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Reproductive phenotype predicts adult bite-force performance in sex-reversed dragons (*Pogona vitticeps*)

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Bite-force in sex reversed dragons

RESEARCH HIGHLIGHTS

Male bearded dragon lizards (ZZm) bite more forcefully than either chromosomally concordant females (ZWf) or sex-reversed females (ZZf), and this difference is associated with differences in head size and allometric scaling.

ABSTRACT

Sex related differences in morphology and behaviour are well documented, but the relative contributions of genes and environment to these traits are less well understood. Species that undergo sex reversal, such as the central bearded dragon (*Pogona vitticeps*), offer an opportunity to better understand sexually dimorphic traits because sexual phenotypes can exist on different chromosomal backgrounds. Reproductively female dragons with a discordant sex chromosome complement (sex reversed), at least as juveniles, exhibit traits in common with males (e.g., longer tails, greater boldness). However, the impact of sex reversal on sexually dimorphic traits in adult dragons is unknown. Here we investigate the effect of sex reversal on bite-force performance, which may be important in resource acquisition (e.g., mates and/or food). We measured body size, head size, and bite force of the three sexual phenotypes in a colony of captive animals. Among adults, we found that males (ZZm) bite more forcefully than either chromosomally concordant females (ZWf) or sex-reversed females (ZZf), and this difference is associated with having relatively larger head dimensions. Therefore, adult sex-reversed females, despite apparently exhibiting male traits as juveniles, do not develop the larger head and enhanced bite force of adult male bearded dragons. This is further illustrated in the full sample by a lack of positive allometry of bite force in sex-reversed females that is observed in males. The results reveal a close association between reproductive phenotype and bite force performance, regardless of sex chromosome complement.

1. INTRODUCTION

Sexual differences, most notably of secondary sexual characteristics, are a key aspect of within-species variation impacting on anatomy, behaviour, physiology, and life history (Stauber & Booth, 2003; Thompson & Withers, 2005; Wotherspoon & Burgin, 2011; Chen, Stuart-Fox, Hugall, & Symonds, 2012; McLean, Chan, Dickerson, Moussalli, & Stuart-Fox, 2016; Hoops et al. 2017; Littleford-Colquhoun et al., 2019). Sex determination mechanisms in reptiles are incredibly diverse, exhibiting a rich evolutionary history of repeated independent transitions between sex determination modes (Sarre, Ezaz, & Georges, 2011; Gamble et al., 2015; Pokorna & Kratochvil 2016; Alam, Sarre, Gleeson, Georges, & Ezaz, 2018). The accumulating evidence of species that employ both primary cues (genes and environment) to determine sex (transitional systems, Shine, Elphick, & Donnellan, 2002; Radder, Quinn, Georges, Sarre, & Shine, 2008; Holleley et al., 2015; Holleley, Sarre, O'Meally, & Georges, 2016; Hill, Burridge, Ezaz, & Wapstra, 2018), points to the existence of a continuum of states from complete genetic control via sex chromosomes to complete dependence on environmental influence over sex (Sarre, Georges, & Quinn, 2004). The potential for naturally occurring sex reversal (Jiménez, Burgos, Caballero, & De La Guardia, 1988; Holleley et al., 2015; Baroiller & D'Cotta, 2016; Ginot, Claude, Perez, & Veyrunes, 2017) is the hallmark of transitional systems. A small number of studies of terrestrial vertebrates indicate that the de-coupling of chromosomal and phenotypic sex, via sex reversal, can result in individuals bearing a mixture of male-like, female-like, or novel traits (Li, Holleley, Elphick, Georges, & Shine, 2016; Deveson et al., 2017; Ginot et al., 2017). Such differences in phenotype may be associated with significant differences in fitness, and thus be informative for predicting the evolutionary stability of the sex determination system (Holleley et al., 2015; Schwanz, Georges, Holleley, & Sarre, in press). Therefore, to interpret the evolution of sex determination and associated life histories, we need to better understand how sexually dimorphic traits are manifested in sex-reversed individuals with discordant genotypic and phenotypic sex.

The central bearded dragon (*Pogona vitticeps*) provides an excellent model organism for studying sexually dimorphic phenotypic traits. The species is sexually dimorphic (Badham 1976), with a transitional mode of sex determination, exhibiting simultaneous genetic and environmental influence over sex (Ezaz et al., 2005; Quinn et al., 2007). Heterogametic individuals (ZW) are always female (ZWf). Homogametic individuals (ZZ) develop as males at moderate egg incubation temperatures (22˚C-32˚C) (ZZm), but at higher incubation temperatures (≥32˚C) a proportion of chromosomal males will develop into functional, reproductive females (ZZf) (Quinn et al., 2007; Holleley et al., 2015). This temperature induced sex reversal is known to occur in the wild and may be increasing in frequency as a result of climate change (Holleley et al., 2015).

The performance capacities of sex-reversed females is of great interest, because they may afford a positive fitness advantage that could further increase the proportion of ZZf individuals in the wild and accelerate the progressive loss of the female specific W sex chromosome. Under such (directional) selection, the W chromosome could be driven to local extinction and thus the population would transition to temperature dependent sex determination (Holleley et al., 2015). As juveniles, the sex-reversed female phenotype (ZZf) exhibits some male-like traits relative to the chromosomally concordant female phenotype (ZWf), such as a relatively longer tail, greater boldness, higher activity levels, and warmer thermoregulatory preference (Li et al., 2016). As adults, sex reversed ZZf individuals lay more eggs than their ZWf counterparts (Holleley et al., 2015). However, individuals of the two female groups can only be identified with certainty using genetic/chromosomal data (Whiteley et al., 2017). To date, potential differences in whole-organism performance characteristics among the sexual phenotypes have not been examined.

Bite force is an important measure of whole-organism performance that may differ between males and females (Gvozdík & Van Damme, 2003; Lappin & Husak 2005; Lappin et al., 2006a; Lappin & Jones 2014; McLean et al., 2016; Lailvaux, Cespedes, & Houslay, 2019). Some male iguanian lizards use their jaws as a weapon to fight other males or to intimidate and restrain female lizards (Lappin & Husak 2005; Lappin et al., 2006ab; McLean & Stuart Fox 2015; McLean et al., 2016; Lailvaux et al., 2019). In eastern collared lizards (*Crotaphytus collaris*), the bite force of adult males is a strong predictor of access to females, mating success, and genetic paternity, whereas body size is not; the greater bite force of males is associated with considerably greater head dimensions (likely related to having larger jaw muscles) than females (Lappin & Husak 2005; Husak et al., 2009). However, aggression between female lizards also is known to occur in some species, particularly when resources (such as perching or nesting sites) are patchy or limited (Rauch 1988; Baird & Sloan 2003). Therefore, having greater bite-force performance than other individuals of the same sex also can be beneficial for female lizards.

Bite-force performance is unknown for *Pogona vitticeps*, but males reach greater size and have larger and relatively wider heads than females (Badham 1976; Witten, 1994). This pattern might indicate that males bite more forcefully and use biting to compete for access to females (Witten, 1994). Sexual dimorphism in head size (and possibly shape) may be common among agamid lizards (e.g., Stauber & Booth, 2003; Thompson & Withers, 2005; Kuo, Lin, & Lin, 2009; Wotherspoon & Burgin, 2011; Hoops et al. 2017; Littleford-Colquhoun et al., 2019), and greater bite force associated with greater head size has been found in male eastern water dragons (*Intellagama lesueurii*, Baxter-Gilbert & Whiting 2019). Two species of spiny tailed agamid *Uromastyx* do not show obvious sexual differences in head dimensions or bite force but comparisons may be limited by sample size: n = 7:5 and 1:5 (Herrel, Castilla, Al-Sulaiti, & Wessels, 2014). The only other reports of bite force in agamid lizards either include only males (McLean & Stuart-Fox 2015) or do not explicitly report how individual data points correspond to sex or species (e.g., Herrel, Schaerlaeken, Meyers, Metzger, & Ross 2007; Schaerlaeken, Herrel, Aerts, & Ross, 2008; Herrel & De Vree, 2009; Porro et al. 2014). The paucity of detailed bite force performance for Agamidae restricts comparisons within Agamidae as well as broader comparisons between Agamidae and other reptile groups such as Rhynchocephalia (Jones and Lappin 2009).

Here we analyse head dimensions and bite-force performance in *Pogona vitticeps*. Owing to the expected sexual dimorphism in head size and the likely association with male reproductive success, we predict that large heads and forceful bites are associated with only the male phenotype (ZZm), and that both concordant (ZWf) and sex reversed females (ZZf) display similar, smaller heads and weaker bite force.

2. MATERIALS AND METHODS

2.1 Study taxon

The central bearded dragon (*Pogona vitticeps*) is an agamid lizard commonly found in the arid and semiarid regions of central Australia (Badham 1976; Rej & Joyner, 2018) and internationally as a common pet (Raiti 2012; Johnson & Adwick 2019). It is also increasingly used as a reptilian model organism in a wide range of biological disciplines. Recent examples of research focused on this taxon include those related to thermal physiology (Smith et al., 2016; Couture, Monteiro, Aymen, Troncy, & Steagall, 2017), niche modelling (Rej & Joyner 2018), behaviour (Cadena & Tattersall, 2009; Cadena et al., 2017; Kis, Huber, & Wilkinson, 2015; Li et al., 2016), veterinary herpetology (Schmidt-Ukaj, Hochleithner, Richter, Brandstetter, & Knotek, 2017), development (Melville et al., 2016; Whiteley et al., 2017; Ollonen, Da Silva, Mahlow, & Di-Poï, 2018; Haridy 2018), comparative brain anatomy (Tosches et al., 2018), gene expression (Capraro et al. 2019), and genomics (Georges et al., 2015; Deakin et al., 2016). The species *Pogona vitticeps* is also frequently included in broader comparative studies (e.g., Witten, 1985, 1994; Geist 2000; Herrel et al., 2007; Woltering, et al., 2009; Schaerlaeken et al., 2008; Herrel & De Vree, 2009; Holliday, Gardner, Paesani, Douthitt & Ratliff, 2010; Ross et al., 2010; Chen et al., 2012; Head & Polly 2015; Cubo, Hui, Clarac, & Quilhac, 2017; Gray, McDowell, Hutchinson, & Jones, 2017; Gray, Hutchinson, & Jones, 2019; Gray, Sherratt, Hutchinson, & Jones 2019; Littleford-Colquhoun et al., 2019; Macrì, Savriama, Khan, & Di-Poï, 2019; Watanabe et al., 2019).

2.2 Specimens

Our sample comprised 91 captive individuals (39 adults and 52 juveniles) of the central bearded dragon, *Pogona vitticeps*, from a previously studied breeding colony (at the Institute for Applied Ecology at the University of Canberra) (Figure S1; Table S1). The genotypic and phenotypic sex had been characterised for all individuals (Holleley et al., 2015; Li et al., 2016). Animals represent three groups: 26 discordant sex-reversed females (ZZf), 33 genotypically and phenotypically concordant females (ZWf), and 32 males (ZZm) ranging from juveniles to large adults (SVL = 58.5 - 228 mm). A snout-vent length of 150 mm or greater was used to define adults. This value is 20 mm greater than previous observations of sexual maturity (Badham 1976) but helps ensure that the adult sample exhibit adult head proportions (see figure 2 in Witten, 1994). Therefore, our adult sample comprised: 17 sex-reversed females (ZZf), 11 concordant females (ZWf), and 11 males (ZZm). We focus most of our analyses on adult animals to remove the influence of ontogeny, but we also examine scaling of bite force and head dimensions in the full samples to provide additional insight into differences among the groups.

2.3 Morphometrics

For each individual, we measured mass, snout-vent length (SVL), head length directly from the tip of the rostrum to the posterior end of the retroarticular process (HLrr), head length from the tip of the rostrum to the posteroventral corner of the tympanum (approximately the position of the quadrate-articular jaw joint) (HLrq), head width at the base of the tympanum (HWqu), head width at the midpoint of the temporal region (HWmt), and head depth at the midpoint of the temporal region including the lower jaw with the mouth closed (HDmt). Mass was measured using a digital scale, snout-vent length (SVL) was measured using a transparent plastic ruler, and head dimensions were measured using digital callipers.

2.4 Bite-force performance

Bite force was measured using a Kistler piezoelectric force transducer (type 9203, Kistler, Switzerland) attached between two stainless steel bite plates and connected to a charge amplifier (type 5995, Kistler, Switzerland). One plate is straight whereas the other is sigmoid (cf. Herrel, Spithoven, Van Damme & De Vree 1999). To accommodate the size range of specimens tested, two different sized sets of bite plates were used (Figure S2) that differed in their width: 25 mm (also used here: Lappin & Husak 2005; Lappin et al., 2006a; Jones & Lappin, 2009; Lappin & Jones, 2014) and 20 mm wide (used here for the first time). The bite plates of the smaller transducer were shallower and more closely opposed so that the smallest animals did not have to use excessive gape angles, which might restrict maximum performance (Figure 1). Strips of leather (about 5 mm wide) were added to the outer tips of the bite plates to protect the animal's jaws and teeth, to provide a naturalistic surface to bite, and to ensure that the bite force being applied was at a consistent point along the bite plates (Figure 1; Lappin & Jones 2014). The output voltage of the devices was calibrated to bite force (Newtons) by hanging a series of weights by a string positioned at the centre of the leather strip (cf. Dechow & Carlson, 1983; Lappin et al., 2006a).

Prior to bite-force trials, we gave animals an opportunity to thermoregulate under heat lamps for at least 30 min to achieve preferred body temperature. We measured body temperature using a Digitech QM-7221 digital temperature gun with dual laser targeting between the first and second trial. The surface readings recorded (~31°C) were only slightly lower than the preferred or optimal body temperatures reported elsewhere (34-36°C, e.g., Cadena & Tattersall, 2009; Johnson & Adwick 2019). Adult males tended to be slightly warmer than adults of the other two genotypes (Figures S3: ZZf = 30.59°C ± 0.32, ZWf = 30.08°C ± 0.71, ZZm = 31.57°C ± 0.72) but there is no obvious relationship between surface temperature and bite force (Figures S4).

Each lizard was given the opportunity to bite during four trials with a brief period of rest between each successive trial (~30 seconds). Four trials is similar to the number used in previous studies (five, e.g., Herrel et al. 2014: page 172; three, e.g., Baxter-Gilbert & Whiting 2019: page 257). Lizards were encouraged to gape by touching the side or underside of the lower jaw. Bites were elicited by carefully placing the transducer in the animal's mouth. Of the 364 trials only nine resulted in no bite. All trials were digitally video-recorded in lateral view to document the position along the jaws at which each bite was applied. The location along the jaws where the bite was applied to the leather strips was used to measure the proportional bite out-lever (the distance between the bite location and jaw joint along the jaw divided by the full length of the lower jaw). Voluntary bites were delivered with a range of proportional bite out-levers (mean = 0.85, maximum = 0.97, minimum = 0.68). However, we standardised each bite to what it would be at the tips of the jaws, represented by a proportional bite out-lever of 1.0 (Lappin & Jones 2014). As is convention, the greatest bite force among the trials was used in analyses (e.g., Lappin & Husak 2005; Husak et al., 2009; Lappin & Jones 2014; Herrel et al. 2014; Baxter-Gilbert & Whiting 2019). We performed analyses using both the greatest standardised (BF) and greatest raw bite force (non-standardised for lever, BFnolever) values but we focus on standardised bite force in the main text.

2.5 Analyses

All statistical analyses were done using R version 3.6.1 (R Core Team 2019) and code to reproduce the analyses is available at https://github.com/MarcEHJones/sex-reverse-pv.

2.5.1 Bite force and morphometric comparison

To compare body size, dimensions, and bite force measures among adults of the three groups (ZZf, ZWf, ZZm), we calculated the mean ± SE for each untransformed variable (Table 1). We tested for significant differences among the groups using analysis of variance (ANOVA fixed model) natural log transformed variables (Table 2; Table S3). We used standard model checks for linear models (Q-Q plot, histogram of residuals, residuals vs. linear predictors, response vs. fitted values) to assess model fit.

2.5.2 Allometry and scaling

To test the scaling of bite force for allometry across ontogeny (including juveniles and adults) for each of the three groups (ZZf, ZWf, ZZm) we fitted linear models of log10-transformed bite force as a function of log10-transformed SVL, mass, and each of the head dimensions to each group separately (Table 3). Maximum bite force performance should scale isometrically with the cross-sectional area of the jaw muscles contributing to the bite (Gans 1982; Erickson, Lappin, & Vliet, 2003; Santana, Dumont, & Davis, 2010; Gröning et al. 2013; Sellers, Middleton, Davis, & Holliday, 2017). Therefore, if bite force performance in *Pogona* scales isometrically with size it should scale to the square of linear measurements (e.g., SVL) with a slope of 2.000 and to the two-thirds power of volumes (e.g., body mass) with a slope of 0.667 (Erickson et al. 2003). We used the standard error to estimate upper and lower limits for the slope and to evaluate whether there was adequate evidence of negative or positive allometry (respectively) (Table 3 and S3). To compare the scaling relationships between bite force and measures of body and head size (all natural log-transformed) among the three groups, we used linear regressions again checking model fit as described above (Table S4).

2.5.3 Head shape comparison

To compare head shape among the three groups (ZZf, ZWf, ZZm) controlling for isometric size differences, the five head dimensions were converted to log shape ratios (Mossiman variables). This approach involves dividing each of the five head dimensions by the geometric mean of the five dimensions for each specimen (e.g., see Sakmoto & Ruta 2012). These new values were examined with a principal components analysis using the princomp function in R. We performed this analysis for both the full sample (e.g. Figure S7) and adults (e.g. Figure 5).

3 RESULTS

For the entire sample (juveniles and adults, 58.5 - 228 mm SVL) bite force standardised at the tips of the jaws ranged from 5.59 to 98.73 N and is positively correlated with body size (Tables 1, 2, 3).

3.1 Adult bite force and morphometric comparison

At least among adult animals, males (ZZm) bite with greater force than both groups of phenotypically female individuals (ZZf, ZWf) (Figure 2, Table 1, Table 2). The difference between males (ZZm) and sex reversed females (ZZf) is significant ($p = 0.014$, Table 2) but between males (ZZm) and concordant females (ZWf) it is not ($p = 0.052$, Table S2). We did not observe any difference in bite force when comparing sex-reversed females and concordant females ($p = 0.832$, Figure 2; Table 2). Males (ZZm) have greater head dimensions than both sex-reversed (ZZf) and concordant females (ZWf) (Table 1). The difference is significant for head length between the rostrum and tympanum (HLrq), head width at the tympanum (HWqu), head depth at the mid-temporal region (HDm) (Table 2; Table S2), and, with sex reversed females (ZZf) only: head length between the rostrum and posterior end of the retroarticular process (HLrr) (Table 2). Sex-reversed females and concordant females do not differ significantly from each other in any measure of head size (Figure 3, Table 2). Mass and SVL do not differ significantly across the three groups (Figure 2; Table 2; Table S2).

3.2 Allometry and scaling comparison

Sex-reversed females (ZZf) and concordant females (ZWf) exhibit negative allometry of standardised bite force on body size (SVL and mass) whereas males (ZZm) exhibit positive allometry of bite force on body size (Table 3). The positive allometric scaling of males (ZZm) is driven by the relatively greater bite force of adult males (Figures 4, S5, and S6). Using the standard error to estimate upper and lower limits for the slope, males (ZZm) show positive allometry of bite force on every head dimension, concordant females (ZWf) only show positive allometry for head length

(HLqu), and sex reversed females (ZZf) show no obvious allometry with respect to any head dimension (Table 3). Standardised bite force scales with body size (SVL and mass) with a significantly lower slope in sex-reversed females (ZZf) than it does in males (ZWf) (p = 0.035 and p = 0.039 respectively, Table S4), but no significant difference was found between the slopes of sex-reversed females (ZZf) and concordant females (ZWf) (Table S4). We did not detect any significant differences among the three groups in the scaling of bite force with respect to any head dimension (Table S4).

If non-standardised bite force is used, males (ZZm) still show positive allometry of bite force on both measures of body size and all head dimensions (Table S3). Sex-reversed females (ZZf) and concordant females (ZWf) show negative allometry of bite force on body mass. Sex-reversed females (ZZf) show positive allometry on head width at the mid-temporal region whereas concordant females (ZWf) show positive allometry on head width at the mid-temporal region and head length between the rostrum and base of the tympanum (Table S3). We did not detect any significant differences among the three groups in the scaling of un standardised bite force with respect to body size or head dimension (Table S4).

3.4 Isometric head shape

There was no clear difference in head shape (isometric size removed) among the three groups (Figure 5 and Figure S7). For the full sample, PC1 (34.1% of total variation) mainly reflects relative head depth, PC2 (26.7%) mainly reflects relative head width, and PC3 (21.8%) reflects differences in head width at the tympanum (quadrates) and head width at the mid-temporal region. The distribution of the three genotypes extensively overlap (Figure S7). Individuals with greater bite forces appear to plot with lower PC1 scores and higher PC3 scores: heads that are relatively deep at the mid-temporal region and wide at the base of the tympanum (Figure S8). This pattern parallels body size: adults plot with lower PC1 scores and high PC3 scores (Figure S9 and S10). In the analysis of just adults, PC1 (36.6%) mainly reflects relative head width, PC2 (31.7%) mainly reflects relative head depth, and PC3 (21.2%) reflects differences in head width and length at the tympanum (quadrates) relative to the other dimensions. Again the distribution of the three groups overlaps but the distribution of sex-reversed females (ZZf) tends to overlap concordant females (ZWf) more than concordant males (ZZm) (Figure 5). The sex reversed females (ZWf) tend to plot with narrower heads compared to males. However, this distribution does not obviously reflect body size or bite force (Figure 5).

4 DISCUSSION

We show that adult male (ZZm) bearded dragons (*Pogona vitticeps*) can bite more forcefully and in general have larger heads than adult females whether they are sex-reversed (ZZf) or concordant (ZWf) females. This result is consistent with our prediction based on sexually dimorphic head size in this species. It suggests that male bearded dragons, like some other iguanian lizards, use their jaws and bite force to secure access to females at the expense of other males (Lappin & Husak 2005; Lappin et al., 2006ab; Baxter-Gilbert & Whiting 2019). This enhanced bite-force performance may also incidentally allow males access to a wider range of food items.

Despite sex-reversed *Pogona vitticeps* (ZZf) reportedly exhibiting several male (ZZm) traits as juveniles (Li et al., 2016), we found no evidence that they possess the enhanced bite force and larger heads of adult males (ZZm) (Table 2). Concordant (ZWf) and sex-reversed female (ZZf) bearded dragons have similar bite-force performance, despite the two groups of females being initiated by different primary mechanisms (sex chromosomes and temperature, respectively) enacted on different genetic backgrounds (ZW and ZZ, respectively). The adult phenotype of this sexually dimorphic trait reflects reproductive phenotype, not sex chromosome complement, suggesting that either environmental cues are pivotal to the development of this trait, and/or (more likely) that bite force (and head size) is controlled exclusively by a common downstream sexual differentiation cascade and sex hormone production. Inter-individual levels of testosterone in the (non-iguanian) lizard *Gallotia gallotia* have been found to correlate with bite force performance (Huyghe et al., 2010). More work is required to determine the relative fitness of sex-reversed females, which will allow us to predict the evolutionary stability of sex chromosomes and the likelihood of transitions to temperature dependent sex determination.

The only other study of intraspecific bite-force performance in a species with sex reversal was conducted on adults of the African pygmy mouse (*Mus minutoides*) (Ginot et al., 2017). In contrast to the situation in *Pogona vitticeps*, sex-reversed female mice (X*Y) were found to have greater bite-force performance than both concordant females and males (Ginot et al., 2017). However, as in *Pogona vitticeps*, the greater bite force is associated with greater head size, thus highlighting the close relationship between head size and bite force (related to jaw muscle physiological cross-sectional area, e.g., Gans 1982; Erickson et a. 2003; Santana et al. 2010; Gröning et al. 2013; Sellers et al. 2017). The results for both taxa hint at the diversity of phenotypic responses to sex reversal that may exist in nature. A full understanding of the fitness of sex reversed individuals in a suite of phenotypic traits will be crucial to our understanding of key evolutionary processes, such as the birth and death of sex chromosomes in response to rapidly changing environmental stimuli.

The positive allometry of bite force performance in male *Pogona vitticeps* relative to SVL and mass is similar to that reported in other reptiles (Erickson et al. 2003, 2014; Herrel and O'Rielly 2006; Jones and Lappin 2009) but it is greater than would be expected given that bite force should scale according to muscle cross-sectional area. This result likely reflects a range of factors (Erickson et al. 2003; Herrel and O'Reilly 2006; Jones and Lappin 2009) including a disproportional increase in jaw muscle size (=cross-sectional area). Correspondingly, the postorbital region of the skull (which houses the jaw muscles) lizards does increase disproportionally during ontogeny in *Pogona* (Gray et al. 2019b) as it does in many lepidosaurs (e.g., Jones 2008; Jones and Lappin 2009). Other factors may include ontogenetic changes in muscle quality and skeletal stiffness involved in the transmission of muscle forces (Erickson et al. 2003).

The result that males generally have greater head dimensions compared to females matches the results of previous studies (Badham 1976; Witten 1994). In particular, males tend to have deep heads that are relatively wide at the quadrates. However, substantial intraspecific variation limits distinguishing between individuals of the three genotypes using isometric head shape alone. As previously reported, the ontogeny of various head dimensions (e.g., head width, head depth) relative to body size are not necessarily linear or similar to each other (Witten 1994: figure 2). This variation clouds comparisons using isometric shape alone. Therefore, although some head dimensions differ on average between males and females, head shape and size are unlikely to be a reliable predictor of sex for an individual unless that individual is a very large male (where differences have the potential to be most exaggerated).

The new bite force data here permits some limited comparisons with other agamids and lepidosaurs. Our bite force values for *Pogona vitticeps* do not appear to be substantially different from others reported for agamids (Herrrel et al. 2007: figure 4; Herrel & De Vree, 2009: figure 2). Our results suggest that among agamids, *Pogona vitticeps* has a bite force similar to that of *Uromastyx* sp. (Herrrel et al. 2014), greater than *Ctenophorus maculosus* (McLean and Devi Stuart Fox 2015), but much less than *Intellagama lesueurii* (Baxter-Gilbert & Whiting 2019). This pattern broadly reflects differences in body and head size, with larger taxa having greater bite force. However, such broad comparisons remain problematic due to differences in exact method used (e.g., standardisation of out lever, biting substrate, number of trials, rest period between trials). Perhaps more importantly, comparisons are inhibited by non-disclosure (publication) of raw measurement data for individual animals which means comparisons are limited to plots of values from a range of taxa (Herrrel et al. 2007; Herrel & De Vree, 2009), mean values (Baxter-Gilbert & Whiting 2019: page 259) or maxima (McLean and Devi Stuart Fox 2015: table 1). In the spirit of open data and reproducibility, we advocate that future studies should provide explicit methods and publication of raw individual measurement data.

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Competing interests

The authors declare no competing interests.

Data availability

The raw data as well as the code to reproduce the analyses is available at https://github.com/marcehjones/sex-reverse-pv (Jones & Cooper, 2020).

Ethics

All research was conducted under the University of Canberra animal ethics protocol CEAE 15-01 and the University of Adelaide animal ethics protocol 2013/37.

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TABLES

TABLE 1 Mean body size and head shape measures (with standard error) of adult *Pogona vitticeps* used in this study. ZZf, sex-reversed females (n = 17), ZWf, concordant females (n = 11), ZZm, males (n = 11). Mass, body mass (g); SVL, snout-vent length (mm); HLrr, head length directly from the tip of the rostrum to the posterior end of the retroarticular process (mm); HLrq, head length from the tip of the rostrum to the posteroventral corner of the tympanum (approximately the position of the quadrate-articular jaw joint) (mm); HWqu, head width measured at the base of the tympanum (mm); HWmt, head width measured at the midpoint of the temporal region (mm); HDmt, and head depth measured at the midpoint of the temporal region (mm); BF at tips, bite force at the tips corrected for out lever (N) (Lappin and Jones 2014); BF no lever, bite force without lever correction (N)

	ZZf	**ZWf**	**ZZm**	**units**
Mass	228.07 ± 19.73	236.38 ± 23.1	234.68 ± 13.71	g
SVL	183.85 ± 4.93	184.64 ± 6.09	187.82 ± 4.24	mm
HLrr	50.54 ± 1.55	52.20 ± 1.74	56.31 ± 1.23	mm
HLrq	42.51 ± 1.32	42.23 ± 1.31	46.37 ± 0.96	mm
HWqu	37.55 ± 1.30	37.89 ± 1.38	41.48 ± 0.96	mm
HWmt	36.12 ± 1.04	36.50 ± 1.20	38.64 ± 1.23	mm
HDmt	23.54 ± 0.75	24.62 ± 1.11	28.11 ± 0.83	mm
BF at tips	50.28 ± 4.04	52.66 ± 5.98	71.46 ± 6.46	N
BF no lever	62.20 ± 5.28	65.93 ± 8.27	90.37 ± 9.59	N
n	17	11	11	

TABLE 2 Results from analysis of variance (ANOVAs) testing for differences in variables across three genotypes in adults. ZZf, sex-reversed females (n = 17), ZWf, concordant females (n = 11), ZZm, males (n = 11). Degrees of freedom 2, 36 for all tests. * Significant at p = 0.05. ** Significant at p = 0.01. SE, standard error

			ZZf		ZZf and ZWf difference				ZZf and ZZm difference			
response	**F**	**p**	**Mean**	**± SE**	**Mean**	**± SE**	**t**	**p**	**Mean**	**± SE**	**t**	**p**
log(Mass)	0.161	0.852	5.370	0.078	0.047	0.124	0.376	0.709	0.067	0.124	0.538	0.594
log(SVL)	0.197	0.822	5.208	0.025	0.004	0.040	0.112	0.911	0.024	0.040	0.612	0.544
log(Bite Force)	3.710	0.034*	3.865	0.087	0.030	0.138	0.214	0.832	0.356	0.138	2.581	0.014*
log(Bite Force nl)	3.388	0.045*	4.066	0.095	0.043	0.152	0.284	0.788	0.377	0.152	2.492	0.017*
log(HLroret)	3.669	0.035*	3.916	0.026	0.034	0.042	0.807	0.425	0.113	0.042	2.695	0.011*
log(HLroqu)	3.133	0.056	3.743	0.025	-0.004	0.041	-0.106	0.916	0.092	0.041	2.258	0.030*
log(HWqu)	2.749	0.077	3.616	0.030	0.012	0.047	0.247	0.806	0.106	0.047	2.241	0.031*
log(HWmt)	1.258	0.297	3.580	0.028	0.011	0.044	0.260	0.797	0.068	0.044	1.541	0.132
log(HDmt)	6.830	0.003**	3.151	0.031	0.042	0.049	0.851	0.400	0.180	0.049	3.642	0.001**

Abbreviations: SE, standard error.

TABLE 3 Bite force scaling results in full sample of *Pogona vitticeps* (n = 91). Adult and juvenile ZZf, sex-reversed females (n = 26), ZWf, concordant females (n = 33), ZZm, males (n = 32). Standard error (SE) was used to define upper and lower limits. Allometry is considered positive when the lower limit of the slope is greater than 0.667 for mass and 2.0 for linear measurements (the isometric predictions) and negative when the upper limit of the slope is lower than 0.667 for mass and 2.0 for linear measurements.

genotype	predictor	slope	SE	upper limit	lower limit	df	t	p	Predicted isometry	adj.r2	allometry
ZZf	SVL	1.747	0.186	1.933	1.560	24	9.37	<0.001	2.000	0.78	negative
ZWf	SVL	1.799	0.137	1.937	1.662	31	13.11	<0.001	2.000	0.84	negative
ZZm	SVL	2.222	0.130	2.352	2.092	30	17.11	<0.001	2.000	0.90	positive
ZZf	Mass	0.567	0.052	0.619	0.515	24	10.81	<0.001	0.667	0.82	negative
ZWf	Mass	0.581	0.039	0.620	0.541	31	14.73	<0.001	0.667	0.87	negative
ZZm	Mass	0.729	0.035	0.764	0.694	30	20.83	<0.001	0.667	0.93	positive
ZZf	log(HLroret)	1.983	0.187	2.170	1.795	24	10.58	<0.001	2.000	0.82	-
ZWf	log(HLroret)	1.939	0.144	2.082	1.795	31	13.50	<0.001	2.000	0.85	-
ZZm	log(HLroret)	2.262	0.117	2.379	2.145	30	19.33	<0.001	2.000	0.92	positive
ZZf	log(HLroqu)	2.094	0.190	2.284	1.903	24	11.01	<0.001	2.000	0.83	-
ZWf	log(HLroqu)	2.206	0.162	2.369	2.044	31	13.62	<0.001	2.000	0.85	positive
ZZm	log(HLroqu)	2.433	0.113	2.545	2.320	30	21.58	<0.001	2.000	0.94	positive
ZZf	log(HWqu)	1.895	0.143	2.038	1.752	24	13.26	<0.001	2.000	0.87	-
ZWf	log(HWqu)	1.882	0.152	2.034	1.730	31	12.38	<0.001	2.000	0.83	-
ZZm	log(HWqu)	2.233	0.095	2.328	2.137	30	23.41	<0.001	2.000	0.95	positive
ZZf	log(HWmt)	2.095	0.174	2.269	1.921	24	12.01	<0.001	2.000	0.85	-
ZWf	log(HWmt)	2.106	0.159	2.265	1.946	31	13.23	<0.001	2.000	0.84	-
ZZm	log(HWmt)	2.422	0.128	2.550	2.294	30	18.88	<0.001	2.000	0.92	positive
ZZf	log(HDmt)	2.003	0.187	2.269	1.921	24	10.73	<0.001	2.000	0.82	-
ZWf	log(HDmt)	1.922	0.148	2.265	1.946	31	12.97	<0.001	2.000	0.84	-
ZZm	log(HDmt)	2.190	0.109	2.550	2.294	30	20.11	<0.001	2.000	0.93	positive

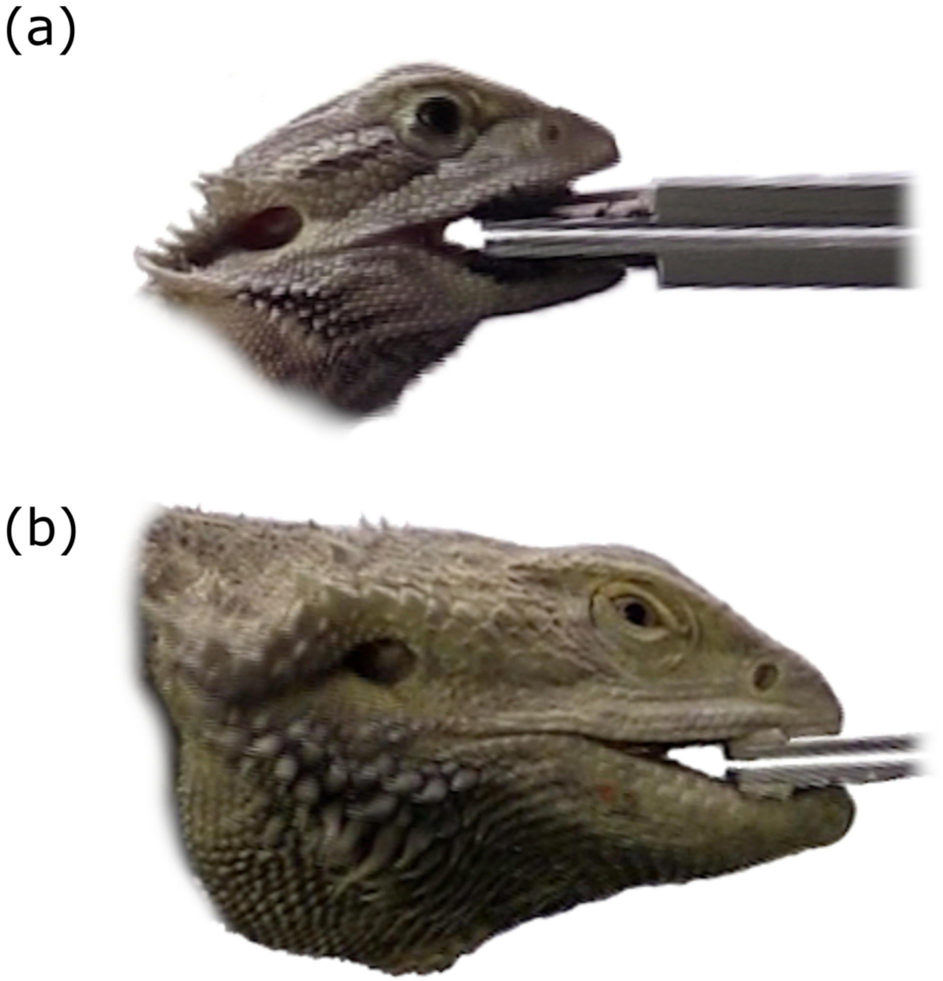

FIGURE 1 *Pogona vitticeps*biting the bite plates of the force transducer in lateral view: juvenile 008031 ZWf concordant female (45 g Mass, 110 mm SVL, HLqu = 27.1 mm) (a), and adult ZZf sex reversed female 002919 (365 g mass, 228 mm SVL, HLqu = 51.9 mm) (b)

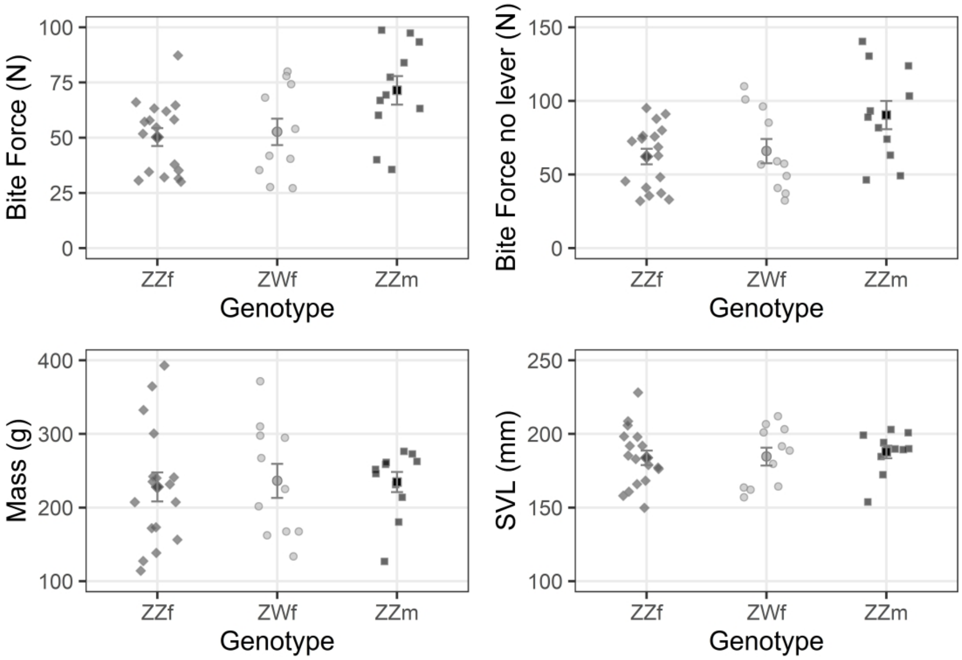

FIGURE 2 Bite force, mass and snout vent length (SVL) in adult *Pogona vitticeps* (n = 39) according to genotype ZZf, sex-reversed females (n = 17), ZWf, concordant females (n = 11), ZZm, males (n = 11)

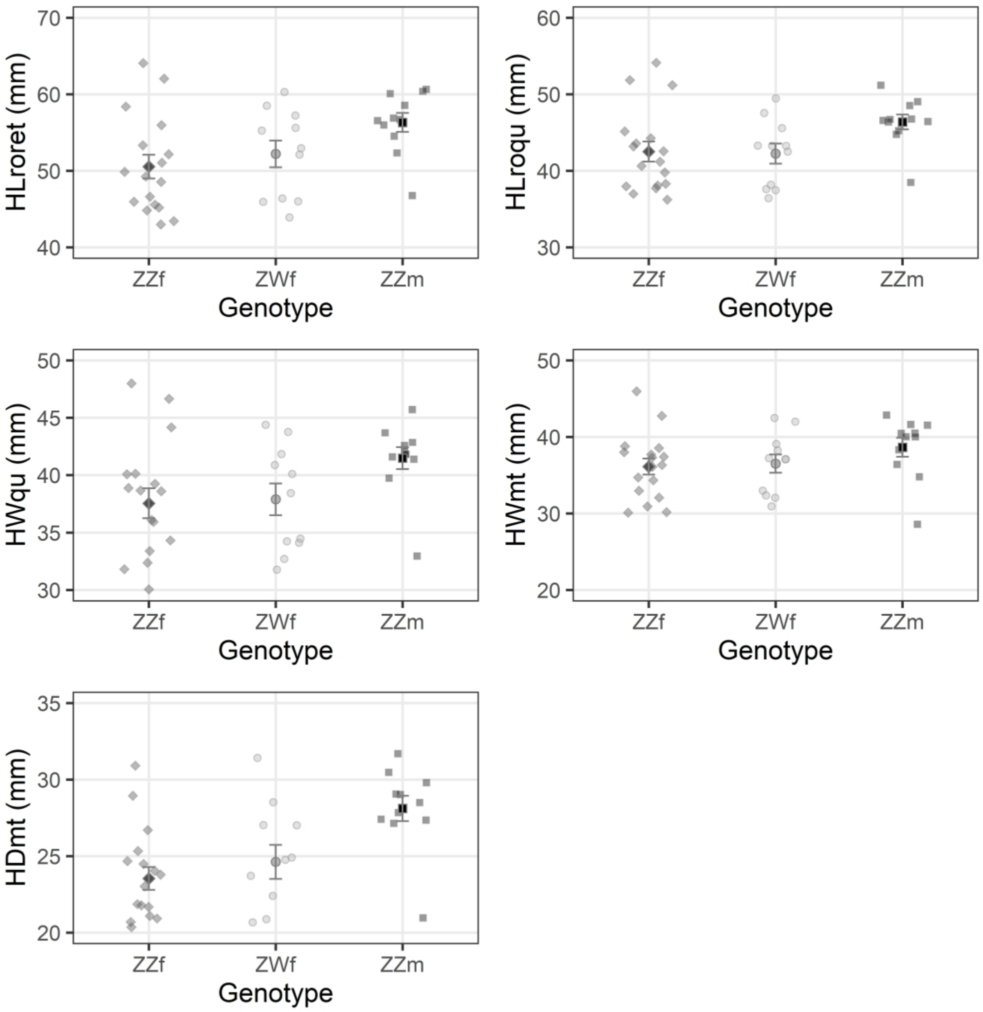

FIGURE 3 Basic head dimensions in adult *Pogona vitticeps* (n = 39) according to genotype: ZZf, sex-reversed females (n = 17), ZWf, concordant females (n = 11), ZZm, males (n = 11). HLrr, head length directly from the tip of the rostrum to the posterior end of the retroarticular process (mm); HLrq, head length from the tip of the rostrum to the posteroventral corner of the tympanum (approximately the position of the quadrate-articular jaw joint) (mm); HWqu, head width measured at the base of the tympanum (mm); HWmt, head width measured at the midpoint of the temporal region (mm); HDmt, and head depth measured at the midpoint of the temporal region (mm)

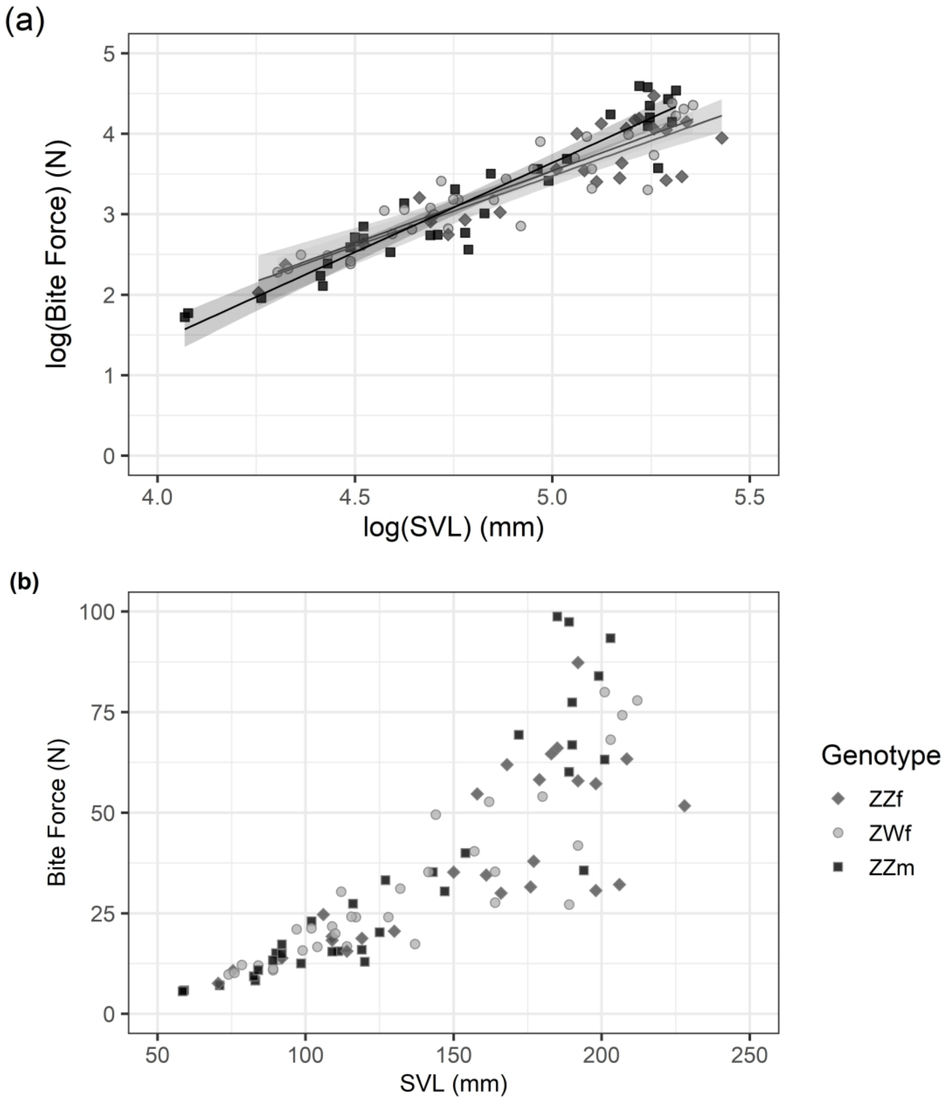

FIGURE 4 Relationship between snout-vent length (SVL) and bite force in *Pogona vitticeps* (n = 91) from three different genotypes shown in log (a) and non-log (b). Adult and juvenile ZZf, sex-reversed females (n = 26), ZWF, concordant females (n = 33), ZZm, males (n = 32)

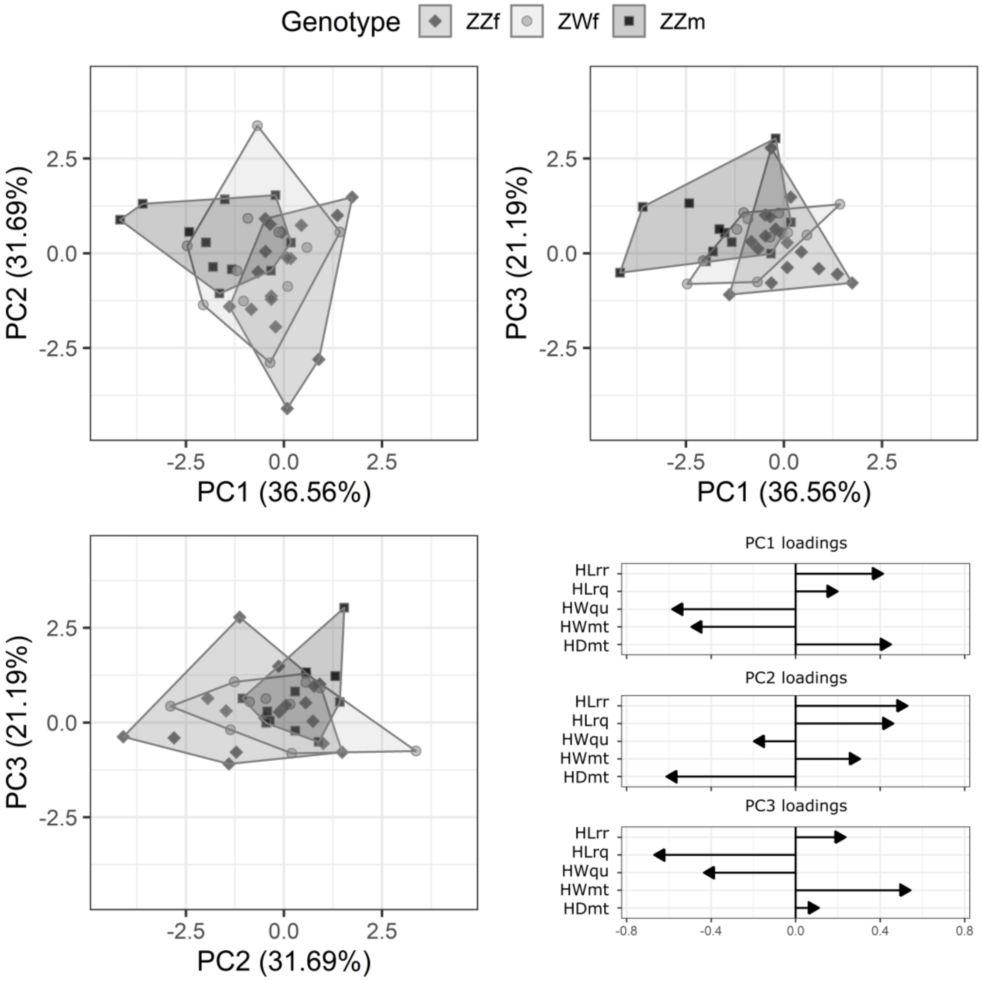

FIGURE 5 Isometric head shape variation among adult *Pogona vitticeps* (n = 39) of known genotypes (>150 SVL mm, n = 39) according to a principal components analysis of log shape ratios of five head dimensions. Arrows indicate the relative loading of each head dimensions for each PC axis. ZZf, sex-reversed females (n = 17), ZWF, concordant females (n = 11), ZZm, males (n = 11)