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GTP cyclohydrolase II (*gch2*) and axanthism in ball pythons: A new vertebrate model for pterin-based pigmentation

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Abstract

Pterin pigments are responsible for many of the bright colors observed across the animal kingdom. However, unlike melanin, the genetics of pterin-based pigmentation has received relatively little attention in animal coloration studies. Here, we investigate a lineage of axanthic ball pythons (*Python regius*) found in captivity as a model system to study pterin pigmentation in vertebrates. By crowdsourcing shed skin samples from commercial breeders and applying a case–control study design, we used whole-genome pool sequencing (pool-seq) and variant annotation. We identified a premature stop codon in the gene *GTP cyclohydrolase II* (*gch2*), which is associated with the axanthic phenotype. GCH2 catalyzes the first rate-limiting step in riboflavin biosynthesis. This study provides the first identification of an axanthism-associated gene in vertebrates and highlights the utility of ball pythons as a model to study pterin-based pigmentation.

KEYWORDS

axanthism, gch2, genomics, GTP cyclohydrolase, pigmentation, pterin, reptile, riboflavin, xanthophore

INTRODUCTION

Animal pigmentation has long served as a valuable model for understanding genetics, development, and evolution (Caro, 2017; Cott, 1940; Endler & Mappes, 2017; Kronforst et al., 2012; San-Jose & Roulin, 2017). Vertebrate pigmentation arises from the differential absorption of light by pigments within specialized cells called melanophores (melanocytes in endothermic vertebrates) and xanthophores. All vertebrates produce a brown/black form of melanin derived from the amino acid tyrosine (Wakamatsu & Ito, 2021). In addition, poikilothermic vertebrates (fish, amphibians, and reptiles) display bright coloration through yellow/red pigments found in cells called xanthophores. Xanthophores derive their vibrant yellow or red coloration from pterin or carotenoid pigments. While vertebrates acquire carotenoids exclusively from their diet (Maoka, 2020), pterin pigments are synthesized de novo from guanosine triphosphate (GTP).

Structural coloration in poikilothermic vertebrates arises from guanine nanocrystals in iridophore cells, which manipulate light through interference. When regularly arranged, these crystals produce iridescence (Kuriyama et al., 2006) or act as narrowband reflectors, generating angle-dependent color shifts (Saenko et al., 2013). In contrast, disordered crystals function as broadband reflectors, contributing to white skin (Saenko et al., 2013). While melanin-based pigmentation is well studied, other pigment classes, such as pterins, remain less explored.

The mouse has been instrumental in melanocyte biology, identifying genes involved in melanocyte development and patterning (*kit*, *pax3*, *mitf*, *edn3*, *ednrb*, and *sox10*; Steingrímsson et al., 2006), melanin synthesis (*tyr*, *tyrp1*, *dct*; McNamara et al., 2021; Wakamatsu & Ito, 2021), and its regulation (*mc1r*; García-Borrón et al., 2005). Meanwhile, zebrafish have provided insights into poikilothermic vertebrate pattern formation

(Singh & Nüsslein-Volhard, 2015), showing that local, short- and long-range interactions between chromatophores shape patterns (Frohnhöfer et al., 2013). Zebrafish mutants lacking a single chromatophore type develop spots instead of stripes, while double mutants with only one chromatophore type display uniform pigmentation (Frohnhöfer et al., 2013; Owen et al., 2020). Gap junction proteins mediate these interactions, and mutations in these genes can produce labyrinthine patterns (Irion et al., 2014). However, the extent to which zebrafish cell–cell interactions apply across vertebrates remains unclear.

First discovered in butterfly wings (Hopkins, 1895), pterin-based pigmentation is widespread in nature but has received less attention than melanin-based pigmentation (Andrade & Carneiro, 2021). The pterin pigment synthesis pathway has been largely elucidated through *Drosophila* studies (Courtright, 1967; Evans & Howells, 1978; Glassman & Mitchell, 1959; Goto & Sugiura, 1971; Kim et al., 2013; Reaume et al., 1991; Spradling & Rubin, 1983; Ziegler & Harmsen, 1970). Notably, *Drosophila*'s first described mutant, white, lacked eye pterin (drosopterin) and ommochrome pigments (Morgan, 1910). While melanin is often linked to adaptive traits such as cryptic coloration (Baltazar-Soares et al., 2024; Barrett et al., 2019; Harris et al., 2020), pterins play key roles in signaling within and between species (Andrade & Carneiro, 2021; Kikuchi et al., 2014; Kikuchi & Pfennig, 2012). In vertebrates, zebrafish have been the primary model for xanthophore biology, including their specification from neural crest cells (Kelsh et al., 1996; Minchin & Hughes, 2008; Odenthal et al., 1996; Parichy et al., 2000) and pigment synthesis (Lister, 2019; Ziegler, 2003).

Studying pigment-lacking mutants provides insights into the genetics of bright coloration, a strategy pioneered by Thomas Hunt Morgan in fruit flies. Axanthic vertebrates, which lack yellow and red pigments but retain melanin, are rare, with only a few cases reported in amphibians and reptiles (Bechtel, 1991; Borteiro et al., 2021; Cattaneo, 2015; Cavalcante & Bruni, 2018; Jablonski et al., 2014; Kolenda et al., 2017; Schluckebier et al., 2022). Among pigmentation research models, the axanthic axolotl, first described by Lyerla and Dalton (1971), remains a rare exception (Bukowski et al., 1990; Dunson, 1974; Frost et al., 1986; Masselink & Tanaka, 2021). These axolotls lack both xanthophores and iridophores, indicating that axanthism affects chromatophore development rather than pigment synthesis. However, their low survivability complicates research efforts, including breeding programs, population genetics studies, and comparative analyses.

We investigate axanthic ball pythons to explore the genetics of yellow pigmentation in reptiles. Based on vertebrate pattern formation models (Frohnhöfer et al., 2013; Singh & Nüsslein-Volhard, 2015), we hypothesize that axanthism results from defects in pterin synthesis (Ziegler, 2003), not carotenoid metabolism or xanthophore development. Since axanthic ball pythons retain wild-type melanin patterning, we expect xanthophore development to remain unaffected, indicating intact cell–cell interactions. Furthermore, we anticipate that yellow pigmentation (or its absence) is linked to pterin-based pigmentation, rather than carotenoids, since ball pythons are born with yellow coloration before acquiring carotenoids from their diet. This suggests that pterins, not carotenoids, underlie the color difference, unless possible carotenoids in yolk—though primarily studied in birds and, to a lesser extent, lizards—have been absorbed (Blount et al., 2000; Dierenfeld et al., 2001; Weiss et al., 2011). This suggests that pterins, not carotenoids, underlie the coloration difference.

METHODS

Sample collection, study design, DNA extraction, and sequencing

We collected shed skin samples from breeders, including five VPI Axanthic ball pythons (Table S1). This recessive morph originates from a wild-caught axanthic snake acquired by Vida Preciosa International (VPI) and is the source of all VPI Axanthics in captivity. As a control, we sampled 38 Clown pythons (Table S2) to identify genetic variants linked to axanthism. Additional control samples included Spider ($n=26$, Table S3), Enchi ($n=7$, Table S4), Ivory ($n=15$, Table S5), and Lavender ($n=14$, Table S6) morphs, none of which carry axanthism. To reduce population structure effects, breeders provided shed samples from unrelated individuals, though some relatedness may persist within our pools.

Similarly, the axanthic samples were confirmed to lack any variation associated with the Clown, Spider, Enchi, Ivory, or Lavender morphs (i.e., inferred to be homozygous for the reference alleles at those loci). We identified candidate variants by comparing genetic variation in the axanthic pool with multiple reference sample sets known, through pedigree analysis, to not carry the axanthic variant.

Wild-type ball pythons have a black background with tan saddles and traces of yellow (Figure 1a). In non-axanthic individuals, yellow pigment overlaps tan saddles but is absent from dark regions (Figure 1b), whereas axanthic pythons lack yellow but retain wild-type melanin patterns (Figure 1c). Xanthism increases yellow brightness or coverage (e.g., 'Enchi'), while morphs such as Tri-Stripe suggest that xanthophores and melanophores repel each other, similar to zebrafish (Frohnhöfer et al., 2013). Breeders report that wild types have brown, golden, or yellow eyes with a distinct

FIGURE 1 Mendelian phenotypes (color morphs) in ball pythons. Compared to the (a) wild type, (b) the albino color morph lacks melanin pigment which allows the display of xanthophore (pterin) pigmentation and patterning, occupying the tan-colored parts of the wild type. (c) The VPI Axanthic is characterized by a lack of yellow pigmentation on a wildtype pattern. (d) Combining color morphs with axanthism (VPI Axanthic + Pastel + Fire is shown) can create different forms of the color anomaly. (e) Some color morphs like Enchi (Albino + Enchi is shown) are associated with changes in xanthophore migration (e.g., xanthism). (f) Tri-Stripe color morph exhibits altered melanin patterning. (g) The Albino form of Tri-Stripe shows the effects of this color morph on xanthophore pigmentation. (h) Melanic color morphs like Cinnamon do not have xanthophore pigmentation, as shown by an (i) all-white snake in the Albino form. Photo credit: worldofballpythons.com.

pupil, while axanthic individuals have black eyes with a silvery sheen, making the pupil less distinct. Axanthism affects both sexes equally, unlike other morphs (Mallery Jr & Carrillo, 2016).

We extracted and sequenced DNA from the shed skins using a standard 3-day phenol–chloroform procedure. DNA quantification and quality checks were performed with an Infinite® 200 Nanoquant (Tecan Group Ltd., Männedorf, Switzerland). After normalizing the DNA from each sample, we combined them into pools in equimolar amounts. These pooled samples (case and controls) were then sent to the McGill Genome Centre for whole-genome sequencing. Library preparation was performed using a PCR-based method, and sequencing was carried out with 150 bp paired-end reads on one lane of the Illumina NovaSeq 6000 platform.

Bioinformatics

We used the trim-fastq.pl. program from Popoolation (Kofler, Orozco-terWengel, et al., 2011) to filter raw reads based on quality (--quality-threshold 20) and length (--min-length 50). This step ensured that each read had a minimum quality score of 20 and a minimum length of 50 bp. We then mapped the filtered reads to a reference genome using the bwa-mem program with default parameters (Li & Durbin, 2009). Since a ball python reference genome is not available, we aligned the reads to both the draft Burmese python reference genome *Pmo2.0* (Castoe et al., 2013) and the more recent chromosome-length Burmese python assembly (*Python molurus bivittatus*-5.0.2_HiC), available at DNAzoo.org. This chromosome-length assembly, based on Hi-C data (Dudchenko et al., 2017; Dudchenko et al., 2018), is aligned to the annotated draft genome (Castoe et al., 2013) and has been used in previous studies to identify color-related loci (García-Elfring et al., 2023; García-Elfring et al., 2025).

Next, we converted SAM files to BAM format using SAMtools (Li et al., 2009) and removed reads with a mapping quality below 20 (-q 20). We then generated mpileup and sync formats using SAMtools and Popoolation2 (Kofler, Pandey, et al., 2011), respectively, before proceeding to variant calling. Variants were called and filtered with bcftools (vcfutils.pl. varFilter) using a minimum depth of five (-d 5) and a mapping quality of 20 (-Q 20).

We performed genome scans to identify the genomic region of interest, using F_{ST} and Fisher's exact test (FET) for significant changes in allele frequency as metrics for genomic differentiation. F_{ST} (fixation index) measures genetic differentiation between populations by comparing allele frequency differences. Values range from 0 (no differentiation, indicating complete mixing) to 1 (complete differentiation, where populations share no alleles). We calculated single nucleotide variant (SNV)-specific F_{ST} and FET values using fst-sliding.pl. and fisher-test.pl. from Popoolation2 (Kofler, Pandey, et al., 2011), respectively. FET *p*-values were adjusted using the Benjamini–Hochberg method. We also applied a sliding-window approach for F_{ST} estimation (window size of 10 kb and a step size of 2 kb). We visualized genome scans between VPI Axanthic ($n=5$) and control pools (Spider $n=26$; Enchi $n=7$; Ivory $n=15$; and Lavender $n=14$). Since the control pools lacked axanthic variation, we expected the derived allele to be fixed in the axanthic samples and the reference allele to be fixed in the control pool. A threshold F_{ST} value of 1.0 was used to identify candidate single nucleotide variants.

Variant annotation and identification of polymorphisms and fixed differences

We used the software SnpEff (Cingolani et al., 2012) to annotate variants mapped to both the draft (Castoe et al., 2013, accession: GCF_000186305.1) and chromosome-length (www.dnazoo.org) assemblies. SnpEff uses a genome assembly and its corresponding annotation file (GFF3 or GTF) to assess the functional impact of variants on genomic features. For genomic regions showing elevated F_{ST} values ("F_{ST} peaks"), we focused on individual variants with an F_{ST} of 1.0, that mapped to protein-coding regions and were predicted to affect gene products. These included nonsense variants and small indels classified as "high impact," and missense variants classified as "moderate impact," which were considered candidate variants. We mapped gene annotations from the draft assembly to the chromosome-length assembly using the program Liftoff (Shumate & Salzberg, 2021).

To visualize the location and impact of candidate genetic variants on the protein, we used Protter and the protein sequence as input (Omasits et al., 2014). We confirmed the candidate variant by PCR amplification and Sanger sequencing of the locus using samples that were not included in the pooled libraries. We validated the candidate loci by Sanger sequencing samples with genotypes inferred from breeder pedigrees. These included inferred heterozygotes ($n=10$), homozygotes for the derived axanthism-causing allele (i.e., axanthic individuals, $n=6$), and inferred homozygotes for the wild-type allele ($n=10$).

Primers used to amplify the region surrounding the candidate locus were designed with Primer3 (Kõressaar et al., 2018; Kõressaar & Remm, 2007). We used the following primers for PCR amplification:

- Forward (2: 281145975 to 281145997): 5′-GAAACTTT TGACCAGACTCAGTC-3′
- Reverse (2: 281146837 to 281146858): 5′-CTTCAATC ACCACAGCCACTCC-3′

Domains, synteny, and phylogenetic analysis of *gch1* and *gch2*

We analyzed gene protein domains using InterPro (Blum et al., 2025). InterPro offers functional analysis of proteins by categorizing them into families and predicting their domains and key functional sites. We constructed a phylogenetic tree for the *GCH1*/*gch1* and *gch2* genes across multiple species: Burmese python (*Python bivittatus*), brown anole (*Anolis sagrei*), coelacanth (*Latimeria chalumnae*), and the elephant shark (*Callorhinchus milii*). The human genome does not have a *GCH2* annotation. To ensure focus on conserved regions, we trimmed the gene sequences (555 bp) before alignment. Due to the evolutionary divergence of these species, we used MAFFT for sequence alignment (Katoh et al., 2002) and constructed the phylogenetic tree using FastTree (Price et al., 2010), which estimates maximum-likelihood phylogenies, allowing us to infer the evolutionary relationships of these genes across species. We used the NCBI Sequence Viewer and Genome Data Viewer (Rangwala et al., 2021) to observe what genes flanked *GCH1*/*gch1* (human, coelacanth, Burmese python) and *gch2* (coelacanth and Burmese python).

Gene nomenclature

Gene names are written in lowercase to ensure clarity, except for human gene symbols, which are written in uppercase, following the established convention. We adhere to the Human Genome Variation Society (HGVS) guidelines for variant nomenclature. Accordingly, we avoid the use of the terms "mutation" and "polymorphism" due to their implications of pathogenicity and benignity, respectively. Instead, we use "variant" or "change," which are more precise terms defined within the HGVS nomenclature framework (Richards et al., 2015).

RESULTS

Our whole-genome sequencing and variant calling identified 26 449 779 SNVs across all samples received from ball python breeders. Analysis of allele frequency differences between the axanthic pool and the control pool (Clown color morph) demonstrates overall high levels of background F_{ST} (Figure S1), probably resulting from the low sample size for the axanthic phenotype ($n=5$). Using the windowed approach, we find a genome-wide F_{ST} average of 0.171 (SD = 0.058) between axanthic and the Clown control pool, which is higher than the differentiation observed in other datasets with higher sample size (e.g., Clown and Ivory controls, Table S7). We find two areas of high differentiation (Figure 2, Figure S1). One region on chromosome 2 (Figure 2a) and a second peak on chromosome 3, corresponding to the region containing the Clown variant (García-Elfring et al., 2025). Comparing the axanthic data with additional control pools (Spider, $n=26$; Enchi, $n=7$; Ivory, $n=15$; Lavender, $n=14$) confirms that the region of high differentiation on chromosome 2 is associated with axanthism (Figure S2). Figure S2 shows additional peaks on chromosomes 7 (top), 14 (second from top), and 4 (bottom), corresponding to regions with the putative causal gene for Spider, Enchi, and for the Lavender albino color morph, which has been identified as LOC103059581/*oca2* (Brown et al., 2022).

Within the peak on chromosome 2, one variant (2: 281146407) has high levels of differentiation ($F_{ST}=1$, Figure S1), highly significant changes in allele frequency with p-values ranging from 4.71×10^{-15} to 7.54×10^{-28} after adjusting for multiple hypothesis testing (Figure 2, Figure S2), and is predicted to have a high impact on the gene product. This change is annotated as a nonsense variant, mapping to exon 5 of LOC103050242 (*GTP cyclohydrolase 1-like*) in the draft assembly. The candidate SNV involves a C to T nucleotide substitution (c.520C>T), leading to the replacement of an arginine residue (CGA) with a TGA stop codon (p.Arg174*). This variant is anticipated to produce a truncated protein (Figure 2b) that lacks the following sequence (Figure S4): RLTKQIAVAI TEVLKPVGVAVVIEASHMCMIMRGVQKMNS TTVTSSMFGILQEDSKTREEFLALIKN. This region contains an N-glycan motif and key domains, including the GTP cyclohydrolase I (IPR020602) and GTP cyclohydrolase I, C-terminal/NADPH-dependent 7-cyano-7-deazaguanine reductase (IPR043133).

We validated the axanthic variant found in poolseq data by PCR amplification and Sanger sequencing of the locus of interest in additional individuals (Table S8: inferred heterozygotes: $n=10$; homozygotes for the derived axanthism-causing allele: $n=6$; inferred homozygotes for the wild type allele: $n=10$). All individuals tested had the genotype that was inferred from pedigrees by breeders. However, our methods preclude detection of larger structural variants.

Based on the annotation of *gch1* in the Burmese python reference genome and the absence of an annotated *gch2*, we hypothesized that the gene LOC103050242 (*GTP cyclohydrolase 1-like*) corresponds to the paralog *gch2*. A phylogenetic analysis shows that the Burmese python gene LOC103050242 is more closely related to the brown anole LOC132761499 and coelacanth *gch2* than to Burmese python *gch1* (Figure S2). Synteny analysis further supports the identification of LOC103050242 as *gch2*. In humans, coelacanth, and python, *GCH1*/*gch1* is flanked by *SAMD4A*/*samd4a*, while in coelacanth and python, *gch2* (LOC103050242 in python) is flanked by *pigf* and *cript* (Figure 3, Figure S4).

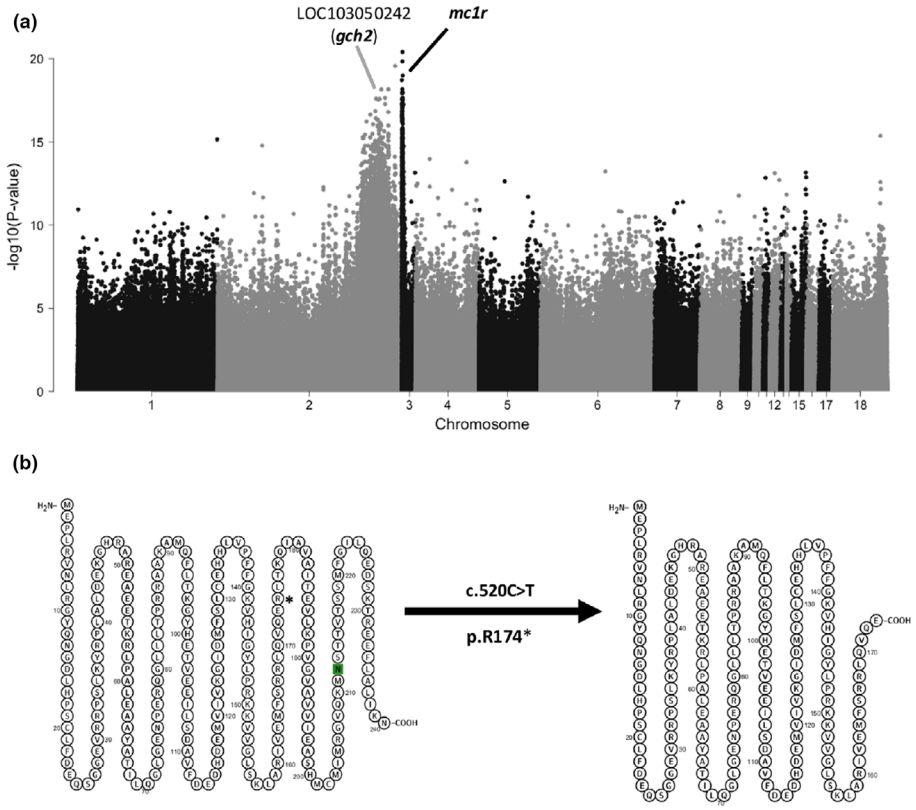

FIGURE 2 (a) Manhattan plot of Fisher's exact test values for single nucleotide variants mapped to the chromosome-length assembly. Genomic differentiation between VPI axanthic and a reference pool (Clown color morph) shows a peak on chromosome two housing the axanthism candidate variant on LOC103050242 (*gch2*). The peak on chromosome three corresponds to the region housing the candidate causal gene for the Clown color morph (Garcia-Elfring et al. 2025). (b) Burmese python amino acid sequence of LOC103050242, with the VPI Axanthic locus indicated by an asterisk (left panel). A nonsense mutation at the 174th residue (p.Arg174*) probably leads to a defective truncated protein (right panel) and axanthism. Green square represents a N-glycosylation motif.

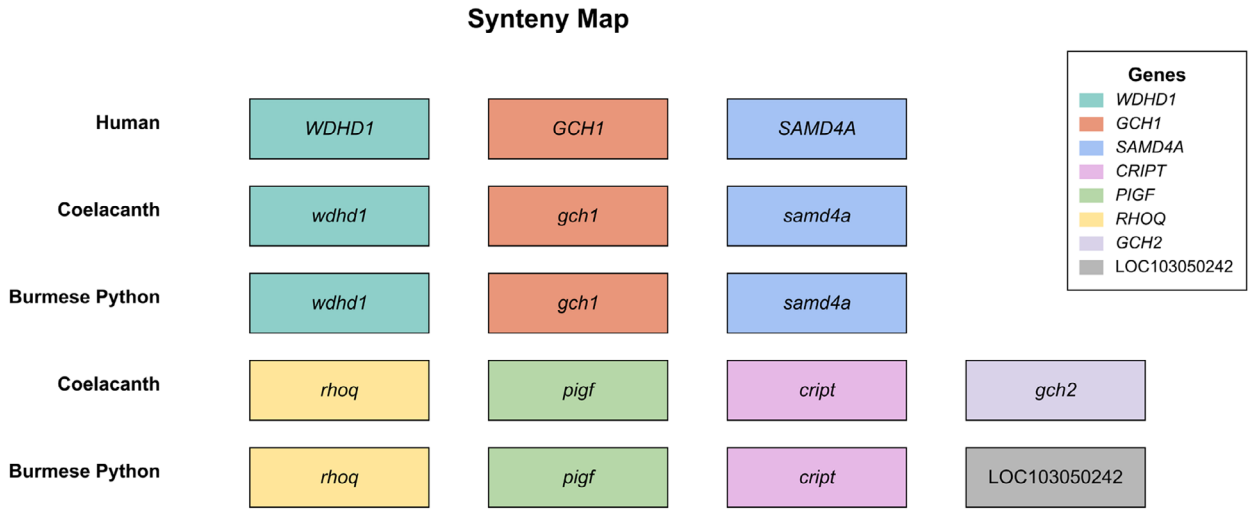

FIGURE 3 The map displays the genomic arrangement and genes flanking *GCH1*/*gch1*, *gch2*, and LOC103050242 in human, coelacanth, and Burmese python. Gene names are italicized and color-coded.

DISCUSSION

Captive-bred ball pythons have become a valuable model for studying pigmentation biology, providing unique opportunities to explore the genetics of coloration in reptiles (Brown et al., 2022; Dao et al., 2022; García-Elfring et al., 2023; García-Elfring et al., 2025; Lederer et al., 2023). This study focuses on an axanthic line to

investigate the genetic basis of yellow pigmentation in ball pythons. While yellow and red hues in vertebrates can result from dietary carotenoids or endogenous pigments, the carnivorous diet of snakes and the yellow coloration seen in wild-type ball pythons suggest that endogenous pigments are the primary source of yellow in this species. Given the normal melanin pattern in axanthic ball pythons, we hypothesized that any candidate variant would be likely to be found in a gene related to pigment biosynthesis, rather than xanthophore cell-fate specification.

Our analysis revealed a genetic variant in *gch2*, a gene known as ribA in bacteria, including *Escherichia coli*, where it was first isolated and extensively studied (Averianova et al., 2020; Richter et al., 1993). *gch2* plays a crucial role in riboflavin (vitamin B2) biosynthesis and the production of flavin nucleotide coenzymes, essential for various enzymatic reactions (Bacher et al., 2000; Ren et al., 2005). Unlike animals, bacteria are capable of synthesizing riboflavin de novo via an ancestrally conserved pathway, similar to those in plants and fungi (Anam et al., 2020; Averianova et al., 2020; Nasuno et al., 2022; Tian et al., 2022). In bacterial systems, *gch2* catalyzes the conversion of GTP to 2,5-diamino-6-ribosyl-amin o-4(3H)-pyrimidinedione 5′-phosphate (DARPP), with formate and pyrophosphate as byproducts (Averianova et al., 2020; Klein et al., 2023). In animals, although *gch2* can also catalyze this GTP-to-DARPP reaction, its physiological role is considered non-essential because the riboflavin pathway is not fully functional (García-Angulo, 2017).

The precise function of *gch2* in animals remains less clear compared to that of *GCH1*/*gch1* (*GTP cyclohydrolase I*), which is essential for the synthesis of tetrahydrobiopterin (BH4), a cofactor for enzymes involved in neurotransmitter production (e.g., dopamine, serotonin) and nitric oxide synthesis (Larbalestier et al., 2022; Lister, 2019). It also serves as a marker for the xanthophore lineage (Hamied et al., 2020; Nagao et al., 2014; Pelletier et al., 2001; Thöny et al., 2000; Wu et al., 2022; Ziegler et al., 2000). In humans, changes in *GCH1* lead to severe metabolic and neurological disorders (Fanet et al., 2021; Himmelreich et al., 2021; Longo, 2009; Nagatsu & Ichinose, 1996), such as phenylketonuria (Trujillano et al., 2014) and DOPA-responsive dystonia (Bradley et al., 2021; Hirano & Ueno, 1999; Kostić et al., 2020; Yoshino et al., 2018). *GCH1*/*gch1* and BH4 are also crucial for embryonic development, with homozygous variants being lethal in mice and zebrafish (Douglas et al., 2015; Larbalestier et al., 2022).

Although *gch2* and *GCH1*/*gch1* share similar functions, such as guanine ring opening, formate release, and zinc binding, they catalyze different reactions (Auerbach et al., 2000; Kaiser et al., 2002; Ren et al., 2005). While the role of *gch2* in vertebrate pigmentation is less understood, it has been identified as an early marker for xanthophores (Miyadai et al., 2023; Parichy et al., 2000). In zebrafish, *gch2* is essential for larval xanthophore pigmentation but not for adults (Lister, 2019). In contrast, studies in flounder show that *gch2* is required for adult melanophore and xanthophore differentiation (Watanabe et al., 2008). Similarly, in adult mutant tilapia, altered color patterns in the trunk were observed, with an irregular distribution of melanophores and xanthophores in disrupted pattern regions (Wang et al., 2021). These fish findings differ from our observations, where *gch2* sequence changes lead to pigmentation alterations without affecting the overall pattern, as seen in ball python albino mutants (Brown et al., 2022).

We discovered a nonsense variant at residue 174 (p.Arg174*), probably resulting in a non-functional protein, occurring 16 residues upstream of the conserved histidine believed to be critical for producing DARPP from GTP (Yadav et al., 2022). Our findings indicate that xanthophore pigmentation in ball pythons arises from the riboflavin biosynthesis pathway, rather than the biopterin pathway. Additionally, since loss-of-function changes in *gch1* often lead to severe developmental defects, the involvement of *gch2*—rather than *gch1*—is consistent with the viability and fertility of axanthic ball pythons. Although no obvious pleiotropy leading to large structural changes is observed, more subtle deleterious effects may occur as a result of nonfunctional *gch2*.

The extensive phenotypic variation in ball python color morphs and the wide range of color combinations breeders have produced in captivity provide an opportunity to explore and test hypotheses regarding the mechanisms behind pattern formation. Recent studies suggest that all ball pythons lack iridophores, one of the three chromatophore types (Tzika, 2024). This makes ball pythons natural analogs to Shd mutant zebrafish, which also lack iridophores (Frohnhöfer et al., 2013).

In zebrafish double mutants (Shd;Pfe), where two of the three chromatophore types (e.g., iridophores and xanthophores) are absent, the remaining chromatophore type (e.g., melanophores) uniformly covers the body, uninhibited by cell–cell interactions. Similarly, we hypothesize that the genetic basis of all-black melanic ball python morphs, such as 'Cinnamon' (Figure 1h), is probably due to genetic changes in genes essential for xanthophore development, allowing melanophores to spread across the body. This contrasts with genetic variants in melanin production genes, such as agouti, which are more common in mammals (e.g., Sasamori et al., 2017; Reissmann et al., 2020). Further support for a repulsive interaction between xanthophores and melanophores comes from the Albino form of the Cinnamon morph (Figure 1i). Instead of revealing an underlying pterin pigment pattern, which might be expected if melanism was driven by excessive melanin production, the 'Cinnamon Albino' phenotype presents as an entirely white ball python with no pterin pigmentation (Figure 1i).

Other recessive lines of axanthism exist in captive-bred ball pythons, such as the 'MJ' line. Notably, VPI Axanthic and other axanthism lines are not allelic with one another, meaning crosses between different lines result in double heterozygotes with wildtype coloration, suggesting distinct genetic causes for each axanthism lineage. These various axanthism lines, when combined with other color variants (Figure 1d), provide valuable models tofurther explore the pterin pigment biosynthesis pathway and identify new axanthism-causing genes in reptiles. Consistent with our findings, we demonstrated in the VPI lineage that pterin pigment synthesis, rather than carotenoid metabolism, is associated with yellow coloration in ball pythons.

In summary, our study highlights a significant link between *gch2* and axanthism in ball pythons, suggesting that the initial stages of the ancestral riboflavin pathway are crucial for yellow pigmentation. Further research is necessary to identify the specific xanthophore pigments involved in ball python pigmentation. Nonetheless, our findings enhance the understanding of the genetics of pigmentation in reptiles.

CONCLUSION

We used ball python color morphs to study the genetic basis of xanthophore pigmentation in snakes. We found that axanthism is associated with a key gene in the folate biosynthesis pathway, providing the first evidence that pterins, not carotenoids, produce the yellow pigment observed in ball pythons. To our knowledge, *gch2* is the first axanthism-associated gene identified in vertebrates. Our results provide new research avenues for the study of axanthism and xanthophore pigmentation in snakes and highlights the utility of using captive-bred ball pythons in pigmentation research.

AUTHOR CONTRIBUTIONS

Alan García-Elfring: Conceptualization; formal analysis; investigation; methodology; project administration; writing – original draft. **Heather L. Roffey:** Data curation; resources; validation; writing – review and editing. **Jaren M. Abergas:** Methodology; writing – review and editing. **Andrew P. Hendry:** Conceptualization; funding acquisition; writing – review and editing. **Rowan D. H. Barrett:** Conceptualization; funding acquisition; project administration; resources; supervision; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this manuscript have been uploaded to the NCBI Sequence Read Archive (SRA) with the accession PRJNA1122856.

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SUPPORTING INFORMATION

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