

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

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20 *Short running title:* Energetic consequences of sex-reversal
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22 *Keywords:* energetics, sex determination, sex-reversal, Pogona vitticeps, Bassiana duperreya

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27 **Summary statement:**
28 *Does phenotypic sex or genotypic sex drive differences in metabolism, growth, and survival*
29 *in two species that can reverse sex?*
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33 **Abstract**
34 Vertebrate sex is typically determined genetically, but in many ectotherms sex can be
35 determined by genes (Genetic Sex Determination: GSD), temperature (Temperature-
36 dependent Sex Determination: TSD), or interactions between genes and temperature during
37 development. Temperature dependent sex determination may involve GSD systems with
38 either male or female heterogamety (XX/XY or ZZ/ZW) where temperature overrides
39 chromosomal sex determination to cause a mismatch between genetic sex and phenotypic sex
40 (sex-reversal). In these temperature-sensitive lineages, phylogenetic investigations point to
41 recurrent evolutionary shifts between genotypic and temperature-dependent sex
42 determination. These evolutionary transitions in sex determination can occur rapidly if
43 selection favours the reversed sex over their concordant phenotypic sex. To investigate the
44 consequences of sex-reversal on offspring phenotypes, we measured two energy-driven traits
45 (metabolism and growth) and 6-month survival in two species of reptile with different
46 patterns of temperature-induced sex-reversal. Male sex-reversal occurs in *Bassiana duperreyi*
47 when chromosomal females (femaleXX) develop male phenotypes (males_{SR}XX), while female
48 sex-reversal occurs in *Pogona vitticeps* when chromosomal males (maleZZ) develop female
49 phenotypes (females_{SR}ZZ). We show metabolism in males_{SR}XX was like that of maleXY, that
50 is, reflective of phenotypic sex and lower than genotypic sex. In contrast, for *Pogona*
51 *vitticeps*, females_{SR}ZZ metabolism was intermediate between maleZZ and femaleZW
52 metabolic rate. For both species, our data indicate that differences in metabolism become
53 more apparent as individuals become larger. Our findings provide some evidence for an
54 energetic advantage from sex-reversal in both species but do not exclude energetic processes
55 as a constraint on the distribution of sex-reversal in nature.
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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, 1980). 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, 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 evenly across ecotypes in natural systems, suggesting free-living animals may experience costs associated with sex-reversal that are not accounted for in theoretical models (Bókonyi et al., 2021; Castelli et al., 2021; Mikó et al., 2021; Wild et al., 2022). Quantifying costs and benefits of sex-reversal will help clarify patterns of sex-reversal in wild populations and 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 which can be estimated by measuring metabolic rates. In both empirical and theoretical studies, estimates for metabolism have been shown to be linked to individual patterns of growth, reproduction and survival (Peterson et al., 1999; Burton et al., 2011; White et al., 2022). Metabolism (and associated energy expenditure) thus provides a crucial link between individual life history traits (somatic growth, developmental rates, and age at maturity) and population processes (population growth, 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; Coddington et al., 2011; Geffroy, 2022) and may contribute to energetic differences in sex-reversed individuals and their phenotypic and genotypic counterparts. Exploring how sex-reversal impacts metabolism and other traits that relate to energy use will provide insight into observed patterns of sex-reversal in natural populations.

Here, we test whether and to what degree sex-reversed individuals differ in metabolism, growth, and survival compared to their phenotypic and genotypic counterparts using two species of lizard, *Pogona vitticeps* and *Bassiana duperreyi*, that undergo sex-reversal in the wild (Dissanayake et al., 2021a; Holleley et al., 2015; Wild et al., 2022). Sex-reversal in *B. duperreyi* occurs when chromosomal females (female XX) develop male phenotypes [males_{SR} XX] (Dissanayake et al., 2021a; Quinn et al., 2009), whereas sex-reversal in *P. vitticeps* occurs when chromosomal males (male ZZ) develop female phenotypes [females_{SR} ZZ] (Holleley et al., 2015; Quinn et al., 2007). Three

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255 plausible phenotypic/genetic patterns may manifest that can influence the evolution of
 256 sex-reversal in nature (Fig. 1 – e.g., metabolism):

- 257 (1) there is no difference in metabolism, growth, or survival among different
- 258 genotype-phenotype combinations such that males, females, and sex-reversed
- 259 individuals are indistinguishable (Null);
- 260 (2) sexes are phenotypically similar with discordant sex-reversed individuals (e.g.
- 261 females_{SR} ZZ or males_{SR} XX) and concordant individuals of the same phenotypic sex
- 262 (e.g. female ZW, males_{SR} XY) exhibiting similar metabolic rate, growth, and/or
- 263 survival (Like Phenotype); or
- 264 (3) sexes are phenotypically different with discordant sex-reversed individuals (e.g.
- 265 females_{SR} ZZ or males_{SR} XX) and concordant individuals of the same chromosomal
- 266 sex (e.g. male ZZ, female XX) exhibiting similar metabolic rate, growth, and/or
- 267 survival (Like Genotype).

268 Evidence for the Like Phenotype hypothesis would suggest that metabolic differences
 269 between phenotypic sexes (i.e., male vs. female) may be driven by hormonal mechanisms
 270 or sexually-antagonistic selection that leads to sexual dimorphism in traits such as
 271 morphology or physiology (Cox et al., 2017; Eyer et al., 2019; Lipinska et al., 2015; van
 272 Doorn and Kirkpatrick, 2010). Support for the Like Genotype hypothesis would imply
 273 that sex-linked genes may be involved in the expression of traits associated with
 274 metabolism, energy use, and potentially other fitness-related endpoints (Charlesworth and
 275 Charlesworth, 1980; Fisher, 1931; Harrison et al., 2015). To date, no studies have
 276 explored how energetic components (i.e. metabolism, growth, maintenance) are affected
 277 by sex-reversal, even though sex-specific strategies of energy allocation have been
 278 documented between phenotypic males and phenotypic females (Geffroy, 2022; Somjee et
 279 al., 2022).

283 2 | Materials and methods

284 2.1| Lizard collection and husbandry

285 *Bassiana duperreyi* –Twenty-five *B. duperreyi* nests with a total of 40 eggs (1-4 eggs per
 286 nest) were opportunistically located in November 2020 by flipping rocks, logs, and other
 287 cover objects at two field locations within the Brindabella Range (Mount Ginini – 1640 m
 288 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
 289 148°48'12.5"E). These sites were selected because of high frequencies of sex-reversal
 290 previously documented within these populations (Dissanayake et al., 2021a). The number of
 291 eggs per nest was recorded, and temperature dataloggers (iButton® model DS1921G;
 292 accuracy ± 1°C) were placed at the core of each nest to monitor nest temperatures. Each nest
 293 was maintained in natural conditions for 9-10 weeks at each location, and the mean nest
 294 temperatures (Mount Ginini – 18.94°C ± 0.98 & Piccadilly Circus – 20.42°C ± 0.84; Fig. S1)
 295 were monitored to ensure approximately 90% of the development period passed in natural
 296 conditions (Shine et al., 2002). Therefore, sex-reversal in *B. duperreyi* occurred in natural nest
 297 sites due to exposure to sex-reversing low temperatures (<20°C) *in situ*. The eggs were then
 298 collected, placed in moist vermiculite, and transported back to the University of Canberra.
 299 Eggs were placed in incubators (LabWit, ZXSDR1090) that maintained 23°C, which

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produces a balanced sex ratio (Shine et al., 2002). For the study site description and further detail regarding general egg collection methods see (Dissanayake et al., 2021b). 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 transillumination after 5 weeks (Dissanayake et al., 2021b). 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 (male ZZ) to either three sex-reversed females (female_{SR} 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 ($\dot{V}O_2$, 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

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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 O₂ 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 scale (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;

$$VO_2 \text{ mL min}^{-1} = \frac{\%O_2(V_{\text{chamber}} - V_{\text{lizard}})}{t}$$

where the rate of O₂ is the maximum percentage of O₂ a sample below that baseline; V_{chamber} is the volume of the chamber (*B. duperreyi*: 23.56 mL; *P. vitticeps*: 251.33 mL); V_{lizard} 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

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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). 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:

$$MR_{ijk} = (\beta_0 + id_j + d_k) + \beta_1 \cdot Sex_{Female} + \beta_2 \cdot Sex_{Male} + \beta_3 \cdot Sex_{SR} + (\beta_4 + \beta_{(id_{ij})} \cdot time_z) + \beta_5 \cdot \log Mass_{sc} \cdot Sex_{Female} + \beta_6 \cdot \log Mass_{sc} \cdot Sex_{Male} + \beta_7 \cdot \log Mass_{sc} \cdot Sex_{SR} + e_{ijk}$$

where MR_{ijk} is the metabolic rate ($\log VO_2 \cdot mL^{-1} \cdot min^{-1}$) for measurement i ($i = 1$ to N_m , number of measurements) on individual j ($j = 1$ to N_{id} , number of individuals) and day k ($k = 1$ to N_d , number of days). Contrasts for the different sex classes ($\beta_1 - \beta_3$), where Sex_{Female} and Sex_{Male} are for concordant sexes and Sex_{SR} sex-reversed animals, respectively. A linear slope β_4 was estimated for measurement time ($time_z$, z-transformed) and a random intercept (id_j) and slope for $time_z$ ($\beta_{(id_{ij})}$) were included for individual j across measurement occasions.

A linear slope for log transformed mass ($\log Mass_{sc}$, centered on mean, sc) and mass scaling relationships were estimated separately for the different sex classes (i.e., $\beta_5 \cdot \log Mass_{sc} \cdot Sex_{Female}$, $\beta_6 \cdot \log Mass_{sc} \cdot Sex_{Male}$, and $\beta_7 \cdot \log Mass_{sc} \cdot Sex_{SR}$ respectively). Deviations were sampled from a multivariate normal distribution ($\sim MVN([0,0], ID)$, where ID is a (co)variance matrix with a random intercept and slope variance and their covariance. A random-effect for day (d_k) ($\sim N(0, \sigma_k^2)$) 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 VO₂ 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 four MCMC chains, and we present posterior means and their 95% credible intervals. To test for the Like Genotype

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(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 were less than 0.05. 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_{SR} 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 - male_{SR} XX; pMCMC = 0.33; Table 3) and lower than their genotypic counterparts (female XX - male_{SR} XX; pMCMC < 0.01). For phenotypic males (male_{SR} 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 (female_{SR} 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* (female_{SR} ZZ) had a mass-specific metabolic rate that was overall higher than their genotypic counterparts (male ZZ - female_{SR} ZZ; pMCMC < 0.01), but lower than their phenotypic counterparts (female ZW - female_{SR} ZZ; pMCMC = 0.04; Table 3). The mass scaling relationship of metabolism for female_{SR} ZZ was more like ZZmales than ZW females (Fig. 2D; Table S3). As a consequence, large female_{SR} 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 detectable 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 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

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636 concordant females (83%) and concordant males (95%), but this relationship was also not
 637 significant ($p = 0.06$).
 638 **4 | Discussion**
 639 We examined two species with different modes of sex-reversal to test whether **metabolism**,
 640 growth, and survival differed **between** sex-reversed individuals **and others of the same**
 641 **phenotypic and genotypic sex**. Metabolic responses differed between the two species, with
 642 clear support for the Like Phenotype **hypothesis** when males reverse sex (males_{SR} XX;
 643 *Bassiana duperreyi*) and equivocal support for each **hypothesis** when females reverse sex
 644 (females_{SR} ZZ; *Pogona vitticeps*). For both species, regardless of whether individuals reversed
 645 sex, phenotypic females required more energy than phenotypic males as individuals grew
 646 larger. While sex-reversed animals appeared to have reduced survival, albeit not significantly
 647 so, there is no clear evidence in either species for growth advantages over their phenotypic
 648 sex. **Together our results suggest that traits associated with energy use and growth may not be**
 649 **strongly tied to genes on the sex chromosomes. Other mechanisms, such as hormonal**
 650 **pathways or differences in immune function, may better explain the stronger signal for**
 651 **phenotypic sex differences** (Cox et al., 2017; Kelly et al., 2018; van Doorn & Kirkpatrick,
 652 2010). **Assuming similar** patterns occur in natural populations, energetic processes may have
 653 varying impacts on the species' life-history traits, which could provide insight into what
 654 constrains the distribution of sex-reversal in nature.
 655 **Regardless of the sex-determining system, we show that females had higher mass**
 656 **scaling relationships for metabolism than males (Tables 1 & 2). Hormone-mediated effects,**
 657 **such as responses to elevated levels of thyroxine or corticosterone, have been responsible for**
 658 **increasing metabolic rates for female lizards, and these same hormones are important**
 659 **regulators of phenotypic sex differences in adults** (DuRant et al., 2008; John-Alder, 1990;
 660 Meylan et al., 2010). **Such differences in hormonal pathways between sexes may be**
 661 **responsible for the observed concordant sex differences in metabolism, but hormonal**
 662 **responses may transpire differently depending on the phenotype that undergoes sex-reversal.**
 663 **However, how endogenous hormone levels shift during early ontogeny for male and female**
 664 **lizards remains poorly understood (but see Lovern et al., 2001) and requires further attention**
 665 **when accounting for sex-reversed individuals as they mature.**
 666 **We showed that metabolic scaling relationships of sex-reversed individuals differed**
 667 **depending on the GSD system. In the ZZ/ZW system of *P. vitticeps*, larger sex-reversed**
 668 **females (females_{SR} ZZ; > +1.5SD above mean mass) have lower metabolism (15%) than**
 669 **concordant females (female ZW) of similar size (Fig. 2D; Table S3), whereas we observed no**
 670 **such differences for small sized hatchlings. Given that selection for larger hatchling lizards in**
 671 **the wild is common in lizards (i.e. 'bigger is better' hypothesis; Ferguson and Fox, 1984;**
 672 **Sinervo et al., 1992; Warner and Andrews, 2002), this would imply energetic differences**
 673 **between adult sex-reversed and concordant female *P. vitticeps*. As such, we predict that adult**
 674 **females_{SR} ZZ may have more residual energy than female ZW to allocate towards storage,**
 675 **production, or activity after resting metabolic costs have been paid. Such surplus in energy**
 676 **reserves for females_{SR} ZZ may explain why sub-adult (<1 year) and adult females_{SR} ZZ *P.***
 677 ***vitticeps* are more similar to male ZZ in behaviour and morphology, including higher activity,**
 678 **levels of aggression, and larger body size in captivity (Holley et al., 2015; Li et al., 2016).**
 679 **However, further work is needed to investigate if these different strategies of energy**
 680 **allocation exist and how they translate to the observed differences between phenotypic**
 681 **females in body mass, body size, and fecundity in wild populations of *P. vitticeps* (Wild et al.,**
 682 **2022). Given that our results indicate that the magnitude of metabolic differences varies**

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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.

In contrast to *Pogona vitticeps*, *B. duperreyi* showed strong support for the like-phenotype hypothesis. One simple explanation for this finding is that traits linked to metabolism are of little or no consequence for males. Alternatively, traits linked to metabolism for sex-reversed males (male_{SR} XX) in this species may not be associated with sex chromosomes and are linked to hormonal levels relevant to the phenotypic sex. This hypothesis is plausible if phenotypic males share similarities in their gonadal steroid levels, specifically testosterone. If this hypothesis is true, then it is likely that steroid levels would have a comparable effect on their metabolism compared to females, and the strengths of these signals could differ across life stages or seasons (Marler and Moore, 1989; Oppliger et al., 2004; Zena et al., 2019). Some support for this idea exists in *Anolis carolinensis*. Plasma testosterone concentrations in males are upwards to 4 times higher than similar-sized females 2 weeks post-hatch, and this difference in testosterone persists throughout juvenile growth where male testosterone can be 3 to 10 times higher than females (Lovern et al., 2001). If these hormonal differences were to exist between phenotypes in *B. duperreyi* this may provide a mechanism for why male_{SR} XX are more like their phenotypic sex.

Overall, there has been little attention focused on how growth or survival differs in sex-reversed individuals compared to their phenotypic or genotypic sex. While we did not detect a significant difference in growth or survival, 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). The lack of clear evidence for differences in metabolism, growth, and survival for sex-reversed individuals (male_{SR} XX or female_{SR} ZZ) over their concordant phenotypic sex (male XY or female ZW) in our study provides insight into the factors that may explain the occurrence of sex-reversal in the wild. 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 to impact the relative differences we observed between sex-reversed and concordant individuals in these two species. In both species, incubation temperatures mimicked nest temperatures documented in the wild (Castelli et al., 2021; Dissanayake et al., 2021b), and all hatchlings were reared under common laboratory conditions for the first 6-months of life when all measurements were taken. 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 and Wedekind, 2009). Overall, the lack of explicit support in our data for the Like Genotype hypothesis in metabolism, growth, or survivorship reveals clues on the mechanisms that drive sex-reversal in nature.

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Competing interests

We declare we have no competing interests.

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1215 Ezaz, Paul Waters and Jennifer Marshall Graves.
1216 **Data accessibility**
1217 Data, code, and additional resources are available on GitHub:
1218 https://github.com/danielInoble/energy_sex_reversal.git
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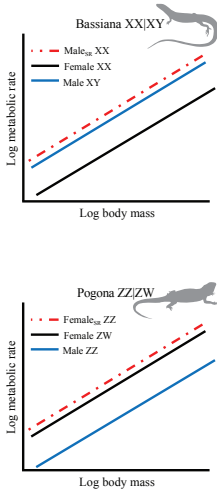
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Like phenotype hypothesis



Like genotype hypothesis

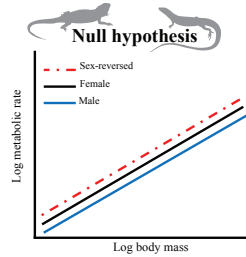
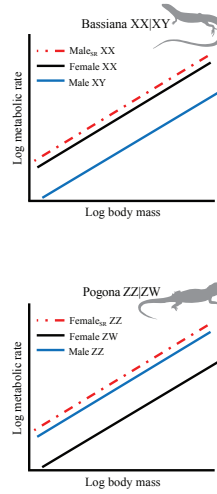
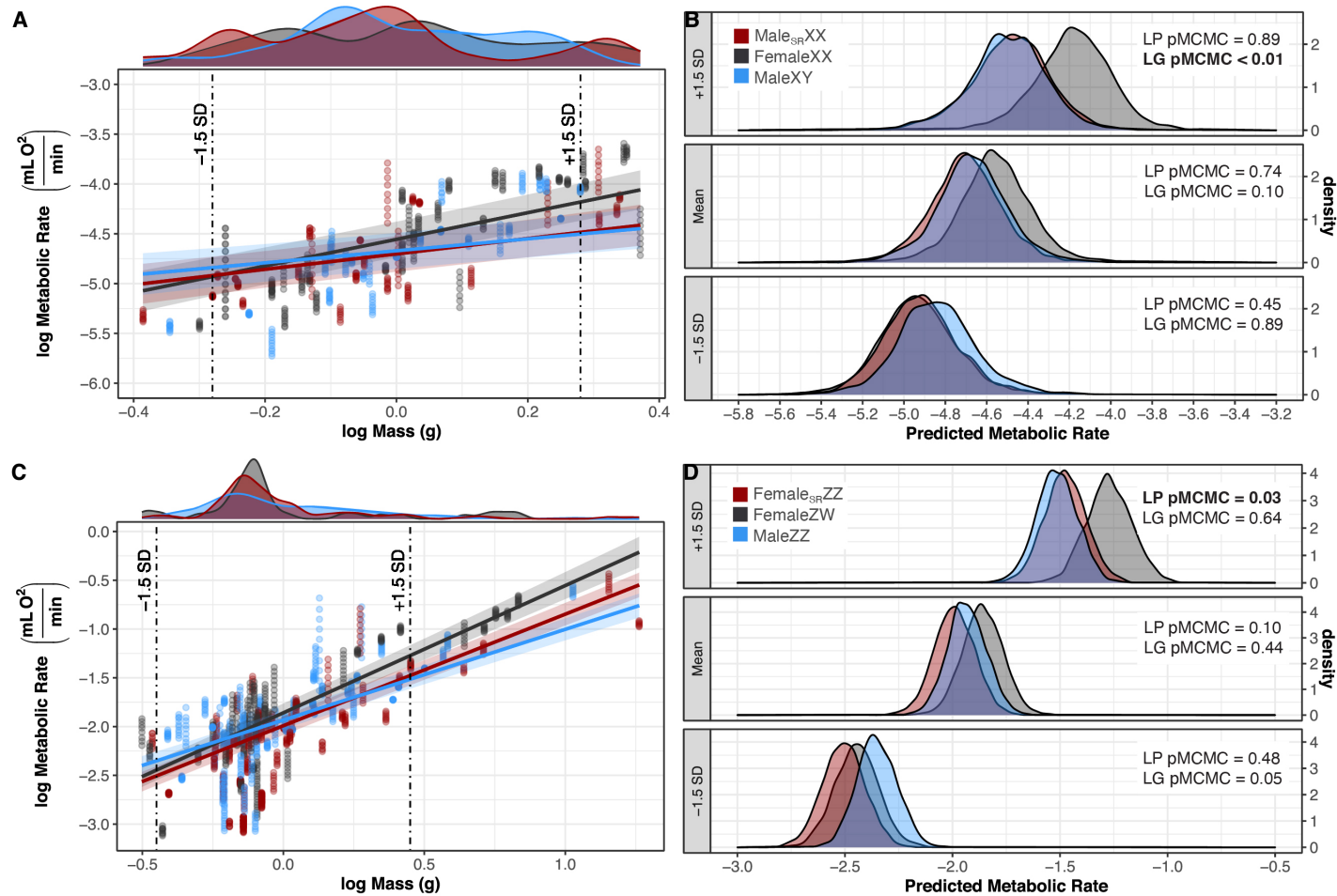


Figure 1. The Like Phenotype/Genotype Framework for testing the metabolic consequences of sex-reversal for ZZ/ZW and XX/XY genetic sex determination systems with different patterns of genetic sex determination. Colours indicate sex class for each species. Body mass and metabolic rates have been log transformed 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.



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Figure 2. Comparison of log metabolic rate ($\dot{V}O_2$ mL min⁻¹) across log body mass (g) by sex class for *Bassiana duperreyi* (A-B; [n = 40](#)) and *Pogona vitticeps* (C-D; [n = 96](#)). Sex-reversed individuals (male_{SR} XX or female_{SR} ZZ) are denoted by red colour, phenotypic females (female XX or female ZW) are denoted in black, phenotypic males (male XY or male ZZ) 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.](#)

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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
<i>Fixed Effects</i>			
Intercept (FemaleXX)	-4.56	-4.90	-4.20
Males _{SR} XX	-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
Males_{SR}XX:logMass	-0.56	-0.90	-0.23
MaleXY:logMass	-0.74	-1.07	-0.41
<i>Random Effects</i>			
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 from 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
<i>Fixed Effects</i>			
Intercept (FemaleZW)	-1.86	-2.04	-1.67
Females _{sr} ZZ	-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
Females_{sr}ZZ:logMass	-0.16	-0.32	-0.01
MaleZZ:logMass	-0.37	-0.55	-0.21
<i>Random Effects</i>			
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. [Growth rate models \(SVL and mass\) posteriors were extracted while accounting for log metabolic rate on each growth estimate by sex. Full model results can be found in Tables S5 & S6.](#)

Species	Test	Contrast	Estimate	l-95% CI	u-95% CI	pMCMC Value
<i>B. duperreyi</i>	Log MR	Males _{SR} XX - Male XY	0.18	-0.17	0.53	0.33
		Males_{SR} XX - Female XX	-0.56	-0.90	-0.23	< 0.01
	SVL (mm/d)	Males _{SR} XX - Male XY	3.77	8.53	15.57	0.52
		Males _{SR} XX - Female XX	4.06	15.80	7.71	0.47
	Mass (cg/d)	Males _{SR} XX - Male XY	-0.43	-4.92	3.88	0.85
		Males _{SR} XX - Female XX	-2.59	-6.54	1.13	0.18
<i>P. vitticeps</i>	Log MR	Females_{SR} ZZ - Female ZW	-0.16	-0.32	-0.01	0.05
		Females_{SR} ZZ - Male ZZ	0.21	0.09	0.32	< 0.01
	SVL (mm/d)	Females _{SR} ZZ - Female ZW	1.50	4.60	1.78	0.37
		Females _{SR} ZZ - Female ZW	1.16	3.99	1.68	0.43
	Mass (g/d)	Females _{SR} ZZ - Female ZW	0.91	4.44	2.80	0.61
		Females _{SR} ZZ - Male ZZ	1.81	5.00	1.25	0.25

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