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LETTER

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Sex reversal triggers the rapid transition from genetic to temperature-dependent sex

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Sex determination in animals is amazingly plastic. Vertebrates display contrasting strategies ranging from complete genetic control of sex (genotypic sex determination) to environmentally determined sex (for example, temperature-dependent sex determination)[1]. Phylogenetic analyses suggest frequent evolutionary transitions between genotypic and temperature-dependent sex determination in environmentally sensitive lineages, including reptiles[2]. These transitions are thought to involve a genotypic system becoming sensitive to temperature, with sex determined by gene–environment interactions[3]. Most mechanistic models of transitions invoke a role for sex reversal[3–5]. Sex reversal has not yet been demonstrated in nature for any amniote, although it occurs in fish[6] and rarely in amphibians[7,8]. Here we make the first report of reptile sex reversal in the wild, in the Australian bearded dragon (*Pogona vitticeps*), and use sex-reversed animals to experimentally induce a rapid transition from genotypic to temperature-dependent sex determination. Controlled mating of normal males to sex-reversed females produces viable and fertile offspring whose phenotypic sex is determined solely by temperature (temperature-dependent sex determination). The W sex chromosome is eliminated from this lineage in the first generation. The instantaneous creation of a lineage of ZZ temperature-sensitive animals reveals a novel, climate-induced pathway for the rapid transition between genetic and temperature-dependent sex determination, and adds to concern about adaptation to rapid global climate change.

Sex determination is the regulatory process that initiates differentiation of the gonads in the early embryo to form either testes or ovaries. Very many reptiles have temperature-dependent sex determination (TSD), whereby the temperature that eggs experience in the nest determines the sex of offspring. Others have male or female heterogamety, either XX/XY systems as in mammals or ZZ/ZW systems as in birds, with or without strongly heteromorphic sex chromosomes[9]. Mounting evidence suggests that genotypic and environmental modes of sex determination are not mutually exclusive dichotomous strategies[1]. Many species have differentiated sex chromosomes, but also show a temperature override, where genes and environment interact to determine sex[10–14]. Furthermore, the great diversity of sex-determining mechanisms seen in reptiles, matched in amphibians and fishes, but not mammals and birds, shows poor respect for phylogeny, implying a complex evolutionary history of multiple transitions among sex determination modes[2,9].

The widely distributed Australian central bearded dragon (*P. vitticeps*) was one of the first reptile species in which a temperature override was observed[10–12,14], and the first in which it was demonstrated genetically[12]. *P. vitticeps* has a female heterogametic system of sex determination with ZZ males (ZZm) and ZW females (ZWf)[15], but high incubation temperatures experimentally feminize chromosomally male animals and produce sex-reversed females (ZZf)[12]. To identify sex-reversed females, we developed a new robust sex-specific molecular marker from previously characterized *P. vitticeps* sex chromosome sequences[16,17] and validated the test against a panel of unrelated individuals incubated at temperatures where phenotypic sex and genotypic sex are concordant (20 ZZm and 20 ZWf). Absence of a W chromosome in putatively sex-reversed ZZ females was confirmed cytogenetically to eliminate the possibility of low-frequency recombination being mistakenly interpreted as sex reversal (Extended Data Figs 1–3).

Animals were sampled from several widely distributed populations. Application of the PCR sex marker to 131 wild-caught individuals identified 11 sex-reversed ZZ females occurring towards the northern end of the *P. vitticeps* range, near the border of Queensland and New South Wales (Fig. 1). Although sex reversal in reptiles has been demonstrated under laboratory conditions[10–12], this is the first time that sex reversal has been shown to occur naturally in a wild population of reptiles, or indeed any amniote. Sex reversal was widespread in this population, with instances distributed over a total area of 23,650 km^2 in remote semi-arid Australia. Among wild phenotypic females, the proportion of ZZ sex-reversed females increased each year over the study, from 6.7% in 2003, to 13.6% in 2004, to 22.2% in 2011 (Fig. 1), suggestive of a trend but not significant ($\chi^2 = 1.65$, d.f. $= 2$, $P = 0.44$).

The sex-reversed females were viable and fertile. In fact, our sex-reversed females laid significantly more eggs per year (mean

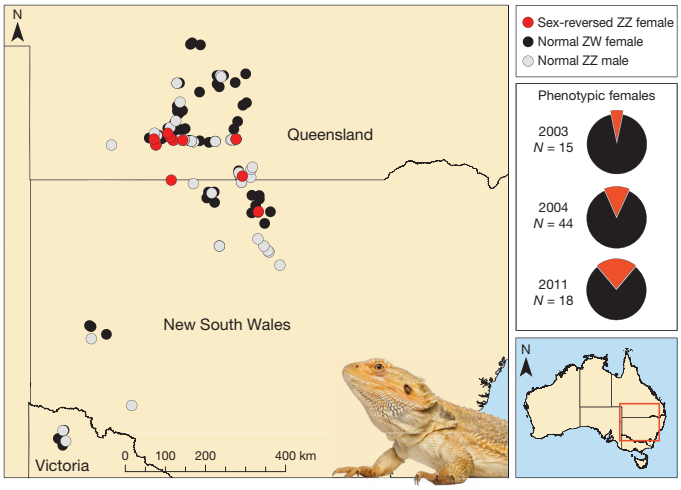

Figure 1 | Geographical distribution of sex reversal in wild populations of *P. vitticeps*. Location of sex-reversed ZZ females (ZZf) across years is indicated by red circles ($N = 11$), normal ZW females (ZWf) by black circles ($N = 72$) and normal ZZ males (ZZm) by grey circles ($N = 48$). Pie charts indicate the relative proportions of ZZf and ZWf in years where sample size exceeded 15 phenotypically female individuals. The temporal trend is suggestive, but not significant ($\chi^2 = 1.65$, d.f. $= 2$, $P = 0.44$).

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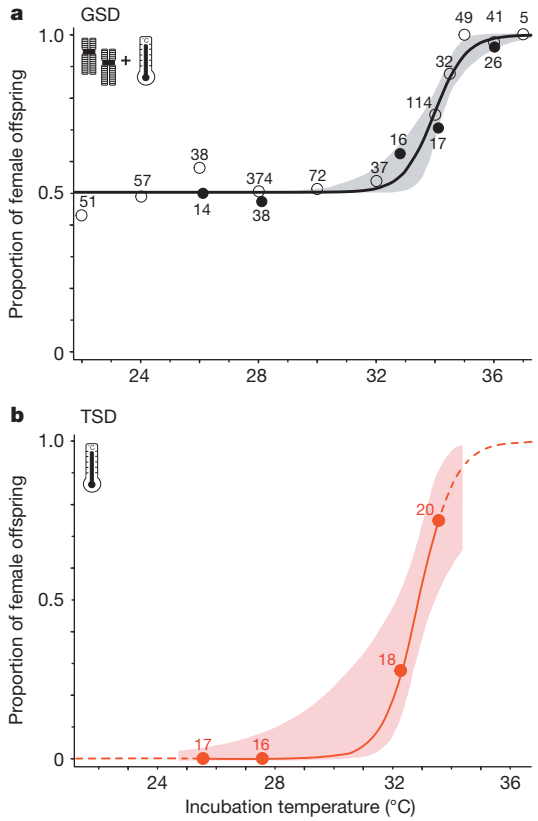

Figure 2 | Offspring sex ratio as a function of egg incubation temperature in *P. vitticeps*. **a**, GSD system of sex determination with a high-temperature override. Data are from ref. 12 (open circles) and this study (filled circles). Proportion of phenotypically female offspring from control ZZm × ZWf crosses is a function of constant incubation temperature T, given by $\Pr\{f\} = 0.5033 + \frac{(1-0.5033)e^{1.8723T-63.5904}}{1+e^{1.875T-63.5904}}$. **b**, Functional TSD by sex reversal. Proportion of phenotypically female offspring from ZZm × ZZf crosses is given by $\Pr\{f\} = \frac{e^{-52.9999+1.5866T}}{1+e^{-52.9999+1.5866T}}$. Dashed lines, extrapolation of the fitted curve beyond the data. Shaded regions, 95% confidence limits. The number of individuals in each treatment is shown.

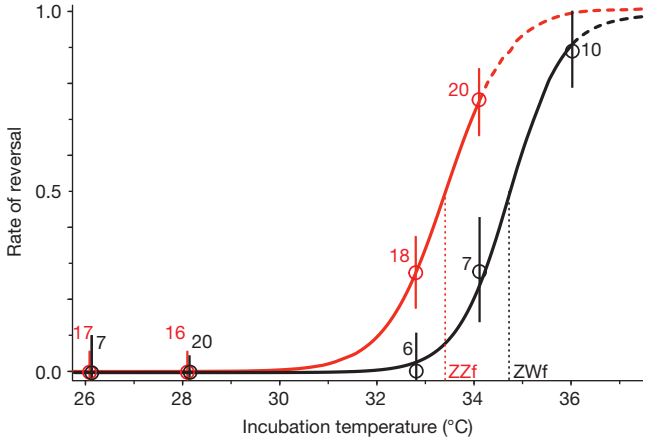

Figure 3 | Rate of sex reversal as a function of egg incubation temperature in *P. vitticeps*. Offspring of sex-reversed mothers (ZZf shown in red) are reversed more frequently and at a lower temperature than the offspring of control mothers (ZWf shown in black), implying that temperature sensitivity is variable in the population and heritable. Vertical bars, standard error of the observed proportion. Dashed lines, extrapolation of the fitted curve beyond the data (see Methods for equations). Dotted lines, the pivotal temperature at which half of ZZ offspring are reversed. Sample size (numbers) is the total number of ZZ individuals.

ZZf = 47.3 eggs, $N = 6$ females) than ZW females of an equivalent age (mean ZWf = 24.5 eggs, $N = 11$ females) ($P < 0.05$).

Four wild-caught and three captive-bred[12] sex-reversed females (ZZf) were mated with normal males (ZZm) under controlled laboratory conditions, to yield 389 eggs from 21 clutches. As expected from ZZm × ZZf matings, eggs incubated at the low temperature of 28 °C produced ZZ hatchlings, which were all male ($N = 35$ hatchlings, two complete clutches, 87.5% hatching success). Parentage analysis using 2,229 single nucleotide polymorphism (SNP) markers in a subset of individuals confirmed that eggs laid by sex-reversed females were the product of sexual reproduction and not facultative parthenogenesis (Supplementary Table 1), a phenomenon that is uncommon, but known in some squamates[18–20].

Following our experiments at the low incubation temperature of 28 °C, we then compared the offspring sex ratio of sex-reversed females (74 ZZf eggs) and control females (130 ZWf eggs) across a range of temperatures. Hatching success was high both for sex-reversed and for control matings (95.9% and 85.4%, respectively) (Supplementary Table 2). Offspring sex ratios from the control matings confirmed the existence of a ZW chromosomal temperature override as previously described[12] (Fig. 2a) (probit regression, Wald $\chi^2 = 14.13$, d.f. = 1, $P < 0.0002$). Specifically, chromosomal influence over sex determination was dominant from 22 °C to 32 °C, where the proportion of males and females was equal (estimate of unconstrained lower asymptote = 0.503; Fig. 2a). Temperature began to interact with and override chromosomal sex determination (causing sex reversal) above 32 °C, reaching almost complete female bias at high temperatures (proportion female at 36 °C = 0.96) (Fig. 2a; Supplementary Table 2). In contrast to the control matings, the offspring sex ratios produced by sex-reversed females followed the pattern of a TSD species with no chromosomal influence over sex (logistic regression, Wald $\chi^2 = 8.09$, d.f. = 1, $P < 0.005$) (Fig. 2b and Supplementary Table 2). In the absence of a W chromosome, the female phenotype is possible only via sex reversal[12] (Fig. 3 and Supplementary Table 3). Thus, sex-reversed mothers produced only male hatchlings at low and intermediate incubation temperatures (26 °C and 28 °C), but at 33 °C produced female as well as male offspring (proportion female = 0.28) and at 34 °C produced offspring that were predominantly female (proportion female = 0.75) (Fig. 2b).

Logistic regressions of rate of sex reversal against temperature were significant for the offspring of both normal ZZm × ZWf crosses (Wald $\chi^2 = 8.14$, d.f. = 1, $P < 0.005$) and sex-reversed ZZm × ZZf crosses (Wald $\chi^2 = 8.09$, d.f. = 1, $P < 0.005$) (Fig. 3 and Supplementary Table 3). However, offspring of sex-reversed mothers have themselves a greater temperature sensitivity, compared with offspring of normal ZW mothers ($\chi^2 = 55.39$, $P < 0.0001$) (Fig. 3). The pivotal temperature for offspring of ZW mothers was 34.7 °C whereas for the offspring with sex-reversed ZZf mothers it was lower, at 33.5 °C (Fig. 3).

Our discovery of naturally occurring sex reversal has afforded us the unique opportunity to conduct ZZm × ZZf matings that can yield only ZZ offspring, whose sex is determined entirely by incubation temperature. The W chromosome was thus eliminated from our sex-reversed lineage in one generation, and sex of the offspring was determined by a mode indistinguishable from TSD. Thus, we have used high-temperature incubation to experimentally induce, within a lineage, a transition from a predominantly genotypic system with heteromorphic sex chromosomes[15] to a temperature-dependent sex determination system. The homomorphic sex chromosomes in ZZ male and female offspring have effectively become autosomal. Although loss of the W chromosome can be induced in a single generation under laboratory conditions, in wild populations the W chromosome is expected to persist for multiple generations and the transition in sex determination modes be more gradual.

This experimental transition from genotypic sex determination (GSD) to TSD demonstrates a novel transitional pathway, in which TSD can evolve rapidly in response to extreme environmental conditions (high temperatures), without requiring that there be sex-specific selective advantages. This observation challenges conventional theory on the drivers of transitions from GSD to TSD. Our stochastic model of the transition from GSD to TSD provides an alternative mechanism to the prevailing view that TSD evolves in response to a fitness advantage to the offspring to be female at some temperatures and male at others (invoking the Charnov–Bull model)[21–23]. Until now it has not been possible to disambiguate sex-specific fitness differences that could cause a GSD to TSD transition from fitness differences that arose after the transition. The Charnov–Bull model still is key in explaining the maintenance of TSD in populations; however, we show here that there are multiple pathways for populations to achieve this transition (stochastic and evolutionary). In our case, optimization of male and female fitness at the different temperatures they experience probably follows and reinforces the GSD to TSD transition, rather than being the proximal cause of the evolutionary transition.

Our demonstration that TSD can evolve rapidly in response to extreme environmental conditions (high temperatures) predicts that in the wild TSD may become fixed in small demes in which sex-reversed ZZf females mate, and the W is lost stochastically. Drift effects may be especially important if the sensitivity of ZZ males to sex reversal (the pivotal temperature for sex determination) has limited opportunity to evolve, owing to a small number of generations or low effective heritability[24]. Alternatively, the transition to TSD may be driven by positive selection[21]. Our extraordinary finding that sex-reversed females laid nearly twice as many eggs per year than normal ZW females of an equivalent age suggests an immediate fitness advantage to sex reversal[25] which could drive transitions. Indeed, the greater fecundity of sex-reversed females may well combine with their heritable increased propensity to reverse (Fig. 3), exacerbating the overproduction of females and accelerating the loss of the W chromosome.

In the absence of drift and positive selection, the W chromosome may still be eliminated from populations by Fisher's frequency-dependent selection[3,26]. An increase in the frequency of ZZ females by high-temperature reversal will be favoured by selection for mothers who produce only sons (the rarer sex at higher temperatures). Modelling this response (see Methods) shows a precipitous decline in the frequency of the ZW genotype with increasing incubation temperature. The W chromosome frequency reduces at temperatures above 32.0 °C with complete loss, enforced by frequency-dependent selection alone, at temperatures above 33.4 °C (Extended Data Fig. 4). Our wild population resides on the precipice between GSD with sex reversal and TSD arising from the loss of the W chromosome. Thus, under climatic conditions where extreme high temperatures are experienced at an increasing rate, the relatively rapid loss of the W chromosome and the adoption of TSD become increasingly likely via the combined effects of drift, positive selection and/or Fisherian sex-ratio selection. Consistent with these predictions we observed increased rates of sex reversal among wild phenotypic females over the course of this study (Fig. 1).

Our observation that offspring from sex-reversed mothers are more frequently reversed and at a lower temperature than the offspring of normal ZW mothers (Fig. 3) implies that the eggs of ZZ sex-reversed females are more sensitive to temperature. This strongly suggests that heritable variation (genetic or epigenetic) exists at the locus that controls sex determination in *P. vitticeps*, providing a mechanism for sex determination thresholds to evolve. Additional evidence for heritable selectable variation in thermosensitivity was observed in a single sex-reversed female that produced 100% male offspring at all temperatures (28–36 °C; $N = 32$ eggs; Supplementary Tables 2 and 3; data excluded from analyses).

One of the most important questions for the near future is whether organisms will be sufficiently resilient to withstand a rapidly changing climate, or so vulnerable that they will succumb to extinction[27,28]. We provide here an example of how climatic extremes can rapidly and fundamentally alter the biology (switches in sex determination mode) and the genome (loss of the W chromosome) of climate-sensitive reptiles. Thus adverse evolutionary responses, specifically a switch to TSD if global temperatures rise, can occur through a combination of temperature sensitivity and stochastic processes. This is important because temperature-induced extreme sex-ratio bias is thought to be an extinction driver exclusively in species with TSD[29]. However, we show here that these risks may extend more broadly. Exposure to high temperatures can perturb apparently stable GSD systems, induce a rapid transition to TSD and then proceed inexorably towards a highly feminized population and thus a greater risk of extinction.

The key to determining how important transitions in sex-determining mode are to species' survival will be to discover how thermal sensitivities can adapt after the transition to TSD. If adaptation is rapid, TSD could produce stable unbiased sex ratios, and may even be favoured by climate change. For example, a temperature-dependent strategy might afford greater control of sex ratio manipulation in an unpredictable climate. In this way, reptiles may have greater capacity to cope and compensate for climate change than previously appreciated[27]. A high degree of flexibility in sex-determination mode could be a powerful and, until now, unappreciated weapon in the arsenal of evolutionary responses to an unpredictable climate.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions C.E.H. and A.G. designed the study. C.E.H. conducted breeding experiments, egg incubations, parentage SNP analysis and prepared figures. D.O'M. collected the animals from the field. C.E.H. and X.Z. conducted the molecular sex testing. B.A. and K.M. undertook the cytogenetic analysis and prepared extended data figures, under the supervision of T.E. A.G. and C.E.H. undertook the statistical analyses and A.G. conducted the modelling of ZW genotype frequency with temperature. All authors contributed to writing the manuscript.

Author Information The W chromosome sequence has been deposited in GenBank under accession number KM508988. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.E.H. (clare@holleley.net) or A.G. (georges@aerg.canberra.edu.au).

METHODS

Field collection. We collected samples (tail snips or blood) from 131 wild adult *P. vitticeps* individuals, during sampling trips conducted in October 2003, 2004, 2008, September 2009 and March 2010, 2011. Phenotypic sex was determined by the presence or absence of hemipenes visible as two latero-ventral lumps on the tail immediately behind the vent and subsequent eversion of hemipenes if present, and presence or absence of eggs by palpation, with confirmatory but not definitive presence or absence of enlarged femoral pores and larger head size characteristic of males.

Breeding experiments. Captive females were allowed to lay eggs naturally in a sand substrate. Eggs were recovered from cages within 14 h and transferred to plastic boxes filled with vermiculite with a water potential of −200 kPa (120.0% water to vermiculite, by mass)[23]. Allocation of clutches to experiments was not randomized and the investigators were not blinded to allocation during experiments and outcome assessment. In the first experiment, two whole clutches (21 and 19 eggs) from a sex-reversed female (ZZf) were selected as they became available and incubated at a constant 28 °C (28.1 °C, s.d. ±0.6) to evaluate reproductive viability. In the second experiment, clutches laid both by sex-reversed (ZZf) and by control (ZWf) females were chosen randomly then systematically allocated across four constant temperature incubation treatments: 26 °C, 28 °C, 33 °C, 34 °C (Supplementary Table 2). An additional 36 °C treatment was conducted only for the eggs of control females because of lower numbers of available eggs from ZZf. Sample sizes were determined by availability of clutches from the captive breeding programme. No statistical methods were used to predetermine sample size. We have not interpreted non-significant results, where considerations of power are more crucial. Eggs were removed from the experiment if they were physically damaged (crushed or pierced) or inviable (no white patch or vascularization) before entering the thermosensitive period when sex is determined[30] (Supplementary Table 4). Whole clutches with egg mortality rates of at least 40% were excluded from analysis. The phenotypic sex for all captive-bred animals was established by hemipene eversion upon hatching[31], hemipenal transillumination[32] at 1–4 months and by gross external morphology at 4–9 months of age. Female reproductive fitness was estimated as the total number of eggs produced per season, a measure that summarizes the effects of clutching frequency and clutch size. We compared the reproductive fitness of 6 sex-reversed (ZZf) and 12 control (ZWf) females using unpaired *t*-tests.

Molecular detection of sex reversal. All individuals collected from the wild and bred in captivity were genotypically sexed using two PCR primers: H2, GCCCATATCTCACTAGTTCCCCTCC; F, CAGTTCCTTCTACCTGGGAGT GC, which flank two W-chromosome-specific deletions (150 base pairs and 14 base pairs) that we identified in published *P. vitticeps* anonymous sex chromosome sequence[16] (GenBank accession numbers EU938138.1 and KM508988). PCR conditions for the novel test were 1× MyTaq HS Red mix (Bioline), 4 μM each primer and 50 ng of genomic DNA. Cycling conditions were 95 °C for 5 min; (95 °C for 20 s, 70–65 °C for 20 s, 72 °C for 1 min) × ten cycles with annealing temperature decreased 0.5 °C per cycle; (95 °C for 20 s, 65 °C for 20 s, 72 °C for 1 min) × 30 cycles; 72 °C for 10 min. PCR products were visualized on a 1.5% agarose gel using SYBR Safe (Life Technologies). Two bands amplified in ZW individuals, whereas a single control band amplified in ZZ individuals. Individuals showing genotype–phenotype discordance were classed as sex-reversed. The rate of sex reversal was calculated as the proportion of ZZ individuals with a female phenotype. All molecular sex tests were conducted with the investigator blinded to the identity and phenotypic sex of the samples.

Cytogenetics. The accuracy of the PCR sex test was validated using C-banding (Extended Data Fig. 1), comparative genomic hybridization (Extended Data Fig. 2) and by physically mapping a *P. vitticeps* W-chromosome-linked microsatellite motif (Extended Data Fig. 3). Two wild-type ZW females (001003386049, 001003342236), two wild-type ZZ males (001003338787, 001003387339) and a putative sex reversal female (001003344224) of *P. vitticeps* were used for cytogenetic analyses. Metaphase chromosome spreads were prepared from fibroblast cultures of tail tissue following ref. 33. Metaphases for all individuals were stained with 4′,6-diamidino-2-phenylindole (DAPI), the chromosome number identified and compared with the normal *P. vitticeps* karyotype[34], to eliminate the possibility of chromosome abnormality. C-banded chromosomes were obtained by the CBG method (C-bands by barium hydroxide using Giemsa)[15,35]. Comparative genomic hybridization was conducted as previously described[15,36] using fluorescently labelled male and female genomic DNA. Physical mapping of the W-chromosome-linked microsatellite $(AAGG)_8$ was conducted using fluorescence *in situ* hybridization, following ref. 37. For all cytogenetic analyses, the presence or absence of W-chromosome-specific signal was scored in eight to ten metaphases per individual.

Statistical analysis. Curves of best-fit offspring responses to incubation temperature were estimated by applying logistic regressions to the raw data (male = 0; female = 1) using PROC LOGISTIC in SAS Software version 9.1 or, in the case of the proportion of females varying from 0.5 to 1, by fitting a logistic regression with unconstrained lower asymptote using PROC PROBIT. In both cases, the SAS ODS output was used to generate 95% confidence limits around the estimated regression lines. Logistic regressions were compared using PROC LOGISTIC with the addition of a CLASS variable for maternal genotype (ZZ × ZZ versus ZZ × ZW). These analyses have an appropriate error structure for data in the form of counts of males and females, and counts of sex-reversed versus concordant individuals.

Parentage analysis. Parentage analysis was conducted to confirm that the eggs produced by sex-reversed females (ZZf) were the product of sexual reproduction and not asexual reproduction by parthenogenesis. We genotyped 18 individuals at 2,229 SNPs using a proprietary reduced-representation sequencing approach called DArTseq (Diversity Arrays Technology), alternatively referred to as double-digest restriction-site-associated DNA markers (RAD-seq)[38–41]. Four methods of complexity reduction were tested in *P. vitticeps* (data not presented) and the PstI–SphI method was selected. Approximately 2,000,000 sequences per barcode per sample were identified and used in marker calling. We subsequently applied further stringent quality control measures, requiring 100% reproducibility over two independent runs, a minimum of 5× read depth (mean read depth = 32×) and complete data for all individuals. Specifically, we sequenced three parents (two sex-reversed females mated to the same male), nine offspring from the first pairing, two offspring from the second paring, three unrelated ZW individuals and one unrelated ZZ individual. We used these data to estimate mother–offspring sequence identity (clonal parthenogenesis hypothesis), the percentage of heterozygous loci in offspring (non-clonal parthenogenesis hypothesis) and the percentage of parent–offspring allelic mismatches (sexual reproduction hypothesis). For comparison, these statistics were also calculated comparing four unrelated individuals to the parental genotypes. Parentage hypotheses were tested using *t*-tests (Supplementary Table 1).

Modelling the decline of the ZW genotype. We modelled the decline of the ZW genotype resulting from frequency-dependent selection because of overproduction of females through sex reversal with increasing temperature[3]. Fitness within a sex is the same for all genotypes. Let the starting frequency of ZW among zygotes be y, the starting frequency of ZZ be $z = 1 - y$, and let a fraction $P_1[T]$ of ZZ become reversed to a female phenotype if they have a ZW mother, $P_2[T]$ if they have a ZZ mother. The equations for $P_1[T]$ and $P_2[T]$ are the functions of temperature T given in Fig. 3. In any given generation n, we have the proportion of female phenotypes (f_n) equal to the sum of the numbers of normal ZW females (y_n), of sex-reversed ZZ females with ZW mothers (r_n) and of sex-reversed ZZ females with ZZ mothers (r_n'),

$$f_n = y_n + r_n + r_n'$$

The frequency of ZW zygotes and the frequency of ZZ zygotes with ZW mothers are equal and both given by

$$y_{n+1} = z_{n+1} = \frac{y_n}{2f_n}$$

The frequency of sex-reversed ZZ zygotes with ZW mothers thus given by

$$r_{n+1} = P_1[T] z_{n+1}$$

and the frequency of sex-reversed ZZ zygotes with ZZ mothers is given by

$$r'_{n+1} = P_2[T] \frac{(f_n - y_n)}{f_n}$$

Determined from our experimental data, the probability of sex reversal for offspring with ZW mother (Fig. 3, shown in black) is given by

$$P_1[T] = \frac{e^{-63.1402 + 1.8184T}}{1 + e^{-63.1402 + 1.8184T}}$$

and the probability of sex reversal for offspring with ZZ mothers (Fig. 3, shown in red) is given by

$$P_2[T] = \frac{e^{-52.9999 + 1.5866T}}{1 + e^{-52.9999 + 1.5866T}}$$

We iterate for an equilibrium solution for y for various values of temperature T. Overlapping generations will delay the rate of convergence to equilibrium, but will not affect the equilibrium value for a particular temperature.

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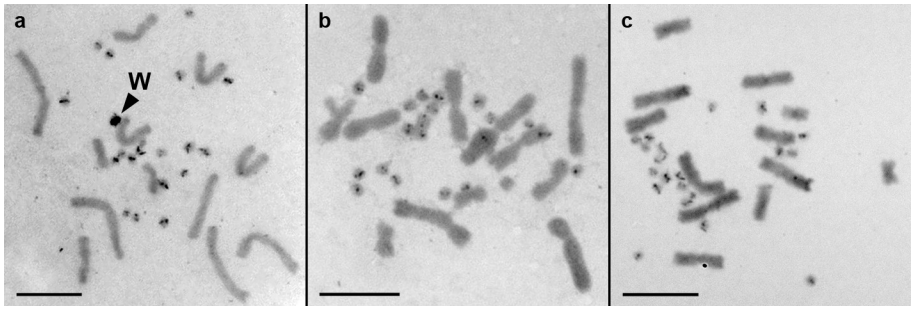
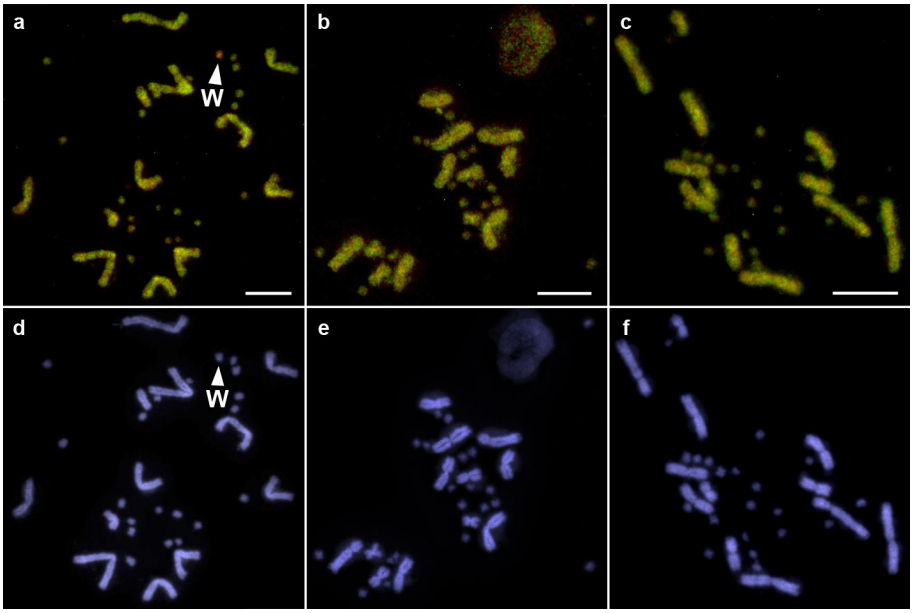
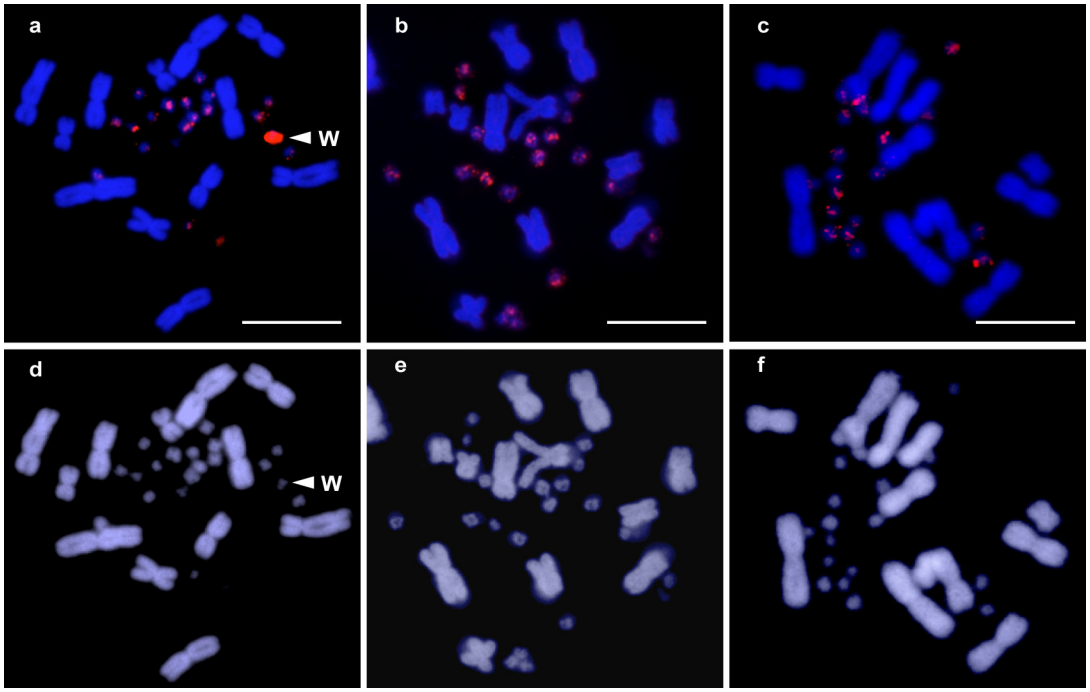


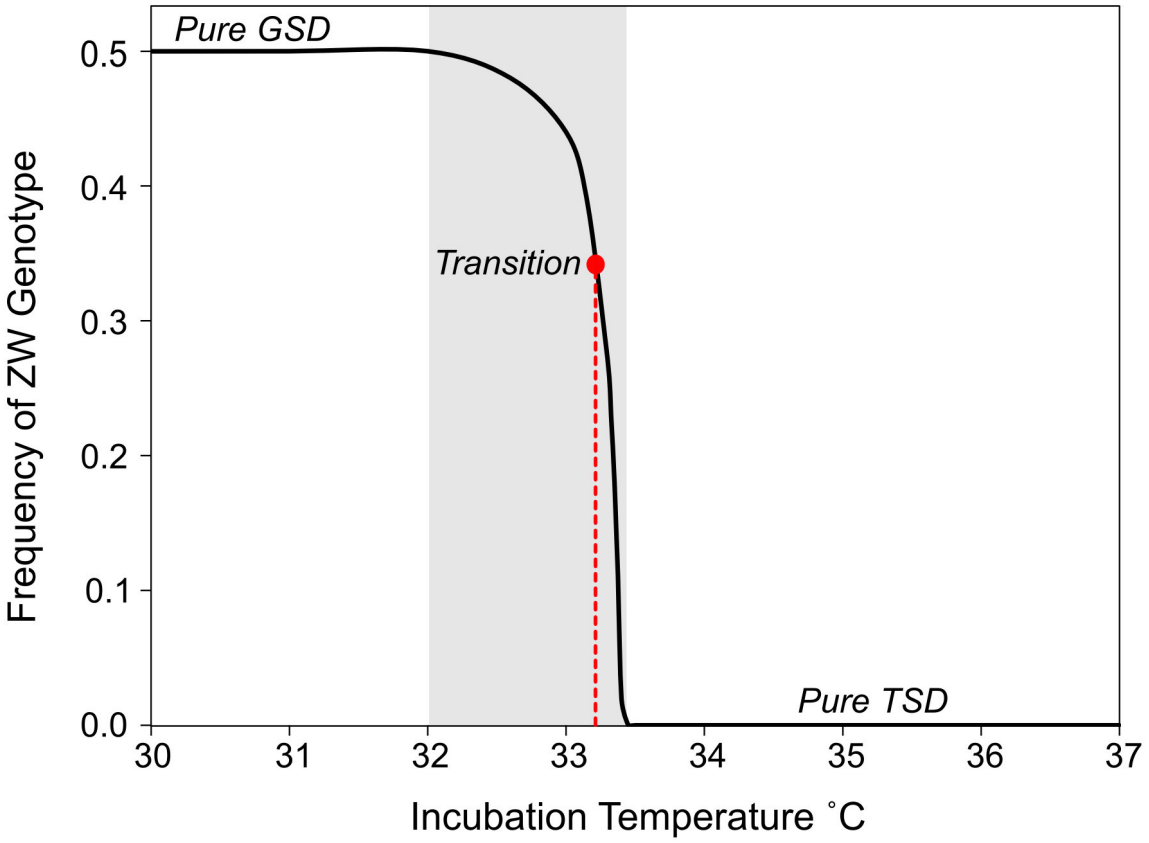
Extended Data Figure 1 | C-banded *P. vitticeps* chromosomes. **a**, Mitotic metaphase chromosomes of a ZW control female individual. Arrowhead indicates the presence of a W chromosome identified by dense black staining of a single microchromosome. **b**, Mitotic metaphase chromosomes of a female putative ZZ sex-reversed individual. No evidence of a W chromosome was detected. **c**, Mitotic metaphase chromosomes of a control ZZ male individual. No evidence of a W chromosome was detected. Scale bar, 10 μm.



Extended Data Figure 2 | Comparative genomic hybridization in *P. vitticeps*. Genomic DNA was labelled by nick translation incorporating SpectrumGreen-dUTP for males and SpectrumOrange-dUTP for females. **a**, Mitotic metaphase chromosomes of a ZW control female individual. Arrowhead indicates the presence of a single W microchromosome identified by the enriched orange fluorescence of female specific genomic DNA labelled with SpectrumOrange-dUTP. **b**, Mitotic metaphase chromosomes of a female putative ZZ sex-reversed individual. No evidence of a W chromosome was detected. **c**, Mitotic metaphase chromosomes of a control ZZ male individual. No evidence of a W chromosome was detected. **d**–**f**, DAPI staining of the same metaphases, control ZW female, sex-reversed ZZ female and control ZZ male, respectively. Scale bar, 10 μm.



Extended Data Figure 3 | Physical mapping of a W-chromosome-linked microsatellite motif in *P. vitticeps*. **a**, Mitotic metaphase chromosomes of a ZW control female individual. Arrowhead indicates the presence of a W chromosome identified by a strong hybridization of $(AAGG)_8$-Cy3 florescence (orange) on a single microchromosome. **b**, Mitotic metaphase chromosomes of a female putative ZZ sex-reversed individual. No evidence of a W chromosome was detected. **c**, Mitotic metaphase chromosomes of a control ZZ male individual. No evidence of a W chromosome was detected. **d**–**f**, DAPI staining of the same metaphases, control ZW female, sex-reversed ZZ female and control ZZ male, respectively. Scale bar, 10 μm.



Extended Data Figure 4 | Modelling the decline of the ZW genotype resulting from frequency-dependent selection. Frequency of the ZW genotype declines precipitously with increasing incubation temperature. Our wild population (shown in red, 14.3% sex reversal) resides on the precipice between GSD and TSD and requires only a small change in environmental temperature to precipitate loss of the W chromosome.

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Table S1. Summary statistics calculated for 2,229 single nucleotide polymorphisms to test three hypotheses on asexual (parthenogenetic) and sexual reproduction in *Pogona vitticeps*.

				Hypothesis 1: Clonal parthenogenesis	Hypothesis 2: Non-clonal parthenogenesis	Hypothesis 3: Sexual reproduction
Relatedness Group	Offspring ID	Putative Mother ID	Putative Father ID	Percent identity mother-offspring	Percent heterozygous loci	Percent allelic mismatches parent-offspring
Family 1	ZZ_5002997	ZZ_P344224	ZZ_P387339	78.85	31.81	3.43
	ZZ_5002901	ZZ_P344224	ZZ_P387339	78.56	32.88	3.95
	ZZ_5002967	ZZ_P344224	ZZ_P387339	79.27	32.75	3.50
	ZZ_5003363	ZZ_P344224	ZZ_P387339	79.12	31.36	3.50
	ZZ_5002907	ZZ_P344224	ZZ_P387339	79.32	31.27	3.48
	ZZ_5002938	ZZ_P344224	ZZ_P387339	78.87	31.72	3.32
	ZZ_5002919	ZZ_P344224	ZZ_P387339	80.28	32.48	3.21
	ZZ_5002920	ZZ_P344224	ZZ_P387339	78.17	30.10	3.66
	ZZ_5002995	ZZ_P344224	ZZ_P387339	77.34	29.79	3.81
Family 2	ZZ_AA61722	ZZ_P351766	ZZ_P387339	79.79	27.37	2.94
	ZZ_AA61723	ZZ_P351766	ZZ_P387339	79.30	26.74	3.01
ZW unrelated	ZW_5002949	ZZ_P344224	ZZ_P387339	65.88	15.97	16.20
		ZZ_P351766	ZZ_P387339	70.66	15.97	14.36
	ZW_5002982	ZZ_P344224	ZZ_P387339	66.94	15.39	15.43
		ZZ_P351766	ZZ_P387339	71.80	15.39	13.55
	ZW_AA61694	ZZ_P344224	ZZ_P387339	66.35	16.91	15.70
		ZZ_P351766	ZZ_P387339	71.58	16.91	13.64
ZZ unrelated	ZZ_AA61721	ZZ_P344224	ZZ_P387339	65.63	14.67	16.24
		ZZ_P351766	ZZ_P387339	70.73	14.67	14.24
			Null expectation	% ID = 100.00	% Heterozygosity = 0.00	Unrelated mean =14.92 ; SD = 1.10
			Test statistic	362.60	30.75	29.40
			***P*-value**	< 0.00001	< 0.00001	< 0.00001
			Conclusion	Mother and offspring are not genetically identical, thus NOT clonal.	Offspring heterozygous, thus NOT the product of non-clonal parthenogenesis.	Allelic mismatches occur at a significantly lower rate when putative parents are related to offspring. **Consistent with sexual reproduction.**

Table S2. Sexual phenotypes, mortality and sex ratios resulting from a range of constant egg incubation temperature treatments in *Pogona vitticeps*.

Cross Category	Treatment Temperature ˚C	Number of Males	Number of Females	Died During Incubation	Total Surviving Hatchlings	Proportion Female	Lower 95% CI	Upper 95% CI	Data Source
Control	22	29	22	31	51	0.4314	0.303	0.566	Quinn et al. 2007
ZWf x ZZm	24	29	28	14	57	0.4912	0.366	0.618	Quinn et al. 2007
	26	16	22	8	38	0.5790	0.424	0.724	Quinn et al. 2007
	26.1	7	7	2	14	0.5000	0.268	0.732	This study
	28	185	189	50	374	0.5054	0.455	0.556	Quinn et al. 2007
	28.1	20	18	4	38	0.4737	0.325	0.627	This study
	30	35	37	35	72	0.5139	0.401	0.626	Quinn et al. 2007
	32	17	20	7	37	0.5405	0.384	0.692	Quinn et al. 2007
	32.8	6	10	4	16	0.6250	0.385	0.816	This study
	34	29	85	68	114	0.7456	0.661	0.819	Quinn et al. 2007
	34.1	5	12	2	17	0.7059	0.466	0.870	This study
	34.5	4	28	14	32	0.8750	0.735	0.959	Quinn et al. 2007
	35	0	49	29	49	1.0000	0.942	1.000	Quinn et al. 2007
	36	1	40	28	41	0.9756	0.891	0.999	Quinn et al. 2007
	36.0	1	25	7	26	0.9615	0.796	1.000	This study
	37	0	5	9	5	1.0000	0.607	1.000	Quinn et al. 2007
Temperature Sensitive	26.1	17	0	0	17	0.0000	0.000	0.216	This study
Sex-Reversed	28.1	16	0	1	16	0.0000	0.000	0.227	This study
ZZf x ZZm	32.8	13	5	2	18	0.2778	0.122	0.512	This study
	34.1	5	15	0	20	0.7500	0.528	0.892	This study
Temperature Insensitive	28.0	1	0	0	1	0.0000	0.000	0.8325	This study
Sex-Reversed	29.9	6	0	0	6	0.0000	0.000	0.4428	This study
ZZf x ZZm	31.9	8	0	0	8	0.0000	0.000	0.3722	This study
	33.5	7	0	2	7	0.0000	0.000	0.4044	This study
	36.0	7	0	1	7	0.0000	0.000	0.4044	This study

Table S3. Rates of reversal and mortality rates resulting from a range of constant egg incubation temperature treatments in *Pogona vitticeps*.

Cross Category	Treatment Temperature ˚C	Number of ZZ Males	Number of ZZ Females	Died During Incubation	Total Surviving ZZ individuals	Rate of Reversal	Lower 95% CI	Upper 95% CI
Control	26.1	7	0	2	7	0.0000	0.000	0.404
ZWf x ZZm	28.1	20	0	4	20	0.0000	0.000	0.190
	32.8	6	0	4	6	0.0000	0.000	0.443
	34.1	5	2	2	7	0.2857	0.076	0.648
	36.0	1	9	7	10	0.9000	0.574	1.000
Temperature Senstitive	26.1	17	0	0	17	0.0000	0.000	0.216
Sex-Reversed	28.1	16	0	1	16	0.0000	0.000	0.227
ZZf x ZZm	32.8	13	5	2	18	0.2778	0.122	0.512
	34.1	5	15	0	20	0.7500	0.528	0.892
Temperature Insenstive	28.0	1	0	0	1	0.0000	0.000	0.8325
Sex-Reversed	29.9	6	0	0	6	0.0000	0.000	0.4428
ZZf x ZZm	31.9	8	0	0	8	0.0000	0.000	0.3722
	33.5	7	0	2	7	0.0000	0.000	0.4044
	36.0	7	0	1	7	0.0000	0.000	0.4044

Table S4. Temperature dependent developmental rates and the estimated thermosensitive period (TSP) for *Pogona vitticeps.*

Treatment Temperature ˚C	Observed Temperature ˚C	In oviduct development* (Days from conception)	Mean incubation duration (Days from lay)	Total developmental duration (Days from conception)	Thermosensitive Period (Days from conception)	Thermosensitive Period (Days from lay)
26	26.1 ± 0.2 SD (*N* = 57458)	19	94.6 ± 3.3 SD (*N* = 35)	114	38 – 76	19 – 57
28	28.1 ± 0.2 SD (*N* = 3639)	18	73.0 ± 3.5 SD (*N* = 76)	91	30 – 61	12 – 43
32	32.8 ± 0.5 SD (*N* = 8192)	16	50.6 ± 1.8 SD (*N* = 37)	66	22 – 44	6 – 28
34	34.1 ± 0.2 SD (*N* = 39571)	14	47.9 ± 2.8 SD (*N* = 35)	62	21 – 42	7 – 28
36	36.0 ± 0.2 SD (*N* = 103018)	13	46.7 ± 1.6 SD (N = 33)	60	20 – 40	7 – 27

*Calculated as: $y = -0.571x + 34$, where y is the duration of development in the oviduct, and x is incubation temperature.