Supplementary Information for

The functional genetic architecture of egg-laying and live-bearing reproduction in common lizards

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Materials and Methods

Sampling

Sampling was performed in a contact zone between oviparous and viviparous common lizards in Carinthia, Austria[15,21]. The oviparous lineage corresponds to eastern oviparous (EO; ancestrally oviparous) lineage, and the viviparous lineage to the central viviparous II (CVII; derived viviparous) lineage[15]. Adult common lizards were caught in the years 2014 – 2017 between April and August. Males and females were distinguished by the presence of bulge at the base of the tail in males. For each individual, non-lethal sampling by tail clip was taken for later use in the genetic analyses. Individuals were then released at site of capture. Female pregnancy was assessed by a presence of a bite mark on the female's flank resulting from mating.

Pregnant females were maintained in 20cmx35cmx15cm terraria and food was provided ad libitum. For more detailed housing conditions see[61]. Females were checked for the presence of a clutch on a daily basis and kept until parturition or oviposition [from here on this time point is referred to as 'parition'[76]], followed by their release at the point of capture. All females (N = 480) that laid eggs or gave birth were phenotyped for parity mode using three measures: i) number of external incubation days after parition, ii) embryonic stage at parition, and iii) eggshell thickness. In addition, three females of each parity mode were lethally sampled at three time points during pregnancy (early, mid, and after gestation; total N = 18 females). From these, uterine glands were extracted and preserved in RNAlater (Supplementary Table 7).

Clutches were weighed using a smart weigh high precision scale (to the nearest 0.001 g), and one egg was removed from each clutch and fixed in formalin (24h in 10% buffered formalin solution) and stored in 70% EtOH for later embryonic staging and eggshell analysis. The remaining eggs were embedded in moist vermiculite before being incubated at 24ºC in an ExoTerra reptile egg incubator. The number of external incubation days was recorded for every hatchling or neonate of a clutch. The average across all offspring was then calculated and used for genetic mapping analyses. All clutches were checked daily for appropriate moisture, presence of infertile or dead embryos, or any hatching offspring. The number of external incubation days per clutch was calculated as the average number of incubating days for the clutch offspring that successfully hatched. Clutch survival was measured as the proportion of successfully hatched individuals within a clutch. Offspring were released at the mother's point of capture.

Egg characteristics:

Thickness: To prepare and dehydrate eggshells for scanning electron microscopy (SEM), half of each eggshell was removed and washed once in 90% EtOH, twice in 100% EtOH, then twice in hexamethyldisilazane (HMDS) before being transferred to a desiccator overnight. Under a light microscope, the dry eggshells and membranes were cut with a sharp scalpel and mounted on aluminium mounts using double-sided sticky copper tape (AGG3397, AGAR Scientific Ltd). Samples were stuck to the copper tape with the straight cut edges facing upwards. The samples were then coated with gold-platinum for 250 sec using a LEICA EM SCD005 machine to a thickness of about ~15nm. To ensure conductivity across the sample, silver paint was carefully added to the base of each eggshell sample. Images were taken of intact, straight edges of each sample at a magnification of 500x (for most oviparous eggshells) or 1000x (for most viviparous membranes) on a Leica EM SCD005 scanning electron microscope (SEM). Each eggshell was fully inspected along its ridge to only record measurements from eggshell edges at an angle of approximately 90°. If no satisfactory edge could be identified across the whole sample, the copper tape with the eggshell sample on was bent under a light microscope. The sample was then re-inserted into the SEM and re-inspected. This was repeated until eggshell samples were at a straight angle. Five measures were taken across each image using the linear measurement tool and recorded. The average and standard deviation across the five measurements were recorded for each sample. For a subset of samples (N = 42), a second image from a different part of the eggshell was taken to assess the consistency across different regions across the shell. These

repeated measures showed that eggshell thickness does vary across different regions of the shell, but is generally strongly correlated within a sample (R^2= 0.88, P < 0.0001).

Chemical composition (Ca content): Part of each fixed egg sample was removed and mounted on an aluminium mount with an adhesive double-sided carbon coated disc (Agar Scientific Ltd, UK) such that the outside of the eggshell/membrane was facing outwards. Samples were then sputter-coated in vacuum with gold using a LEICA EM SCD005 for 160sec. Chemical composition of eggshells was assessed using energy-dispersive X-ray spectroscopy (EDX). The relative contribution of carbon (C), oxygen (O), sodium (Na), phosphorus (p), sulfur (S), calcium (Ca), magnesium (Mg) and potassium (K) were extracted. Chemical composition of eggshells was measured on six different points of each sample's image and averaged. For subsequent admixture mapping analyses, the relative amount of Ca only was extracted. For a set of samples (N=53), we extracted a second sample from the same individual's eggshell and repeated the whole process to estimate measurement error. Overall, there was a strong correlation between the first and second Ca measurement (R^2= 0.79, P < 0.0001).

An 'eggshell' score was calculated for each individual. Thickness and Ca traits were first normalized by re-scaling values such that they varied between 0 (lowest viviparous value) and 1 (highest oviparous value) (min-max normalization)[77]. Because the two measures differed in consistency and eggshell thickness proved to be more reliable both within samples (R^2= 0.88 vs R^2= 0.79) and compared to the genomic background (R^2= 0.86, vs R^2= 0.56; Supplementary Fig. 3), we weighted the two individual traits according to their Pearson correlation coefficient with genomic background. For individuals that had only one of the two measurements, only that single normalized score was retained. This resulted in a total of 434 clutches phenotyped for eggshell characteristics.

Gestation time: Embryonic stage at parition and number of incubation days

Embryonic stage at parition was identified in the lab using a light microscope (Wild M3Z dissecting microscope fitted with an eyepiece scale) and a staging table of common lizards following Dufaure and Hubert (1961)[62]. Embryos were imaged with a Nikon D5100 DSLR camera with a Nikkor 40 mm lens camera at 6.3x to 40x magnification.

Similar to summarizing eggshell characteristics in a score, we calculated a gestation time score by normalizing the embryonic stage at parition and the number of external incubation days both to 1 and averaging across these two scores. Because the two phenotypes were very similar in their correlation with genomic background (R^2= 0.93 vs R^2= 0.96, Supplementary Fig. 3) and also showed strong correlation with each other (R^2= 0.94; Supplementary Fig. 11), these were not weighted. This resulted in a total of 403 individuals phenotyped for gestation time.

Genotyping by ddRADseq

DNA was extracted from tail samples using the Macherey-Nagel NucleoSpin® Tissue kit at a minimum of 20 ng/ul. Eight double-digest RAD Sequencing (ddRADSeq) libraries with 105 individuals each (N = 798 [557 females and 241 males]) were then prepared with restriction enzymes *PstI* and *MspI* following[15]. Each library was sequenced at Edinburgh Genomics on an Illumina HiSeq 4000 at 2x150bp read length to ~5M reads per individual. Technical replicates (N = 36) were included to calculate genotyping error rates. A total number of 5.9 billion reads were obtained.

After sequencing, all reads were de-multiplexed with the barcode- and enzyme-correction options in STACKS version 1.44[63]. De-multiplexed reads were aligned to the *Z. vivipara* reference genome[22] using Burrows-Wheeler transform[78] and SAMtools[79] and sorted into loci in Stacks allowing up to three mutations per locus and a minimum stack depth of three reads.

We removed multiple replicates of individuals sampled more than once across years and retained the sample with the highest sequencing coverage. This was performed by extracting genotypes from STACKS with a minimum coverage of 8x, presence in at least 50% of all individuals, and a minor allele frequency of 10%, and then running the software KING vers. 2.1.5[80] using the --duplicate option. Out of the 798 individuals, 89 (11.1%) were inferred as individuals that were sampled at least twice across years and excluded from all following analyses.

Female (N = 495) and male (N = 222) genotypes (total N = 717) were extracted from STACKS, with the same parameters as stated above. The genomic ancestry of each individual was then inferred by a structure clustering method, ADMIXTURE vers.1.3[64] with two genetic clusters (K = 2) and performing 10-fold cross-validation. The degree of admixture was estimated from inferred membership values (*Q*). In cases where reproductive data from females was present, the correlation between genotype and phenotype was assessed. For each phenotype, the correlation coefficient with the genome-wide degree of admixture (*Q*-value) was estimated.

Genotyping from whole genome sequencing

Fifty-nine females with hybrid genomic backgrounds, and three females with purely oviparous and viviparous backgrounds, respectively, were chosen for low-coverage whole genome re-sequencing (total N = 65; average of 4.1x coverage; Supplementary Table 1). Genomic libraries were prepared using a modified protocol based on DNA tagmentation using the Nextera DNA Flex Library Prep Kit and library amplification using the KAPA Library Amplification Kit for Illumina[81]. In brief, high molecular weight DNA (> 25ng/ul) was extracted using the Macherey-Nagel NucleoSpin® Tissue kit and then fragmented using transposon cleavage, followed by tagging of the double-stranded DNA ends. Adapters and sequencing primers were added to the tagged ends and amplified. This was followed by a cleanup and size selection of DNA fragments ranging from 400-700 bp (average library size: 568 bp). Seven genomic libraries with a pool of 5 to 12 individuals at equimolar concentrations were sequenced on a HiSeqX with 150 bp paired-end reads at BGI Tech Solutions (Hong Kong). In total, ~4.74 billion reads were sequenced.

Individuals were de-multiplexed and aligned to a draft version of the *Z. vivipara* reference genome[22] using BWA-mem[78] with the -M flag and resulting BAM files were sorted with SAMtools[79]. PCR duplicates were removed with GATK 4[82]. To call genetic variants we used the bcftools[83] with multi-sample calling (-m) of all BAM files simultaneously for improved identification of heterozygous variants in low-coverage data. Reads with a mapping quality below 40 (phred-scaled) and nucleotides with quality scores lower than 25 were excluded from variant calling (-q 40, -Q 25). The final VCF file was filtered using vcftools[84] to remove variants with phred-scale quality less than 300 (--minQ 300) or with more than 45 missing genotypes or completely monomorphic.

Regions with approximately two-fold higher coverage in homo- relative to heterogametic sex were identified as sex chromosomal regions comparing a male (ELT04800) and female (ELT07038) whole genome sequences.

Genetic mapping

All females (N = 458) with reproductive data and genotypes from ddRADSeq were used for admixture mapping. For individuals sampled repeatedly across years (determined from genotype data), phenotypic values were averaged. Individuals were categorized as oviparous, admixed, or viviparous by pre-assessed genomic background. Individuals with more than 90.0% oviparous or viviparous genomes were assigned into those respective classes, whereas all individuals in between those two classes were considered admixed. This was done to balance the number of individuals with different genomic backgrounds irrespective of coverage differences that can result from library preparation (e.g. slight differences in size selection) and sequencing. Genotypes that were present in all three classes at a minimum of 60% per class and a coverage of at least 8x were extracted for further analyses (a total of 80,696 SNPs). The genotype matrix was phased and missing genotypes imputed using BEAGLE vers. 06.2017[85]. Phased genotypes were used as input for GEMMA vers. 0.98[65]. In GEMMA, a relationship matrix between all individuals is first calculated to correct for population stratification (in this case the two evolutionary lineages, oviparous and viviparous). First, a Bayesian sparse linear mixed model (BSLMM) was used to infer associations between genotypes and the two phenotypic scores: gestation time and eggshell characteristics. The BSLMM was run 20 times, with 50 million iterations using a burn-in of 35 million. Runs were visually inspected for convergence and the ten runs showing poorest mixing were excluded[86]; the remaining ten runs were combined and the estimates averaged. From these estimates heritability and the phenotypic variance explained by genotypic

variance was inferred. Second, for association mapping a linear mixed model (LMM) was performed on all four reproductive phenotypes and the two combined phenotype scores for gestation time and eggshell characteristics. The number of individuals included for each trait that was mapped differed (N external incubation days = 390, N embryonic stage = 222, N eggshell calcium = 146, N eggshell thickness = 422, N gestation time score = 403, N eggshell traits score = 434). Because not all traits were measured in each year and some incubating offspring failed to hatch, the number of recorded traits per female differs.

The whole-genome dataset (N = 65) was used to assess regional and genome-wide linkage disequilibrium. First, genotype likelihoods of filtered SNPs (min. depth = 90, max. depth = 543, min. individuals = 49) were extracted for each chromosome. These were then imported into ngsLD, and correlation coefficients were estimated for SNPs within a maximum distance of 100kb. A random sample of 0.1% was exported from these analyses for each chromosome. To assess linkage disequilibrium in candidate regions compared to genome-wide background, candidate regions were derived using all SNPs significantly associated (adjusted P value < 0.01) with gestation time or eggshell traits. We then extracted R^2 values of LD for each candidate region and compared it to the genomic background.

To assess which genes were associated with the SNPs identified in the mapping, we first inferred average linkage block size for each chromosome. For each chromosome, we estimated LD decay (which follows an exponential decay function) and extracted the half-life for each chromosome's LD curve. We used the chromosome-specific half-life as linkage boundary for each SNP on its respective chromosome. Next, using these boundaries, we extracted all genes lying within boundaries of all significantly associated SNPs. On this set of genes we performed pathway enrichment analyses using the Protein Annotation THrough Evolutionary Relationship (PANTHER) option in WebGestalt[66]. The RefSeq protein dataset for the chicken genome (GCA_000002315.5) was used as a reference.

Genomic analyses of divergence, linkage and selection

A genotype matrix restricted to purely (>99% from the admixture analysis) oviparous (N = 222) and viviparous (N = 238) individuals was extracted from STACKS using genotypes present in at least 66% of individuals within both the oviparous and viviparous lineage and a minimum allele frequency of 10%. Nucleotide diversity (pi) was calculated in sliding windows of 50,000 sites using the 'popgenWindows.py' script[67] available on GitHub. Haplotype-based F_{ST} values were extracted from STACKS (Fst' option). Outliers in nucleotide diversity were identified by estimating the top 5% and lowest 5% quantiles across the genome of each parity mode. F_{ST} outliers were identified as the top 5% quantiles.

In addition, PCAdapt[45] was used to identify genomic loci under selection between oviparous and viviparous common lizards. In contrast to other methods such as scans of selection using F_{ST}-based approaches that require a-priori definition of two groups for comparison, this approach can handle substantially admixed individuals[45] and was therefore run on the full dataset of 717 individuals. We tested for up to $K = 20$, but the first principal component (PC) was the main predictor for divergence between the oviparous and viviparous lineages and was therefore used for subsequent selection analysis. Genomic loci that were significantly associated with PC1 were considered candidates under selection. We chose a threshold of q = 0.01 for the determination of outlier loci for selection. Next we tested for linkage of outlier loci with genes using the same LD block inference method as for admixture mapping. We extracted all genes tested for enriched PANTHER pathways. In addition, we extracted all SNPs (and the genes linked to those SNPs) that were both significantly associated with the two mapped reproductive phenotypes and showed signals of selection and performed pathway enrichment analyses on these.

Gene expression analyses

RNA was extracted from glandular uterus tissue of the 18 females during different reproductive and developmental stages (Supplementary Table 7). The first sample ('early') was taken after females emerged from hibernation and mating had occurred in early June, as evidenced by the presence of bite marks on the female flank. It was also ensured that samples were taken after vitellogenesis, as evidenced during dissection by the eggs being present in the glandular uterus. The second sample ('mid') was taken two weeks after the first sample was taken[87]. Samples were taken at the same time points for both oviparous and viviparous

females at the ‘early’ and ‘mid’ reproductive stages. The final sample (‘after’) was taken three days after parition for each female. This time point was chosen to ensure that females returned to a pre-pregnancy state, with previous research showing that female metabolism returns to the pre-reproductive stage three days after parition[88]. Because tissues were stored under suboptimal conditions in the field and RIN values after RNA extraction varied between 3-6, we used the NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® in combination with the NEBNext® rRNA Depletion Kit to remove ribosomal RNA during library preparation for RNA sequencing. Libraries were sequenced in three runs (run 1: N = 2; run 2: N = 7; run 3: N = 9) on an Illumina NextSeq 500 at Glasgow Polyomics using paired-end sequencing at 150 bp.

Prior to quality filtering, ribosomal RNA was removed from the sequenced RNA. This was achieved by first constructing a reference database of *Z. vivipara* ribosomal RNA by screening the reference genome[22] for rRNA like sequences using barrnap vers. 0.6[89]. All rRNA sequences were then removed from the sequenced RNA using tagdust vers. 2.33[90]. The rRNA cleaned reads were then processed with Trimmomatic vers. 0.36[91] to remove any low quality sequences prior to alignment. Leading and trailing bases with Phred quality score below 20 were removed and a sliding window approach (4 bp window size) was used to trim reads at positions with Phred scores below 20, allowing for a minimum read length of 50 bp after trimming. Fastqc vers. 0.11.8[92] was used to assess read quality before and after processing. Processing removed 40% of all reads, resulting in a dataset of 494 million cleaned reads (range: 6.06M - 48.55M; Supplementary Table 7). Cleaned reads from each library were aligned against the reference genome[22] using STAR vers. 2.5.2b[68], with default parameters, and gene expression was quantified with HTSeq vers. 0.11.2[93] using the parameters: --stranded = no, --order = pos, --type = CDS and idattr = Parent.

Given the varying degree of RNA degradation among our samples (indicated by RIN values between 3-6) and the expected heterogenous pattern of degradation among transcripts within samples, we quantified degradation across all transcripts and all samples using the degradation normalisation pipeline DegNorm[69]. DegNorm normalisation is based on a non-negative matrix factorization over-approximation algorithm that corrects for gene-by-gene biases in degradation while simultaneously controlling for sequencing depth[69]. Briefly, the algorithm works in two main steps; 1) it utilises the information from the alignment files for each sample to generate coverage curves for expected and observed amounts of degradation for each gene individually. This is then used to estimate a degradation index (DI) score for each sample. 2) The generated DI scores are used to adjust the raw read counts for all genes across all samples, while simultaneously normalising for sequencing depth. Both steps are repeated until the algorithm converges.

In the current dataset, DegNorm highlighted one (out of 18) samples as having major expression bias due to degradation. This sample (ELT08545, Supplementary Table 7) was excluded from all further analyses. The degradation adjusted read counts for the remaining 17 samples were then filtered to remove genes with less than 10 read counts across 90% of samples in the R package DESeq2 vers. 3.5[94].

To identify the major axes of expression variation between samples from different parity modes and reproductive stages, we performed a single principal component analysis (PCA) on the full dataset (N = 17). The PCA was performed using the svd approach in the R package pcaMethods. Expression patterns were assessed further by applying a single conditioned redundancy analysis (RDA) on all individuals, using the R package vegan. Reproductive stage was specified as a ‘condition’ within the model to control for expression variation associated with differences between reproductive stages. All count data were $\log_2$ scaled using the rlog function in DESeq2 prior to conducting the PCA and RDA analyses.

To identify genes associated with parity mode, we applied two approaches. First, we performed separate differential expression analyses with DESeq2, on the degradation adjusted read counts, to identify sets of differentially expressed genes (DEGs) between parity modes, both within and across stages. Finally, we only considered genes that were significantly differentially expressed (adjusted p-value: 0.05) during a reproductive stage (‘early and/or ‘mid’, but not ‘after’ gestation) as candidates of functional relevance. Therefore, genes that were differentially expressed in the glandular uterus between parity modes during both the reproductive and non-reproductive (‘after’) stage were excluded. This was done to ensure that genes not functionally active during reproduction but differentially

expressed between parity modes (presumably due to gene expression differentiation as a result of the divergence time) were discarded. However, it is possible that this approach may exclude some functionally relevant genes that are expressed after pregnancy, and genes that are consistently differentially expressed in uterine tissue, irrespective of pregnancy status. Second, we used a Weighted Gene Co-Expression Network Analysis (WGCNA) to identify modules of co-expressed genes associated with parity mode[70]. The R package WGCNA was used to construct a single network for all 17 individuals, based on the $\log_2$ scaled count data. Network modules were defined using the dynamic treecut algorithm, with a minimum module size of 30 genes and a cut height of 0.981. The module eigengene distance threshold was set to 0.25 to merge highly similar modules. Pearson's correlations were calculated between module eigengenes (the first principal component of the expression profile for a given module) and trait measurements (parity mode and reproductive stage) to identify modules that were significantly associated with parity mode. All P-values were Benjamini-Hochberg corrected (FDR < 0.05). The direction of correlation (i.e. positive or negative) determined whether modules were associated with expression in oviparous or viviparous individuals. Trait values for parity mode were specified in binary format (oviparous = 0 and viviparous = 1), therefore positive correlations represent up-regulation in the viviparous individuals (vs. oviparous), and negative correlations represent up-regulation in oviparous individuals (vs. viviparous).

To further identify genes which may act as key regulators of parity mode evolution, we identified a set of hub genes (i.e., the most highly connected genes) for all modules significantly associated with parity mode. Hub genes were selected based on two criteria. 1) Module membership (MM) scores, which represents the correlation between the expression levels of a gene and the module eigengene value. 2. Gene significance (GS) scores, which represents the correlation between the expression levels of a gene and the biological trait of interest. Genes within the top 10% quantile of both MM and GS scores were considered hub genes.

Functional characterization of parity-associated gene sets

To identify molecular pathways associated with the different parity modes we performed separate functional enrichment analyses on the parity-associated genes sets identified using differential expression and WGCNA analyses, using the PANTHER classification tool[71]. Gene Set Enrichment Analyses (GSEAs)[66] were used to identify significantly enriched pathways for DEG gene sets using ranked expression scores ($-\log_{10}$(p-value)$*\log_2$FoldChange) to calculate enrichment scores. To identify pathways that were enriched within parity-associated co-expression modules, we performed individual Over-Representation Analyses (ORAs) for each module. The background gene set for all GSEA and ORA analyses was specified as the full set of 21,187 genes present within the genome[22]. P-values obtained for enrichment analyses were adjusted for multiple testing using Bonferroni correction (FDR < 0.05).

Developmental pathways between parity modes across vertebrates

We first assessed the overlap between differentially expressed genes in systems with oviparous-viviparous species pairs[9,10]. This included two lizard systems: the bimodal system in *Saiphos equalis* and oviparous-viviparous sister species in the genus *Phrynocepahlus*. We also extracted all genes that were differentially expressed between oviparous and viviparous *Z. vivipara* during reproductive stages. This resulted in three lists of gene symbols – one for each oviparous-viviparous model – between which we then assessed the overlap using the R package 'SuperExactTest'[73]. We assumed a total gene set equalling 20,000 genes, which is a good approximation across genomes of vertebrate species[72]. We then performed statistical tests to assess if intersections between gene sets were larger than expected by change for each possible intersection as implemented in the package 'SuperExactTest'.

In addition, we compiled a dataset that was composed of differentially expressed genes in reproductive tissues of pregnant viviparous amniote species and seahorse relative to the same tissue in their non-pregnant states (uterus for amniotes, pouch for seahorse; Supplementary Table 23). For simplicity, in the following we will refer to all viviparous amniotes and the seahorse exhibiting male pregnancy as viviparous vertebrates, although sea horse pregnancy is not structurally homologous to amniote viviparity[50]. This included the

mammalian species *Bos taurus* (cow), *Capra aegagrus* (goat), *Canis lupus* (wolf), *Equus caballus* (horse), *Homo sapiens* (human), *Monodelphis domestica* (opossum), and *Sus scrofa* (boar). Gene sets of the three more closely related even-toed ungulates (*Bos taurus, Capra aegagrus*, and *Sus scrofa*) were combined into a single gene set. We included the three gene sets from the previously extracted DE genes in the three bimodal lizard systems (genes differentially expressed during pregnancy, excluding DE gene during non-pregnant state), and the two independently derived viviparous species *Chalcides ocellatus* and *Pseudemoia entrecasteauxii* within the family of skinks to focus on genes in viviparous reproduction specifically. This ensures that where closely related groups of oviparous and viviparous species exist, genes that are expressed in uterine tissue generally, but not involved in viviparous reproduction, are excluded. We also highlight that by focusing on functional genes during reproductive stages, any regulatory differences associated with non-pregnant stages (such as underlying structural differences between uterine glands) are ignored. Finally, we included differentially expressed genes in the seahorse *Hippocampus abdominalis* (Supplementary Table 23). The final data set consisted of 11 gene sets (five mammalian, five squamate, and one fish) composed of 13 species spanning vertebrates.

To assess the congruence of the gene lists across species, we used the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8[95]. For each of the 13 gene lists, we determined to which species the most hits were found, and then converted and extracted this gene list to match the *Homo sapiens* and *Gallus gallus* gene symbol format. We chose those two species as their genes are well annotated close relatives of the mammalian and squamate species we used, respectively. We then combined the two gene lists, excluding any duplicates. We found the lists of both species largely matched, with *H. sapiens* generally exhibiting more hits across all species (on average, gene lists matched to *H. sapiens* contained 10.8% more genes than *G. gallus*). The combined lists contained only 1.2% more genes than the *H. sapiens* list. The DAVID filtered gene lists contained 6.9% fewer genes than the gene lists that were not filtered using DAVID. The modest loss was presumably due to i) being private to the query species, ii) being absent in the chicken and human annotation, or iii) matching to another species' (not *H. sapiens* nor *G. gallus*) gene list symbol. We then tested whether filtering affected downstream results by performing the gene overlap analysis for the unfiltered and the DAVID-filtered gene lists using the package 'SuperExactTest'. For each intersection, we recorded the fold enrichment (FE; proportion of observed versus expected gene overlap), and then performed a linear model (LM) to test the correlation between the filtered and unfiltered approaches. We found that FE was highly correlated ($R^2 = 0.997$, $P < 0.0001$; Supplementary Fig. 12) between unfiltered and filtered lists. Because results were highly similar and our interpretations not affected by the filtering, we proceeded with the unfiltered results, as a more conservative approach that considers and retains the possibility of private genes. Next, for the unfiltered list we calculated intersections between gene sets in the same way as the previous analyses using 'SuperExactTest'[73]. We performed three comparisons: i) overlap of genes across mammalian gene sets, ii) overlap of genes across viviparous squamate gene sets, iii) overlap of genes across all vertebrate viviparous gene sets. For genes that were shared by more than four groups within the mammalian gene set and the squamate gene set we performed enrichment analyses of biological pathways in WebGestalt[66].

With the aim of accommodating phylogenetic relatedness in the hypergeometric tests of gene overlaps across species, we assessed if the time since the most recent ancestor (TMRCA), the number of independent transitions to viviparity, and the number of total species affected the degree of gene overlap between viviparous species. We tested if the SuperExactTest fold enrichment (FE; transformed to logarithmic) and the P-value (transformed to negative logarithmic) were associated with TMRCA, the number of independent transitions, the number of species and an interaction between the number of species and number of transitions using a generalized linear model (GLM).

Supplementary Table 1. Bayesian sparse linear mixed model (BSLMM) results for the two reproductive traits a) gestation time and b) eggshell characteristics. Both traits were highly heritable (h^2) and showed a large proportion of phenotypic variance explained by genotypes (PVE) and sparse effects (PGE).

parameter	mean	median	2.50%	97.50%
a) gestation time				
h^2	0.958	0.967	0.873	0.992
PVE	0.971	0.971	0.957	0.983
rho	0.962	0.972	0.866	0.997
PGE	0.944	0.975	0.72	0.998
b) eggshell characteristics				
h^2	0.941	0.944	0.895	0.97
PVE	0.975	0.975	0.964	0.983
rho	0.995	0.997	0.979	1
PGE	0.998	0.999	0.989	1

Supplementary Table 2. Pathways that were enriched for genes associated with reproductive traits. Significantly enriched (P < 0.05) pathways are shown with P-values in bold.

Description	Size	Expect	Ratio	P-Value	FDR
Alzheimer disease-presenilin pathway	30	0.996	6.03	**0.0002**	0.012
Wnt signalling pathway	67	2.224	3.15	**0.0028**	0.100
Cadherin signalling pathway	38	1.261	3.96	**0.0052**	0.123
T-cell activation	18	0.597	5.02	**0.0179**	0.318
Fas signalling pathway	8	0.265	7.53	**0.0257**	0.365
Huntington disease	40	1.327	3.01	**0.0352**	0.416
Nicotinic acetylcholine receptor signalling pathway	25	0.830	3.62	**0.0438**	0.430
Cytoskeletal regulation by Rho GTPase	26	0.863	3.48	**0.0485**	0.430
Alzheimer disease-amyloid secretase pathway	16	0.531	3.77	0.0943	0.744
Inflammation mediated by chemokine and cytokine signalling pathway	41	1.361	2.20	0.1465	0.968

Supplementary Table 3. Biological processes that were significantly enriched (P < 0.01) for genes that showed association with gestation time.

Description	Size	Expect	Ratio	P - Value	FDR
response to growth factor	253	7.9	2.0	0.005	0.518
positive regulation of cell death	188	5.9	2.2	0.006	0.518
positive regulation of cell proliferation	302	9.4	1.9	0.006	0.518
negative regulation of cell proliferation	211	6.6	2.1	0.006	0.518
regulation of cell cycle	390	12.1	1.7	0.010	0.567
cell junction organization	96	3.0	2.7	0.010	0.567

Supplementary Table 4. Biological processes that were significantly enriched (P < 0.01) for genes that showed association with eggshell traits.

Description	Size	Expect	Ratio	P - Value	FDR
negative regulation of cell communication	477	1.7	4.6	0.0002	0.032
negative regulation of signaling	478	1.7	4.6	0.0002	0.032
cytokine production	201	0.7	6.9	0.0007	0.076
positive regulation of catalytic activity	473	1.7	3.5	0.0057	0.436
taxis	209	0.8	5.3	0.0063	0.436
negative regulation of molecular function	362	1.3	3.8	0.0086	0.496

Supplementary Table 5. Samples collected for RNA sequencing and their respective stages during gestation. Sequencing effort for each sample with raw reads, filtered reads and the percentage of reads that mapped to the *Zootoca vivipara* reference genome are shown.

ID	Sampling date	stage	parity mode	eggs (N)	Number of raw reads	Number of cleaned reads	% reads mapped
ELT08543	08/06/2017	early	oviparous	6	57,387,536	34,089,830	56.64
ELT08544	08/06/2017	early	oviparous	8	62,201,602	23,813,398	40.24
ELT08593	08/06/2017	early	oviparous	10	57,719,308	56,257,371	67.01
ELT08526	08/06/2017	early	viviparous	8	45,203,318	42,765,373	74.38
ELT08527	08/06/2017	early	viviparous	6	45,000,467	40,536,529	76.75
ELT08528	08/06/2017	early	viviparous	8	51,547,225	40,609,297	73.42
ELT08628	18/06/2017	mid	oviparous	6	47,401,105	40,848,251	76.31
ELT08630	20/06/2017	mid	oviparous	5	22,574,287	14,278,997	70.59
ELT08545	18/06/2017	mid	oviparous	11	48,494,343	16,327,194	74.48
ELT08529	18/06/2017	mid	viviparous	3	16,276,083	8,761,328	69.16
ELT08532	18/06/2017	mid	viviparous	5	43,348,318	35,674,242	74.37
ELT08534	18/06/2017	mid	viviparous	5	42,335,556	36,759,557	74.96
ELT08636	15/07/2017	after	oviparous	4	65,425,714	64,720,844	75.01
ELT08637	07/07/2017	after	oviparous	6	44,968,452	40,268,336	71.36
ELT08638	30/06/2017	after	oviparous	8	53,914,941	50,105,353	71.53
ELT08585	16/07/2017	after	viviparous	4	66,889,404	61,662,615	61.83
ELT08533	09/07/2017	after	viviparous	8	66,889,404	40,315,756	77.27
ELT08659	16/07/2017	after	viviparous	8	66,889,404	48,301,789	80.10

Supplementary Table 6. Pathways enriched for differentially expressed (DE) genes between oviparous and viviparous common lizards during a) reproductive stages (N = 2160 genes) and b) after pregnancy (N = 507). Significantly enriched ($P < 0.05$) pathways are shown with P-values in bold.

Description	Size	Expect	Ratio	P Value	FDR
a) during reproductive stages					
Apoptosis signaling pathway	21	2.93	2.39	**0.017**	0.65
Parkinson disease	26	3.62	2.21	**0.018**	0.65
Alzheimer disease-amyloid secretase pathway	16	2.23	2.24	0.057	1.00
Angiogenesis	46	6.41	1.56	0.087	1.00
B cell activation	11	1.53	1.96	0.188	1.00
Metabotropic glutamate receptor group I pathway	6	0.84	2.39	0.198	1.00
Axon guidance mediated by semaphorins	6	0.84	2.39	0.198	1.00
p38 MAPK pathway	6	0.84	2.39	0.198	1.00
TGF-beta signaling pathway	29	4.04	1.48	0.203	1.00
VEGF signaling pathway	20	2.79	1.43	0.299	1.00
b) after pregnancy					
Integrin signalling pathway	166	5.64	2.30	**0.003**	0.36
B cell activation	58	1.97	3.04	**0.013**	0.48
Plasminogen activating cascade	15	0.51	5.89	**0.013**	0.48
Axon guidance mediated by Slit/Robo	18	0.61	4.90	**0.021**	0.60
Alzheimer disease-presenilin pathway	112	3.81	2.10	**0.034**	0.76

Supplementary Table 7. Pathways that were enriched for genes associated with overexpression in viviparous and oviparous modules ($P < 0.05$) identified from a Weighted Gene Co-Expression Network Analysis (WGCNA).

Description	Size	Expect	Ratio	P - Value	FDR
Parity DarkGreen Module - up-regulated in viviparous					
Angiogenesis	44	3.47	2.0	0.045	1.0
Parity LightCyan Module - up-regulated in viviparous					
Beta2 adrenergic receptor signaling pathway	7	0.33	6.0	0.039	0.59
Beta1 adrenergic receptor signaling pathway	7	0.33	6.0	0.039	0.59
VEGF signaling pathway	18	0.86	3.5	0.048	0.59
Parity DarkRed Module - up-regulated in oviparous					
TGF-beta signaling pathway	27	0.26	7.8	0.022	1.0

Supplementary Table 8. Enrichment of all annotated hub genes (N = 51 genes) for biological processes (P < 0.01).

Description	Size	Expect	Ratio	P - Value	FDR
establishment or maintenance of cell polarity	85	0.30	13.54	0.0002	0.065
Notch signaling pathway	77	0.27	11.21	0.0023	0.395
renal system process	29	0.10	19.84	0.0044	0.514
epithelium development	382	1.33	3.76	0.0089	0.617
biological adhesion	400	1.39	3.60	0.0107	0.622
second-messenger-mediated signaling	153	0.53	5.64	0.0153	0.653
protein localization to membrane	171	0.59	5.05	0.0206	0.653

Supplementary Table 9. Pathways showing enrichment of genes (N = 1,621 genes) linked to SNPs under selection (q < 0.01). Significantly enriched pathways (P < 0.05) are shown in bold.

Description	Size	Expect	Ratio	P - Value	FDR
Fas signalling pathway	8	0.90	3.32	**0.050**	0.745
Angiogenesis	46	5.19	1.73	0.059	0.745
Cytoskeletal regulation by Rho GTPase	26	2.93	2.05	0.060	0.745
Oxidative stress response	9	1.02	2.95	0.070	0.745
Alzheimer disease-amyloid secretase pathway	16	1.81	2.22	0.094	0.745
De novo purine biosynthesis	5	0.56	3.55	0.100	0.745
EGF receptor signalling pathway	30	3.39	1.77	0.108	0.745
Cadherin signalling pathway	38	4.29	1.63	0.121	0.745
Nicotinic acetylcholine receptor signalling pathway	25	2.82	1.77	0.138	0.745
p38 MAPK pathway	6	0.68	2.95	0.139	0.745

Supplementary Table 10. Overlap of genes differentially expressed between oviparous and viviparous related squamate systems. All intersections are shown, and significant intersections are shown in bold. All intersections were significant.

Intersections	Degree	N genes (shared)	N genes (expected)	FE	P-value
Zootoca	1	2150	NA	NA	NA
Saiphos	1	605	NA	NA	NA
Phrynocephalus	1	458	NA	NA	NA
Saiphos & Zootoca	2	68	65	1.05	0.37
Phrynocephalus & Zootoca	2	99	49.2	2.01	**<0.0001**
Phrynocephalus & Saiphos	2	15	13.9	1.08	0.41
Phrynocephalus & Saiphos & Zootoca	3	8	1.5	5.37	**<0.0001**

Supplementary Table 11. Overlap of DE genes shared by viviparous squamates during pregnancy. All intersections are shown, and significant intersections are shown in bold.

Intersections	Degree	N genes (shared)	N genes (expected)	FE	P-value
Zootoca	1	2150	NA	NA	NA
Phrynocephalus	1	458	NA	NA	NA
Chalcides	1	6602	NA	NA	NA
Pseudemoia	1	2599	NA	NA	NA
Saiphos	1	362	NA	NA	NA
Chalcides & Zootoca	2	1011	709.7	1.42	**<0.0001**
Chalcides & Phrynocephalus	2	229	151.2	1.51	**<0.0001**
Pseudemoia & Zootoca	2	396	279.4	1.42	**<0.0001**
Pseudemoia & Chalcides	2	1025	857.9	1.19	**<0.0001**
Phrynocephalus & Zootoca	2	99	49.2	2.01	**<0.0001**
Pseudemoia & Phrynocephalus	2	85	59.5	1.43	**0.0004**
Saiphos & Zootoca	2	60	38.9	1.54	**0.0004**
Saiphos & Pseudemoia	2	64	47	1.36	**0.0061**
Saiphos & Chalcides	2	131	119.5	1.1	0.1078
Saiphos & Phrynocephalus	2	10	8.3	1.21	0.318
Pseudemoia & Chalcides & Zootoca	3	227	92.2	2.46	**<0.0001**
Chalcides & Phrynocephalus & Zootoca	3	48	16.3	2.95	**<0.0001**
Pseudemoia & Chalcides & Phrynocephalus	3	52	19.6	2.65	**<0.0001**
Pseudemoia & Phrynocephalus & Zootoca	3	27	6.4	4.22	**<0.0001**
Saiphos & Pseudemoia & Chalcides	3	42	15.5	2.7	**<0.0001**
Saiphos & Chalcides & Zootoca	3	36	12.8	2.8	**<0.0001**
Saiphos & Pseudemoia & Zootoca	3	15	5.1	2.97	**0.0002**
Saiphos & Pseudemoia & Phrynocephalus	3	5	1.1	4.64	**0.0048**
Saiphos & Phrynocephalus & Zootoca	3	4	0.9	4.49	**0.0128**
Saiphos & Chalcides & Phrynocephalus	3	6	2.7	2.19	0.0584
Pseudemoia & Chalcides & Phrynocephalus & Zootoca	4	17	2.1	8.05	**<0.0001**
Saiphos & Pseudemoia & Chalcides & Zootoca	4	13	1.7	7.79	**<0.0001**
Saiphos & Pseudemoia & Chalcides & Phrynocephalus	4	3	0.4	8.44	**0.0057**
Saiphos & Chalcides & Phrynocephalus & Zootoca	4	2	0.3	6.8	**0.0355**
Saiphos & Pseudemoia & Phrynocephalus & Zootoca	4	1	0.1	8.64	0.1094
Saiphos & Pseudemoia & Chalcides & Phrynocephalus & Zootoca	5	1	0	26.16	**0.0375**

Supplementary Table 12. List of core differentially expressed (DE) genes between pregnant and non-pregnant viviparous squamates. Only genes shared by at least four viviparous squamates are shown. Presence of a DE gene is indicated by '1', absence by '0'.

gene symbol	*C. ocellatus*	*P. vlangalii*	*P. entrecasteauxii*	*S. equalis*	*Z. vivipara*	sum squamates shared	sum shared all excl. *Zootoca*
CDH5	1	1	1	1	1	5	5
ELL2	1	0	1	1	1	4	6
RASEF	1	1	1	0	1	4	6
SGK1	1	0	1	1	1	4	6
RHOU	1	0	1	1	1	4	6
ASAH1	1	0	1	1	1	4	5
B4GALT3	1	0	1	1	1	4	5
EDEM3	1	1	1	0	1	4	5
ATP8B1	1	1	1	0	1	4	5
DIO2	1	1	1	1	0	4	6
COL4A2	1	0	1	1	1	4	5
KCNK1	1	1	1	0	1	4	5
MCOLN3	1	1	0	1	1	4	5
CTSA	1	0	1	1	1	4	5
PLCB4	1	1	1	0	1	4	5
SLC7A1	1	1	1	0	1	4	5
ATP8A1	1	1	1	0	1	4	4
ITPK1	1	1	1	0	1	4	4
CLCN3	1	1	1	0	1	4	4
SCNN1A	1	0	1	1	1	4	4
SLC38A2	1	1	1	0	1	4	4
SLC9A2	1	0	1	1	1	4	4
SMAD6	1	0	1	1	1	4	4
CYP51A1	1	1	1	1	0	4	5
B4GALNT3	1	1	1	0	1	4	4
ACVR2B	1	1	1	0	1	4	3
LMBRD2	1	1	1	0	1	4	3
NR4A2	1	0	1	1	1	4	3
PXDN	1	1	1	0	1	4	3
SCNN1B	1	0	1	1	1	4	3
SLC7A11	1	1	1	0	1	4	3
TMEM181	1	1	1	0	1	4	3

Supplementary Table 13. Overlap of differentially expressed genes shared by viviparous mammals during pregnancy. All intersections are shown, and significant intersections are shown in bold. All intersections were significant.

Intersections	Degree	N genes (shared)	N genes (expected)	FE	P-value
Bos	1	5095	NA	NA	NA
Canis	1	1439	NA	NA	NA
Equus	1	342	NA	NA	NA
Homo	1	2310	NA	NA	NA
Monodelphis	1	2152	NA	NA	NA
Bos & Homo	2	838	588.5	1.42	**<0.0001**
Monodelphis & Homo	2	418	248.6	1.68	**<0.0001**
Bos & Canis	2	545	366.6	1.49	**<0.0001**
Monodelphis & Bos	2	754	548.2	1.38	**<0.0001**
Bos & Equus	2	170	87.1	1.95	**<0.0001**
Monodelphis & Equus	2	86	36.8	2.34	**<0.0001**
Monodelphis & Canis	2	245	154.8	1.58	**<0.0001**
Equus & Homo	2	89	39.5	2.25	**<0.0001**
Equus & Canis	2	48	24.6	1.95	**<0.0001**
Canis & Homo	2	195	166.2	1.17	**0.0087**
Monodelphis & Bos & Homo	3	203	63.3	3.21	**<0.0001**
Monodelphis & Bos & Equus	3	53	9.4	5.65	**<0.0001**
Monodelphis & Bos & Canis	3	107	39.4	2.71	**<0.0001**
Bos & Equus & Homo	3	42	10.1	4.17	**<0.0001**
Monodelphis & Equus & Homo	3	27	4.3	6.35	**<0.0001**
Monodelphis & Canis & Homo	3	55	17.9	3.08	**<0.0001**
Bos & Canis & Homo	3	89	42.3	2.10	**<0.0001**
Bos & Equus & Canis	3	28	6.3	4.47	**<0.0001**
Equus & Canis & Homo	3	15	2.8	5.28	**<0.0001**
Monodelphis & Equus & Canis	3	13	2.6	4.91	**<0.0001**
Monodelphis & Bos & Equus & Homo	4	16	1.1	14.78	**<0.0001**
Monodelphis & Bos & Canis & Homo	4	26	4.6	5.71	**<0.0001**
Monodelphis & Equus & Canis & Homo	4	8	0.3	26.16	**<0.0001**
Monodelphis & Bos & Equus & Canis	4	8	0.7	11.86	**<0.0001**
Bos & Equus & Canis & Homo	4	8	0.7	11.05	**<0.0001**
Monodelphis & Bos & Equus & Canis & Homo	5	5	0.1	64.18	**<0.0001**

Supplementary Table 14. List of the core differentially expressed (DE) genes between pregnant and non-pregnant viviparous mammals. Only genes shared by at least four viviparous mammals are shown. Presence of a DE gene is indicated by '1', absence by '0'.

gene symbol	*E. caballus*	Artiodactyla	*M. domestica*	*C. lupus*	*H. sapiens*	sum mammals shared
UPK1B	1	1	1	1	1	5
CDO1	1	1	1	1	1	5
SLCO2A1	1	1	1	1	1	5
THBS2	1	1	1	1	1	5
FGL1	1	1	1	1	1	5
AR	0	1	1	1	1	4
ADAMTS1	0	1	1	1	1	4
PHGDH	1	1	1	0	1	4
CA12	0	1	1	1	1	4
CXCL14	0	1	1	1	1	4
GLUL	0	1	1	1	1	4
SLC1A1	0	1	1	1	1	4
TNC	0	1	1	1	1	4
DACT2	0	1	1	1	1	4
FLT1	0	1	1	1	1	4
IER3	1	1	1	1	0	4
PLXDC2	1	1	1	0	1	4
TACSTD2	1	1	1	0	1	4
GARNL3	1	1	1	0	1	4
GSN	1	1	1	0	1	4
PROCR	1	1	0	1	1	4
ATP6V0A4	1	0	1	1	1	4
ATP13A4	0	1	1	1	1	4
MFSD4	0	1	1	1	1	4
OAS3	0	1	1	1	1	4
PALMD	0	1	1	1	1	4
PCSK6	0	1	1	1	1	4
NOS3	0	1	1	1	1	4
TNFRSF12A	1	1	1	1	0	4
VLDLR	1	1	1	1	0	4
DMBT1	1	1	1	0	1	4
ENPP1	1	1	1	0	1	4
LTBP1	1	1	1	0	1	4
MET	1	1	1	0	1	4
SFRP1	1	1	0	1	1	4
RARRES2	1	0	1	1	1	4
ACSL5	0	1	1	1	1	4
HLF	0	1	1	1	1	4
IGDCC4	0	1	1	1	1	4
KCNE3	0	1	1	1	1	4
OSMR	0	1	1	1	1	4
SLC39A14	0	1	1	1	1	4
CRYAB	1	1	1	0	1	4
GNAI1	1	1	1	0	1	4
CP	1	1	0	1	1	4
SERPINA1	1	0	1	1	1	4

Supplementary Table 15. Enrichment of gene sets of genes that were shared by at least four viviparous mammals for biological processes ($P < 0.01$).

Description	Size	Expect	Ratio	P Value	FDR
morphogenesis of a branching structure	196	0.54	9.21	0.0002	0.05
regulation of vasculature development	313	0.87	6.92	0.0002	0.05
response to antibiotic	316	0.87	6.86	0.0002	0.05
urogenital system development	326	0.90	6.65	0.0003	0.05
response to alcohol	231	0.64	7.82	0.0004	0.07
response to ketone	189	0.52	7.64	0.0018	0.20
regulation of supramolecular fiber organization	329	0.91	5.49	0.0020	0.20
angiogenesis	487	1.35	4.45	0.0021	0.20
positive regulation of cell motility	493	1.37	4.40	0.0022	0.20
response to toxic substance	499	1.38	4.34	0.0023	0.20
regulation of actin filament-based process	362	1.00	4.99	0.0031	0.21
extrinsic apoptotic signaling pathway	220	0.61	6.57	0.0031	0.21
transition metal ion transport	109	0.30	9.94	0.0034	0.21
epithelial cell proliferation	372	1.03	4.85	0.0035	0.21
regulation of apoptotic signaling pathway	385	1.07	4.69	0.0040	0.23
regulation of cellular response to growth factor stimulus	256	0.71	5.64	0.0053	0.28
sex differentiation	266	0.74	5.43	0.0061	0.30
reproductive system development	428	1.19	4.22	0.0063	0.30
gland development	434	1.20	4.16	0.0066	0.30
tissue migration	283	0.78	5.10	0.0076	0.30
trabecula morphogenesis	49	0.14	14.74	0.0081	0.30
cell chemotaxis	289	0.80	5.00	0.0082	0.30
negative regulation of transport	458	1.27	3.94	0.0083	0.30
positive regulation of response to external stimulus	293	0.81	4.93	0.0086	0.30

Supplementary Table 16. Generalized linear model on the effect of time of most recent common ancestor (TMRCA), number of independent transitions from oviparity to viviparity, and number of species on the degree of gene overlap. Gene overlap was measured as logarithm of the fold enrichment (see Supplementary Data 5) for each individual intersection.

character	Estimate	Std.Error	t-value	P-value	
TMRCA	-0.001	0.000	-5.9	<0.00001	***
N transitions	0.090	0.035	2.6	0.0108	*
N species	0.134	0.025	5.4	<0.00001	***
N transitions: N species	-0.037	0.005	-6.9	<0.00001	***

Supplementary Table 17. List of viviparous species with data on differentially expressed genes in reproductive tissue between pregnant and non-pregnant states. Species are ordered by class. Embryonic developmental stage is indicated for viviparous and oviparous species when available. Stages were separated into three classes: early (blastocyst till auditory vesicle formation), mid (onset of eye pigmentation till start of sex differentiation) and late (scalation in squamates visible, pigmentation visible, embryo close to parturition). False discovery rate (FDR) thresholds implemented by the referred studies from which data were extracted are displayed.

Species	Order	Class	pregnancy state		False discovery rate	N diff. genes	Reference
			oviparous	viviparous			
Hippocampus abdominalis	Syngnathiformes	Actinopterygii		early - late	< 0.05	311	[96]
Bos taurus	Artiodactyla	Mammalia		early	< 0.1	195	[97]
Capra aegagrus	Artiodactyla	Mammalia		early	< 0.01	3231	[98]
Sus scrofa	Artiodactyla	Mammalia		early	< 0.01	2223	[99]
Canis lupus	Carnivora	Mammalia		mid	<0.1	1439	[100]
Monodelphis domestica	Didelphimorphia	Mammalia		late	< 0.05	2152	[101]
Equus caballus	Perissodactyla	Mammalia		early	< 0.05	342	[102]
Homo sapiens	Primates	Mammalia		early	< 0.05	2310	[103,104]
Chalcides ocellatus	Squamata	Reptilia		late	< 0.1	6602	[8]
Pseudemoia entrecasteauxii	Squamata	Reptilia		late	< 0.05	2599	[105]
Phrynocepahlus vlangalii	Squamata	Reptilia	early - mid	mid - late	< 0.05	458	[9]
Saiphos equalis	Squamata	Reptilia	mid - late	mid - late	< 0.05	362	[10]
Zootoca vivipara	Squamata	Reptilia	early - mid	early- mid	< 0.05	2150	this study

**Monodelphis domestica* pregnancy state reported as 'late pregnancy'

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