Evolutionary stability inferred for a free ranging lizard with sex-reversal

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Abstract

The sex of vertebrates is typically determined genetically, but reptile sex can also be determined by developmental temperature. In some reptiles, temperature interacts with genotype to reverse sex, potentially leading to transitions from a chromosomal to a temperature-dependent sex determining system. Transitions between such systems in nature are accelerated depending on the frequency and fitness of sex-reversed individuals. The Central Bearded Dragon, *Pogona vitticeps*, exhibits female heterogamety (ZZ/ZW) but can have its sex reversed from ZZ male to ZZ female by high incubation temperatures. The species exhibits sex-reversal in the wild and it has been suggested that climate change and fitness of sex-reversed individuals could be increasing the frequency of reversal within the species range. Transitions to temperature-dependent sex determination require low levels of dispersal and high (>50%) rates of sex-reversal. Here, we combine genotype-by-sequencing, identification of phenotypic and chromosomal sex, exhaustive field surveys, and radio telemetry to examine levels of genetic structure, rates of sex-reversal, movement, space use, and survival of *P. vitticeps* in a location previously identified as a hot spot for sex-reversal. We find that the species exhibits low levels of population structure (FST ~0.001) and a modest (~17%) rate of sex-reversal, and that sex-reversed and nonsex-reversed females have similar survival and behavioural characteristics to each other. Overall, our data indicate this system is evolutionary stable, although we do not rule out the prospect of a more gradual transition in sex-determining mechanisms in the future in a more fragmented landscape and as global temperatures increase.

KEYWORDS
dispersal, movement, sex determination, sex ratio, sex-reversal, survival

1 | INTRODUCTION

The sex of vertebrates is typically determined by the set of sex determining genes within the sex chromosomes, but in many species, sex can also be influenced by environmental factors experienced during development (Bull, 1983). Some species do not have sex chromosomes, and in such cases, sex is likely to be determined entirely by environmental factors (Schwanz & Georges, 2021; Valenzuela et al.,

2003). Reptiles exhibit a wide variety of sex determining mechanisms, including genotypic sex determination (GSD) with multiple chromosomal forms, temperature-dependent sex determination (TSD), and some in which environment and genes interact to determine sex (Dissanayake et al., 2021; Phillips, 2013; Quinn et al., 2007; Radder et al., 2008; Sarre et al., 2004; Van Doorn, 2014). Given the variety of sex determining mechanisms that occur even in closely related species, reptiles have proven to be useful vertebrate models with which to examine the evolution of sex determining systems, including transitions between GSD and TSD (Bull, 1983; Gamble et al., 2015; Holleley et al., 2015; Quinn et al., 2011).

Species in which environmental factors interact with sex chromosomes to reverse the sex of some individuals provide an opportunity to study how transitions from GSD to TSD might occur (Georges et al., 2021; Whiteley et al., 2021). For example, sex-reversal of animals with ZZ male genotypes (ZZ male/ZW female system) would result in three classes of sex, namely heterogametic/concordant (ZW) females, homogametic/concordant (ZZ) males, and homogametic/discordant sex-reversed (ZZ) females (Holleley et al., 2015, 2016; Quinn et al., 2011). This system of sex-reversal makes possible the overproduction of the female phenotype (concordant ZW females complemented by ZZ females) which, under frequency-dependent selection for a balanced sex ratio, reduces the proportion of individuals with ZW genotypes (Fisher, 1930; Schwanz & Georges, 2021). Ultimately, the W chromosome could be eliminated if reversal occurs with sufficient frequency, changing a species with GSD to one without sex chromosomes and resulting in a temperature-dependent mode of sex determination (Grossen et al., 2011; Schwanz et al., 2013, 2020).

Sex-reversal has been documented in recent studies of lizards (Dissanayake et al., 2021; Holleley et al., 2015; Wiggins et al., 2020) and for some species, there is a suggestion that this phenomenon may increase with a warming climate (Holleley et al., 2015). However, the extent to which sex-reversal occurs in a population, and whether or how quickly this will lead to full transitions to TSD remains unclear (Schwanz et al., 2020; Stelkens & Wadekind, 2010). Multiple factors, including the magnitude of fluctuations in nest temperature, local threshold temperatures for sex-reversal, and the behaviours and fitness of sex-reversed individuals relative to concordant individuals will influence the likelihood of a transition occurring (Boyle et al., 2014; Castelli et al., 2021; Grossen et al., 2011; Schwanz et al., 2020). In particular, high rates (>50%) of ZZ reversal from male to female and low (<1%) levels of immigration (dispersal) between populations (demes) are likely to be required in order for chromosome loss in a sex-reversing species, although strong fitness advantages for sex-reversed females could induce transitions to TSD with a much lower rate of sex-reversal (Hurley et al., 2004; Schwanz et al., 2020).

The rate of sex-reversal and its behavioural and fitness consequences remain untested in wild populations, but this ecological information is essential for understanding evolution of sex-determining mechanisms in natural systems. The Australian Central Bearded Dragon, *Pogona vitticeps*, is a species which exhibits sex-reversal in the laboratory (Quinn et al., 2007) and in the wild (Castelli et al.,

2021; Holleley et al., 2015). Laboratory incubation studies of *P. vitticeps* show that high incubation temperatures result in reversal of ZZ genotype embryos (normally male) to female phenotypes with a ZZ genotype (Quinn et al., 2007). Subsequent matings between ZZ females (ZZf) and ZZ males (ZZm) produce only chromosomal male offspring when incubated at intermediate incubation temperatures. In such matings, females can arise only from temperature-induced sex-reversal (ZZf) (Holleley et al., 2015). In laboratory studies, sex-reversed females are larger, more fecund, bolder and have higher activity than their ZZm or concordant (ZWf) female counterparts (Holleley et al., 2015; Li et al., 2016). Simulation modelling based on this species suggests that the rate of philopatry between phenotypic females (ZZf and ZW) and the possible fitness advantages for ZZf over their ZWf counterparts could accelerate localised transition from GSD to TSD (Schwanz et al., 2020). An increase in the proportion of phenotypic female *P. vitticeps* that are ZZ (sex-reversed) from 6.7% to 22.2% between 2003 and 2011 in south-western Queensland (Holleley et al., 2015) further supports that proposition, but it is also possible that local genetic adaptation in the temperature threshold for sex-reversal could counteract the sex-reversing influence of high incubation (Castelli et al., 2021).

Here, we combine population genetics, exhaustive field surveys, and radiotelemetry to examine *P. vitticeps* in south-western Queensland. This area of focus was recently identified as containing the highest proportion of sex-reversed individuals within the geographic range of *P. vitticeps* (Castelli et al., 2021). Our aim was to examine the factors that are key to the transition from GSD to TSD (and subsequent loss of the W chromosome) within a wild population. Specifically, we applied genotype-by-sequencing analyses to samples collected across the range of sex-reversed animals to examine rates of gene flow for the three sex classes of *P. vitticeps*. If genetic distance is high (and therefore gene flow is low) for both ZWf and ZZf types, then the loss of the W chromosome and a transition to TSD is more plausible than if the reverse is true. Second, we used broad-scale field sampling of *P. vitticeps* to calculate the rate of sex-reversal in order to determine if rates of reversal are changing over time, and if they are high enough to facilitate a transition from GSD to TSD. A sex-reversal rate greater than 50% is typically required for the localised loss of a W chromosome unless ZZf have a fecundity advantage (Schwanz et al., 2020). Finally, we applied radio-telemetry to wild *P. vitticeps* individuals to determine if sex-reversed females (ZZf) have different survival rates, behaviours, or morphometric characteristics compared with concordant females (ZWf) and males (ZZm). Our expectations, based on laboratory experiments (Holleley et al., 2015, Li et al., 2016), are that ZZf will have similar behaviour, survival, and morphology to ZZm, but different than their ZWf counterparts. Conversely, if ZWf demonstrate moderate population structure, suggestive of moderate gene flow, and show higher fitness related traits (i.e., survivorship or fecundity) in comparison to ZZf, we predict that the presence of the W chromosome would retard a rapid transition from GSD to TSD. These observations will determine whether the greater productivity, activity, and boldness seen in laboratory ZZf animals manifest themselves similarly in free

living individuals and will help determine whether the current extent of sex-reversal is leading a transition from GSD to TSD modes of sex determination in *P. vitticeps*.

2 | MATERIALS AND METHODS

2.1 | Specimen collection, phenotypic and molecular sex identification

Tissue and blood sampling was restricted to the south-eastern part of *P. vitticeps* range, where 82% of documented cases of sex-reversal have occurred (Castelli et al., 2021). To determine the rate and extent of sex-reversal across the study area and how it has changed over time, samples were collected from field trips in this area over a 16-year period (2003–2019; Figure 1). Lizards were captured ad hoc by driving gravel tracks. Once lizards were captured, a small portion of the tail tip (5–6 mm) was removed with medical grade scissors and then placed into 1.5 ml tubes containing 95% ethanol. Blood samples were obtained by applying the blood drop from the cut tail to a Whatman FTA Elute Micro Card (Qiagen). The tail tissues were placed on ice at the time of collection and transported to the

Wildlife Genetics laboratory at the University of Canberra where they were stored frozen (–80°C). The blood samples were stored at room temperature. Phenotypic sex of each lizard was determined by hemipene eversion (Harlow, 1996) and, where available, confirmed with other information such as nesting behaviour, gravidity, or presence of femoral pores. All animals were then released immediately at their point of capture.

Genotypic sex (ZZ or ZW) was determined in the laboratory using a PCR test from extracted DNA collected from tail tips or blood. We followed the PCR based molecular sex test described by Holleley et al. (2015) using sex linked markers identified by Quinn et al. (2010). PCR products were visualized on a 1.5% agarose gel where ZW females had two bands and ZZ individuals only one band. Internal ZZ and ZW control samples were run on every gel. Animals showing genotype-phenotype discordance were classified as sex-reversed (Holleley et al., 2015). The occurrence of sex-reversal is reported in two ways: (1) The sex-reversal rate, as the proportion of all ZZ individuals that are female (pZZ) (Grossen et al., 2011; Schwanz et al., 2020), and (2) the proportion of all females that are sex-reversed ZZf (pf) Holleley et al. (2015) and Castelli et al. (2021). Chi-square tests were used to determine if there was an association between sex-reversal (i.e., pZZ) and sampling year, with samples aggregated
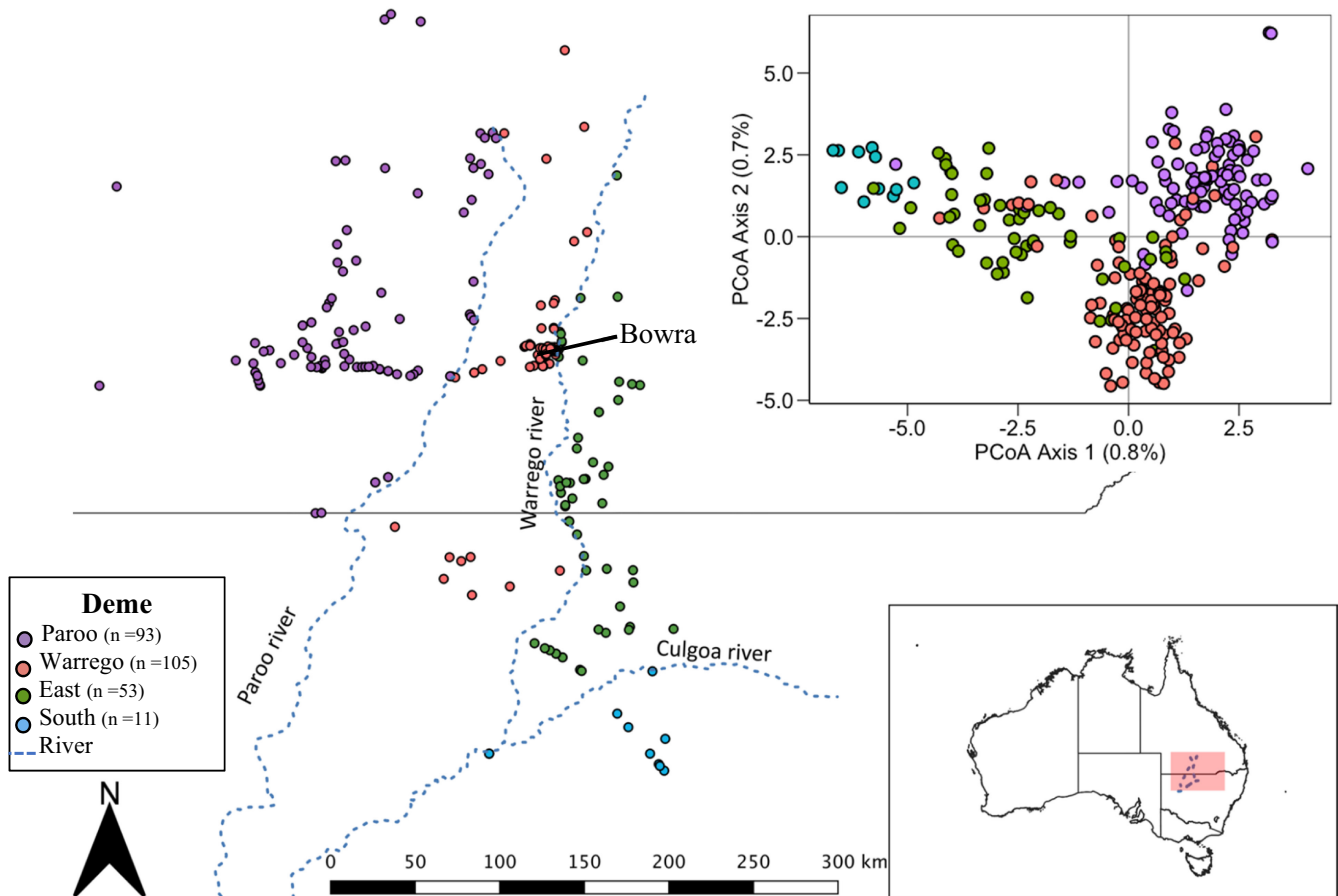

FIGURE 1 PCoA (top right panel) among individuals of *Pogona vitticeps* individuals sampled within 300 km of the focal population (Bowra Wildlife Sanctuary). Colour of points indicate what deme individuals were assigned to. PCoA show some level of clustering by deme for all individuals across 300 km range; however, axis 1 and 2 explain only 0.8% and 0.7% variation, respectively

into 8-year periods (2003–2011 or 2012–2019) across the 16-year sampling period. This 8-year window was chosen because this was in the middle of the 16-year sampling window and was roughly the midpoint for half of our samples during each period (2003–2011: $n = 58$; 2012–2019: $n = 50$).

2.2 | Genotype by sequencing and population genetic analysis

Reduced representation sequencing (Kilian et al., 2012) was used to genotype individuals in order to determine the levels of population genetic structure (as an indicator of gene flow) across the study area. Genomic DNA from tail tissue and blood samples was extracted using two DNA extraction methods appropriate for each sample type: (1) Qiagen's Gentra Puregene DNA purification kit for tail tips, or (2) Whatman Elute quick extraction protocol for blood on Whatman FTA Elute Cards. Specifically, DNA was extracted and sequenced and informative SNP markers identified using the commercial provider Diversity Arrays Technology (DArT Pty Ltd., www.diveristyarrays.com). Briefly, SNP genotyping was performed using a combination of complexity reduction using restriction enzymes, implicit fragment-size selection and next-generation sequencing (Kilian et al., 2012). The restriction enzyme combination of PstI (recognition sequence 5′-CTGCA|G-3′) and SphI (5′-GCATG|C-3′) was used for complexity reduction by double digestion of genomic DNA. Sequences were processed using proprietary DArT analytical pipelines (Kilian et al., 2012) to yield SNPs that were polymorphic within the set of samples. Calling quality was assured by high average read depth per locus (medium coverage, average ca 10×). In addition, approximately one-third of samples were processed twice from DNA to allelic calls as technical replicates. These are technical replicates in that the samples are not extracted twice, but the remainder of the workflow is repeated, including the preparation of libraries. Scoring consistency (repeatability) was used as the main selection criteria for high-quality and low error-rate markers. For further detail refer to Georges et al. (2018). Sequences were then filtered using the R package dartR (v.1.1.11; Gruber et al., 2018). Loci were removed with a call rate of less than 95%, repeatability less than 99%, and if they were sex-linked across all individuals. Secondary SNPs, that is, SNPs residing on a single sequence tag, were filtered, retaining only one at random. After filtering loci, individuals with a call rate of less than 95% were removed.

The R package dartR (Gruber et al., 2018) was used for population genomic analyses of the DArTseq SNP data. Principal coordinates analysis (PCoA) and F statistics (F_{IS}, F_{IT}, F_{ST}) were used to identify population structure across the study area, particularly those areas bounded by the three rivers (likely to be the most significant landscape barriers for this generalist species) that flow through the study area (Figure 1). Previous broadscale work has shown that this species exhibits a stepping-stone mode of dispersal across its range (Castelli et al., 2021), so isolation by distance was used to assess the relationship between genetic and geographic distance. It

has been shown previously that provided dispersal occurs at the juvenile stage and that genetic sampling is conducted on adults, the dispersing sex should be less genetically structured than the philopatric sex even with interbreeding among dispersing and philopatric individuals (Goudet et al., 2002; Rassmann et al., 1997). We used this approach to test for differences in genetic structure among the three sexes (ZZm, ZZf, ZWf). Genetic distance between demes was estimated using F_{ST} among the sexes and using the proportion of shared alleles between pairs of individuals. A Mantel test in the R package adegenet (Jombart, 2008) used to test for an association between genetic distance and geographic distances. We also used pairwise analysis of F_{ST} to examine our populations for sex biased dispersal. Isolation by distance in one sex relative to another would suggest lower levels of dispersal by that sex. Finally, we applied a redundancy analysis (RDA) to decompose the contribution of geographical coordinates (latitude and longitude) and sex on allele frequencies (Meirmans, 2015).

2.3 | Study site, frequency of sex-reversal, and morphometrics

We expanded on previous reporting of sex-reversal in this species with an exhaustive survey of P. vitticeps at one site within the area described in Section 2.1. We studied a population of P. vitticeps continuously from September 2018 to March 2020 at Bowra Wildlife Sanctuary (hereafter, Bowra): a 14,000 ha property located in our broader study area of south-west Queensland (Figure 1). This enabled us to quantify the operational sex ratio and infer the rates of sex-reversal with greater confidence than has previously been possible. Vegetation type at Bowra was determined using a stereoscopic photo interpretation of 2015 aerial photography (1:30,000, resolution 0.21 m), with ground truthing by vegetation surveys recorded by the Australian Wildlife Conservancy (Stanton et al., 2019). Vegetation was classified into six types (woodland, grassland, mixed forest woodland, claypan/herbland, shrubland, and other) based on dominant vegetation (Table S1) and surveys for lizards were conducted by foot and by vehicle within the property. The surveys were timed to match the diurnal activity patterns of the lizards during their most active months (October–December). Both foot and road surveys were standardized by the number of hours and observers. To ensure that we sampled the population within this sanctuary thoroughly, foot surveys included all vegetation types at least once each week and all roads driven at least once each week (Figure S1). Capture effort for each lizard was estimated as the sum of total search hours (or driving hours) divided by the total number of lizards captured. Capture effort was then compared by survey method (search/vehicle count) and compared across sex classes. Chi-square tests were used to estimate if sex-ratio was biased in the population between males (ZZm) and females (ZZf +ZWf).

Following procedures outlined in Section 2.1, upon capture each lizard was sexed phenotypically and a blood sample taken for genotyping. Snout-vent length (SVL) was measured using a ruler (± 1 mm)

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and mass was measured using an Optek digital scale (±0.5 g). Body condition index (BCI) was calculated for each lizard as the residual from an ordinary least squares (OLS) regression of SVL and mass (after Schulte-Hostedde et al., 2005). ANOVA was used to compare how morphometric data (SVL, mass, and body condition index) varied among sex classes.

2.4 | Radiotelemetry and GPS tracking

To determine how movement rates, space use, survival, and growth vary among sex classes, adult lizards were tracked from September 2018 to March 2020. Each lizard was fitted with a Pinpoint Beacon 250 (Lotek Ltd., Havelock North, New Zealand) using a custom-fit backpack harness (after Price-Rees & Shine, 2011). Each unit housed a GPS logger, a single stage VHF transmitter 150–151 Hz) and 3-axis accelerometer. The unit and harness did not exceed 5% of the animal's body mass. Dilution of precision (DOP; Langley, 1999) was recorded for every GPS fix, and this measurement was used as an indicator of quality of GPS position (≤5 m accuracy). Estimates of movement and space use were calculated from GPS data only (DOP ≤3; Jones et al., 2016; Price-Rees et al., 2014). Lizards were located as required using a VHF receiver (Biotracker, Lotek Ltd, Havelock North, New Zealand) and Yagi antenna. GPS points were acquired every day in spring (September–November), summer (December–February), and autumn (March–May), and once every three days in winter (June–August). The status of animals (dead or alive) was determined by relocating all individuals once per week (September–December) and a minimum of once per month for the reminder of the year (January–August). To determine reproductive status, females were palpated every two weeks during the breeding season (October–December) and monthly when lizards were handled to change telemetry equipment.

2.5 | Movement and space use estimates

Movement rate (metres moved per day, m/d) was calculated as the straight-line distance between consecutive GPS locations divided by the number of days elapsed between relocations. A linear mixed-effects model was used to test the effect of season, sex class, and their interaction on movement rate with individual lizard as a repeated (random) effect. To determine if reproductive status influenced female movement, within-individual variability of movement between gravid and non-gravid periods was compared by recording distance moved per day for gravid females (2 weeks pre/post lay date) with the distances moved while non-gravid (2–6 weeks post lay date). We then tested for the effect of reproductive status on distance moved per day (response variable), with reproductive status (gravid or not gravid) as a fixed effect, and female ID as a repeated effect.

We used two home range estimates to compare space use, including 100% minimum convex polygons (MCP; Burt, 1943; Row

& Blouin-Demers, 2006) and 95% fixed kernel density estimators (KDE; Worton, 1989; Young et al., 2018) using the *adehabitatHR* package (Calenge, 2006). The MCP method provides an outline that encloses all locations and was selected to estimate the maximum potential area an individual occupied, whereas KDE was selected to estimate variation in intensity of space use among individuals (Seaman & Powell, 1996). Least square cross validation was used to select a kernel-smoothing parameter for KDE (Horne & Garton, 2006). These estimates were defined as "space use" and not home range because of the duration of tracking and the length of our study. We estimated space use only for animals that had a minimum of 15 GPS locations, and estimates were log_{10}-transformed for both 100% MCP and 95% KDE to fit normality assumptions. A linear mixed-effects model was used to test the effect of season, sex class, body size (SVL) and their interaction on space use estimates with individual lizard as a repeated (random) effect. When results were significant, we followed with pairwise comparisons from *emmeans* package in R (Lenth et al., 2018). Interactions (sex class×season) or covariates (SVL) for movement and space use estimates that were not significant were removed from further analysis. Equations for each movement and space use model used can be found in Supplementary Table S2.

2.6 | Survival and growth rate estimates

In cases where a tracked lizard was found dead, the date of death was determined using the last movement recorded by the accelerometer housed on the GPS units or abnormal large-scale movements (>1 km) over a short priod of time (consistent with predation by birds of prey). Accelerometers recorded average changes in acceleration over a minimum of 120 s intervals. Maximum likelihood survival probabilities were estimated using known fate models in the program MARK (White & Burnham, 1999). These models estimate survival probability (φ) between a set time interval when fates (i.e., dead or alive) of individuals can be determined with certainty when animals are continuously tracked with telemetry equipment. Time intervals were set seasonally (spring, summer, autumn, winter) and sex class was defined as the genotype and phenotype combination (ZZf, ZZm, ZWf). Akaike Information Criteria (AICc) was used to correct for small sample sizes and models with delta AICc of <2.0 from the best model were considered to have support. We started with a fully saturated model in which survival probability was dependent on sex class, body size (SVL), season, and their interaction, then we fitted a series of reduced-parameters models.

Growth rates were calculated by dividing the change in SVL between the initial capture and subsequent re-captures by the total number of days elapsed (>20 days). Differences in SVL growth rates across sex classes were determined using an analysis of covariance (ANCOVA) with sex class as a fixed (3-level) factor, growth rate as the response variable and initial log_{10}-transformed SVL as a covariate.

3 | RESULTS

3.1 | Frequency of sex-reversal across populations

A total of 207 lizards were captured during sampling trips across the study area (excluding individuals captured at Bowra) and once individuals were genotyped for their chromosomal sex (see example gel Figure S2) this amounted to: ZZf ($n = 18$), ZWf ($n = 98$), ZZm ($n = 91$). We documented sex-reversal in three out of four demes that were sampled (Figure 2). Overall, the proportion of females (*pf*) that were sex-reversed was 16%, while the rate of sex-reversal (*pZZ*) was 17% when considering all animals captured during the 16-year sampling period. However, we observed a decline in the rate of sex-reversal (*pZZ*) from 24% to 6% ($\chi^2 = 5.36$, $df = 1$, $p < .05$) between the first half of that sampling period (2003–2011) to the second (2012–2019).

3.2 | Population genetic structure

The DArTseq genotype by sequencing generated 167,396 SNP loci prior to filtering for 272 individuals (all samples including Bowra) with a missing data rate of 31.9% overall. Those individuals whose PCoA coordinates exceeded six standard deviations from the

centroids of the first 10 principal components were considered to be extreme outliers ($n = 6$; Figure S3) (after DeSaix et al., 2019). Because of the possibility that these 10 outliers would exert undue leverage in subsequent analyses, they were removed from further analysis. Preliminary PCoA quality control analyses showed two discrete groups occurring within the study area (Figure S4). These groups were unrelated to sampling year, sex class or geography and showed the same pattern of relatedness across the study site (Figure S4). Subsequent analyses revealed that a small number of highly linked loci ($n = 40$) caused this dichotomy and removal of those loci removed the dichotomy (Figure 1). We interpret this as a linked nonrecombining haplotype of unknown origin and note that it represented <1% of our total remaining loci once filtering was completed. We therefore removed these 40 loci from all subsequent analyses. Once filtering and quality control was completed, the data set was reduced to 13,871 SNPs for 262 individuals with a missing data rate of 1.91%. Overall F_{IT} (0.232) and F_{IS} (0.226) values indicated that our sample population was not in Hardy-Weinberg Equilibrium and that inbreeding is evident within demes. Pairwise comparisons of F_{ST} values were low (0.006–0.015; Table 1) regardless of sex suggesting low levels of population structure among the demes and sex. However, the PCoA (Figure 1) indicated the presence of some structure across the drainage barriers. We also
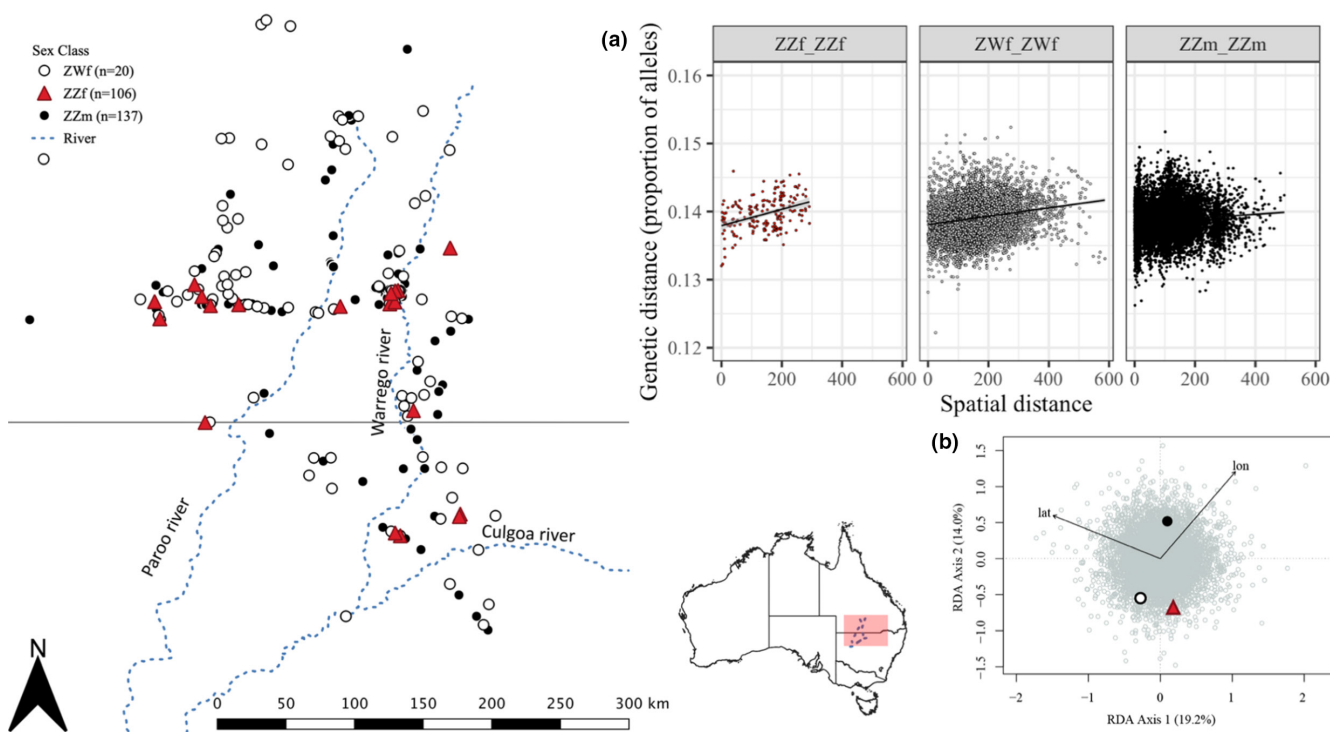

FIGURE 2 Genetic isolation by distance among *Pogona vitticeps* individuals sampled within 300 km radius of focal population (Bowra Wildlife Sanctuary). (a) Shows individual pairwise genetic distance, measured by the proportion of shared alleles, to geographic distance (km) by sex class. We detected a significant positive relationship between genetic distance and geographic distance (km) for ZW females (Mantel's test: $r = 0.047$, $p = .001$) and ZZ females (Mantel's test: $r = 0.119$, $p = .016$), suggesting genetic similarity decreases with increasing distances. Whereas males show no relationship between genetic distance and geographic distance (Mantel's test: $r = 0.001$, $p = .384$). (b) A biplot of redundancy analysis (RDA) to assess the influence of geographical coordinates (latitude and longitude) and sex (ZZf, red triangle; ZWf, white circle; ZZm, black circle) on allele frequencies (grey circles). Axis for RDA biplot are Eigenvalues for constrained axes. Together both geographic coordinates and sex significantly explained 2% of total genetic variance across all our samples

detected isolation by distance (pairwise $F_{ST}/(1-F_{ST})$) in pairwise comparisons among the four demes (Mantel test; $r = 0.930$, $p = .042$. When we investigated the relationship between genetic distance (proportion of shared alleles) and geographic distance (Figure 2), we detected a significant positive relationship for females (ZZf: Mantel test; $r = 0.119$; $p = .016$; ZWf: Mantel test; $r = 0.047$, $p = .001$; Figure 2a), but did not detect a relationship for males only (Mantel test; $r = 0.001$, $p = .38$). The RDA analysis detected a significant relationship of geographic coordinates (lat|lon: $p < .001$) and sex ($p = .026$) on population allele frequencies ($r^2 = 0.02$; Figure 2b).

3.3 | Sex-reversal rate, morphometrics, and reproduction within the Bowra population

A total of 3,295 (by foot) and 3,832 (by vehicle) observer hours were spent surveying for lizards at Bowra between September 2018 and March 2020. These surveys yielded 65 lizards (50 ZZm, 12 ZWf and 3 ZZf) requiring ~137 survey hours on foot and ~98 h by vehicle to find one lizard regardless of sex (Table S3). Once the chromosome sex was determined from individuals at bowra, the proportion of females that were sex-reversed (*pf*) was 20%, and a sex-reversal rate (*pZZ*) of 6%. We detected a significant ($\chi^2 = 18.85$, $df = 1$, $p < .05$) male-biased (3.3:1) sex ratio within the Bowra population. If, despite our extensive search effort, we missed capturing the number of females that would equilibrate the sex ratio (35 females), we estimate

we missed 7 ZZf (20% of 35 missed phenotypic females), which would indicate a sex-reversal rate of 17%.

Length ($F_{2,63} = 12.58$; $p < .05$) and mass ($F_{2,63} = 13.58$; $p < .05$) varied by sex class, with ZWf being smaller and lighter than ZZm and ZZf (Table S4). In contrast, residual body condition did not differ significantly among the sex classes (ZZf, −13.9; ZWf, −12.3; ZZm, 3.8; $F_{2,63} = 0.94$; $p = .40$; Table S3). Eleven out of 12 ZWf (~92%) were gravid during the study, and two of those individuals were gravid twice within one calendar year. No ZZf were gravid at any point.

## 3.4 | Movement and space use

We radio-tracked 63 adult *Pogona vitticeps* (ZZm $n = 48$; ZWf $n = 12$; and ZZf $n = 3$) over an average of 119 days (range: 10–431 days) per lizard for a total of 6,205 GPS locations. Movement rates (m/d) differed among the sex classes ($F_{2,6203} = 5.55$, $p < .05$), with post-hoc tests indicating that ZZm had higher movement (49.5 m/day) than ZWf (24.6 m/day) and ZZf (27.6 m/day), but that there were no differences between ZW and ZZ females (Figure 3). Movement rates also differed across seasons ($F_{3,6202} = 29.09$, $p < .05$), where overall movement was highest across sex class during the reproductive season (spring; Figure 3). We detected a sex × season interaction ($F_{6,6199} = 2.81$, $p < .05$), where ZZm had higher movements than females during each season, respectively. We also detected within-individual variability between gravid and nongravid periods, with gravid ZWf making longer daily movements (40 m/day) compared to nongravid

TABLE 1 Pairwise comparisons of F_{ST} values by sex and between demes of *Pogona vitticeps*. F_{ST} values range from 0 (complete panmixia) to 1.0 (breeding units fixed for alternative alleles)

| Deme | ZZf | | ZWf | | | ZZm | | |
	Warrego	East	Warrego	East	South	Warrego	East	South
East	0.006	–	0.002	–	–	0.003	–	–
Paroo	0.007	0.008	0.003	0.006	0.019	0.003	0.004	0.013
South	–	–	**0.015**	**0.010**	–	**0.014**	0.008	–

Note: Bold values indicate significant difference in pairwise comparisons within each sex class. For geographical origin of demes see Figure 1. We did not have all combinations for pairwise comparisons for ZZf category because no samples were collected in the South deme.

FIGURE 3 Seasonal mean daily (±SE) movement rates for *Pogona vitticeps* across sex classes at Bowra wildlife sanctuary, Queensland. Connecting letters indicate significant difference on a pairwise basis derived from a Tukey's test when comparing sex for each season
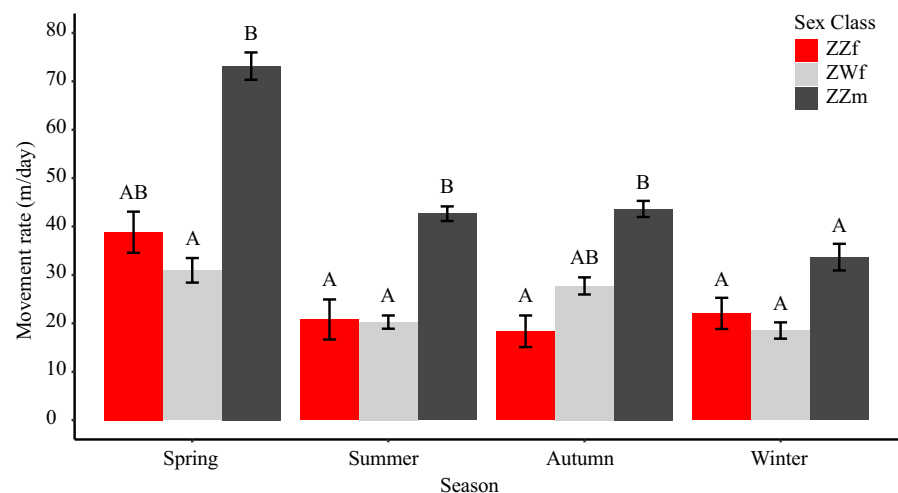
females (20 m/day; $F_{1,282} = 15.44$, $p < .05$). Space use for both estimates was significantly higher (100% MCP: $F_{2,120} = 8.18$, $p < .05$; 95% KDE $F_{2,120} = 7.89$, $p < .05$) for ZZm compared with ZWf and ZZf, and no differences between ZWf and ZZf were detected (Table S5). Space use differed across seasons for both estimates (100% MCP: $F_{3,119} = 4.85$, $p < .05$; 95% KDE $F_{3,119} = 6.89$, $p < .05$), where spring and summer had the highest space use estimates in comparison to winter and autumn (Tables S5 and S6). For space use estimates, we did not detect a sex class × season interaction (100% MCP: $F_{6, 116} = 0.87$, $p = .52$; 95%; KDE: $F_{6,116} = 0.79$, $p = .58$) or significant effect of body size (100% MCP: $F_{1,121} = 4.03$, $p = .06$; 95% KDE: $F_{1, 121} = 3.06$, $p = .08$).

3.5 | Survival and growth

Thirty-three lizards died over the 18-month study period, including all ZZf ($n = 3$), eight of 12 ZWf, and 22 of 48 ZZm. Most mortality events were from predation by non-native cats (*Felis catus*) and predatory birds, such as species of Falconidae and Accipitridae. The primary factors influencing survival probability was the interaction between season and sex class (ΔAICc = 0.00, weight = 0.75; Figure 4). Sex class survivorship probabilities (mean, SE) over the duration of the study were 0.78 ± 0.04 for ZZm, 0.71 ± 0.17 for ZZf, and 0.65 ± 0.09 for ZWf. Overall seasonal survivorship probabilities were 0.62 ± 0.09 for the spring, 0.80 ± 0.06 for the summer, 0.74 ± 0.13 for autumn, and 0.88 ± 0.08 for the winter. All other competing models had little support (Table S7). Growth rates ranged from 0 to 34 mm/year, but did not differ among sex classes ($F_{1,32} = 0.24$, $p = .79$).

4 | DISCUSSION

Here, we report on a natural population of *P. vitticeps* where sex-reversal occurs at its highest rates within its range and below we demonstrate that our data is consistent with an intermediate system of localised sex-reversal that is evolutionarily stable. We document sex-reversal in three of the four sampled demes (Figure 2) over a 16-year period and find a decline in rates of sex-reversal (*pZZ*) from 24 to 6% between the first and second halves of that sampling period. In a small but intensively surveyed area of the study system (Bowra), we similarly found low rates of sex-reversal (*pZZ* = 6%) in 2018–2019. Even an assumption of equal sex ratios at Bowra would lead to a sex-reversal rate of only 17%. These rates of sex-reversal for Bowra and across multiple demes are considerably less than the *pZZ* threshold of 50% predicted by simulations to be necessary for the rapid loss of the W chromosome and a transition from GSD to TSD (Schwanz et al., 2020). Moreover, the proportion of sex-reversed females (*pf*) has not increased substantially, if at all, since 2011 (22%, Holleley et al., 2015), and is relatively low (12%) across the species' range (Castelli et al., 2021). Thus, we argue that rates of sex-reversal in this system are not progressing towards a system of TSD and rather that

this intermediate system of localised sex-reversal is evolutionarily stable (Smith, 1979).

Sex-biased differences in vagility are known to effect rates of gene flow in vertebrates (Li & Kokko, 2019) and may contribute to lower-than-expected rates of sex-reversal across our demes. Our estimates for genetic distance indicate that gene flow is being contributed by ZZm, while phenotypic females (ZZf & ZWf) exhibit greater structure among demes. Immigration as low as 1% per generation (including ZW individuals) provides a major buffering effect in the loss of the ZW genotype within a population, even where sex-reversal occurs at high frequencies (Schwanz et al., 2020). The patterns of genetic structure across four demes indicate that transitions from GSD to TSD are unlikely to occur, in part, owing to the modest-but-sufficient dispersal capabilities of ZWf. It is therefore possible that frequency of ZZf individuals is driven more by local extrinsic factors such as resource availability (Dupoué et al., 2020), environmental conditions of nest selection (Schwanz et al., 2020), or local adaptation of temperature sensitivity (Castelli et al., 2021).

There are two components of gene flow that could facilitate a rapid spread of sex-reversal across natural populations: (1) dispersal of sex-reversed (ZZf) individuals away from their site of origin and (2) subsequent reproduction and introduction of genes from ZZf individuals into a new gene pool. Our data show ZZf have higher F_{ST} values (i.e., lowest dispersal) compared to other sex classes (Table 1), and demonstrate a positive relationship between geographic distance and genetic distance, respectively (Figure 2). These patterns of gene flow are consistent with the vagility of ZZf within our sub-population, where daily distance moved and space use suggest they are unlikely to move long distances or occupy large areas. Lastly, none of the ZZf became gravid at any point, whereas 11 of 12 (92%) ZWf individuals were gravid at least once during the study. This runs counter to previous laboratory findings (Holleley et al., 2015) and our own expectations that reproductive potential would be higher among ZZf than ZWf individuals. While the movement and reproductive data were limited by sample size for ZZf, they are consistent with the broader genetic data where we provide multiple lines of evidence that suggests this species demonstrates sex-bias dispersal (Rassmann et al., 1997). The low rates of gene flow, lack of long-distance movements and low reproductive success, suggests adult ZZf carrying thermosensitive genes are unlikely to move to adjacent demes. However, we cannot rule out the possibility that ZZm offspring from sex-reversed females disperse to adjacent demes taking with them thermosensitive genes that decrease the temperature threshold for sex-reversal and thereby increase the rate of sex-reversal in adjacent demes (Castelli et al., 2021; Quinn et al., 2007).

There is strong evidence that sex-reversed and concordant females differ in several fitness related traits in laboratory experiments on wild caught individuals or offspring of wild caught individuals (Holleley et al., 2015; Li et al., 2016), but such variation is not evident in the wild population studied here. In particular, we found little evidence for differences in fitness proxies or activity between ZZf and ZWf. For example, while captive ZZf display "male-like" behaviours in their activity and territory exploration (Li et al., 2016),

FIGURE 4 Seasonal survival probabilities by sex for *Pogona vitticeps* at Bowra Wildlife Sanctuary, Queensland (2018–2020). Estimates were derived from known fate models in program MARK. Values are mean ±1 SE
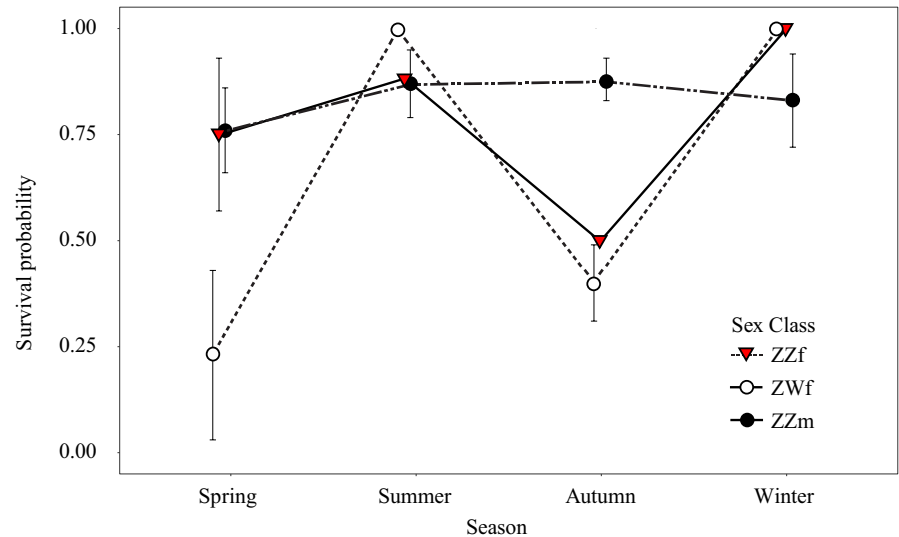
we found no evidence for this in either the radiotelemetry or genetic dispersal data in the wild. Moreover, while ZZf animals were larger than ZWf animals in both wild and captive populations, we found no evidence in our wild animals of the greater reproductive output (Holleley et al., 2015) and body condition seen in captive ZZf juvenile animals (Li et al., 2016). Overall, our data indicate differences in fitness-related traits, and movement among sex class in wild *P. vitticep*s, but counter to our predications, these differences were strongest between reproductive phenotypes (male or female) and not their sex chromosomes.

Depending on an individual's behaviour and reproductive strategies, sex-specific differences in survivorship may shape selection pressures across sex classes (Sillett and Holmes, 2002; York & Baird, 2017) and could influence the stability of ZW genotypes within a population. The ZWf cohort had the lowest survival rate of the three sex classes. Seasonal survivorship for ZWf animals was lowest during the reproductive season (spring) when movement was highest, and we found gravid females doubled their daily movements during this same period. High rates of mortality for females (both ZWf and ZZf) coincides with an increase in conspicuous behaviours associated with mate acquisition or nesting, in this case corresponding with their highest seasonal movements, and perhaps increasing exposure to predators (Stuart-Fox et al., 2003). However, one point of interest is we did not document any sex-reversed females to be gravid, calling into question why their movement and survivorship were like ZWf. One important question to understand is how these behaviours may shape different selection processes between females. It is possible that sex-reversed females may have other "male -like" behaviours, such as display signaling or boldness/ agressivness (Li et al., 2016), which could not be detected by telemetry data. Such behaviours, should they occur in the wild, may explain low rates of survivorship and low mating success for sex-reversed females (Bremner-Harrison et al., 2004; Marler and Moore, 1988).

Our results run contrary to key expectations arising from laboratory experiments and highlight the importance of testing predictions drawn from mechanistic approaches to improve our understanding of the evolutionary significance of sex-reversal in natural, wild populations. Our results indicate that gene flow, movement patterns, rates of survival and body condition of sex-reversed individuals are more reflective of their reproductive phenotype than their chromosome complement (Jones et al., 2020). In the absence of an obvious positive fitness advantage to sex-reversal, our data demonstrate that moderately-high rates of dispersal combined with low rates of sex-reversal may act as a buffer against a rapid localised transition to TSD in *P. vitticeps* and would therefore require significant perturbations to enable a full transition to a TSD form of sex determination. These perturbations could take the form of severe climate warming that causes sex-reversal rates to increase substantially, or significant habitat fragmentation that decreases gene flow among demes and thereby enables sex-reversal to locally increase in frequency. It is also possible that behavioural aspects, such as nest site selection and timing (Doody et al., 2006), contain further buffering mechanisms that limit the frequency of sex-reversal. During the 16-year sampling period, 14 out of 16-years the average ambient temperature exceeded temperatures above the threshold temperature (33°C; Figure S5) at which sex-reversal occurs (Holleley et al., 2015). With increasing temperatures associated with climate change having the potential to accelerate sex-reversal within a few generations (Schwanz et al., 2020), further information is needed on the nesting temperatures of this species and the other reptile species with similar systems (Dissanayake et al., 2021; Whiteley et al., 2021; Wiggins et al., 2020) to determine how robust reptiles are to transitions in sex determining systems in the future. Our research places a new imperative on determining the ecological mechanisms driving sex-reversal and the roles that sex-reversed individuals play in fluctuating sex-determining systems.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

The sampling design, collection materials, laboratory work, and figures were organised by Kristoffer H. Wild, with the support of Stephen D. Sarre, John H. Roe, Lisa Schwanz, and Arthur Georges. The first draft of the manuscript was written by Kristoffer H. Wild. Comments from Stephen D. Sarre, John H. Roe, Lisa Schwanz, and Arthur Georges contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

All data and materials are presented in the main study and Supporting Information. The SNPs data, movement data, program MARK files, and custom scripts used in this study have been deposited are located on the Dryad Digital Repository: https://doi.org/10.5061/dryad.4tmpg4fbd.

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SUPPORTING INFORMATION

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