental temperature of embryos in the simulation was determined by the following equation:

$$td = T_{ann} + (T_{base} - T_{ann})relNb + Nb \qquad (1)$$

Where td is the developmental temperature, T_{ann} is the annual temperature, T_{base} is the baseline climate (28°C), Nb is maternal nesting behaviour after variation in heritability (H_{var}) has been applied, and $relNb$ is nesting plasticity. $relNb$ multiplies the difference between the climate of the current breeding season and the climate of initial adaptation;

this fraction is then added to the female's genetic predisposition for nesting behaviour. Thus, when *relNb* = 0, maternal nesting behaviour is not influenced by annual climate; and when *relNb* = 0.3 or 0.6, plasticity compensates for a portion of the divergence in annual climate from the baseline climate.

Temperature-dependant Sex Determination

In this model, we used Girondot's (1999) equation to describe the logistic curve of the Type 1a. TSD reaction norm (Fig. 1):

$$r = \frac{1}{1+e^{-\left(td-\left(T_{piv}\right)\right)slope}} \qquad (2)$$

Where r is the chance of developing as a male, td is the developmental temperature, T_{piv} is the pivotal temperature after heritability variation (H_{var}), and *slope* is the gradient of the logistic curve. Embryo sex was determined by comparing r to a randomly chosen number between 0 and 1. Embryos with higher values of r were designated male and lower values of r were designated female.

Temperature-dependant Survival

The influence of nest temperature on embryonic survival varied between scenarios (Fig. 2). The chance of survival is determined by a probability density function, scaled by a peak survival rate to ensure that survival probability at the optimum temperature = 1. The following equations determine the peak survival rate and survival probability:

$$peakSurv = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(\mu-\mu)^2}{2\sigma^2}} \qquad (3)$$

$$P_{surv} = \frac{\left(\frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(td-\mu)^2}{2\sigma^2}}\right)}{peakSurv} \qquad (4)$$

Where σ is the survival probability constant for the scenario, μ is the mean for the base climate (28°C), and *td* is the developmental temperature of the embryo. We used two levels of σ in the simulation, 1e10 and 3, where σ = 1e10 meant survival was not dependent on temperature and σ = 3 meant survival decreased by 50% when nest temperatures were approximately 3.5°C above or below the optimum temperature (Fig. 2). This survival curve is informed by two reviews of temperature-dependent embryonic survival in reptiles and is an approximate of the curve described for testudines in these studies (Du and Shine 2015; Noble et al. 2021).

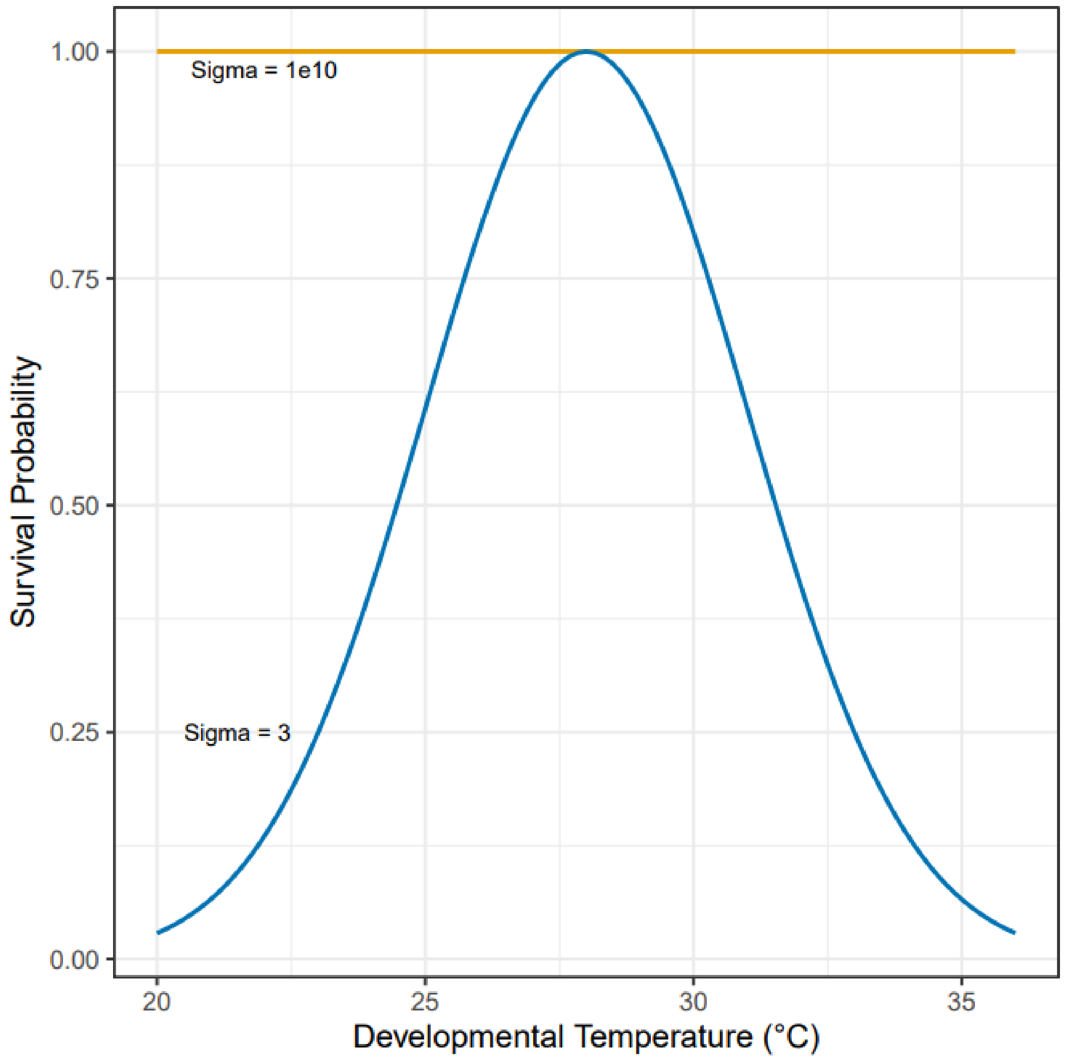

Figure 2. Diagram showing the probability of embryonic survival at different incubation temperatures. Two survival curves with different slopes are shown, the value of sigma determines the slope of the curve. The sigma = 3 and sigma = 1e10 curves are used in the simulation to compare temperature-dependent and temperature-independent survival.

Population Cycle

At the start of each simulation replicate, an initial population of 500 individuals was generated. These individuals developed in the base climate (28°C), and possessed traits adapted to that environment, with some variation (see ‘Genetics and Heritability’ above). The global temperature (T_{glob}) was randomly selected from the three climate scenario options (see ‘Climate’ above).

At the beginning of each reproductive cycle (iteration), the annual temperature (T_{ann}) was randomly selected from a normal distribution with a mean of T_{glob} and a standard deviation of SD_{bw}. Adults in the population then reproduced. Females were assigned a mate, selected randomly with replacement from the male population, and produced a clutch. A female’s clutch size was exponentially related to her own developmental temperature, which created sex-differential fitness in nest temperature, replicating the biological conditions that result in selection for TSD. Additionally, clutch size was limited by the number of males in the population, to prevent unrealistic persistence of a population with an extremely female-biased sex ratio. Clutch size was calculated with this equation:

$$\mathrm{CS} = (F_{scale}(td)^{Phi})(\frac{OSR}{OSR+M_{lim}}) \qquad (5)$$

Where CS is the clutch size, and F_{scale} and Phi are fertility scalers that modify the developmental temperature, td, to produce a clutch size of 10-25 embryos, over nest temperatures of 22°C -35°C. We used Rankin and Kokko’s (2007) male limitation equation, where OSR is the operational sex ratio and M_{lim} is the limiting effect of male population proportion on clutch size. In this simulation, female fertility was only limited when male proportion was below 10% ($M_{lim} = 0.01$). All individuals in the clutch were assigned the same developmental temperature (determined as per ‘Developmental Temperature’ above).

New embryos inherited one maternal and one paternal allele for each trait (T_{piv} and Nb), randomly selected from their parents’ allelic complement. Embryos had a 2% chance to receive mutations on these alleles. Mutant allele values were randomly selected from a normal distribution with the original trait value as the mean, and a standard deviation of 2% of the trait's biologically realistic range. Mutant embryos received mutations on all alleles of traits that were allowed to evolve in the replicate's scenario. Embryos' sex was then determined by their T_{piv} alleles and developmental temperature, as described in "Temperature-dependent Sex Determination" above.

After embryo traits were determined, juvenile mortality occurred. Juvenile survival was dependent on developmental temperature (see "Temperature-dependent Survival" above) and the size of the adult population. Density-dependent survival reflects the carrying capacity of natural ecosystems to avoid unrealistic population growth, and was determined by the following equation:

$$Surv = e^{(-const(n_{Adults}))} \qquad (6)$$

Where *Surv* is probability of survival, *const* is constant modifying the density of adults, and n_{Adults} is the number of living adults present in the population in that iteration. Following juvenile mortality, adult mortality occurred randomly at a fixed rate of 10% of the population per iteration. Surviving juveniles entered the adult population at the end of the iteration, i.e. time to maturity was one iteration. Replicates reached completion after 10000 iterations, or when no males remained in the population.

Model Validation and Sensitivity Analysis

We examined the sensitivity of T_{piv} and *Nb* heritability to different levels of H_{var}, and found that increasing H_{var} decreased narrow sense heritability, as intended. We assessed the effect of H_{var} on the coevolution of T_{piv} and *Nb* and found no clear effect of varying heritability on evolution. In some 28°C climate scenarios, T_{piv} evolved to be significantly warmer than 28°C and nesting behaviour evolved to be warmer than 0°C. As this directional change was unexpected, we conducted a sensitivity analysis a to determine if trait evolution occurred because temperature-dependent fertility promoted evolution of warmer nest temperatures (Appendix C).

The simulation was designed and executed in MATLAB v.2022b with the Statistics and Machine Learning Toolbox add-on (MATLAB 2022). Graphs were produced in R v.4.0.5, using the R interpreter, RStudio v.2022.12.0, and the Tidyverse and Patchwork packages (Wickham et al. 2019; Pederson 2020; R Core Team 2022).

Results

Survival

The population extinction rate (populations that collapsed before 10000 iterations) for solitary evolution trails was 19.1% (38 of 199 runs). All runs that went extinct were from extreme climate scenarios (32°C), the extinction rate was 13.6% (12/88 runs) for T_{piv} evolution scenarios and 23% (26/111 runs) for *Nb* evolution scenarios (see Appendix C, Table S1 for notes on sample size variation). The extinction rate for coevolution scenarios, including replicates with temperature-dependent survival and nesting plasticity, was much lower (0.2%, 1 of 540 runs). Results in the following sections exclude data from extinct populations.

Solitary Evolution

As expected, at 28°C, there was no long-term directional change in T_{piv} or *Nb* (Fig. 3). In 30°C treatments T_{piv} evolved to match the new mean climate (mean of all replicates =

29.97°C, SD = 0.11) (Fig. 3, panel A), while *Nb* only partially compensated for the 2°C rise in temperature (mean of all replicates = -1.69°C, SD = 0.2) (Fig. 3, panel B). In the 32°C treatments neither T_{piv} nor *Nb* were able to fully adjust to the new mean climate. However, T_{piv} had a greater adaptive capacity, evolving more than 3°C upwards, while *Nb* only evolved 2°C downwards (T_{piv} mean = 31.13°C, SD = 0.26; *Nb* mean = -2.00, SD = 0.45) (Fig. 3). Variation in heritability had no apparent effect on the evolution of T_{piv} or *Nb* in solitary evolution scenarios (Fig. 3).

Co-evolution

In all temperature scenarios there was a strong negative correlation between T_{piv} evolution and *Nb* evolution (note that the trend appears positive on graphs because *Nb* becomes more negative when adapting to warmer climates) (Fig. 4). Populations with greater evolution of T_{piv} had reduced *Nb* evolution and vice versa. In warming scenarios most replicate populations were able to fully compensate for the difference between the environment they were originally adapted to and the new warmer climate, acquiring a combined 2°C (30°C scenarios) or 4°C (32°C scenarios) change in temperatures (Fig. 4, panels B,C, Table 2). T_{piv} evolution accounted for the majority of this compensation (Fig. 4, datapoints are closer to the black target than to the red target). Some replicates showed nearly complete compensation via T_{piv} evolution alone, while other replicates had reduced compensation by T_{piv} and increased *Nb* compensation. In 30°C scenarios, the complete temperature compensation of T_{piv} and *Nb* in combination was equal to the compensation of T_{piv} in solitary evolution scenarios (Fig. 4, panel B). However, in 32°C scenarios, the combined complete compensation of T_{piv} and *Nb* exceeded the incomplete compensation of either factor in the solitary evolution trials (Fig. 4, panel C). Therefore, populations with T_{piv} and *Nb* co-evolving were better adapted to extreme temperatures than populations where only one trait could evolve.

Unexpectedly, replicates in 28°C climates saw a rise in T_{piv} and *Nb* (Fig. 4, panel A). This trend was not observed when temperature-dependent fecundity was removed from the simulation (see Appendix C for sensitivity analysis). As in the solitary evolution trials, there was no observable effect of heritability variation on T_{piv} or *Nb* evolution (Fig. 5, top row, also see sensitivity analysis in Appendix C).

Temperature-dependent Survival

Introducing temperature-dependent survival shifted the balance of the co-evolutionary relationship between T_{piv} and *Nb* in the favour of increased evolution of nesting preferences (Fig. 5, middle row). When incubation temperature influenced offspring survival, the contribution of *Nb* evolution increased in comparison to T_{piv} evolution (Fig 5. panels E, F, Table 2.). Changes in heritability did not influence T_{piv} or *Nb* evolution in the temperature-dependent survival scenarios (Fig. 5, top row).

Plasticity in Nesting Behaviour

When nesting behaviour was plastic in response to annual climate, the overall evolutionary compensation for temperature increase by T_{piv} and *Nb* was reduced (Fig. 5, panels H, I, Table 2). The level of plastic compensation for temperature rise was directly related to the degree of reduction in T_{piv} and *Nb* evolution. When *relNb* = 0.3, plastic nesting behaviour compensates for 30% of the difference between T_{base} (28°C) and T_{ann}. This level of plasticity lead to a 31% and 28% decrease in the average combined compensation of T_{piv} and *Nb*, in the 30°C and 32°C scenarios, respectively (Table 2). At *relNb* = 0.6 compensation for annual temperature variability increases to 60%. This level of plasticity lead to an average combined compensation decrease of 60% and 58%, in the 30°C and 32°C scenarios (Table 2).

Adaptive nesting plasticity had a greater negative influence on Nb evolution than T_{piv} evolution. At 30°C nesting behaviour plasticity significantly reduced the potential for Nb evolution and had a small hindering effect on T_{piv} evolution (Fig. 5, panel H, Table 2. At 32°C the differing effect of plasticity on T_{piv} and Nb evolution was less dramatic, although Nb evolution was still more strongly affected by nesting behaviour plasticity (Fig. 5, panel I, Table 2). H_{var} value did not influence T_{piv} or Nb evolution in the nesting plasticity scenarios (Fig. 5, top row).

Table 2. Mean pivotal temperature, nesting behaviour, individual, and combined compensation values for nesting behaviour plasticity scenarios, temperature-dependant survival scenarios, and scenarios where these variables interacted. Combined compensation is the total adjustment of T_{piv} and nesting behaviour.

Global Temperature (°C)	**Nesting Plasticity (*relNb*)**	**Temperature-dependent Survival**	**Trait**	**Final Value (°C)**	**Individual Compensation (°C)**	**Combined Compensation (°C)**
28	0	No	T_{piv}	28.61	0.61	-0.05
			Nb	0.66	-0.66	
		Yes	T_{piv}	28.34	0.34	0.02

			Nb	0.32	-0.32	
	0.3	No	T_{piv}	28.58	0.58	-0.01
			Nb	0.59	-0.59	
		Yes	T_{piv}	28.24	0.24	0.01
			Nb	0.23	-0.23	
	0.6	No	T_{piv}	28.42	0.42	0.00
			Nb	0.42	-0.42	
		Yes	T_{piv}	28.26	0.26	0.00
			Nb	0.26	-0.26	
30	0	No	T_{piv}	29.52	1.52	1.98
			Nb	-0.46	0.46	
		Yes	T_{piv}	28.7	0.7	2.00
			Nb	-1.3	1.3	
	0.3	No	T_{piv}	29.41	1.41	1.36
			Nb	0.05	-0.05	
		Yes	T_{piv}	28.47	0.47	1.43
			Nb	-0.96	0.96	
	0.6	No	T_{piv}	29.06	1.06	0.81
			Nb	0.25	-0.25	
		Yes	T_{piv}	28.35	0.35	0.81
			Nb	-0.46	0.46	
32	0	No	T_{piv}	30.38	2.38	3.90
			Nb	-1.52	1.52	
		Yes	T_{piv}	29.66	1.66	3.98
			Nb	-2.32	2.32	
	0.3	No	T_{piv}	29.72	1.72	2.79
			Nb	-1.07	1.07	
		Yes	T_{piv}	29.1	1.1	2.82
			Nb	-1.72	1.72	
	0.6	No	T_{piv}	29.43	1.43	1.62
			Nb	-0.19	0.19	
		Yes	T_{piv}	28.44	0.44	1.63
			Nb	-1.19	1.19	

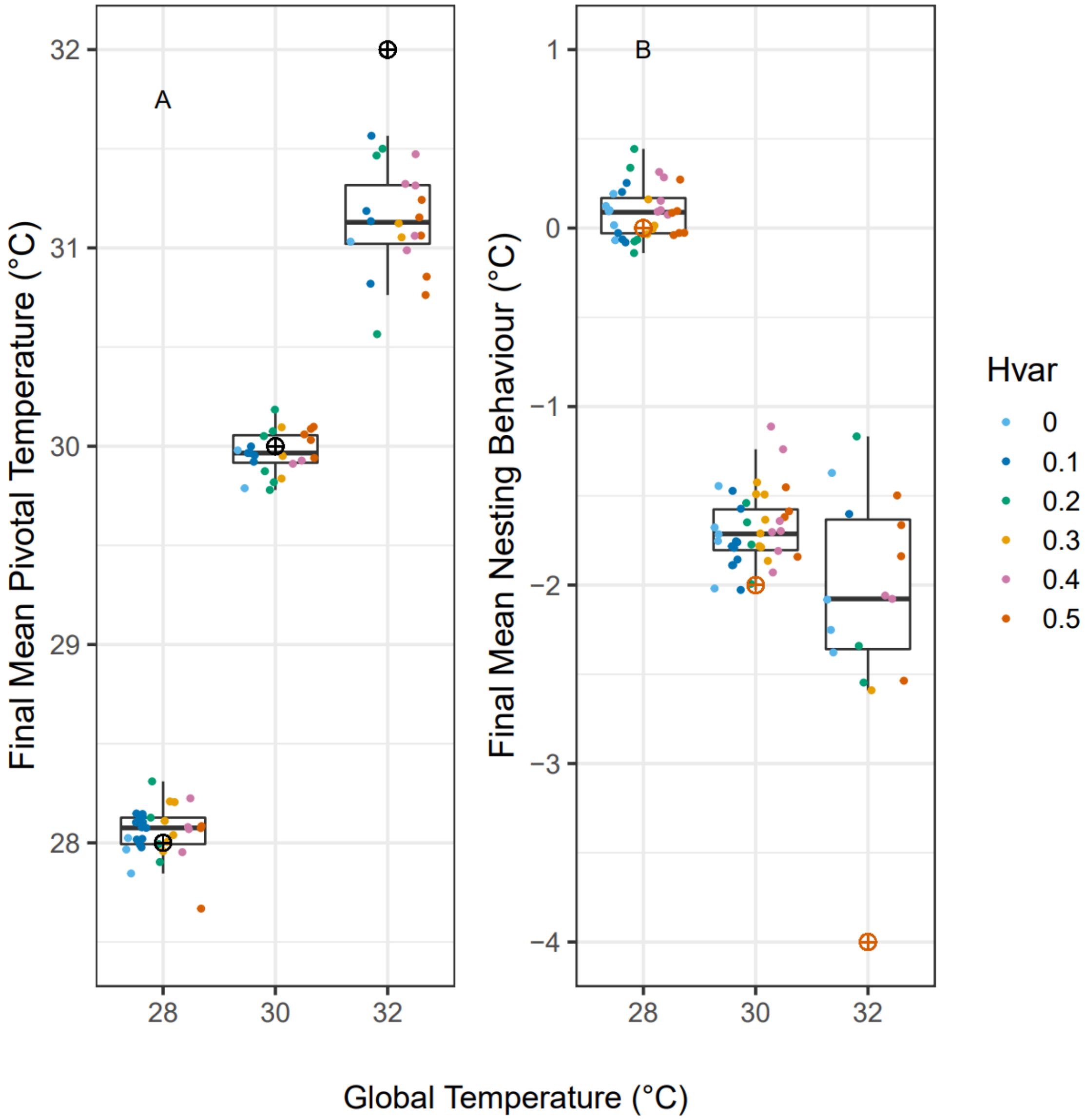

Figure 3. Final values for traits in the solitary evolution sceanrios (mean value of the last 1000 generations), grouped by climate scenario. H_{var} is the variation in trait heritability, higher values correspond to lower heritability (see Table 1.). Panel A. shows final mean T_{piv}, the starting value T_{piv} is 28°C. Populations with pivotal temperatures that equal the mean temperature of the environment are well adapted to their climate scenario. The black target symbols indicate the value of T_{piv} that would fully compensate for the Panel B. shows final mean *Nb*, the starting value of *Nb* is 0°C. Populations with nesting behaviours that result in nest temperatures equal to 28°C (eg. -2°C in a 30°C climate) are well adapted to their environment. scenario temperature rise, and the red target symbols indicate the equivalent value for *Nb*

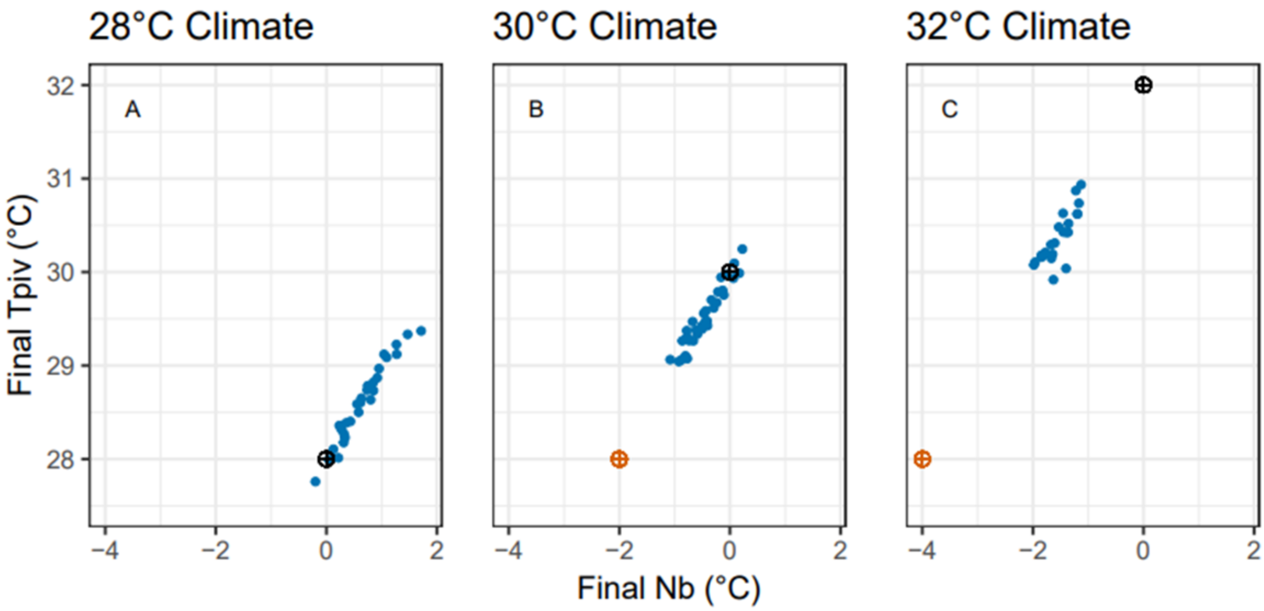

Figure 4. Comparison of the final values of coevolving T_{piv} (y-axis) and Nb (x-axis), in three climate scenarios. The black target symbols indicate the value of T_{piv} that would fully compensate for the scenario temperature rise, and the red target symbols indicate the equivalent value for Nb. The results presented here have varying values of H_{var}, which are visualised in Fig. 5. However, as H_{var} did not influence the coevolution of T_{piv} and Nb, the replicate final means have been presented without distinction here.

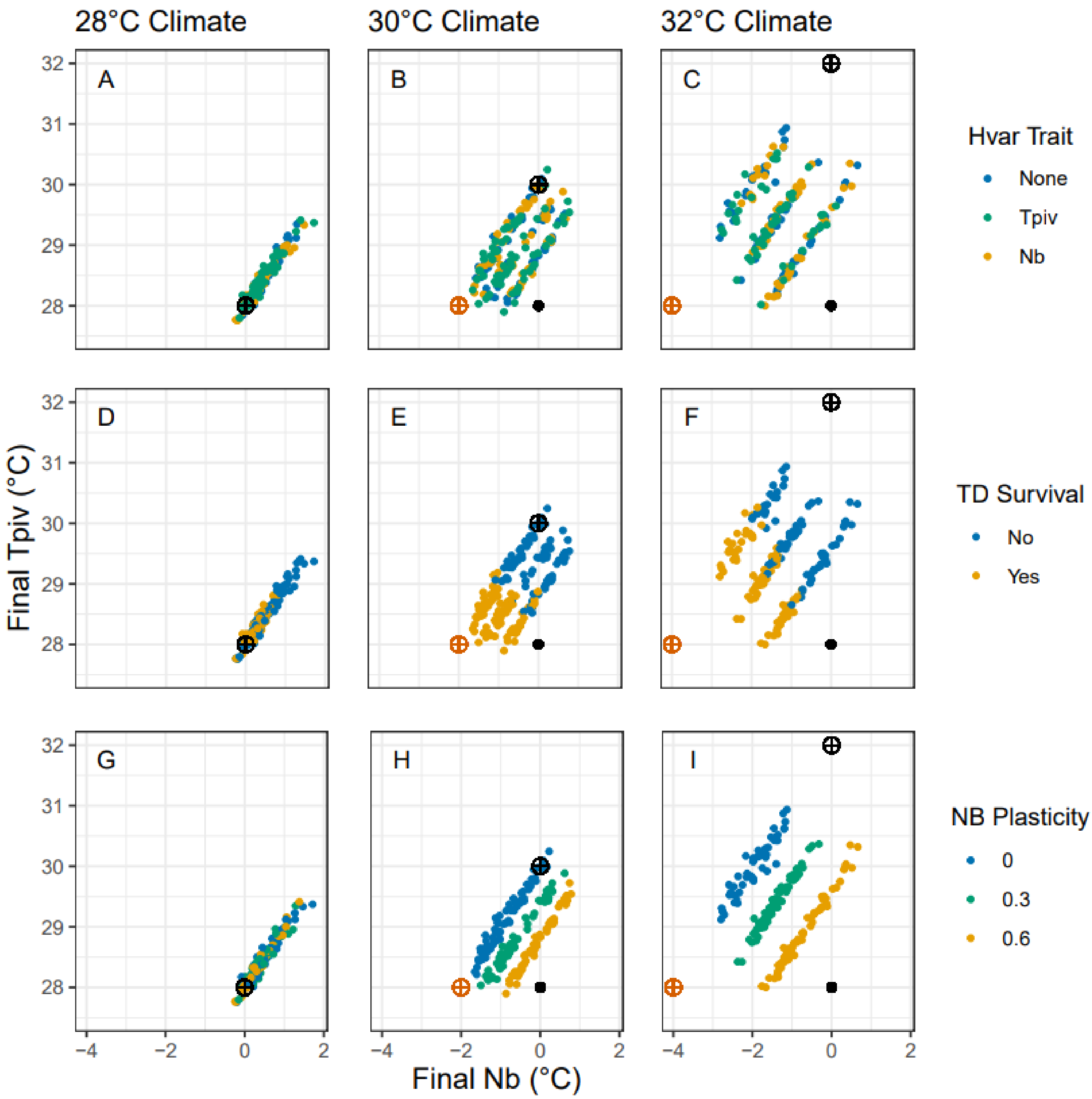

Figure 5. Comparison of the final values of coevolving T_{piv} (y-axis) and *Nb* (x-axis), showing the influence of relative heritability (top row, colour reflects which trait had reduced heritability), temperature-dependent survival (middle row), and nesting behaviour plasticity (bottom row). The same data for each climate scenario (columns) is presented in each row, including the coevolution data from Fig. 4, but different independent variables have been highlighted. The large black points on the 30°C and 32°C graphs indicate the starting value of T_{piv} and *Nb* in these scenarios. The black target symbols indicate the value of T_{piv} that would fully compensate for the scenario temperature rise, and the red target symbols indicate the equivalent value for *Nb*.

Discussion

Temperature has an enormous influence over individual phenotypes and population dynamics in species with TSD, yet these species have adapted to occupy a broad range of climates (Ewert et al. 2005; Refsnider et al. 2014; Carter 2019). In this study, we examined the relative evolvability of the pivotal temperature and maternal nesting behaviour in TSD species' adaptation to environmental change. The capacity of T_{piv} to adapt to changes in temperature is greater than that of *Nb*, though *Nb* does contribute to adaptation to the new environment. However, if embryos experience reduced survival at extreme temperatures, then selection favours behaviour that moderates nest temperatures. Conversely, if individuals with TSD are able to respond to seasonal shifts in climate by changing their nesting behaviour, then selective pressure on underlying nest preferences is reduced.

When all other factors were equal, T_{piv} possessed a greater potential for adaptation to rising temperatures than *Nb*. This conclusion is supported by a previous model that compared the adaptive capacity of T_{piv} and *Nb*, and by estimations of high relative heritability for T_{piv} in comparison to *Nb* (Rhen and Lang 1998; Morjan 2003b; McGaugh et al. 2010; McGaugh and Janzen 2011; McGaugh et al. 2011). In contrast, some studies have reported low effective heritability values for T_{piv} that should reduce its response to selective pressure (Bull et al. 1982; Janzen 1992). One possible explanation for the discrepancy is that the effective heritability of T_{piv} has often been underestimated (McGaugh and Janzen 2011; McGaugh et al. 2010). Additionally, *Nb* heritability is environmentally-specific due to variation in expression with the environment, and therefore challenging to measure (McGaugh et al. 2010). Though T_{piv} and *Nb* had different narrow sense heritability when no H_{var} was applied (see Appendix C), we found that varying the relative heritability of T_{piv} and *Nb* did little to shift the balance of their co-evolution. Possibly the sex-specific expression of *Nb* reduces its adaptive capacity. T_{piv} is expressed by the individual whose fitness will be directly affected by T_{piv}, while *Nb* is expressed by the mother who will only pass one of her alleles on to the impacted offspring. As a result, the correlation between parent and offspring *Nb* phenotypes is reduced compared to the parent/offspring T_{piv} correlation, diminishing the power of *Nb* to evolve (Morjan 2003b).

Despite the relatively reduced capacity for *Nb* to adapt to environmental change when compared to T_{piv}, *Nb* does play an important role in this coevolutionary relationship. The presence of *Nb* has a neutral effect when warming is moderate, as T_{piv} can completely compensate for 2°C warming in solitary evolution, but in extreme warming scenarios the presence of behavioural adaptations has a positive effect as the combined compensatory effect of T_{piv} and *Nb* is greater than T_{piv} evolving in isolation. Given the evidence of significant geographic variation in wild nesting behaviours which suggest that *Nb* plays a crucial role in local adaptation of TSD species, the reduced adaptive role of *Nb* in our simulation is surprising (Janzen and Morjan 2001; Morjan 2003a; St. Juliana et al. 2004; Ewert et al. 2005; Doody 2009; Mitchell and Janzen 2010; Refsnider and Janzen 2012; Refsnider et al. 2014). The discrepancy can be resolved by considering how selective pressures other than sex ratio selection, such as temperature-dependent survival, influence the evolution of *Nb*.

While temperature-dependent survival of embryos has been identified as a potential driver of nesting behaviour in empirical studies, it's influence on the coevolution of T_{piv} and *Nb* is not well understood (Ewert et al. 2005; reviewed in Refsnider and Janzen 2010). When we included thermal limits on the viability of embryos in the model, the evolution of *Nb* became more important to adaptation to rising temperatures, in some cases exceeding the contribution of T_{piv}. This is significant because embryonic survival is influenced by temperature in many species, including those with TSD (Schwarzkopf and Brooks 1987; Weisrock and Janzen 1999; Leslie and Spotila 2001; Ewert et al. 2005; Warner and Shine 2009; Refsnider and Janzen 2010; Du and Shine 2015; Noble et al. 2021). The presence of temperature-dependant survival places a limit on the capacity of T_{piv} evolution to balance sex ratios, as pivotal temperatures beyond the range of embryonic survival cannot produce live offspring of the rare sex. The only alternative for TSD species is to evolve cooler nests, placing increased selective pressure on *Nb*. This extra pressure on *Nb* may be additionally increased if we took into account non-lethal negative effects of incubation temperature on offspring fitness (Burger 1989; Brooks et al. 1991; Van Damme et al. 1992; Shine and Harlow 1996; reviewed in Refsnider and Janzen 2010).

In addition to sex ratio selection and embryonic survival, nesting behaviour is driven by other selective pressures that may not stabilise sex ratios (Refsnider and Janzen 2010). For example, evidence from simulation modelling suggests that natal philopatry reduces the capacity of nesting behaviour to respond to sex ratio selection (Morjan 2003b). It is also possible that conflict exists between nesting mothers and their offspring, for instance, nest sites that promote maternal survival (e.g. safer or easier sites) may not optimise offspring sex (Refsnider and Janzen 2010). Where sex allocation is condition dependent, as is the case in TSD, parent-offspring conflict can influence sex ratio evolution, as offspring evolve to resist parental manipulation of sex ratios (Eshel and Sansone 1994; Kuijper and Pen 2014). It cannot be expected that all selective forces on nesting behaviour will stabilise sex ratios, and resultant parent-offspring conflict could affect the evolution of sex determination in TSD species.

Our model provides additional evidence to the argument that phenotypic plasticity can reduce the capacity for adaptive evolution (Sultan 1987; Sultan 1995; Sultan 2000;

Huey and Kingsolver 1993; Huey et al. 2003; Ghalambor 2007). In particular, fixed nest preferences become far less important in adaptation to rising temperatures when nesting behaviour is allowed to respond to annual climate fluctuations, as nesting plasticity compensates for temperature increase. To understand the impact of this result, it is necessary to quantify the extent of plasticity in the nesting behaviour of species with TSD. There is a great deal of individual, seasonal, and geographic variation in the nesting behaviours of TSD species. Maternal choice of nest timing, vegetation cover, depth, moisture, and substrate conditions vary across a species' geographic range, and in many cases these aspects of nest site choice predict nest temperature (Morjan 2003a; Ewert et al. 2005; Doody et al. 2006; Doody 2009; Telemeco et al. 2009; Mitchell and Janzen 2010; Refsnider and Janzen 2012; Somaweera and Shine 2013; Mitchell et al. 2013; Refsnider et al. 2014; Delaney et al. 2020). However, this variation could result from local adaptation in populations experiencing different climates, rather than plasticity (Refsnider and Janzen 2012). Several studies have demonstrated variation in nest timing and depth with annual changes in climate over a short time scale, suggesting a plastic response to seasonal changes (Telemeco et al. 2009; Schwanz and Janzen 2008; Mitchell and Janzen 2010; Somaweera and Shine 2013). There is debate over whether plasticity can compensate for current rates of climate warming, particularly at extreme temperatures (Doody 2006; Telemeco et al. 2009, Telemeco et al. 2013). This model, and previous work in this thesis suggest that while adaptive plasticity limits evolution, this is only due to the stabilising effect plasticity has on sex ratios, which likely benefits population persistence under environmental change (see Chapter 2 of this thesis).

The presence of coevolution means that T_{piv} and *Nb* are both likely to play a role in any adaptive response of TSD species to rising temperatures. There is an elegant balance between these two traits. When one is shielded from selection, the other increases its pace of adaptation, driven by the strength of negative frequency-dependent selection. This balance allows for multiple pathways toward stable sex ratios, which, while promising for species survival, may complicate comparisons between populations, as single trait measurements are unlikely to provide a complete picture of a species' adaptive strategy. The simultaneous evolution of multiple traits could promote speciation, as separate populations could evolve different adaptations to address sex ratio imbalance. This trend which may occur not just in TSD species, but in other cases of complex trait evolution (Endler 1995). Broadly, our results highlight the challenge of understanding how species adapt to new environments. When populations encounter novel conditions, changes in multiple traits will contribute to species adaptation. These coevolving traits will be influenced by plastic responses to the environment, producing a complex web of interactions that will determine the new direction of the species.

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Chapter 5

General Discussion and Conclusion

Claudia Crowther

Introduction

Phenotypic plasticity is a ubiquitous force that shapes the responses of organisms to their environment across the biosphere (Sultan 2021). Much attention has been directed at the capacity of plasticity to facilitate species' survival through rapid anthropogenic change (Chevin et al. 2010; Crispo et al. 2010; Diamond and Martin 2016; Fox et al. 2019). However, we know far less about how plasticity influences evolution in the long term. Though plasticity may drive the quick development of new phenotypes, the effects of plastic expression are not short lived. Populations that exist today are likely still influenced by plastic expression that occurred during past incidents of environmental upheaval, and it is predicted that current climate change will produce new phenotypes that may affect the fate of species long into the future (Sih et al. 2011; Merlia and Hendry 2014). In order to understand how the species we observe today came to be and predict what may become of them in the future, we must better understand how plasticity influences evolution.

In particular, our understanding of the evolution of discrete plastic traits is lacking. Despite their prevalence, traits that switch between distinct phenotypes in response to environmental cues are still under-investigated (Wright 1934; Charnov and Bull 1977; Moczek and Emlen 1999; Ostrowski et al. 2000; Wells and Pigliucci 2000; Suzuki and Nijhout 2006; Kamakura 2011; Chevin and Lande 2013; Germain et al. 2018; Debes et al. 2020). In this thesis I examined the role of plasticity in the evolution of environmental sex determination (ESD), a discrete trait of great importance to the survival and fitness of a wide range of species (Charnov and Bull 1977; Valenzuela and Lance 2004; Beukeboom and Perrin 2014; Schwanz and Georges 2021). I focused specifically on temperature-dependent sex determination (TSD), a common form of ESD, where the temperature of embryonic development permanently determines an individual's sex. I developed simulation models to test how the evolution of TSD is influenced by plasticity in key targets of sex ratio selection: pivotal temperature (T_{piv}), transitional range of temperatures (*TRT*), and nesting behaviour (*Nb*). In addition, I employed data from a long-term monitoring program to investigate plasticity in a wild population with TSD. My findings demonstrate that the impact of plasticity on the evolution of TSD is strongly shaped by interactions with sex ratio selection. Furthermore, my investigation revealed the capacity of plasticity to control the strength of selective pressure on genotypes. In this final chapter, I will review my findings, first considering each investigation in isolation, and then exploring the implications of my research from a holistic perspective.

Chapter Overview

In Chapter 2, I aimed to determine how phenotypic plasticity influences the evolution of environmentally sensitive discrete traits, such as the male/female switch in species with TSD. I developed an individual-based simulation model to compare how plasticity in the switch point of sex determination (T_{piv}) and the slope of the TSD reaction norm (*TRT*) affect the adaptation of TSD species to novel environments. I found that these two forms of plasticity have different effects on the evolution of T_{piv}, and that this difference was due to their opposite effect on sex ratios. T_{piv} plasticity mitigated sex-ratio bias, reducing selective pressure on T_{piv}, while slope plasticity increased sex-ratio bias and promoted the evolution of T_{piv} in the direction of the new environmental mean. Plasticity that promoted population survival hindered evolution of T_{piv}, while plasticity that increased the rate of T_{piv} evolution was associated with population extinction. Surviving populations achieved stable sex ratios, either through T_{piv} evolution, evolution of increased T_{piv} plasticity, or loss of slope plasticity (transition to genotypic sex determination). This study demonstrates that plasticity can significantly influence the evolution of discrete traits, and that the effect of plasticity on selection pressure determines if trait evolution will be promoted or prevented. Additionally, the extent of environmental change and the genetic composition of a population with TSD will determine the ability of that population to adapt, and the traits that contribute to adaptation.

In order to anticipate the potential evolutionary outcomes of TSD species, simulation results must be interpreted in the context of data from wild populations. To this end, in Chapter 3, I evaluated plasticity in the T_{piv} and *TRT* in a species with TSD. Theory suggests that traits that can influence sex ratios, such as the T_{piv} and *TRT*, will evolve to promote production of the rarer sex (Bulmer and Bull 1982; Bull and Charnov 1988; Schwanz and Georges 2021). The results of Chapter 2 suggest that plasticity in these traits affects sex ratios, and subsequently evolution of the TSD reaction norm. However, it is unclear whether species with TSD have the capacity to detect and respond to environmental fluctuation via plasticity in the T_{piv} and *TRT*. Firstly, this study aimed to investigate evidence that T_{piv} adaptively responds to climate fluctuations in a population of the freshwater turtle, *Chrysemys picta,* and determine if a similar response exists in *TRT* width. I found that the T_{piv} and *TRT* did not covary with annual climate in an adaptive direction when I used constant temperature equivalent as the measure of nest temperature. However, I did detect significant interannual T_{piv} variation. Secondly, I aimed to determine if the annual variation I observed in T_{piv} covaried with two proposed mechanisms of plastic response in TSD species, maternal age, and egg size. I found that maternal body size, a proxy for age, had a marginally non-significant negative influence on the proportion of male offspring produced, which warrants further investigation.

Overall, these results suggest that while plastic variation the TSD reaction norm may be observable under controlled conditions, it is difficult to detect in the wild. Additionally, variation in TSD traits may be non-adaptive, or responding to pressures other than sex ratio selection. Maternal behaviour that changes nest timing or nest temperature variability, rather than maternal physiology, may drive adaptation in species with TSD.

In Chapter 4, I aimed to determine which traits are most likely to adapt when TSD species experience environmental conditions that bias sex ratios and examine how plasticity influences these traits. I created an individual-based model to simulate the coevolution of two targets of sex ratio selection in TSD species: pivotal temperature (T_{piv}) and maternal nesting behaviour (*Nb*). I found that T_{piv} had a greater capacity to adapt to environmental change than *Nb*, due to sex-specific expression reducing *Nb* heritability. This investigation was the first to simulate how two important influences on phenotype in TSD species, temperature-dependent survival and nesting behaviour plasticity, affect the coevolutionary relationship between T_{piv} and *Nb*. I found that when embryonic survival depended on temperature, the contribution of *Nb* to adaptation increased. I hypothesise that this change in the coevolutionary relationship occurred because low survival rates at high temperatures placed a cap on T_{piv} evolution. This investigation also demonstrated that plasticity in *Nb* dampens selection on underlying nest preferences and reduces the adaptive capacity of this trait. As in Chapter 2, surviving populations were able to achieve stable sex ratios through a combination of trait adaptations. Taken together, these results demonstrate that plastic responses to environmental conditions, such as variation in phenotype or survival, have a great deal of influence on the process of adaptation to novel environments. Furthermore, my results show how sex ratio selection interacts with other selective pressures, such as viability selection, in TSD species.

The Diverse Responses to Sex Ratio Selection

Sex ratio selection is central to the ecology and evolution of TSD species, however, they can respond to this selective pressure in different ways. In Chapters 2 and 4, I demonstrate that T_{piv}, T_{piv} plasticity, *TRT* plasticity, and *Nb* are viable targets for sex ratio selection. These traits are capable of coevolving in response to environmental conditions that induce sex ratio biases, and adaptation may be more effective when co-evolution occurs. The diversity of potential responses to selection that I observed in my investigations reflects the known variation of wild populations. Species with TSD display seasonal and geographic variation in reaction norm parameters, such as the T_{piv} and *TRT*, and in nesting characteristics such as clutch number, nest timing, depth, shading, soil moisture, and substrate type (Morjan 2003; Ewert et al. 2005; Tucker et al. 2008; Telemeco et al. 2009; Mitchell and Janzen 2010; Schwanz et al. 2010a; Refsnider and Janzen 2012; Somaweera and Shine 2013; Refsnider et al. 2014; Carter et al. 2019). Because of the diversity of potential targets of sex ratio selection, responses to environmental change are likely to vary between populations and species with TSD, and include both the innovation of new traits and plastic variation in trait expression.

The array of potential avenues by which TSD species may respond to environmental change complicate the process of determining the specific mechanisms of adaptation. For example, in Chapter 3, I found that evidence for adaptive plasticity in the T_{piv} of *C. picta* varied depending on the measure of nest temperature used in the analysis. It is possible that the T_{piv} of this population varies with annual climate in a direction that balances sex ratios. However, an alternate explanation is that variation in nest timing caused the difference between the temperature measures, and that nesting phenology may be responding plastically to sex ratio selection in this species. The results of my simulation models in Chapters 2 and 4 suggest that environmental conditions and existing trait distributions within populations determine which traits contribute to the adaptation of TSD species to novel environments.

The Balance Between Adaptive Plasticity and Trait Innovation

In this thesis, I present several lines of evidence that a trade-off exits between adaptive plasticity and trait innovation in the TSD reaction norm. I hypothesise that this trade-off occurs because adaptive plasticity generates beneficial phenotypes, even when historical phenotypes are poorly adapted. This shields poorly adapted genotypes from selection, allowing these genes to persist in the population. When initiated by behavioural plasticity, this process is called the Bogert effect, or behavioural inertia, (Bogert 1949, 1959; Huey et al. 2003; Muñoz 2021). Additionally, the presence of many individuals with well-adapted plastic phenotypes reduces the fitness benefit of new mutations in underlying traits. These forces combine to reduce trait innovation in populations with adaptive plasticity. In Chapter 2, I demonstrated that adaptive plasticity in T_{piv} hinders the evolution of novel T_{piv} genotypes. Similarly, in Chapter 4 the presence of nesting behaviour that adjusted nest temperatures in response to climate fluctuations hindered the evolution of underlying nest preferences. It is likely that plastic expression varies considerably between TSD populations, and as a result we should expect to see a variety of adaptive responses to environmental change (Telemeco et al. 2009; Schwanz and Janzen 2008; Mitchell and Janzen 2010; Somaweera and Shine 2013; Telemeco et al. 2013). Additional investigations into plasticity in real populations, particularly focusing on less studied taxa, would improve our understanding of the role of plasticity in the evolution of TSD.

In Chapter 2, I noted that extinction is a potential outcome if species invest in plasticity that promotes short term survival over long-term trait innovation. However, my results show that the outcome of this conflict depends on the adaptive capacity of plasticity itself. In Chapter 2, when plasticity in T_{piv} and fixed T_{piv} genotypes coevolved, both traits evolved to the degree necessary to compensate for sex ratio bias. In Chapter 4, T_{piv} and *Nb* evolution was hindered by adaptive plasticity because that plasticity balanced sex ratios, meaning that additional change in T_{piv} and *Nb* was not beneficial to individual fitness. Evidently the presence of variation in plasticity between traits, individuals, populations, and species is evolutionarily significant. However, the extent and causes of this variation are not fully understood. The evolution of plasticity appears to be favoured in variable environments where individuals maximise their fitness by adjusting their phenotype to suit the current environment. However, if environmental stochasticity is too great, then predicting the optimal phenotype becomes impossible, and species may evolve fixed, generalist phenotypes over plastic expression (Moran 1992; Snell-Rood and Ehlman 2021). Plasticity may also evolve when phenotypes have relatively low fitness in their current environment. If a population experiencing strong directional selection needs to cross a fitness valley to reach a more optimal phenotype, then plastic expression that jumps to the next fitness peak will be favoured (Gavrilets and Scheiner 1993; Lande 2009; Chevin and Lande 2010; Chevin et al. 2013; Snell-Rood and Ehlman 2021).

Considering the influence of environmental variation and strong directional selection, I describe four conditions that I predict will promote the evolution of adaptive plasticity in traits that influence offspring sex in TSD species:

1. Generational-scale climate variability

Climate variability that occurs on a scale comparable to the reproductive cycle of a species should promote the evolution of adaptive plasticity, as the offspring's developmental environment is a better indicator of future population sex ratios than the sex ratio of the parents' generation. In this scenario it is better to respond plastically to cues from the current environment, than rely on genes that were successful in a previous environment.

2. High mortality rate

The predictive power of the developmental environment is reduced when generations can interbreed because individuals from different developmental environments are influencing the population sex ratio (Bull and Bulmer 1989; van Dooren and Leimar 2003; Schwanz et al. 2010a). High mortality and quick maturation reduce generational overlap and maintain the correlation between the developmental and reproductive environments, and therefore should promote the evolution of adaptive plasticity.

3. Steep reaction norm slope

When the reaction norm slope, or *TRT*, is steep, there is very little room for genetic variation in T_{piv} to be expressed (Hulin et al. 2009). Depending on the degree of warming, gradual evolutionary change in T_{piv} may not result in changes to individuals' sex, and therefore provide no selectable variation in T_{piv} phenotypes. In this case, adaptive plasticity would be favoured over genetic change in T_{piv}, as it could allow the rapid production of a beneficial phenotype (i.e. the rare sex). This may be a unique property of discrete traits evolving in response to a continuous environmental variable.

4. Rapid warming

> The faster the climate warms, the less likely that genotypic change in traits that influence sex can match the pace of warming, and the stronger directional selection on these traits becomes. Adaptive plasticity is favoured under these conditions as it can respond faster to environmental change than trait innovation can arise.

The costs of plasticity would influence any trade-off between plasticity and trait innovation. In Chapters 2 and 4, I modelled plasticity as a cost-free process; however, there is evidence that adaptive plasticity can incur costs to plastic individuals (Auld et al. 2010; Snell-Rood et al. 2010; Murren et al. 2015; Snell-Rood and Ehlman 2021; Bowden and Paitz 2021). The mechanisms by which organisms detect environmental variation and implement a phenotypic response are likely to involve an investment of resources that will only pay off if sufficient phenotypic shift can be achieved. For example, the difference in findings between my wild survey of the impact of egg resource allocation on sex ratios and artificial egg manipulation studies may be due to the high cost or low benefit of expressing this trait in nature (Bowden and Paitz 2021, Chapter 3).

While trade-offs between adaptive plasticity and trait innovation may affect the genotypic composition of a population, it is phenotypes that ultimately determine survival and reproductive success. Individuals that respond to their environment through a combination of plastic and genotypic adaptations may be just as, if not more likely, to survive environmental change.

The Negative Impacts of Plasticity on Population Survival

My results show that forms of plasticity that increase selective pressure on T_{piv} also reduce the chances of population survival. Strong relationships between temperature and sex (Chapter 2) or survival (Chapter 4) had the potential to reduce population persistence. Essentially, these plastic relationships exert additional selective pressure on T_{piv} or *Nb*, such that most individuals experience significantly reduced fitness (i.e., lacking mates) or cannot survive at all. This pressure drives a rapid change in the proportion of alleles in the population and the proliferation of any beneficial mutations that arise. However, the likelihood of a wild population surviving this extreme bottleneck is uncertain. In Chapter 2, simulated populations with steep TSD slopes experienced very high rates of extinction, especially when they were exposed to extreme levels of warming. In Chapter 4, most populations with temperature-dependent survival persisted; however, this is likely due to increased adaptive capacity arising from the coevolution of T_{piv} and *Nb*. My results suggest that genetic diversity and mutation rate will influence population survival under heavily biased sex ratios. Populations that persist through a period of extreme sex ratio bias are likely to experience reduced genetic diversity, and consequently, increased extinction risk (Mitchell and Janzen 2010). Additionally, the circumstances of adaptation may influence the chances of population survival. Evidence from simulation modelling suggest that sub-populations with biased sex ratios can persist if sufficient source populations for the rare sex are present (Harts et al. 2014; Butka and Freedberg 2018). TSD populations migrating to new environments may have many chances to colonise an area without eliminating the source population, while populations experiencing change in their current environment may be at greater risk, as all individuals are being subjected to extreme pressure simultaneously.

The Strength and Limits of Parental Control

An organism's developmental environment can have an enormous influence on its phenotype that may continue throughout its life (Refsnider and Janzen 2010; Du and Shine 2015; Noble et al. 2018; Noble et al. 2021). Parents may be able to improve offspring survival and fitness by altering the conditions that their offspring experience early in life (Refsnider and Janzen 2010). Because parents can control offspring phenotype, parental physiology and behaviour seem to be likely targets of sex ratio selection in TSD species (Bowden et al. 2000; Bowden et al. 2001; Janzen and Morjan 2001; Elf 2003; Bowden et al. 2004; Doody et al. 2004; Doody et al. 2006; Warner and Shine 2008; Telemeco et al. 2009; Schwanz et al. 2010b; Mitchell et al. 2013; Refsnider et al. 2014; Bowden and Paitz 2021). The results of Chapter 4 suggest that when parents can exert control over offspring sex in TSD species, these effects can respond to selection. Though nesting behaviour had a relatively lower adaptive capacity than pivotal temperature, it did play a role in adaptation to novel environments, and was particularly important under extreme climate change. In contrast, I did not find strong evidence that parental physiological traits exert adaptive control over clutch sex ratios in my investigation of a wild population in Chapter 3. This thesis does not argue that parental effects are unimportant in the evolution of sex determination, merely that they are difficult detect in real populations, due to the many other potential influences on offspring sex.

Because parental effects can influence many aspects of offspring phenotype, parental traits of TSD species may be responding to other selective pressures in addition to sex ratio selection. In both Chapter 3 and 4, I detected evidence that nesting behaviour could influence sex ratios in an adaptive direction. However, the decision to nest in a cooler site during a hot year could be driven by selection for offspring survival or phenotypic outcomes other than sex. In Chapter 4, introducing temperature-dependent survival in embryos drove evolution of cooler nest site choice. Previous studies reflect this finding, suggesting that maternal nest site choice optimises embryonic survival and varies between populations, while T_{piv} is more geographically consistent. (Schwarzkopf and Brooks 1987; Weisrock and Janzen 1999; Leslie and Spotila 2001; Ewert et al. 2005; Refsnider and Janzen 2010; Refsnider and Janzen 2014; but see Mitchell et al 2013). Parental effects have the potential to influence the evolution of sex ratios in TSD species, but they are difficult to observe and the ultimate selective forces driving these behaviours may be more complicated than simply selection for the rare sex.

Conclusion

Despite their potentially precarious ecology, species with environmental sex determination have shown a remarkable ability to adapt to changing environments across time. In this thesis, I have explored the role of plasticity in the evolution of species with ESD and revealed how interactions between plasticity and sex ratio selection determine how these species adapt to new environments. My investigations show that plasticity can shield genotypes from selection, and thereby create a trade-off between adaptive plasticity and trait innovation. ESD species display a variety of potentially conflicting responses to the problem of stabilising sex ratios. My findings suggest that these traits evolve a balance that compensates for weak responses in some traits, or reduction of adaptive capacity by plasticity, due to the strength of selection for the rare sex.

There is still a great deal we do not understand about how plasticity affects evolution, both broadly and in relation to species with ESD. Future investigations could focus on gathering data on how plastic responses vary between traits, individuals, populations, and species with ESD, as this would allow comparative study of the influence of plasticity on the evolution of ESD. Additionally, experiments to determine if adaptive plasticity shields genotypes from selection in real populations would further our understanding of the impact of plasticity on evolution in general. Together, this information could be used to investigate the consequences of trade-offs between adaptive plasticity and trait innovation and determine how species' strategies will affect their ability to stabilise sex ratios in future environments. ESD species currently face a significant challenge in the form of climate change, my thesis has highlighted pathways by which species with ESD may adapt to these new conditions, if we allow them the chance.

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Appendix A

Supplementary Material for Chapter Two

Sample Sizes for ESD Plasticity Model

Table S1. Replicate sample sizes for the 36 different scenarios in the ESD plasticity model. In all scenarios, T_{piv} could evolve. In 'slope scenarios', the slope could evolve, and in 'shift scenarios', shift could evolve.

Plasticity Scenario	**Plasticity Level**	**Global Temperature**	**Climate Variation**	**n**
Slope	Low	28°C	Low	26
			High	33
		30°C	Low	25
			High	27
		32°C	Low	22
			High	27
	Medium	28°C	Low	17
			High	26
		30°C	Low	28
			High	22
		32°C	Low	22
			High	19
	High	28°C	Low	36
			High	21
		30°C	Low	25
			High	23
		32°C	Low	24
			High	25
Shift	Low	28°C	Low	19
			High	28
		30°C	Low	22
			High	31
		32°C	Low	25
			High	34
	Medium	28°C	Low	20
			High	29
		30°C	Low	23
			High	32
		32°C	Low	26
			High	35
	High	28°C	Low	21
			High	30
		30°C	Low	24
			High	33
		32°C	Low	27
			High	36
Total				**900**

Model Validation and Sensitivity Analysis

This supplementary document contains information about the sensitivity of our model to the most relevant parameters. Test conducted are summarized in Table S1. Our sensitivity analysis required data from runs that completed 50000 generations. In general, scenarios were run only five times to gather the presented data, however scenarios with a high likelihood of extinction were run until five surviving runs were produced. When this has occurred, we have indicated it next to the results.

Table S2. Validation tests and sensitivity analyses

Test	Parameter description	Validation / Sensitivity Levels	Main Simulation level
Pivotal temperature evolution	Validation of T_{piv} evolvability at control plasticity levels. Values of shift and slope with the least moderating effect on sex ratios were chosen as the control levels.	Climate: 30°C, 32°C	Same
		Variability: 0.75°C, 1.5°C	
Slope evolution	Validation of slope evolution with different starting slopes.	-1, -2, -3, -4, -5, -6, -7	-5, -1.5, -0.5
Shift evolution	Sensitivity of shift evolution to different starting levels of shift.	0.2, 0.4, 0.6, 0.8, 1	0, 0.38, 0.6666
Mutation range	The standard deviation of new mutational values possible for a trait, normally distributed around the initial trait mean. Expressed here as a percentage of the possible initial values of T_{piv}, slope, and shift in the simulation	1%, 3%	2%

Mortality rate	The rate at which adults leave the population.	0.02, 0.5	0.1

Pivotal Temperature Evolution

When slope and T_{piv} shift were maintained at levels which had the least moderating effect on sex ratios (control values for the simulation), T_{piv} evolved to a final level close to the new mean global temperature (Fig. S1). T_{piv} evolution was not sensitive to temperature variability; however, 32 ± 0.75C scenarios resulted in 31 extinct replicates before 5 successful replicates were accumulated.

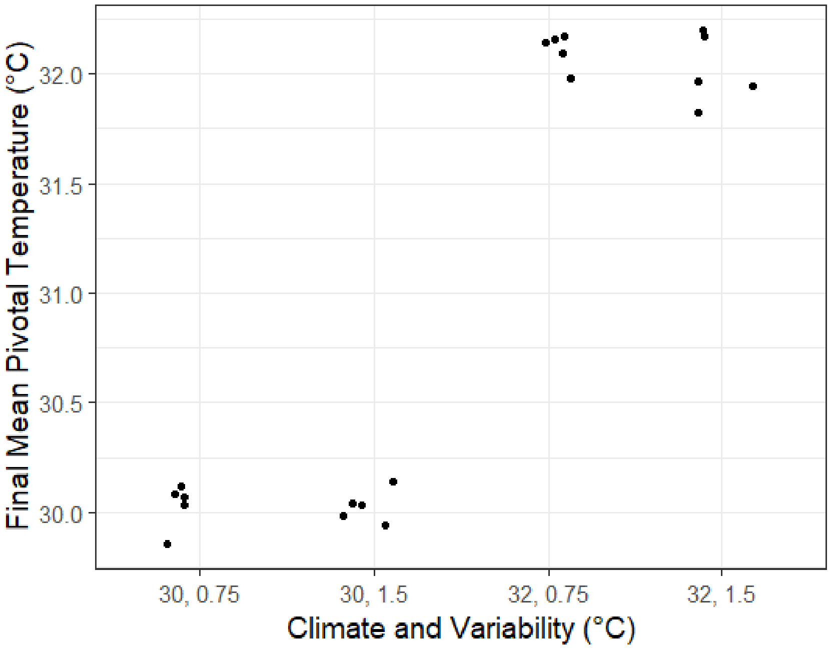

Figure S1. Validation of T_{piv} evolution under two levels of climatic warming (30°C and 32°C) and climate variability (0.75°C and 1.5°C).

Slope Evolution - Validation of selection for ESD

We validated that ESD is selected for under a baseline climate scenario (no climatic warming). T_{piv} shift was set to 0. Neither T_{piv} shift nor T_{piv} could mutate. In these validation replicates, populations with low initial mean slope evolved steeper (more negative) slopes (Fig. S2). When populations were initiated with slopes steeper than -3, their mean slope values generally remained close their starting value. These results suggests that ESD is selected for, but that once the slope is very steep, there is little selection for steeper slopes.

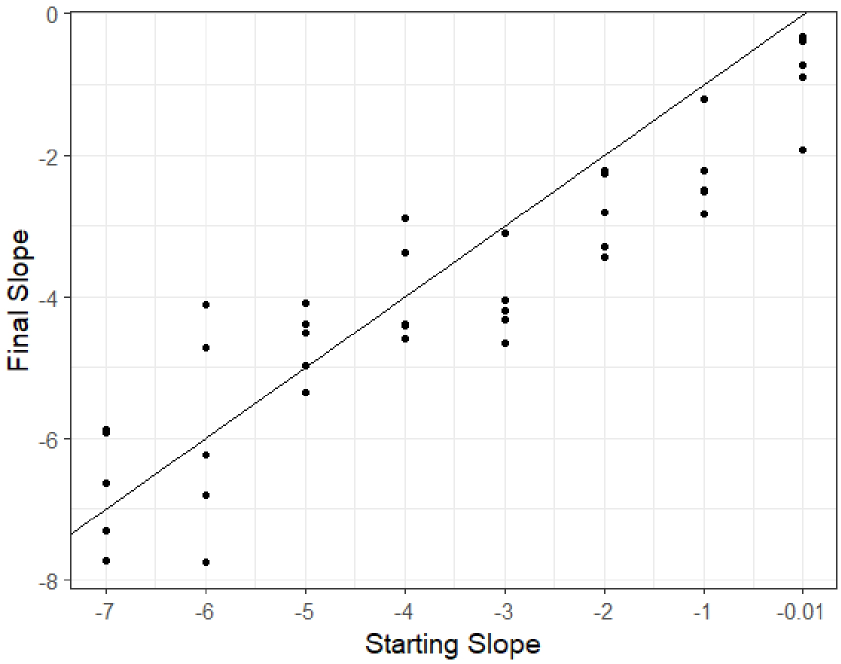

Figure S2. Comparison of final slopes resulting from starting slopes ranging from -0.01 to -7. Scenarios with final slopes steeper than their starting slope fall above the diagonal line, while scenarios with final slopes that are shallower than their starting slope fall below the line. Results are from scenarios with a global temperature of 28°C, climate variability of 0.75°C, and low shift plasticity. Scenarios ran for 50000 iterations. N = 5 for each starting slope value.

T_{piv} Shift Evolution

We validated that shift is selected for in the baseline climate and in the absence of T_{piv} evolution. There was considerable variation in final shift values. In general, scenarios with a starting level of shift at and above 0.4 increased the level of shift over the course of the simulation (Fig. S3). This suggests that shift can evolve if genes for a threshold value of shift exist in the population.

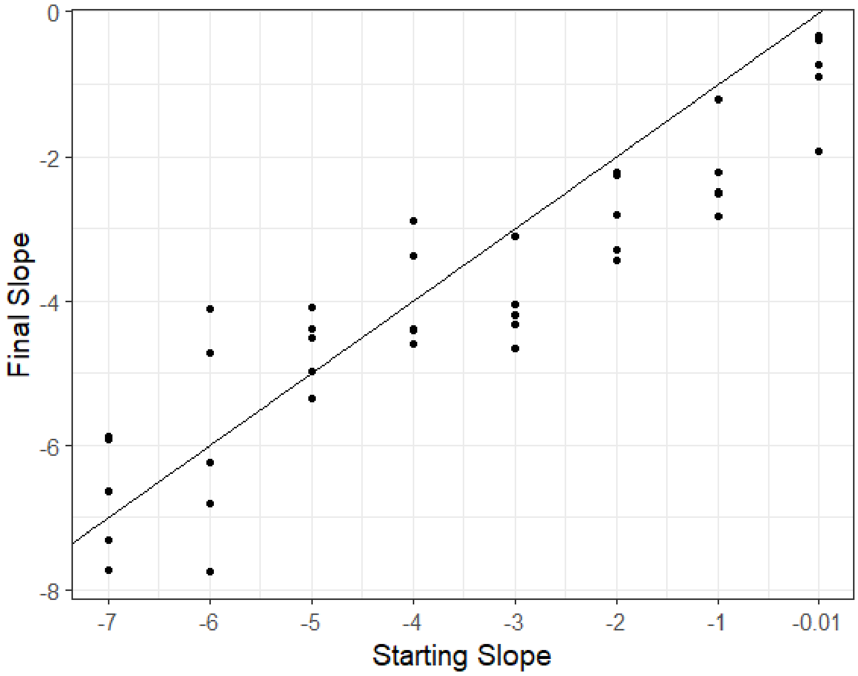

Figure S3. Comparison of final shift values resulting from starting shift values ranging from 0 to 1. Scenarios with final shifts greater than starting shifts fall above the line, while scenarios with final shifts lower than starting shifts fall below the line. Results are from scenarios with a global temperature of 28°C, climate variability of 0.75°C, and high slope plasticity. Scenarios ran for 50000 iterations. N = 5 for each starting shift value.

Sensitivity to Mutation Range

We examined sensitivity of simulation results to mutation by varying the breadth of new mutational values possible for chosen mutants. When the slope of the reaction norm was allowed to evolve, we detected sensitivity to mutation range in both T_{piv} and slope evolution (Fig. S4, Table S3). In low slope plasticity scenarios, broader mutation range was associated with increased evolution of T_{piv} toward the new environmental mean (Fig. S4 first and second row, left). Mutation range did not affect T_{piv} evolution in the high plasticity scenarios. Mutation range affected slope evolution in both the high and low plasticity groups. Steeper slopes were achieved when range was increased (Fig. S4 first and second row, right). In general, as mutation range increased, populations were more likely to retain ESD (steeper slope), and the T_{piv} ended closer to the new climate mean (Table S3). At 3% mutation range populations were less likely to go extinct (Table S3).

In scenarios where shift was able to evolve, there was less sensitivity to mutation range than seen in slope scenarios. Greater mutation range modestly increased the evolvability of T_{piv} in some replicates in the low plasticity scenarios (Fig. S4 third and fourth rows, left). Final shift values were slightly lower in higher mutation scenarios for all run types. Again, this effect was stronger in the low plasticity scenarios (Fig. S4 third and fourth rows, right). In general, as mutation range increased, fewer populations went extinct, populations had lower shifts, and the T_{piv} ended closer to the new climate mean in some of the populations (Table S4).

Overall, it seems that greater mutation range facilitated the directional evolution of T_{piv}, thereby reducing frequency-dependent selection for shallow slopes and large shifts.

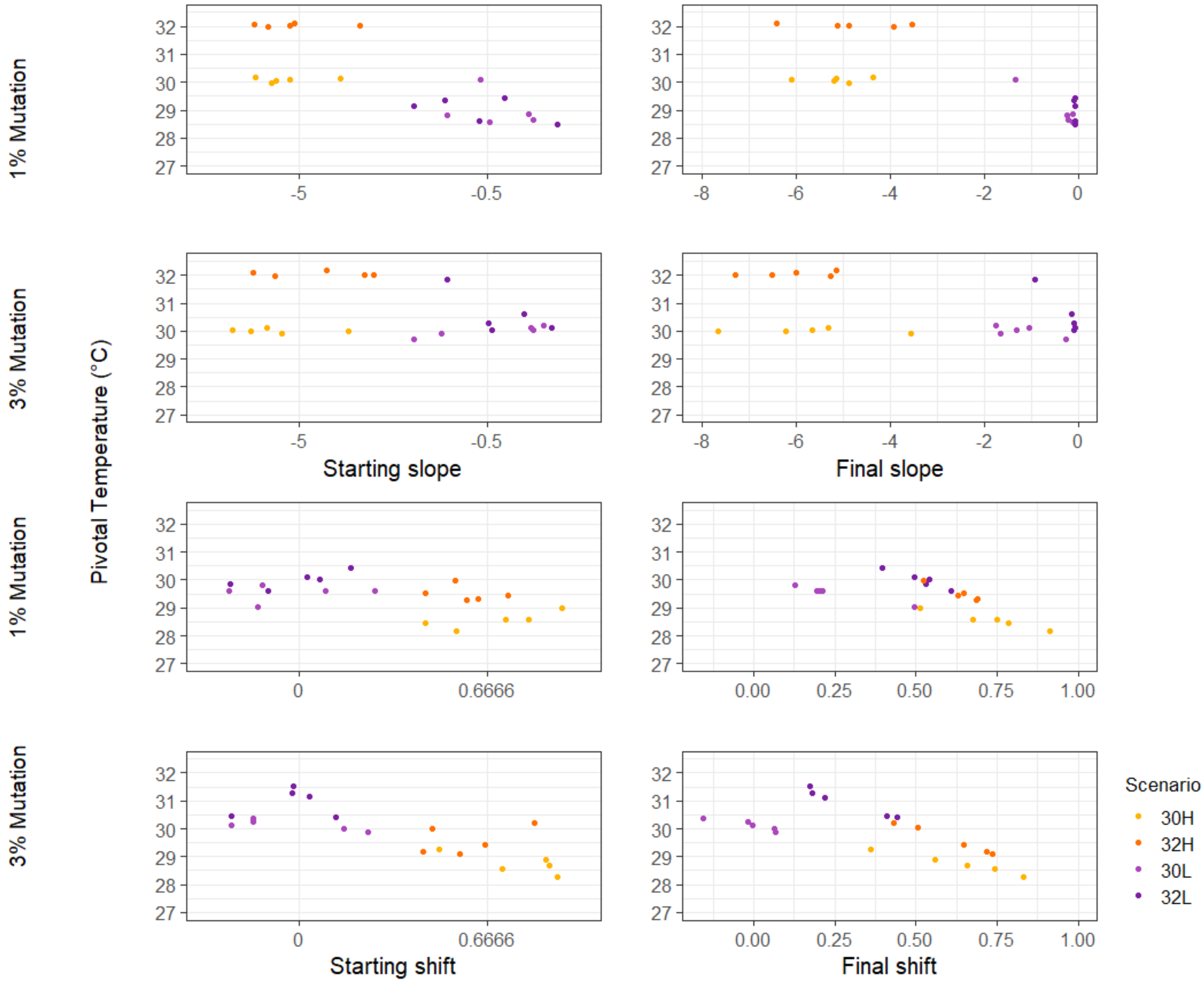

Figure S4. Analysis of the sensitivity of T_{piv}, slope, and shift evolution to two levels of mutation range (1% vs 3% of the trait mean). In the left column, points are jittered around two categorical scenarios on the x-axis.

Table S3. Summary of the sensitivity of T_{piv} and slope evolution to variation of mutation in T_{piv} and slope. The 1% and 3% mutation range results have a sample size of n=5. The 2% mutation range results are presented in the main text and have higher, variable sample sizes. They are presented here again for comparison.

Mutation Variability	**Global Temperature**	**Starting Slope**	**Mean Final T_{piv}**	**Mean Final Slope**
1%	30°C	-0.5	29.0°C	-0.411
		-5	30.1°C	-5.183
	32°C	-0.5	29.0°C	-0.068
		-5*	32.1°C	-4.826
2%	*30°C*	*-0.5*	*29.9°C*	*-1.308*
		-5	*30.0°C*	*-5.050*
	32°C	*-0.5*	*29.2°C*	*-0.076*
		-5†	*32.0°C*	*-4.785*
3%	30°C	-0.5	30.1°C	-1.184
		-5	30.0°C	-5.677
	32°C	-0.5	30.6°C	-0.277
		-5‡	32.0°C	-6.008

*26.3% survival (5/19 runs), †16.7% survival (4/24 runs), ‡ 83.3% survival (5/6 runs)

Table S4. Summary of the sensitivity of T_{piv} and shift evolution to variation of mutation in T_{piv} and shift. All analyses were conducted with a sample size of five replicates. The 1% and 3% mutation range results have a sample size of n=5. The 2% mutation range results are presented in the main text and have higher, variable sample sizes but have been provided for comparison.

Mutation Variability	**Global Temperature**	**Starting Shift**	**Mean Final T_{piv}**	**Mean Final Shift**
1%	30°C	0	29.5°C	0.254
		0.6666	28.6°C	0.728
	32°C	0*	30.0°C	0.502
		0.6666	29.5°C	0.627
2%	*30°C*	*0*	*29.8°C*	*0.121*
		0.6666	*28.8°C*	*0.654*
	32°C	*0†*	*30.4°C*	*0.411*
		0.6666	*29.3°C*	*0.693*
3%	30°C	0	30.2°C	-0.011
		0.6666	28.8°C	0.627
	32°C	0‡	30.9°C	0.2883
		0.6666	29.6°C	0.602

*23.8% survival (5/21 runs), †25.9% survival (7/27 runs), ‡83.3% survival (5/6 runs)

Sensitivity to Adult Mortality Rate

When simulated individuals had shorter lifespans (0.5% = 2-year life expectancy (second and fourth row) vs. 0.02% = 50-year life expectancy (first and third row)), the starting slope had a reduced effect on pivotal temperature evolution. All short-lived populations experienced evolution of the Tpiv to match the mean climate. In long lived populations, only high slope plasticity was associated with pivotal temperature evolution (Fig. S5, first *vs*. second rows, left, Table S5).

In long-lived populations, starting slope influenced slope evolution. Populations with steeper slopes were more likely to maintain ESD-like slopes than populations with shallow starting slopes (Fig. S5, first row, left). In contrast, starting slope and climate interacted to influence slope evolution populations with short life spans. Specifically, high plasticity 30°C replicates maintained steep slopes, and high plasticity 32°C replicates evolved shallower ESD-like slopes while, populations with low plasticity maintained shallow ESD slopes regardless of climate (Fig. S5 second row right panel, Table S3 5).

Increased pivotal temperature evolution and maintenance of ESD in short-lived populations is likely due to strong frequency dependent selection on the pivotal temperature. In long-lived populations, shallow slopes are selected for due to the increased chance of producing a rare male. However, as producing a male in a hot climate is very unlikely, shallow slopes are unlikely to benefit an individual that only breeds once or twice. Therefore, pivotal temperature evolution is a more reliable path to producing the rare sex in short-lived populations. The exception is in high slope plasticity populations in hot climates. These scenarios experience quick pivotal temperature evolution to the new mean temperature, and subsequent evolution of a shallower slope. This suggests that while pivotal temperature evolution is the most important factor for adaptation to rising temperatures in short-lived populations, evolving a shallower slope and therefore a greater chance of producing a mixed-sex nest is also advantageous in extreme climates.

Starting shift had less influence on final T_{piv} in populations with shorter lifespans (Fig. S5, third *vs.* fourth rows, right, Table S6). This was because shorter lifespans led to lower final pivotal temperatures and higher shift across every scenario compared to populations with longer lifespans (Fig. S5, fourth row, right, Table S6). In long-lived populations, the extent of climatic warming impacted shift evolution, 32°C scenarios were more likely to evolve greater shift than 30°C scenarios (Fig S5, third row, dark vs light symbols, Table 6). The relationship between climate and shift evolution is similar to the trend we observed in the main simulation (Fig. 4d, main text).

Overall, for short lived populations, shift evolution was a more advantageous strategy than pivotal temperature evolution. This is probably because being able to respond directly to annual climate is especially useful if individuals have a reduced number of breeding seasons and therefore a reduced chance of producing offspring of the rare sex. Similarly, long-lived 32°C populations may have very few years in which producing a male is possible, despite their longevity. Thus, evolution of shift plasticity was favoured in these populations.

Low mortality and plasticity that ameliorated sex ratio bias (high shift, shallow slope) promoted population survival, while high mortality exacerbated population decline (Tables S5&S6). It is important to note that the extinction rate for short-lived populations with steep slopes or low shift plasticity was very high, so these results are from a subset of unusual populations.

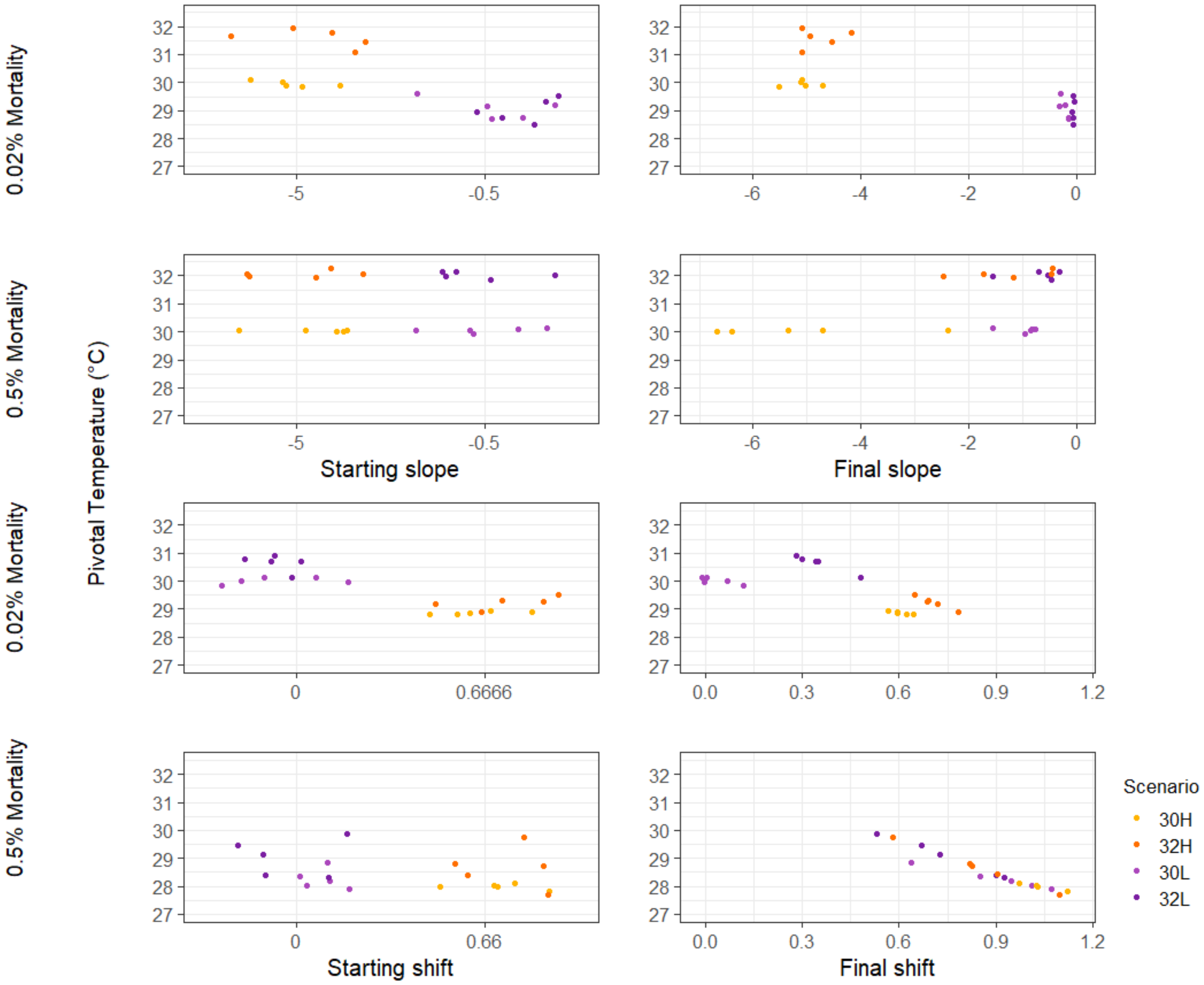

Figure S5. Sensitivity of T_{piv}, slope, and shift evolution to two levels of adult mortality (0.02% and 0.5%).

Table S5. Summary of the sensitivity of T_{piv} and slope evolution to variation in adult mortality rate. The 0.02 and 0.5 mortality rate results have a sample size of n=5. The 0.1 mortality rate results are from the main text and have higher, variable sample sizes but have been provided for comparison.

Adult Mortality	**Global Temperature**	**Starting Slope**	**Mean Final T_{piv}**	**Mean Final Slope**
0.02	30°C	-0.5	29.1°C	-0.235
		-5	29.9°C	-5.076
	32°C	-0.5	29.0°C	-0.054
		-5*	31.6°C	-4.755
0.1	*30°C*	*-0.5*	*29.9°C*	*-1.308*
		-5	*30.0°C*	*-5.050*
	32°C	*-0.5*	*29.2°C*	*-0.076*
		-5†	*32.0°C*	*-4.785*
0.5	30°C	-0.5	30.1°C	-1.046
		-5	29.9°C	-5.193
	32°C	-0.5	32.0°C	-0.834
		-5‡	32.0°C	-1.176

*50% survival (5/10 runs), †16.7% survival (4/24 runs), ‡ (5/91 runs)

Table S6. Summary of the sensitivity of T_{piv} and shift evolution to variation in adult mortality rate. The 0.02 and 0.5 mortality rate results have a sample size of n=5. The 0.1 mortality rate results are from the main text and have higher, variable sample sizes but have been provided for comparison.

Adult Mortality	**Global Temperature**	**Starting Shift**	**Mean Final T_{piv}**	**Mean Final Shift**
0.02	30°C	0	30.0°C	0.034
		0.6666	28.9°C	0.610
	32°C	0*	30.6°C	0.346
		0.6666	29.2°C	0.709
0.1	*30°C*	*0*	*29.8°C*	*0.121*
		0.6666	*28.8°C*	*0.654*
	32°C	*0†*	*30.4°C*	*0.411*
		0.6666	*29.3°C*	*0.693*
0.5	30°C	0	28.2°C	0.934
		0.6666	28.0°C	1.033
	32°C	0‡	29.0°C	0.745
		0.6666	28.6°C	0.843

*38.5% survival (5/13 runs), †25.9% survival (7/27 runs), ‡11.4% survival (5/44 runs)

Results of High Variability (SDbw 1.5°C) Scenarios

The results of scenarios where the standard deviation of inter-annual climate variability was 1.5°C.

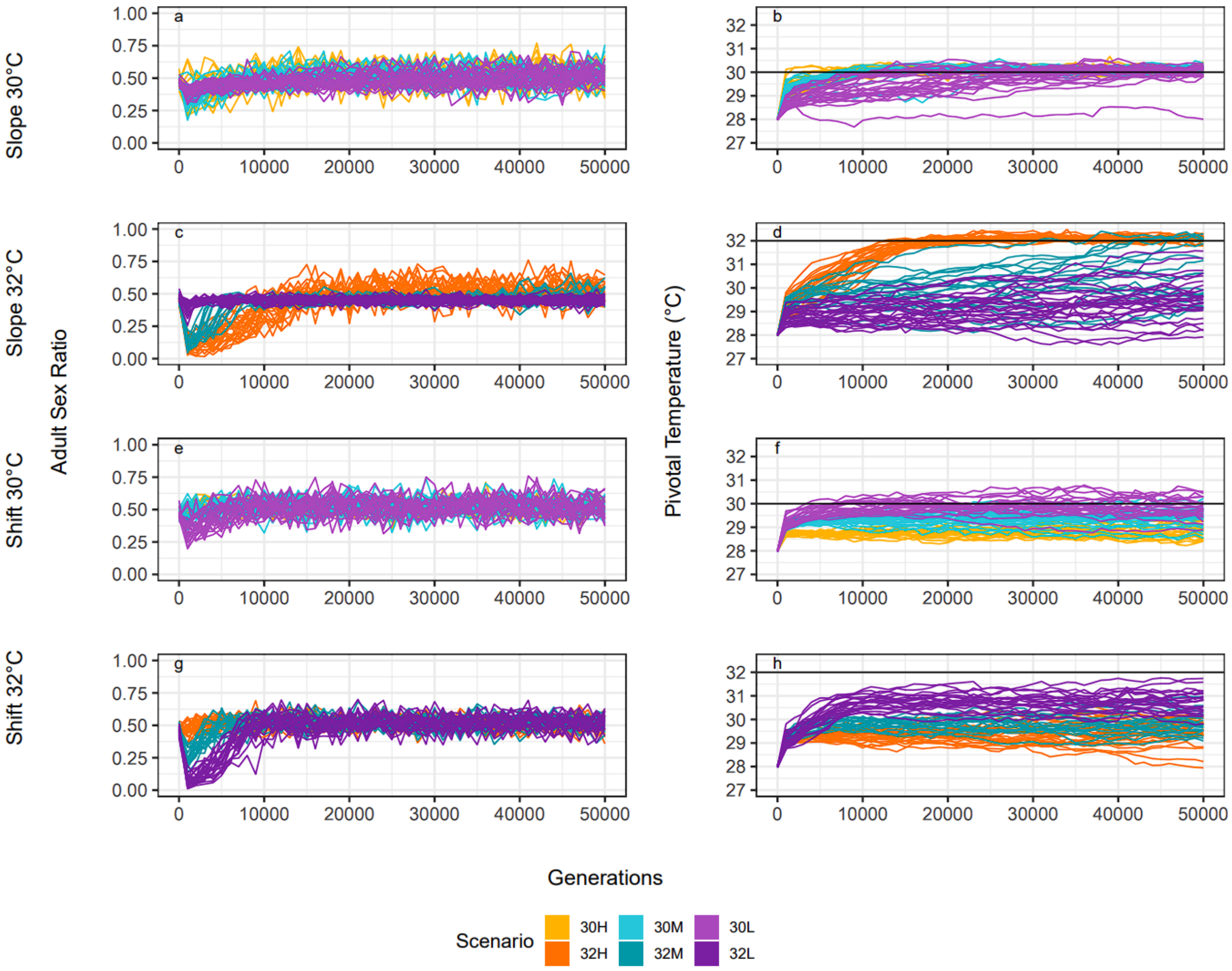

Figure S6. The adult sex ratios and mean pivotal temperatures (T_{piv}) over time for high variability replicate populations at different levels of climate and plasticity. Only results from warmed climate scenarios are presented as there was little change in T_{piv} or adult sex ratio in control climate populations. A ratio of 1 is 100% male, a ratio of 0 is 100% female. Levels of plasticity in the scenario key are: H= High, M= Moderate, L= Low.

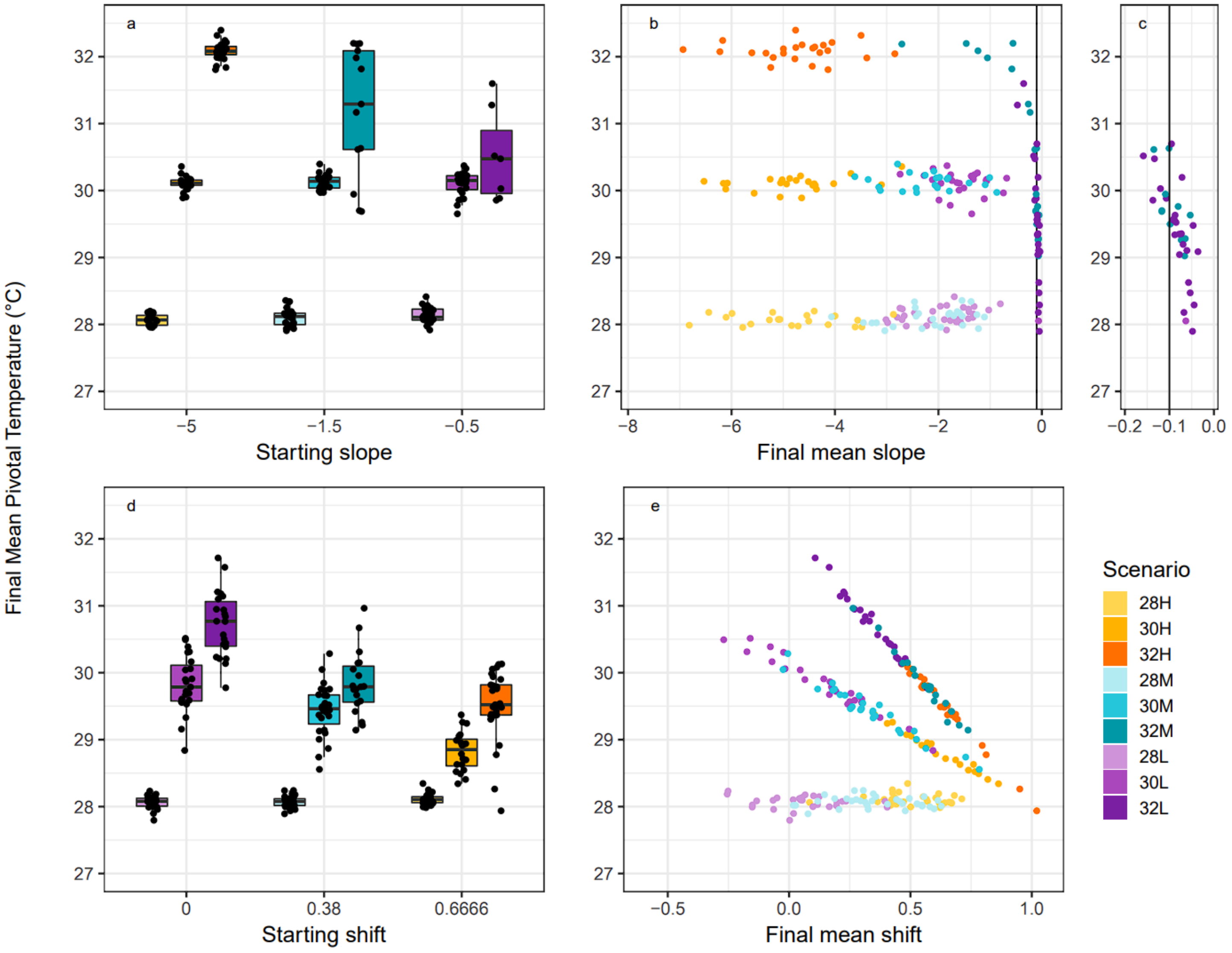

Figure S7. The final mean pivotal temperature of high variability replicate simulations over starting and final mean plasticity levels (mean of the last 1000 generations). Levels of plasticity in the scenario key are: H= High, M= Moderate, L= Low. Results shown are from low climate variability treatments. (a) Final mean pivotal temperatures for the three levels of starting slope plasticity in all climate scenarios. Only replicates that maintained ESD are included in the boxplots. (b) Final mean pivotal temperatures and final mean slopes of replicates in slope plasticity scenarios, the black line indicates the boundary between ESD and a GSD-like slope. (c) An expansion of (b), centered around the black line. It shows the scenarios that lost ESD (slope > -0.1) on the right side of the black line. (d) Final mean pivotal temperatures for the three levels of starting shift plasticity in all climate scenarios. (e) Final mean pivotal temperatures and final mean shifts of replicates in shift plasticity scenarios.

Appendix B

Supplementary Material for Chapter Three

Sample Sizes for Nest Temperature Analyses

Table S1. TSD parameters calculated with Annual average July Nest Temperature.

Year	**N nests**	**TRT Width**	**Lower TRT**	**Upper TRT**	T_{piv}	**Lower CI 95%**	**Upper CI 95%**	**Excluded (Y/N)**	**Reason**
1995	10	5.37	24.47	29.84	27.15	26.71	27.61	N	
1996	22	4.37	23.77	28.12	25.95	24.63	27.30	N	
1997	28	7.14	21.57	28.71	25.13	24.59	25.67	N	
1998	9	15.88	18.91	34.82	26.89	24.90	28.98	N	
1999	9	2.06	26.20	28.26	27.23	26.86	27.59	N	
2000	11	3.36	25.03	28.39	26.70	25.74	27.65	N	
2001	12	2.99	24.90	27.90	26.40	26.04	26.75	N	
2002	29	1.88	25.41	27.29	26.35	26.21	26.49	N	
2003	73	2.34	24.27	26.62	25.45	25.23	25.66	N	
2004	32							Y	No mixed sex ratios
2005	3							Y	Low sample size
2006	2							Y	Low sample size
2007	15	3.49	23.28	26.76	25.03	24.53	25.52	N	
2008	0							Y	Low sample size
2009	11	6.32	22.42	28.76	25.60	23.34	28.42	N	
2010	0							Y	Low sample size
2011	1							Y	Low sample size
2012	0							Y	Low sample size
2013	0							Y	Low sample size
2014	0							Y	Low sample size
2015	2							Y	Low sample size
2016	1							Y	Low sample size
2017	5							Y	No mixed sex ratios

Table S2. TSD parameters calculated with Annual average Nest CTE. Based on these results 1996 and 2009 were excluded from analyses using CTE because the confidence intervals in those years were unusually broad.

Year	**N nests**	**TRT**	**Lower TRT**	**Upper TRT**	T_{piv}	**Lower CI 95%**	**Upper CI 95%**	**Excl (Y/N)**	**Reason**
1995	5	7.14	23.90	31.08	27.49	26.55	28.426	N	
1996	21	11.13	24.94	36.10	30.51	25.91	36.289	Y	Few females, CI too broad
1997	26	7.37	22.89	30.27	26.58	26.07	27.11	N	
1998	9	14.71	20.32	35.09	27.74	26.02	29.60	N	
1999	9	1.95	26.82	28.76	27.79	27.42	28.17	N	
2000	11	2.43	26.44	28.88	27.65	27.03	28.29	N	
2001	12	1.42	27.21	28.64	27.93	27.69	28.16	N	
2002	29	4.03	25.49	29.53	27.51	27.19	27.81	N	
2003	73	4.32	25.10	29.42	27.51	26.83	27.71	N	
2004	32							Y	No mixed sex ratios
2005	3							Y	Sample size too small
2006	2							Y	Sample size too small
2007	15	3.89	24.48	28.35	26.42	25.90	26.94	N	
2008	0							Y	Sample size too small
2009	11	20.18	22.41	42.61	32.58	24.69	44.41	Y	Few females, CI too broad
2010	0							Y	Sample size too small
2011	1							Y	Sample size too small
2012	0							Y	Sample size too small
2013	0							Y	Sample size too small
2014	0							Y	Sample size too small
2015	2							Y	Sample size too small
2016	1							Y	Sample size too small
2017	5							Y	No mixed sex ratio

Regression Analysis of Nest Temperature and Vegetation Cover

Table S3. Relationship between Annual average July Nest Temperature and SWveg for each year.

Year	**N nests**	**Multiple R-squared**	**P-value**	**Slope**	**Intercept**
1995	10	0.47	0.028	-0.02	31.84
1996	22	0.13	0.100	-0.01	24.08
1997	28	0.25	0.006	-0.01	25.21
1998	9	0.39	0.075	-0.02	26.84
1999	9	0.88	<0.001	-0.02	28.67
2000	11	0.33	0.064	-0.02	25.96
2001	12	0.36	0.038	-0.02	28.30
2002	29	0.35	<0.001	-0.02	27.80
2003	73	0.48	<0.001	-0.02	25.55
2007	15	0.59	<0.001	-0.02	26.54
2009	11	0.81	<0.001	-0.03	25.54

Table S4. Relationship between Annual average Nest CTE and SWveg for each year.

Year	**N nests**	**Multiple R-squared**	**P-value**	**Slope**	**Intercept**
1995	5	0.73	0.065	-0.05	34.00
1997	26	0.42	<0.001	-0.02	27.56
1998	9	0.52	0.029	-0.02	28.00
1999	9	0.82	<0.001	-0.02	28.99
2000	11	0.20	0.164	-0.01	26.85
2001	12	0.47	0.015	-0.02	29.17
2002	29	0.28	0.003	-0.01	28.71
2003	73	0.44	<0.001	-0.02	27.01
2007	15	0.48	0.004	-0.03	28.26

Regression Analysis of the TRT and T_{piv} Using Two Temperature Measures

We performed a linear regression of the annual average transitional range of temperatures (TRT) against the annual average pivotal temperature (T_{piv}) to ascertain if any relationship between these factors exists. We found no evidence of correlation between the TRT and T_{piv} in *C. picta*, when either mean July temperature (JTMean) or the constant temperature equivalent (CTE) were used to calculate the TSD parameters (Table S5, Fig. S1 & S2).

Table S5. Results of linear regressions between the TRT and T_{piv} in *C. picta*, using two different temperature measures.

Temperature Measure	SE	DF	F-statistic	P-value	R^2
JTMean	0.65	9		0.79	0.02
CTE	-0.57	7	0.04	0.85	0.005

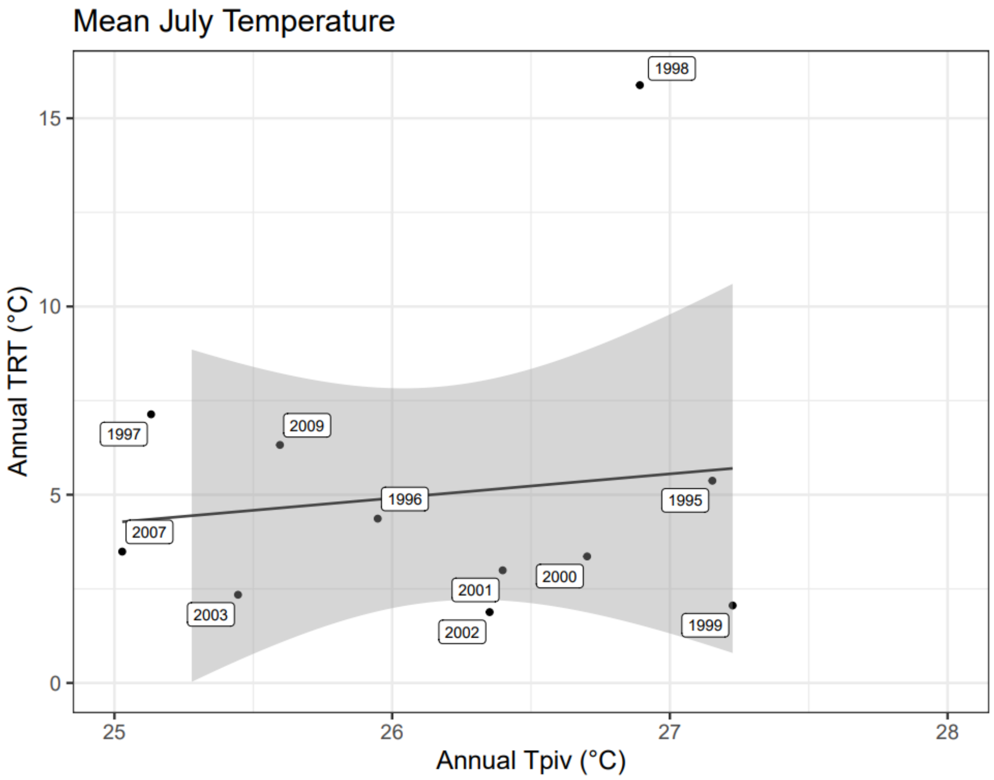

Figure S1. The relationship between the TRT and T_{piv}, when JTMean is used to calculate these TSD parameters. There is no correlation between the TRT and T_{piv}.

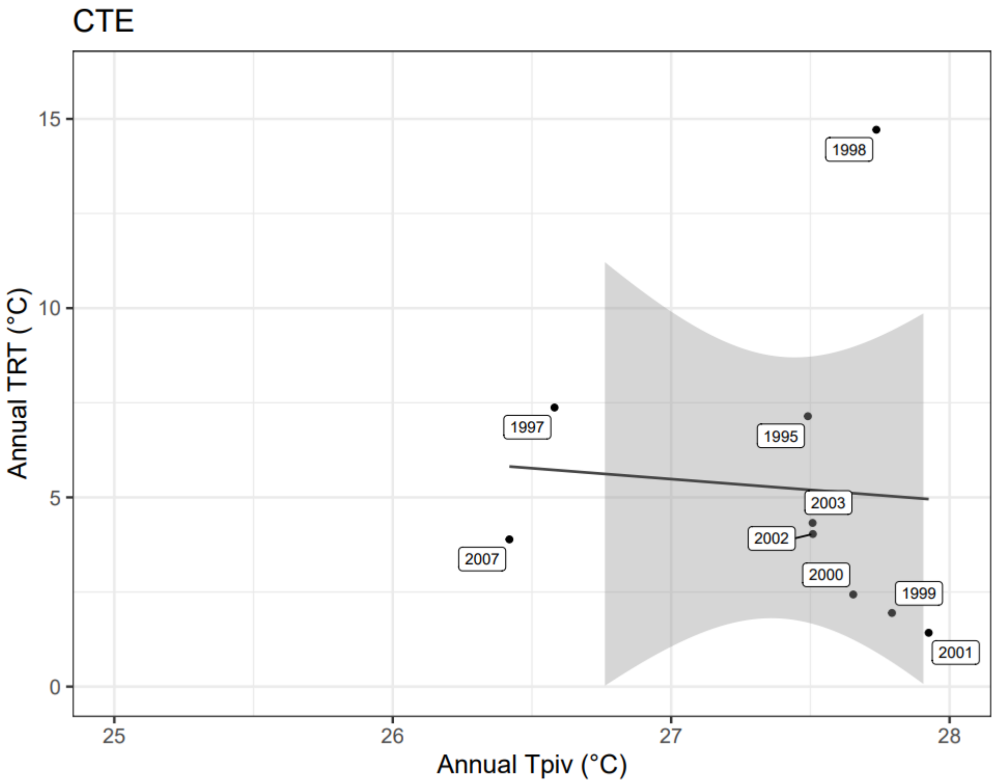

Figure S2. The relationship between the TRT and T_{piv}, when CTE is used to calculate these TSD parameters. There is no significant relationship between the TRT and T_{piv}

Appendix C

Supplementary Material for Chapter Four

Supplement Contents

This supplement contains information about replicate sample sizes of the Chapter 4 models, validation of simulation methods and the sensitivity of the models to the parameters most likely to influence the evolutionary outcomes. Sample sizes and simulation durations vary between tests and have been indicated in each section. Some tests include extinct runs. Where extinct runs are present, the sample size is presented as n = surviving runs/total runs.

Sample Sizes for T_{piv} and *Nb* Evolution Models

Table S1. Sample sizes for replicates in the solitary evolution scenarios. In evolution scenario 1, T_{piv} could evolve, and in evolution scenario 2, *Nb* could evolve. H_{var} = heritability variance (see Table 1. in Chapter 4). Additional runs were conducted in the *Nb* evolution scenarios in order to gather data for the Tglob = 32°C, H_{var} = 0 replicates, which had a comparatively high extinction rate.

Evolution Scenario	**Global Temperature**	H_{var}	**n**
1	28°C	0	3
		0.1	13
		0.2	4
		0.3	6
		0.4	4
		0.5	3
	30°C	0	2
		0.1	5
		0.2	6
		0.3	3
		0.4	2
		0.5	5
	32°C	0	2
		0.1	4/6
		0.2	3/7
		0.3	2/5
		0.4	5/7
		0.5	5

2	28°C	0	6
		0.1	5
		0.2	5
		0.3	4
		0.4	6
		0.5	6
	30°C	0	5
		0.1	10
		0.2	4
		0.3	8
		0.4	7
		0.5	4
	32°C	0	13/20
		0.1	1/5
		0.2	3/4
		0.3	1/2
		0.4	2/4
		0.5	4/6
Total			199

Table S2. Sample sizes for replicates in the T_{piv} and *Nb* coevolution model that included temperature-dependent survival and nesting plasticity. The H_{var} value for the varying trait is 0.3.

Varying Trait	**Global Temperature**	**Temperature-dependent survival**	**Nesting Behaviour Plasticity**	**n**
None	28°C	No	0	11
			0.3	11
			0.6	9
		Yes	0	10
			0.3	10
			0.6	11
	30°C	No	0	10
			0.3	7
			0.6	12
		Yes	0	13
			0.3	9
			0.6	8
	32°C	No	0	11
			0.3	8
			0.6	9

		Yes	0	10
			0.3	17
			0.6	13
T_{piv}	28°C	No	0	9
			0.3	14
			0.6	11
		Yes	0	13
			0.3	7
			0.6	14
	30°C	No	0	13
			0.3	4
			0.6	11
		Yes	0	12
			0.3	17
			0.6	10
	32°C	No	0	5
			0.3	12
			0.6	10
		Yes	0	11
			0.3	13
			0.6	3
Nb	28°C	No	0	8
			0.3	5
			0.6	12
		Yes	0	7
			0.3	10
			0.6	10
	30°C	No	0	11
			0.3	10
			0.6	6
		Yes	0	11
			0.3	5
			0.6	9
	32°C	No	0	7
			0.3	11
			0.6	10
		Yes	0	5
			0.3	9
			0.6	16

Model Validation and Sensitivity Analysis

Table S3. Validation tests and sensitivity analyses.

Test	**Description**	**Validation / Sensitivity Levels**	**Main Simulation level**
Effect of H_{var} on Narrow sense heritability (h_2)	In the simulation, heritability (h_2) is controlled by H_{var}, which is the standard deviation of a normal distribution (with parental traits as the mean) from which offspring traits are drawn.	T_{piv} 0, 0.1, 0.2, 0.3, 0.4, 0.5	T_{piv} 0, 0.3
		Nb 0, 0.1, 0.2, 0.3, 0.4, 0.5	*Nb* 0, 0.3
Effect of H_{var} on coevolution	In the simulation I used a reduced range of H_{var} values in the coevolution scenarios, in order to accommodate the inclusion of temperature-dependent survival and nesting plasticity. The purpose of this test is to examine how a wider range of H_{var} values affect the evolution of T_{piv} and *Nb*	T_{piv} 0, 0.1, 0.2, 0.3, 0.4, 0.5	T_{piv} 0, 0.3
		Nb 0, 0.1, 0.2, 0.3, 0.4, 0.5	*Nb* 0, 0.3
Temperature-independent fertility	In the simulation, female fertility depends on developmental temperature. This test determines if directional change in observed in T_{piv} and *Nb* in control climates treatments is due to Temperature dependent fertility.	Clutch size is *not* related to maternal developmental temperature.	Clutch size *is* related to maternal developmental temperature.

Heritability

In the simulation, I used the constant H_{var} to add environmental noise to trait expression in order to vary the heritability of T_{piv} and Nb. This sensitivity analysis was conducted to establish that increasing H_{var} had the intended effect of reducing the heritability of T_{piv} and Nb in the initial population. To collect the data, I ran the simulation for one generation, in a 28°C climate with no temperature-dependent survival or nesting plasticity. I recorded parental and offspring trait values after H_{var} was applied. To calculate the h_2 of T_{piv} I computed the mid-parent regression of parent T_{piv} and offspring T_{piv}. To calculate the h_2 of Nb I regressed mothers' Nb values against daughters' Nb values, as this trait is only expressed in females. It is important to note that these regressions were performed on T_{piv} and Nb trait values rather than parent/offspring sex.

As expected, increasing H_{var} reduced heritability in both traits (Table S4, S5, Fig. S1, S2, & S3). At $H_{var} = 0$, T_{piv} heritability was approximately double Nb heritability, however T_{piv} heritability decreased at a greater rate than Nb heritability, such that at $H_{var} = 0.5$, the h_2 values of T_{piv} and Nb were closer together than at $H_{var} = 0$ (Fig. S1). H_{var} increase had the greatest effect on h_2 between H_{var} values of 0.1 and 0.5, after which heritability did not decrease further (Fig. S1).

Table S4. Summary of the results of linear regressions of parental and offspring T_{piv} at different levels of H_{var}. Slope coefficient estimates, or h2, are in bold.

H_{var}	**Parameter**	**Coefficient estimate**	**SE**	**t-value**	**P-value**	**R^2**
0	Intercept	1.20	0.556	2.15	0.0313	0.386
	Slope	**0.957**	0.0199	48.2	<2e-16	
0.1	Intercept	1.88	0.458	4.11	4.05e-05	0.449
	Slope	**0.933**	0.0164	57.06	<2e-16	
0.2	Intercept	6.305	0.508	12.4	<2e-16	0.306
	Slope	**0.775**	0.0182	42.7	<2e-16	
0.3	Intercept	11.2	0.532	21.1	<2e-16	0.183
	Slope	**0.599**	0.0190	31.5	<2e-16	
0.4	Intercept	19.3	0.633	30.6	<2e-16	0.0470
	Slope	**0.309**	0.0226	13.7	<2e-16	
0.5	Intercept	19.3	0.623	30.8	<2e-16	0.0457
	Slope	**0.311**	0.0223	13.9	<2e-16	

Table S5. Summary of the results of linear regressions of mothers’ and daughters’ *Nb* values at different levels of H_{var}. Slope coefficient estimates, or h2, are in bold.

H_{var}	**Parameter**	**Coefficient estimate**	**SE**	**t-value**	**P-value**	**R^2**
0	Intercept	0.0381	0.0113	3.37	0.000775	0.211
	Slope	**0.465**	0.0280	16.6	< 2e-16	
0.1	Intercept	-0.00216	0.00660	-0.328	0.743	0.221
	Slope	**0.454**	0.0176	25.8	<2e-16	
0.2	Intercept	0.00367	0.00645	0.569	0.57	0.140
	Slope	**0.363**	0.0154	23.6	<2e-16	
0.3	Intercept	0.0155	0.00917	1.70	0.0901	0.0692
	Slope	**0.263**	0.0206	12.8	<2e-16	
0.4	Intercept	0.0334	0.0179	1.87	0.062	0.0430
	Slope	**0.20**	0.0332	6.02	2.71e-09	
0.5	Intercept	0.0477	0.0230	2.07	0.039	0.0397
	Slope	**0.20**	0.0361	5.54	4.28e-08	

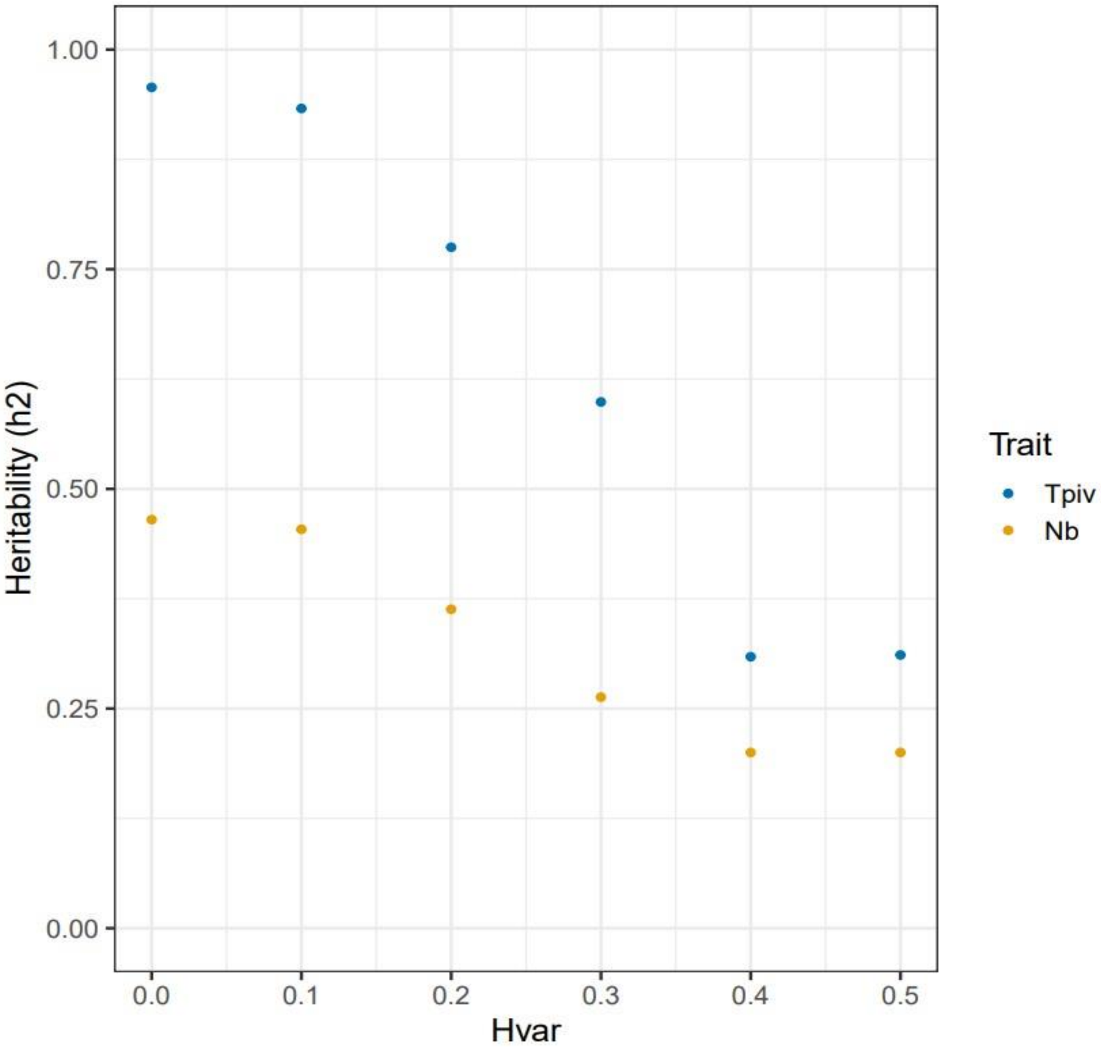

Figure S1. Relationship between *H_{var}* and narrow sense heritability, for *T_{piv}* and *Nb*.

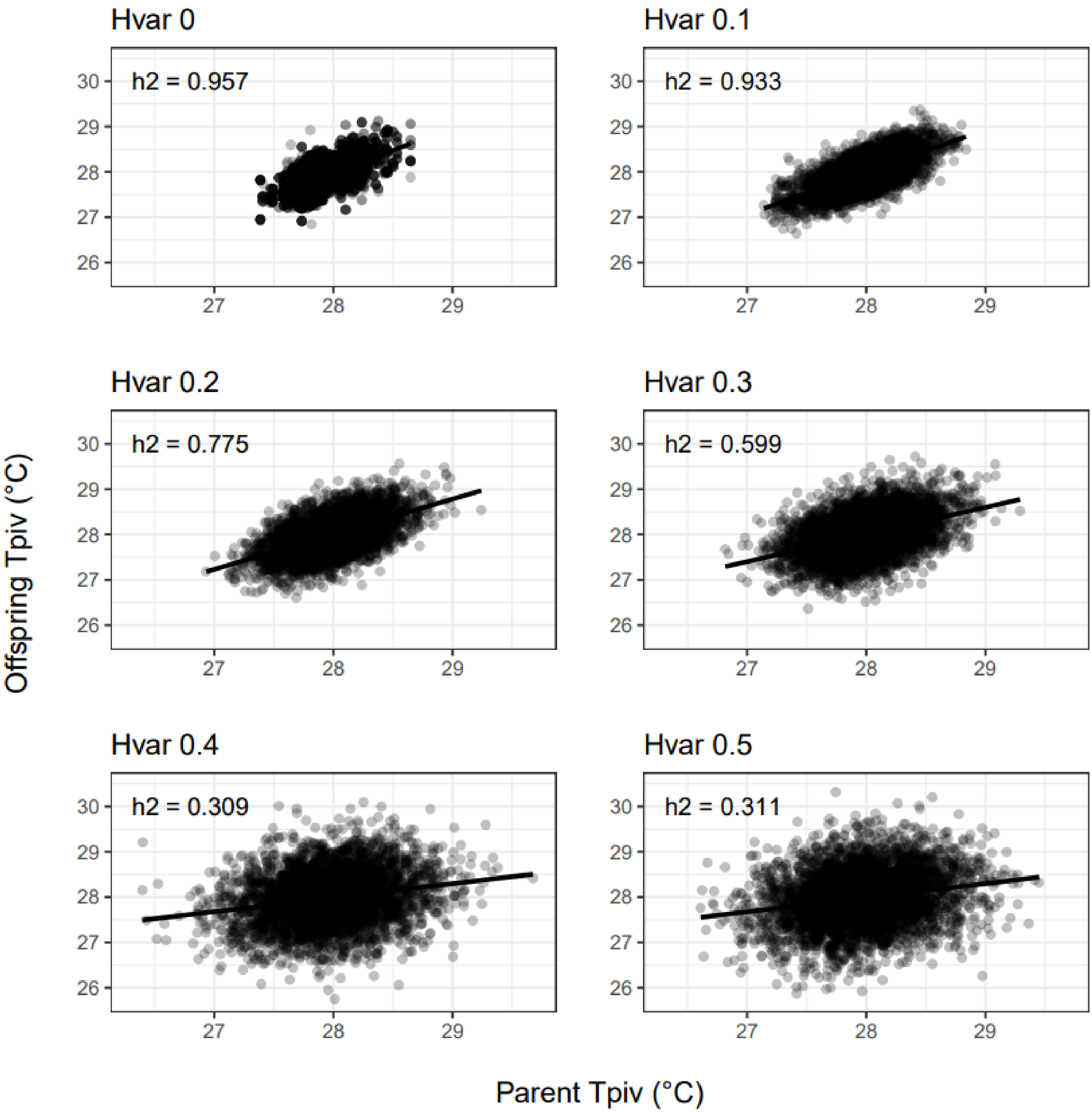

Figure S2. Relationships between parent and offspring T_{piv} at different levels of H_{var}.

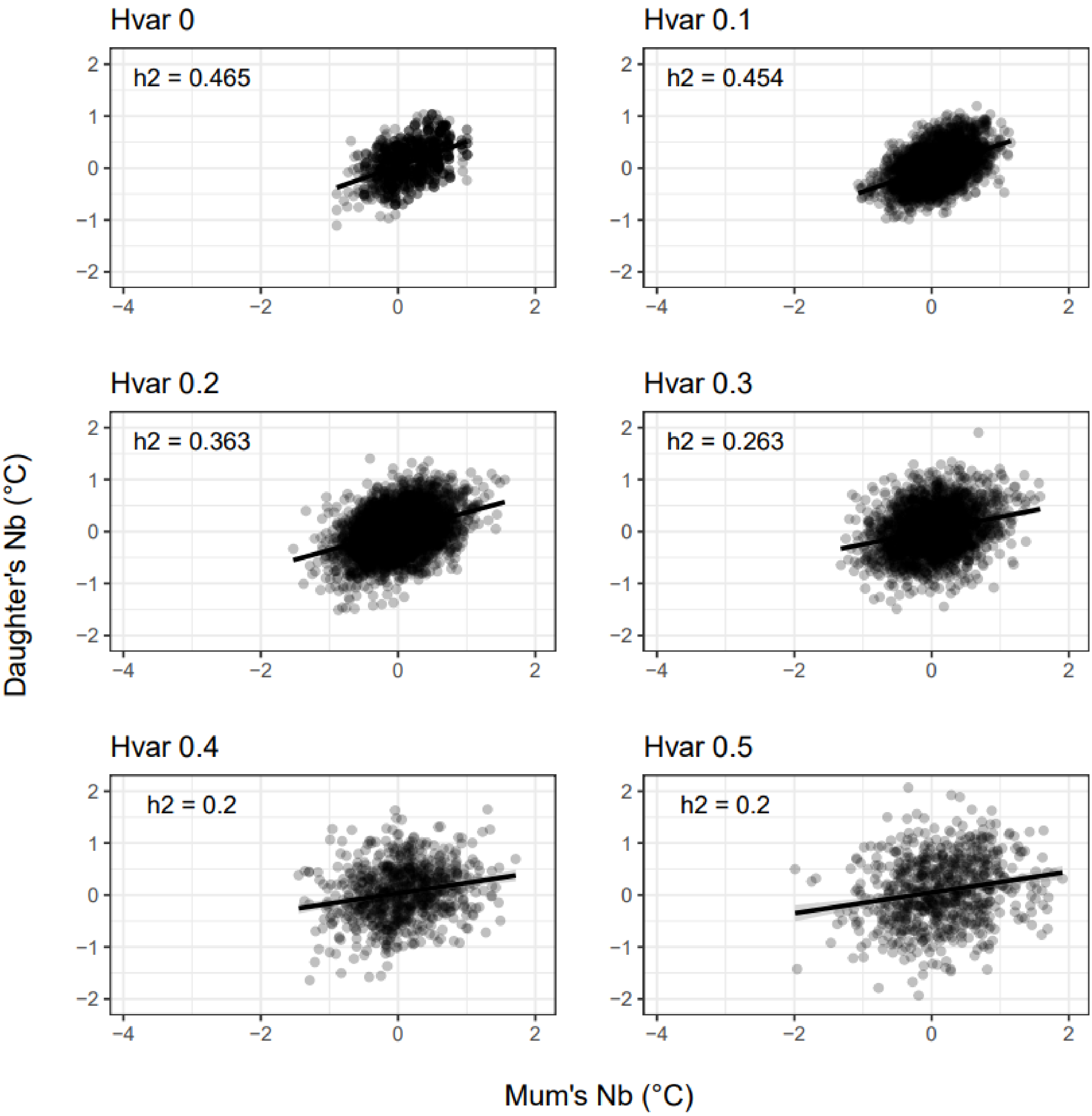

Figure S3. Relationships between mothers' and daughters' *Nb* at different levels of H_{var}.

Effect of H_{var} on Coevolution of T_{piv} and Nb

Varying heritability does not appear to have a strong effect on the coevolution of T_{piv} and *Nb*. There is some variation between mean values T_{piv} and *Nb* from replicates with different levels of H_{var} (Table S6, Fig. S4). However, this variation does not occur in a consistent direction and this effect is not of a magnitude comparable to other factors tested in the main simulation, such as global temperature, temperature-dependent survival, and nesting plasticity.

Table S6. Mean results and sample sizes of coevolution simulation replicates at different levels of H_{var}.

Varying Trait	**Global Temperature (°C)**	H_{var}	**Final Mean** T_{piv} **(°C)**	**Final Mean** Nb **(°C)**	n
None	28	0	28.65	0.70	4
	30	0	29.77	-0.19	1
	32	0	30.49	-1.33	5
T_{piv}	28	0.1	28.81	0.73	6
		0.2	28.55	0.55	8
		0.3	28.70	0.69	8
		0.4	28.44	0.49	5
		0.5	28.47	0.47	5
	30	0.1	29.49	-0.45	3
		0.2	29.55	-0.43	9
		0.3	29.51	-0.43	5
		0.4	29.71	-0.34	6
		0.5	29.60	-0.46	4
	32	0.1	30.49	-1.34	5
		0.2	30.24	-1.66	5
		0.3	30.54	-1.47	7
		0.4	30.57	-1.38	1
		0.5	30.48	-1.38	3
Nb	28	0.1	28.09	0.08	3
		0.2	28.69	0.71	9
		0.3	28.33	0.43	4
		0.4	28.47	0.48	6
		0.5	28.55	0.51	5
	30	0.1	29.39	-0.60	5
		0.2	29.65	-0.29	4
		0.3	29.52	-0.49	6
		0.4	29.55	-0.40	7
		0.5	29.92	-0.18	3
	32	0.1	30.48	-1.42	8
		0.2	30.24	-1.70	4
		0.3	30.41	-1.44	4
		0.4	30.36	-1.48	2
		0.5	30.25	-1.64	4
Total					164

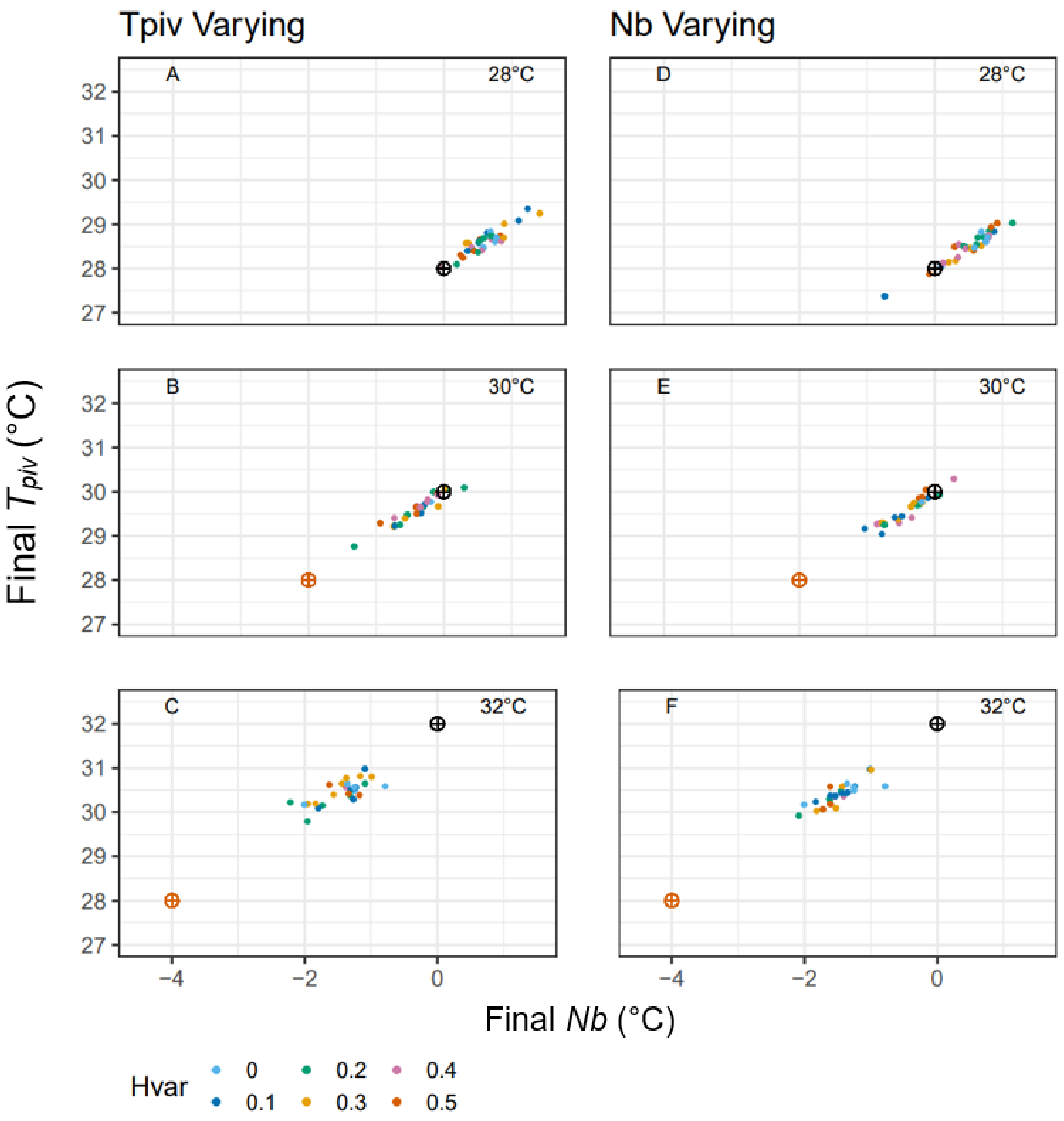

Figure S4. Comparison of final values of T_{piv} (y-axis) and *Nb* (x-axis) in coevolution scenarios (mean value of the last 1000 generations). The lef column shows results from scenarios where T_{piv} heritability varied relative to *Nb* heritability. The right column shows results from scenarios where *Nb* heritability varied. Climate scenario is indicated in the top right corner of each panel. H_{var} is the variation in trait heritability, higher values correspond to lower heritability (see Table 1). The black target symbols indicate the value of T_{piv} that would fully compensate for the scenario temperature rise, and the red target symbols indicate the equivalent value for *Nb*. Anything along the line between these two points represents production of a 50:50 sex ratio, by the evolution of T_{piv} and *Nb*.

Temperature-independent Fertility

In some 28°C scenarios of the main simulation, T_{piv} and Nb evolved to be considerably warmer than expected. We conducted an analysis to determine if this trend was caused by temperature-dependent fertility. Here we compare results from the main simulation with results of replicates were the beneficial effect of warmer developmental temperature on fertility was removed.

Temperature-dependent fertility explained the unexpected pattern of T_{piv} and Nb increase in the 28°C climate (Fig. S5, top row). When warm developmental temperatures increased female fertility, T_{piv} and Nb evolved to be warmer (Fig. S5, Table S7). Nb likely increased because warm nests increased female fitness, and T_{piv} likely increased in response to the resulting sex ratio selection. The strength of this trend decreased with increasing global climate, probably because the fitness benefit gained by producing males at warmer temperatures counteracted selection for increasing T_{piv} (Fig. S5, Table S7). Similarly, when embryonic survival depended on nest temperature, this counteracted the effect of temperature-dependent fertility, and led to lower values of T_{piv} and Nb.

Table S7. Comparing the results of replicates with temperature-dependent fertility (main simulation) and without temperature dependent fertility (model validation, in bold).

Global Temperature	**Temperature-dependent survival**	**Temperature-dependent fertility**	**Final Mean T_{piv} (°C)**	**Final Mean *Nb* (°C)**	**N**
28	No	Yes	28.61	0.66	28
		No	**28.14**	**0.14**	10
	Yes	Yes	28.34	0.32	30
		No	**27.9**	**-0.04**	10
30	No	Yes	29.52	-0.46	34
		No	**29.35**	**-0.73**	5
	Yes	Yes	28.7	-1.3	36
		No	**28.5**	**-1.44**	5
32	No	Yes	30.38	-1.52	22
		No	**30.33**	**-1.64**	5
	Yes	Yes	29.66	-2.32	26
		No	**29.59**	**-2.36**	5

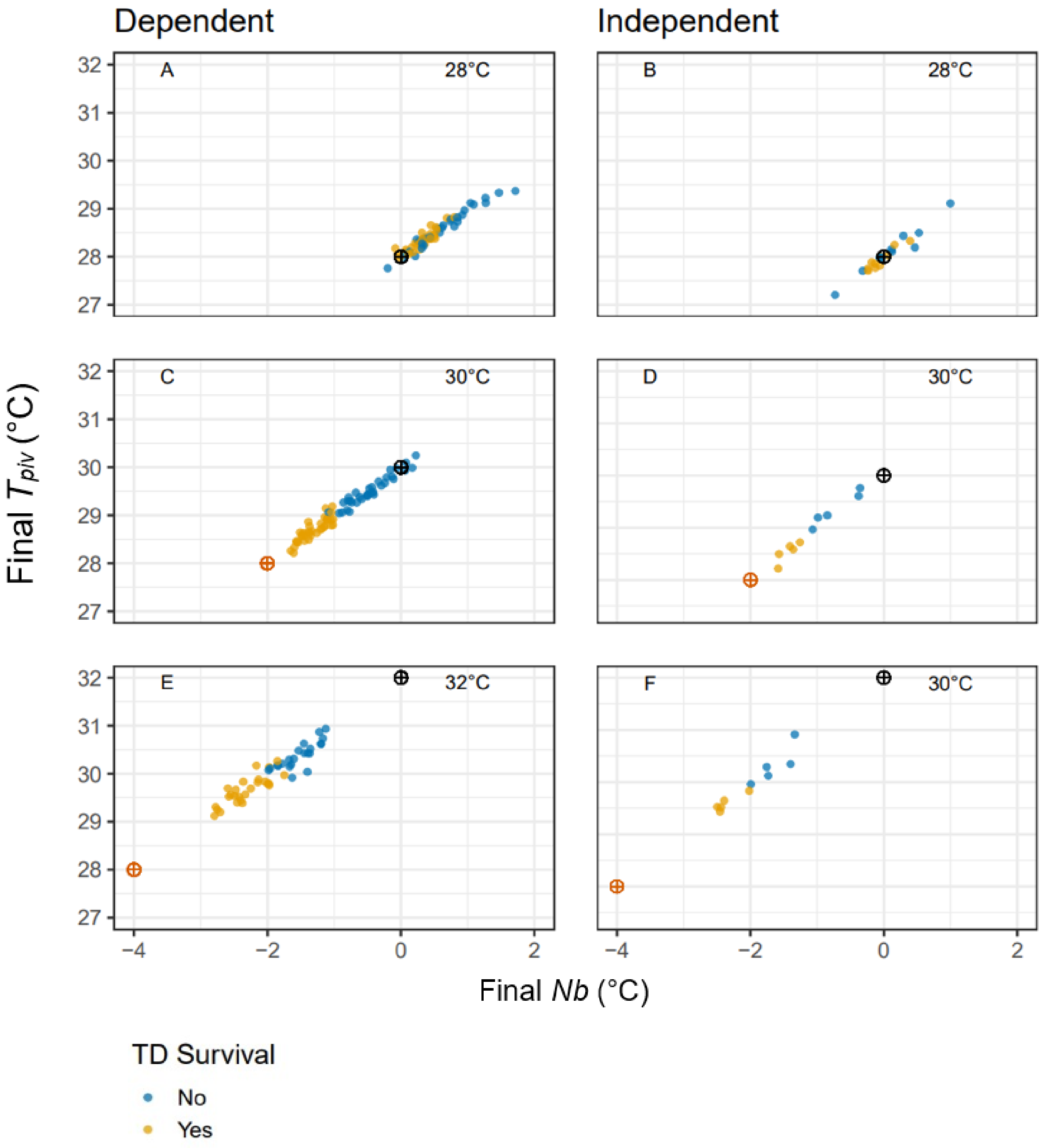

Figure S5. Comparison of final values of T_{piv} (y-axis) and *Nb* (x-axis) in coevolution scenarios (mean value of the last 1000 generations). The left column shows results from scenarios where a female's fertility depended on her developmental temperature. The right column shows results from scenarios where female fertility was independent from developmental temperature. TD survival indicates whether embryonic survival was affected by temperature in the replicate. Climate scenario is indicated in the top right corner of each panel. The black target symbols indicate the value of T_{piv} that would fully compensate for the scenario temperature rise, and the red target symbols indicate the equivalent value for *Nb*.