Metabolic consequences of sex-reversal in two lizard species: a test of the like genotype and like phenotype hypotheses

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*Short running title:* Energetic consequences of sex-reversal

**Abstract**

Vertebrate sex is typically determined genetically, but in many ectotherms sex can be determined by genes (Genetic Sex Determination: GSD), temperature (Temperature-dependent Sex Determination: TSD), or interactions between genes and temperature during development. Temperature dependent sex determination may involve GSD systems with either male or female heterogamety (XX/XY or ZZ/ZW) where temperature overrides chromosomal sex determination to cause a mismatch between genetic sex and phenotypic sex (sex-reversal). In these temperature-sensitive lineages, phylogenetic investigations point to recurrent evolutionary shifts between genotypic and temperature-dependent sex determination. These evolutionary transitions in sex determination can occur rapidly if selection favours the reversed sex over their concordant phenotypic sex. To investigate the consequences of sex-reversal on offspring phenotypes, we measured two energy-driven traits (metabolism and growth-rate) and 6-month survival in two species of reptile with different patterns of temperature-induced sex-reversal. Male sex-reversal occurs in *Bassiana duperreyi* when chromosomal females (femaleXX) develop male phenotypes (maleSRXX), while female sex-reversal occurs in *Pogona vitticeps* when chromosomal males (maleZZ) develop female phenotypes (femaleSRZZ). We show metabolism in maleSRXX was like that of maleXY, that is, reflective of phenotypic sex and lower than genotypic sex. In contrast, for *Pogona vitticeps*, femaleSRZZ metabolism was intermediate between maleZZ and femaleZW metabolic rate. For both species, our data indicate differences in metabolism become more apparent as individuals become larger. Our findings provide some evidence for an energetic advantage from sex-reversal in both species but do not exclude energetic processes as a constraint on the distribution of sex-reversal in nature.

*Keywords****:*** *energetics, sex determination, sex-reversal, Pogona vitticeps, Bassiana duperreyi*

**1 | Introduction**

Sex-determination in vertebrates is highly variable, ranging from genotypic sex determination (GSD) where sex is established by sex chromosomes, to environmental sex determination (ESD) where sex is primarily influenced by prevailing environmental conditions (Bull, 1983). For some species, these pathways of reproductive development are not mutually exclusive but can co-occur (Bachtrog et al., 2014; Sarre et al., 2004). In a few well-studied species, GSD systems with either male (XX/XY) or female heterogamety (ZZ/ZW) are influenced by incubation temperature (Temperature-dependent sex determination; TSD) (Holleley et al., 2015; Noble et al., 2018; Quinn et al., 2009). In these GSD species, when conditions experienced during critical developmental stages exceed a threshold temperature that overrides genetic sex-determining mechanisms. This temperature override, commonly referred to as sex reversal, causes a discordance between phenotypic and genotypic sex (Holleley et al., 2015; Quinn et al., 2009; Radder et al., 2009). Theoretical models predict that when sex-reversed individuals have a greater fitness advantage, populations can rapidly lose the heterogametic sex chromosome (XY or ZW) and result in pure TSD lineages within a few generations (Grossen et al., 2011; Holleley et al., 2015; Schwanz et al., 2020). Consequently, these TSD lineages should become widely established in free-living populations where environmental conditions favour their emergence. However, sex-reversal in some species is not distributed across ecotypes in natural systems as would be, suggesting that free-living animals experience costs associated with sex-reversal that are not accounted for in theoretical models (Bókony et al., 2021; Castelli et al., 2021; Wild et al., 2022). Therefore, quantifying the costs and benefits of sex-reversal will help clarify patterns of sex-reversal in wild populations and will provide insight into the mechanisms that may inhibit or accelerate evolutionary transitions in sex-determining systems (Bachtrog et al., 2014; Sarre et al., 2004).

Of crucial importance for individual growth, reproduction, and survival is energy expenditure (Angilletta, 2009; Bradshaw, 1997), which can be estimated by measuring metabolic rates. In both empirical and theoretical studies, estimates for metabolism have shown to be linked to individual patterns of growth, reproduction and survival (Peterson et al., 1999; Burton et al. 2011; White et al., 2022). Such estimates provide a crucial link between individual life history traits (growth rates, developmental rates, and age at maturity) and population processes (growth rates, carrying capacity, and rates of competition) in a variety of taxa (Brown et al., 2004; Ernest et al., 2003; Burger et al., 2021; Savage et al., 2004). Importantly, strategies used to secure, allocate and expend energy can vary considerably between phenotypic sexes (Arnqvist et al., 2022; Boratyński et al., 2010; Codding et al., 2011; Geffroy, 2022) and may play a driving role in establishing energetic differences in sex-reversed individuals and their phenotypic counterparts. Exploring how sex-reversal impacts metabolism and other traits that relate to energy use will provide insight if costs or benefits exist with sex-reversal and how these responses relate to chromosomal or phenotypic sex. These data will provide valuable information that may help elucidate the distributional patterns of sex-reversal in natural populations.

Identifying the costs and benefits of sex-reversal allows predictions of the conditions under which selective advantages of sex-reversal might lead to rapid transitions in sex-determining mechanisms and help provide an understanding of variation in its frequency across wild populations. Three plausible phenotypic/genetic patterns may manifest that can influence the evolution of sex-reversal in nature (Fig. 1 – e.g., metabolism):

1. there is no difference in metabolism, growth, or survival among different genotype-phenotype combinations such that males, females, and sex-reversed individuals are indistinguishable (Null);
2. sexes are phenotypically similar with discordant sex-reversed individuals (e.g. femaleSR ZZ) and concordant individuals of the same *phenotypic* sex (e.g. female ZW) exhibiting similar metabolic rate, growth, and/or survival (Like Phenotype); or
3. sexes are phenotypically different with discordant sex-reversed individuals (e.g. femaleSR ZZ) and concordant individuals of the same *chromosomal* sex (e.g. male ZZ) exhibiting similar metabolic rate, growth, and/or survival (Like Genotype).

Evidence for the Like Phenotype would suggest that metabolic differences between phenotypic sexes (i.e., male vs. female) are driven by hormonal mechanisms or sexually- antagonistic selection that leads to sexual dimorphism in traits such as morphology or physiology (Cox et al., 2017; Eyer et al., 2019; Lipinska et al., 2015; van Doorn & Kirkpatrick, 2010). Alternatively, support for the Like Genotype would imply that sex-linked genes are involved in the expression of traits linked with metabolism, energy use, and potentially other fitness-related endpoints(Charlesworth & Charlesworth, 1980; Fisher, 1931; Harrison et al., 2015). Under the scenario where selection for the Like Genotype is favoured, an increase in the frequency of XX or ZZ individuals within a population would occur and would rapidly increase the chances of a transition from GSD to TSD (Grossen et al., 2011; Schwanz et al., 2020). To date, no studies have explored how energetic components (i.e. metabolism, growth, maintenance) are affected by sex-reversal, even though sex-specific strategies of energy allocation have been documented between males and females (Geffroy, 2022; Somjee et al., 2022).

To estimate the consequences of sex-reversal and to determine if sex-reversed individuals are more similar to their reproductive phenotype or chromosomal complement, we measured two energy-driven traits (metabolism and growth) and 6-month survival in two lizard species that demonstrate sex-reversal (*Pogona vitticeps*&*Bassiana duperreyi*). Sex-reversal in *B. duperreyi* occurs when chromosomal females (female XX) develop male phenotypes [maleSR XX] (Dissanayake, Holleley, Deakin, et al., 2021; Quinn et al., 2009), whereas sex-reversal in *P. vitticeps* occurs when chromosomal males (maleZZ) develop female phenotypes [femaleSR ZZ] (Holleley et al., 2015; Quinn et al., 2007).In both species, this phenomenon has been documented in the wild(Dissanayake et al., 2020; Holleley et al., 2015; Wild et al., 2022), with evidence indicating both species are sensitive to climate change (Dissanayake, Holleley, & Georges, 2021; Holleley et al., 2015; Schwanz et al., 2020). Similarities between phenotypic forms (femaleSR ZZ and female ZW in *P. vitticeps*; or maleSR XX and male XY in *B. duperreyi*) in these traits would suggest a lack of differences imparted by sex-reversal, while similarities in traits between genotypic forms (femaleSR ZZ and male ZZ in *P. vitticeps*; or maleSR XX and female XX in *B. duperreyi*) would indicate the potential for differences in between discordant and concordant sex. How these traits vary across sex can provide valuable information on the ecological significance of sex-reversed individuals and help determine mechanisms affecting the evolution of sex chromosomes in vertebrate species.

**2 | Materials and methods**

**2.1| Lizard collection and husbandry**

*Bassiana duperreyi* –Twenty-five *B. duperreyi* nests with a total of 40 eggs (1-4 eggs per nest) were opportunistically located in November 2020 by flipping rocks, logs, and other cover objects at two field locations within the Brindabella Range (Mount Ginini – 1640 m a.s.l., 35°31’29.6“S 148°46’58.7”E; Piccadilly Circus – 1240 m a.s.l., 35°21’42.0“S 148°48’12.5”E). These sites were selected because of high frequencies of sex-reversal previously documented within these populations (Dissanayake, Holleley, Deakin, et al., 2021). The number of eggs per nest was recorded, and temperature dataloggers (iButton® model DS1921G; accuracy°C) were placed at the core of each nest to monitor nest temperatures. Each nest was maintained in natural conditions for 9-10 weeks at each location, and the mean nest temperatures (Mount Ginini – 18.94°C 0.98 & Piccadilly Circus – 20.42°C 0.84; Fig. S1) were monitored to ensure approximately 90% of the development period passed in natural conditions (Shine et al., 2002). Therefore, sex-reversal in *B. duperreyi* occurred in natural nest sites due to exposure to sex-reversing low temperatures (<20°C) *in situ*. The eggs were then collected, placed in moist vermiculite, and transported back to the University of Canberra. Eggs were placed in incubators (LabWit, ZXSDR1090) that maintained 23°C, which produces a balanced sex ratio(Shine et al., 2002). *See* Dissanayake et al 2021 for the study site description and further detail regarding general egg collection methods.

Phenotypic sex was determined by squeezing the tail base to evert the hemipenes (Harolow, 1996) for 3-to-7-day old hatchlings and was checked again by hemipene transilluminationafter 5 weeks (Dissanayake, Holleley, & Georges, 2021). Blood from the tail of each individual was collected on Whatman FTATM Elute Micro Card (CAT No. WB120410). Lizards were housed individually in plastic containers (0.35x0.25x0.15m). Each tub contained cardboard tubes and paper egg carton pieces for hides. Full-spectrum UV bulbs and heat bulbs were placed alternating between tubs to create a thermal gradient in each tub (heat from one side, UV from the other). Hatchlings were fed live, gut-loaded crickets once per day *ad libitum* and twice per week the crickets were dusted with calcium powder. Hatchlings were provided with shallow water dishes that were replenished daily, and they were misted twice per day with water.

*Pogona vitticeps* – The University of Canberra (UC) maintains a breeding colony of adult *P. vitticeps*, where breeding enclosures are comprised of one male (maleZZ) to either three sex-reversed females (femaleSR ZZ) or three concordant females (female ZW). During the summer of 2020–2021, females were allowed to lay eggs, which were collected within 2h of deposition. Eggs (n= 96) from 15 clutches were randomly allocated to either 28°C (n= 43; no sex-reversal expected) or 34°C (n = 53; reversal of 50% of ZZ genotypes expected) in temperature-controlled incubators (LabWit, ZXSDR1090) on moist vermiculite. Thus, sex-reversal in *P. vitticeps* occurred because of exposure to sex-reversing high temperatures (> 32°C) during incubation. Once hatchlings emerged, the determination of phenotypic sex and blood sampling followed the same protocols as for *B. duperreyi*. Hatchlings were housed in plastic tubs (0.8x0.5x0.35m; 5 individuals per tub), and in addition to crickets, finely grated vegetables were introduced to the diet beginning at 6-7 weeks post-hatch.

**2.2| Genotyping**

Genotypic sex was determined for both *B. duperreyi* and *P. vitticeps* using polymerase chain reaction (PCR)-based molecular sex tests from extracted DNA collected from tissue samples. DNA was extracted from tissue samples. DNA purity was determined using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and quantified using the Qubit 2.0 Fluorometric Quantitation (Invitrogen, Life technologies, Sydney, N.S.W., Australia). The sex-reversal status was determined for *B. duperreyi* by using PCR as described by Dissanayake et al. (Dissanayake et al., 2020), where the genotypic sex was identified based on Y-specific markers allowing identification of XX and XY samples. No XY females were observed, which is consistent with previous observations that recombination and/or mutation involving these loci is negligible and does not affect the accuracy of genotypic sex assignment. Genotypic sex (ZZ/ZW) for *P. vitticeps* was determined using a PCR-based molecular sex test that amplifies a W-chromosome-specific size polymorphism (Holleley et al., 2015), in which two bands amplify in ZW individuals and one control band amplifies in ZZ individuals. No ZW males were observed. All PCR products were visualized on a 1.5% agarose gel using SYBR Safe (Life Technologies, Carlsbad, USA), and all molecular sex tests were conducted blind to the phenotypic sex of the individuals. For both species, sex class accounted for genotype and phenotype and when genotype–phenotype discordance occurred individuals were classified as sex-reversed (Holleley et al., 2015).

**2.3 | Respirometry**

Metabolic rate (MR) was defined as the rate of oxygen consumption (V̇O2, mL min−1) of post-absorptive animal using a stop flow respirometry system (Stable System FMS, Las Vegas NV, USA). A gas analyser sub-sampler was used to pump outside air scrubbed of CO2 (using soda lime, Chem-Supply, AUS) and water vapour (using Drierite, W. A. Hammond Drierite Co. Ltd) to a mass flow controller that regulated flow rate to a nominal value of 130 ml min−1 (*B. duperreyi*) or 250 ml min−1 (*P. vitticeps*). After passing through the mass flow controller, air was pushed through an airtight cylindrical respirometry chamber, with dimensions designed specifically for each species (*B. duperreyi*: 75x20mm; *P. vitticeps*: 200x40mm). Air was pushed into the chamber and then through a flow meter ensuring that flow rates were constant. Air was then rescrubbed of water vapor, using Drierite, before being passed through H2O and O2 gas analysers. The fractional concentration of O in the ex-current air (FO2) was recorded at a frequency of 1 Hz. Following the manufacture protocols, both H2O and O2 analysers were calibrated prior to experiments.

Metabolic rate was measured within 3 weeks of hatching for all individuals. After a minimum of 24 h fasting period, body mass (0.01g) was measured of each individual lizard using a digital sale (Ohaus SP-202) before and after being placed in the respirometry chamber. Two incubators (LabWit, ZXSDR1090) were used to control the temperature of outside air being pulled into the respirometry system and then flowed through to the second incubator that controlled the temperature ( 1°C) in which animals in chambers were placed. Incubator temperatures were held at a constant temperature relevant to the thermal preference for each species (*B. duperreyi* 34°C (Du et al., 2010); *P. vitticeps* 33°C (Greer, 1989). At approximately 17:00 lizards were placed in respirometry chambers inside a dark incubator and remained in the chambers overnight for the duration of the experiment. As such, these animals were mainly in a quiescent state, but some activity may have occurred within the chamber. Each respirometry trial lasted approximately 14 h, and to ensure that animals were habituated within chambers, the first 2 h of data were discarded from analysis. The system contained seven chambers that lizards were placed in individually and one empty chamber designated as a control. The O2 consumption of each lizard was measured continuously for 5 min over a 70 min sampling window, and this sampling sequence was repeated every 70 min for the duration of the experiment. Immediately following each individual lizard measurement, the control chamber recorded for 5 min as a baseline of O2. During each 70 min sampling window O2 depletion for each individual was identified using the R package “metabR” (github.com/daniel1noble/metabR) and O2 depletion was averaged for each individual across the night to represent MR. The rate of O2 depleted by an individual was calculated following Eq. 4.21 *in* Lighton, 2008):

where the rate of O2 is the maximum percentage of O2 a sample below that baseline; Vchamber is the volume of the chamber (*B. duperreyi*: 23.56 mL; *P. vitticeps*: 251.33 mL); Vlizard was calculated as an average between the pre- and post-measurement mass of each individual, and t is the duration of time the chamber was sealed between air samples taken (70 min). The mass of each lizard was used as a proxy for its volume (1g = 1 ml) because of their high correlation and increased accuracy and precision in mass measurements(Friesen et al., 2017).

**2.4 | Growth and survival**

Measurements of snout-to-vent length (SVL) and mass were used to estimate growth rates. SVL and mass were initially measured during respirometry experiments and remeasured 6 months after the initial measurements. Growth rate was calculated by subtracting initial measurements (SVL or mass) from the final remeasurement and dividing the elapsed time between measurements. SVL growth rate was recorded in mm/d for both species, and mass growth rate was recorded in centigrams (cg/d) for *B. duperreyi* and (g/d) for *P. vitticeps*. The survival rate of hatchlings was determined by documenting the frequency of mortality between the hatch date and 6 months post-hatch date for both species.

**2.5 | Statistical analysis**

All statistical analysis were conducted using the R environment, ver. 4.1.0 ([www.r.-project.org](http://www.r.-project.org)). Bayesian linear mixed effect models from the package *brms* (Bürkner, 2017) were used to analyse O2 data for each species. We used Bayesian modelling approaches because of their flexibility with respect to parameter estimation. It is also easier to interpret and manipulate posterior probabilities for each parameter in the model. Default priors *(See Supplementary Material for Details)* were used and 4 MCMC chains of 5000 were run with a burn in of 1000 and a thinning interval of 5 for the “brms” models. All models were checked for proper mixing and convergence by visually inspecting trace plots. For each species two models were fitted, the first in which homoscedasticity of the data was assumed and the second in which heteroscedasticity was accounted for within the data. The first model for estimating metabolism was fitted using the following structure:

where is the metabolic rate () for measurement *i* (i = 1 to , number of measurements) on individual *j* (j = 1 to , number of individuals) and day *k* (k = 1 to , number of days). Contrasts for the different sex classes (), where and are for concordant sexes and sex-reversed animals, respectively. A linear slope 4 was estimated for measurement time (, z-transformed) and a random intercept () and slope for () were included for individual *j* across measurement occasions. A linear slope for log transformed mass (, centered on mean, sc) and mass scaling relationships were estimated separately for the different sex classes (i.e., , , and respectively). Deviations were sampled from a multivariate normal distribution (~, where ID is a (co)variance matrix with a random intercept and slope variance and their covariance. A random-effect for day () (~ ) was also included in the model to account for variation across days in metabolic rate. In all models, we retained data for each measurement throughout the night to improve analytical power. Given that animals were quiescent, our MR data is expected to be representative of Standard Metabolic Rate (SMR). Nonetheless, some movement did occur in our chambers. As such, we also fit the same models described above but kept the lowest 10% of oxygen consumption values during trials – data that should be quite close to SMR. We found no changes in our results when using the full dataset compared to the dataset that only used the lowest 10% (*see* Fig. S2; Tables S1 & S2 in *Supp*). Therefore, all V̇O2 measurements from trials (MR) were kept for further analysis.

Differences in growth rates were compared across sex class using Bayesian linear models while accounting for individual mean metabolism. This allowed us to test if there was a relationship between metabolism and growth rate (mass or svl) across sex class. Fisher’s exact tests were used to determine if there was an association between sex class and frequency of hatchling mortality after six months.

For all Bayesian models, posterior estimates were from multiple chains, and we present posterior means and their 95% credible intervals. To test for the Like Genotype (genotype - sex-reversed) or Like Phenotype (phenotype - sex-reversed) framework for each species, contrasts were calculated by subtracting the posterior distributions of each sex class. To test if the magnitude of these differences varied significantly, probabilities of parameter estimates were considered statistically significant when the 95% CIs did not include 0, and the pMCMC values calculated by *MCMCglmm* were less than 0.05 (Hadfield, 2010). Data, code, and additional resources are available at <https://github.com/daniel1noble/energy_sex_reversal.git>.

**3 | Results**

**3.1 | Energetic consequences of sex-reversal**

*Bassiana duperreyi* - A total of 760 measurements for 40 individuals (male XX: n = 13, female XX: n = 15, male XY: n = 12) were recorded. There was a strong scaling relationship between log metabolic rate and log mass (Table 1), and scaling slopes varied significantly depending sex class (significant interaction between sex class × logmass – Fig. 2A). Sex-reversed male XX *B. duperreyi* had a mass-specific metabolic rate that was most like their phenotypic counterparts (male XY - maleSR XX; pMCMC = 0.33; Table 3) and lower than their genotypic counterparts (female XX - maleSR XX; pMCMC < 0.01). For phenotypic males (maleSR XX & male XX), the scaling relationship between logmass and metabolism changed similarly across differently sized individuals (Fig. 2B; Table S3). Pairwise comparisons across sex class indicated no differences in body mass across our treatments (Fig. 2A; Table S4). The homogeneous variance model was the most parsimonious ([heteroscedastic model – homoscedastic model] loo: -5.5, SE = 6.87), accounting for 77% (95% CI:0.75 - 0.78) of the variation in metabolic rate.

*Pogona vitticeps* - A total of 1365 measurements for 96 individuals (femaleSR ZZ: n = 28, female ZW: n = 30, male ZZ: n = 38) were recorded. There was a strong scaling relationship between log metabolic rate and log mass (Table 2), and scaling slopes varied significantly depending sex class (significant interaction between sex class × logmass - Fig. 2C). Sex-reversed female *P. vitticeps* (femaleSR ZZ) had a mass-specific metabolic rate that was overall higher than their genotypic counterparts (male ZZ - femaleSR ZZ; pMCMC < 0.01), but lower than their phenotypic counterparts (female ZW - femaleSR ZZ; pMCMC = 0.04; Table 3). The mass scaling relationship of metabolism was more like ZZmales than ZW females (Fig. 2D; Table S3). As a consequence, large female ZZ have significantly lower metabolism compared to female ZW of comparable size (see Figure 2D; Table S3). Pairwise comparisons of body mass across sex class in *P. vitticeps* indicated no differences in body mass across treatments (Fig. 2C; Table S4). The heteroscedasticity variance model was the most parsimonious ([heteroscedastic model – homoscedastic model] loo: -189.8, SE = 33.96), accounting for 84% (95% CI:0.83 - 0.85) of the variation in metabolic rate.

**3.2 | Effects of sex-reversal on growth and survival**

Growth rates for both SVL and mass supported the Null prediction among *B. duperreyi,* where there were no detectible differences across sex class (Table 3). Similarly, in *P. vitticeps* the Null prediction was supported when comparing SVL and mass growth rates across sex class (Table 3). For both species, there was no relationship between individual metabolism and growth rate estimates (Table S5 & S6). Sex-reversed male *B. duperreyi* had the lowest rates of survival (77%; Table S7) in comparison to concordant females (87%) and concordant males (100%), but this relationship was non-significant (p = 0.29). Similarly, sex-reversed *P. vitticeps* individuals had the lowest rates of survival (75%; Table 3) in comparison to concordant females (83%) and concordant males (95%), but this relationship was also not significant (p = 0.06).

**4 | Discussion**

In this study, we examined two species with different modes of sex-reversal to test whether metabolic, growth, and survival differed for sex-reversed individuals and how these individuals compare to their phenotypic or genotypic sex. Metabolic responses differed between the two species, with clear support for the Like Phenotype Hypothesis when males reverse sex (maleSR XX; *Bassiana duperreyi*) and equivocal support for each hypothesis when females reverse sex (femaleSR ZZ; *Pogona vitticeps*). For both species, regardless of whether individuals reversed sex, phenotypic females required more energy than phenotypic males as individuals grew larger. Higher energy requirements for phenotypic females may partly be driven by energy allocation towards reproduction investment (Congdon, 1989; Hayward & Gillooly, 2011). While sex-reversed animals appeared to have reduced survival, albeit not significantly so, there is no clear evidence in either species for growth advantages over their phenotypic sex. Together this suggests traits associated with energy use and growth may not be particularly strongly tied to genes on the sex chromosomes, and other mechanisms, such as hormonal pathways, may explain the stronger signal for phenotypic sex differences (van Doorn & Kirkpatrick, 2010; Cox et al., 2017). If these patterns occur in adult individuals in natural populations, energetic processes may have varying impacts on the species' life-history traits, which could provide insight into what constrains the distribution of sex-reversal in nature.

This is the first study in any vertebrate species to estimate the metabolic consequences of temperature-induced sex reversal. In both GSD systems in this study, concordant females had higher mass scaling relationships for metabolism than concordant males (Tables 1 & 2), but we showed that metabolic scaling relationships of sex-reversed individuals differed depending on the GSD system. In the ZZ/ZW system, larger sex-reversed females (femaleSR ZZ;> +1.5SD above mean mass) have lower metabolism (15%) than concordant females (female ZW) and appear to be more like concordant males (male ZZ; Fig. 2D; Table S3). There is evidence that selection does occur for larger hatchling lizards in the wild, i.e. ‘bigger is better’ hypothesis (Ferguson & Fox 1984; Sinervo et al.,1992; Warner & Andrews, 2002). Together, when investigating larger *P. vitticeps* individuals within a population, we predict that femaleSR ZZ would have more residual energy than female ZW to allocate towards maintenance, growth, or behaviours after resting metabolic costs have been paid. This surplus in energy reserves for femaleSR ZZ may explain why sub-adult (<1year) and adult femaleSR ZZ *P. vitticeps* have similar behaviours and morphologies as male ZZ(Holleley et al., 2015; Li et al., 2015). Different strategies of energy allocation throughout ontogeny may explain previously observed differences in morphology, behaviour, and fecundity differences as sub-adults or adults.

One simple explanation for the lack of differences observed in metabolic rates and growth between male XY and maleSR XX *B. duperreyi* is there being little or no selection on sex-reversal in this species. However, sex-reversal in *B. duperreyi* is linked to changes in elevational gradients where high elevations, with cooler temperatures, increase the frequency of sex-reversed males (Dissanayake, Holleley, Deakin, et al., 2021). Additionally, hatchling phenotypes - morphology, locomotor performance, growth rates, survival, cognitive ability - are significantly influenced by incubation temperatures in *B. duperreyi* (Amiel & Shine, 2012; Flatt et al., 2001; Shine et al., 1997; Shine & Harlow, 1996). Geographic range, habitat, and behaviours can notably affect metabolic rates within species (Angilletta, 2001; Sears, 2005). It is possible that selection on metabolism between sex does occur, but these differences are subtle depending on the environment or population of *B. duperreyi* being sampled*.* For populations at higher elevations, we would predict higher temperature dependence for physiological processes, such as metabolic rate. Additionally, lizard populations at higher elevations would have limited time to achieve body temperatures at physiological optimums or acclimation responses to temperature may differ in lower populations (Jameson Jr et al., 1977; Tsuji, 1988). One alternative explanation for not capturing differences between maleSR XX and male XY in our metabolic measurements is that the temperature selected for our metabolic experiments was not at an ecologically relevant body temperature that hatchling lizards actively select for in natural settings to assimilate energy. Behaviours and physiological processes of hatchling *B. duperreyi* are affected by mean temperature, the variance of temperature within each day, and temperature differences across months (Shine, 2002, 2004; Shine et al., 1997; Shine & Elphick, 2001; Shine & Harlow, 1996). Local adaptations in other physiological traits have been postulated as a possible mechanism for explaining the distribution of sex-reversal in other species (Castelli et al., 2020). Further insight could be made by examining how metabolic rates vary along a gradient of temperatures and how local adaptations in physiological traits influence sex-specific selection processes in *B. duperreyi* and other species that undergo sex-reversal.

The frequency of sex-reversal in *P. vitticeps* occurs across a large part of its range, but neither latitude, climate, or behaviour explain the distribution of sex-reversal (Castelli et al., 2021; Wild et al., 2022). Interestingly, we also found little evidence that in early life stages metabolic rate differed between sex concordant and sex-reversed *Pogona*, expect when comparing the largest individuals born. Among the largest hatchlings, sex reversed animals had lower metabolic rate compared to concordant sex lizards of comparable size (Figure 2D). Given that mortality and selection on body size is often strongest early in life for many reptiles (Sinervo et al.,1992; Warner & Andrews, 2002), energetic differences could help to explain the changes in the frequency of sex-reversal in *Pogona*. The higher survival of larger hatchlings, combined with lower metabolism of sex-reversed females, may impact differences in energy allocation to reproduction or survival of these individuals in the wild. Such differences may be magnified by theunpredictable resource pulses (high rainfall events/high productivity vs. drought/low productivity) in arid or semi-arid environments that are known to shape many demographic processes for other species (Kwok et al., 2016; Letnic & Dickman, 2010; Kearney & Porter, 2004; Congdon, 1989). Locations that experience stochastic fluctuations in resource availability may allow femaleSR ZZ to persist in low frequencies (Burton et al., 2011; Ricklefs & Wikelski, 2002). Future work testing this hypothesis in wild populations will be potentially fruitful in helping to understand the occurrence of sex-reversal in *Pogona*.

There has been little to no attention focused on the energetic and survival consequences associated with genotype and phenotype mismatches and how these sex-reversed individuals compare to their phenotypic or genotypic sex. The lack of clear evidence for differences in metabolism, growth, and survival for sex-reversed individuals (maleSR XX or femaleSR ZZ) over their concordant phenotypic sex (male XY or female ZW) in our study provides insight into possible targets of selection on hatchling phenotypes for species that undergo sex-reversal. In particular, the data indicate that the magnitude of metabolic differences varies across sexes as individuals get larger (Fig. 2). Investigating ontogenetic changes associated with sex-reversal will provide promising insights into the consequences of such effects. While egg incubation differed between the species for logistical reasons – for *B. duperreyi* 90% occurred in the field, while in *P. vitticeps* all eggs were incubated in the laboratory – we do not expect this difference to impact the relative differences we observed between sex-reversed and concordant individuals. However, it may have resulted in some differences in patterns observed between the two species (beyond their different genetic sex-determining systems), although we think this is unlikely. In both species, incubation temperatures mimicked nest temperatures documented in the wild (Castelli et al., 2020; Dissanayake et al., 2021), and all hatchlings were reared under common laboratory conditions for the first 6-months of life when all measurements were taken. Although we did not detect a significant difference in survivorship, in both species, sex-reversed hatchlings had a higher frequency of mortality over a 6-month period than the other sexes. High mortality has been previously observed in sex-reversed individuals in laboratory experiments (Mikó et al., 2021) and in the wild (Wild et al., 2022). Further investigation is required to understand the cause of this low survivorship and the demographic consequences these results have for the emergence of sex-reversal(Cotton & Wedekind, 2009). Overall, the lack of explicit support in our data for the Like Genotype in metabolism, growth, or survivorship reveals clues on the mechanisms that drive sex-reversal in nature.

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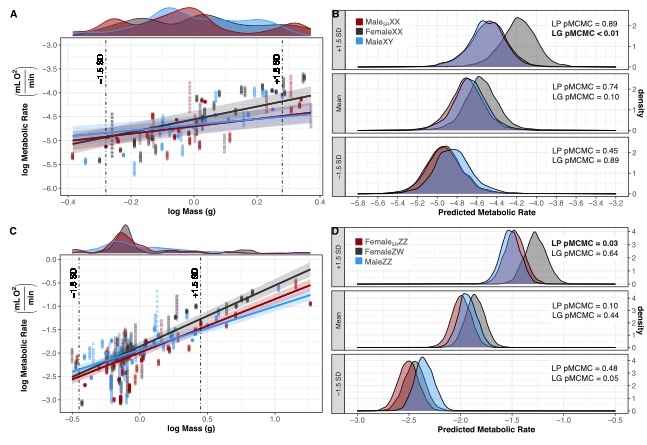
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**Figure 1.** The Like Phenotype/Genotype Framework for testing the metabolic consequences of sex-reversal for ZZ/ZW and XX/XY genetic sex determination systems with different patterns of genetic sex determination. Colours indicate sex class for each species. Body mass and metabolic rates have been log transformed to approximate linear relationships. Each pattern of genetic sex determination contains three competing hypotheses for the relationship between body mass and metabolic rates: Null – no differences; Like Phenotype – similarities between reversed sex and concordant phenotype; Like Genotype – similarities between reversed sex and concordant genotype.



**Figure 2.** Comparison of log metabolic rate (V̇O2 mL min) across log body mass (g) by sex class for *Bassiana duperreyi* (A-B) and *Pogona vitticeps* (C-D). Sex-reversed individuals (maleSR XX or femaleSR ZZ) are denoted by red colour, phenotypic females (female XX or femaleZW) are denoted in black, phenotypic males (male XY or maleZZ) are denoted in blue. Fitted lines were obtained from predicted values from the brms model for each species and confidence bands were constructed from the SE of prediction values for each sex (A,C). Density plots above each regression plot denote the distribution in body mass (log mass) by sex for each species. To visualize how log metabolic rate changes across log body mass, panels (B and D) show the distribution of predicted metabolic rate at three areas of log body mass (mean, +1.5SD, and -1.5SD) denoted by the dash-dot line in panels A and C for each sex and species. In panels A and C pMCMC indicate contrast differences between Like Phenotype (LP) or Like Genotype (LG) for each distribution.

**Table 1**. Model coefficients for testing whether sex affects the slope of metabolic rate for *Bassiana duperreyi*, where the intercept is concordant females. Metabolic rate and mass were log transformed and time was z-transformed. Columns l-95% CI and u-95% CI, are the lower and upper bound of the 95% credible interval for each parameter, estimated from the posterior distribution.

| Parameter | | Estimate | | l-95% CI | | u-95% CI | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *Fixed Effects* |  | |  | |  | |
| **Intercept (FemaleXX)** | **-4.56** | | **-4.90** | | **-4.20** | |
| MaleSRXX | -0.15 | | -0.32 | | 0.02 | |
| MaleXY | -0.12 | | -0.29 | | 0.06 | |
| **logMass** | **1.34** | | **0.87** | | **1.81** | |
| ztime | 0.01 | | -0.02 | | 0.05 | |
| **MaleSRXX:logMass** | **-0.56** | | **-0.90** | | **-0.23** | |
| **MaleXY:logMass** | **-0.74** | | **-1.07** | | **-0.41** | |
| *Random Effects* |  | |  | |  | |
| Lizard Identity (id) |  | |  | |  | |
| **Intercept** | **0.25** | | **0.19** | | **0.33** | |
| **Slope** | **0.09** | | **0.07** | | **0.13** | |
| Sampling Session (day) |  | |  | |  | |
| **Intercept** | **0.38** | | **0.17** | | **0.83** | |
| **Residuals** | **0.26** | | **0.25** | | **0.28** | |

**Table 2**. Model coefficients form hetero testing whether sex affects the slope of metabolic rate for *Pogona vitticeps,* which heteroscedasticity was accounted for within the data. The intercept is concordant females. Metabolic rate and mass were log transformed and time was z-transformed. Columns l-95% CI and u-95% CI, are the lower and upper bound of the 95% credible interval for each parameter, estimated from the posterior distribution.

| Parameter | Estimate | l-95% CI | u-95% CI |
| --- | --- | --- | --- |
| *Fixed Effects* |  |  |  |
| **Intercept (FemaleZW)** | **-1.86** | **-2.04** | **-1.67** |
| FemaleSRZZ | -0.13 | -0.28 | 0.03 |
| MaleZZ | -0.07 | -0.22 | 0.07 |
| **logMass** | **1.30** | **1.11** | **1.49** |
| **ztime** | **0.06** | **0.04** | **0.08** |
| **FemaleSRZZ:logMass** | **-0.16** | **-0.32** | **-0.01** |
| **MaleZZ:logMass** | **-0.37** | **-0.55** | **-0.21** |
| *Random Effects* |  |  |  |
| Lizard Identity (id) | 0.22 | 0.18 | 0.27 |
| **Intercept** | **0.30** | **0.25** | **0.35** |
| **Slope** | **0.07** | **0.06** | **0.09** |
| Sampling Session (day) |  |  |  |
| **Intercept** | **0.28** | **0.19** | **0.42** |
| Residuals |  |  |  |
| **Sigma\_Intercept** | **-1.60** | **-1.64** | **-1.56** |
| **Sigma\_logMass** | **-1.40** | **-1.54** | **-1.26** |
| **Sigma\_ztime** | **0.22** | **0.18** | **0.27** |

**Table 3**. Posterior distributions for log metabolic rate (Log MR) and growth rate (SVL or mass) estimates when testing if sex-reversed individuals show support for Like Genotype or Like Phenotype Framework for *Bassiana duperreyi* and *Pogona vitticeps*. Estimates were comparisons from subtracting the model posterior distribution of the median for sex-reversed individuals by either their phenotypic or genotypic counterparts, depending on the framework being tested.

| Species | Test | Contrast | Estimate | | l-95% CI | u-95% CI | pMCMC Value |
| --- | --- | --- | --- | --- | --- | --- | --- |
| *B. duperreyi* | Log MR | MaleSR XX - Male XY | | 0.18 | -0.17 | 0.53 | 0.33 |
| **MaleSR XX - Female XX** | | **-0.56** | **-0.90** | **-0.23** | **< 0.01** |
| SVL (mm/d) | MaleSR XX - Male XY | | 3.77 | -8.53 | 15.57 | 0.53 |
| MaleSR XX - Female XX | | -4.06 | -15.80 | 7.71 | 0.47 |
| Mass (cg/d) | MaleSR XX - Male XY | | -0.43 | -4.92 | 3.88 | 0.85 |
| MaleSR XX - Female XX | | -2.59 | -6.54 | 1.13 | 0.18 |
| *P. vitticeps* | Log MR | **FemaleSR ZZ - Female ZW** | | **-0.16** | **-0.32** | **-0.01** | **0.05** |
| **FemaleSR ZZ - Male ZZ** | | **0.21** | **0.09** | **0.32** | **< 0.01** |
| SVL (mm/d) | FemaleSR ZZ - Female ZW | | 0.00 | -0.02 | 0.02 | 0.94 |
| FemaleSR ZZ - Female ZW | | -0.01 | -0.02 | 0.01 | 0.48 |
| Mass (g/d) | FemaleSR ZZ - Female ZW | | -1.50 | -4.60 | 1.78 | 0.37 |
| FemaleSR ZZ - Male ZZ | | -1.16 | -3.99 | 1.68 | 0.43 |