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Red carotenoids and associated gene expression explain colour variation in frillneck lizards

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A long-standing hypothesis in evolutionary ecology is that red–orange ornamental colours reliably signal individual quality owing to limited dietary availability of carotenoids and metabolic costs associated with their production, such as the bioconversion of dietary yellow carotenoids to red ketocarotenoids. However, in ectothermic vertebrates, these colours can also be produced by self-synthesized pteridine pigments. As a consequence, the relative ratio of pigment types and their biochemical and genetic basis have implications for the costs and information content of colour signals; yet they remain poorly known in most taxonomic groups. We tested whether red- and yellow-frilled populations of the frillneck lizard, *Chlamydosaurus kingii*, differ in the ratio of different biochemical classes of carotenoid and pteridine pigments, and examined associated differences in gene expression. We found that, unlike other squamate reptiles, red hues derive from a higher proportion of ketocarotenoids relative to both dietary yellow carotenoids and to pteridines. Whereas red frill skin showed higher expression of several genes associated with carotenoid metabolism, yellow frill skin showed higher expression of genes associated with steroid hormones. Based on the different mechanisms underlying red and yellow signals, we hypothesize that frill colour conveys different information in the two populations. More generally, the data expand our knowledge of the genetic and biochemical basis of colour signals in vertebrates.

1. Background

Carotenoid-based coloration is a frequently cited example of a condition-dependent trait, which honestly signals individual quality. The main hypothesized mechanisms maintaining honesty are limited availability, because animals acquire carotenoids through diet, and trade-offs in allocation to ornamentation versus other physiological functions [1–3]. However, some carotenoid metabolites are more likely to reflect condition than others [4]. For example, red ketocarotenoids (carotenoids containing a ketone group, e.g. astaxanthin, canthaxanthin) are rare in the diets of terrestrial species [5–7], though abundant in the marine environment where they are synthesized by microalgae. In some vertebrates, such as birds and turtles, ketocarotenoids are produced by conversion of dietary carotenoids through addition of a double-bonded oxygen (ketolation), a process that may be energetically costly owing to inefficiencies and energy requirements of the conversion process [8,9]. Additionally, allocation trade-offs may be more likely for carotenoids that serve as precursors (i.e. provitamins, e.g. α-carotene, β-carotene, γ-carotene and β-cryptoxanthin) for vitamin A (retinol) [10,11], which plays a role in numerous cellular processes (but see [12,13]). Identifying the specific metabolites used for colour production and their relative levels (rather than total carotenoids) is therefore essential to

understand the potential costs and information content of animal ornaments, but is seldom done.

Although carotenoids are the primary class of pigment generating yellow to red colours in birds, pteridines are the most abundant and widespread class of pigment generating these colours in arthropods and ectothermic vertebrates (fish, amphibians and non-avian reptiles [14]). Unlike environmentally acquired carotenoids, pteridine production appears to be primarily genetically determined. For example, in guppies (*Poecilia reticulata*), environmental variation in carotenoid availability correlates with genetically based differences among populations in drosopterin production, enabling individuals to maintain a constant ratio between the two pigment types, and therefore a constant hue [15,16]. Coloured pteridines within pigment cells (sepiapterin, drosopterin and their derivatives) are synthesized *in situ* within pterinosomes [14,17] from the precursor guanosine triphosphate (GTP). GTP is an abundant cellular building block, including in RNA synthesis, cellular energy transfer and as a precursor to colourless pteridines, which are common cofactors in enzymatic catalysis [18]. Colourless pteridines accumulate in pigment cells in large quantities that greatly exceed their need as an enzymatic cofactor [17], suggesting that GTP and costs of synthesizing coloured pteridines are unlikely to be limiting. Therefore, the expression of pteridine-based ornamental colours is unlikely to be limited by availability or allocation trade-offs, and mechanisms maintaining the honesty of pteridine signals remain poorly understood.

Squamate reptiles (lizards and snakes) produce yellow to red colours using both carotenoids and pteridines, and dietary carotenoids (e.g. lutein, β-carotene) have been linked to individual quality in several species. For example, brighter yellow/orange coloration is associated with higher body condition and lower parasite load in male lacertids [19,20] and female striped plateau lizards [21]. In most squamates studied to date, drosopterin has been identified as the primary pigment generating red and orange colours, sometimes in combination with yellow carotenoids [22–31]. However, few studies have quantified the relative levels of different pigment classes or individual metabolites (but see [30,31]). Ketocarotenoids (primarily astaxanthin and canthaxanthin) have so far been detected in only four lizard species to our knowledge [30,32,33], and in the skin of only two species: *Lacerta vivipara* (Lacertidae [32]) and *Ctenophorus decresii* (Agamidae [30]). Interestingly, the gene CYP2J19, which has recently been implicated to encode the carotenoid ketolase in birds and turtles, is absent in squamates, tuataras and crocodilians [34–36]. This ketolation enzyme probably arose in the common ancestor of birds and turtles, and functioned in colour vision (red retinal oil droplets), but was subsequently repeatedly recruited for integumental coloration [36]. Red retinal oil droplets occur in birds and turtles, but not in other tetrapods, and it is currently unknown whether squamate reptiles use alternative carotenoid ketolation enzymes (and underlying genes). More generally, reptiles represent a major gap in our understanding of the genetic and biochemical basis of colour signals among major vertebrate lineages.

Here, we elucidate the mechanisms underlying colour variation in the iconic frillneck lizard, *Chlamydosaurus kingii*. Specifically, we compare relative levels of specific metabolites and differences in gene expression in red or yellow and grey–brown (hereafter referred to as 'dull') frill skin of two populations that differ in frill colour. The frillneck lizard has one of the largest ornaments relative to body size of any vertebrate [37]. The frill comprises folds of skin around the neck that are raised by the hyoid cartilage to make the lizard appear larger and produce an impressive startle display to ward off predators [37]. Both males and females have similarly coloured frills, but the frills of males are generally larger (as males have larger heads) and prominently displayed during contests [37,38], indicating that frill colour may also convey information on individual quality to conspecifics. Frills have a combination of different colour patches and frill colour varies clinally from red in the west to yellow or white in the east (figure 1). Previous work indicates that carotenoid levels in frill skin decrease from west to east [39], but how populations differ in the ratio of specific metabolites, their genetic basis and implications for colour expression remain unknown. Based on the mechanism underlying colour variation revealed by this study, we develop testable hypotheses regarding costs and information content of red and yellow coloration in frillneck lizards.

2. Methods

(a) Sample collection

Skin samples from 24 adult frillneck lizards (electronic supplementary material, table S1) were collected non-destructively from populations around Wyndham (15.49° S, 128.12° E) and Kununurra (15.87° S, 128.79° E) in Western Australia (red-frilled west form), and Townsville (19.26° S, 146.82° E) and Cairns (16.99° S, 145.48° E) in Queensland (yellow-frilled east form; figure 1), between December 2013 and February 2015. Given that males and females do not differ in coloration [39,40], we included samples from both sexes for pigment identification (18 males and 6 females); however, only males were used for gene expression analysis (6 males). For each lizard, we collected red or yellow and dull (brown–grey) skin from the frill using a hole punch (approx. 5 mm diameter). Tissues for metabolomic analysis were either stored in methanol at −20°C or foil-wrapped at −80°C (electronic supplementary material, table S1), while tissues for transcriptomic analysis were stored in RNAlater (Ambion, Austin, TX, USA) at −20°C. We also measured reflectance of the red/yellow and dull patches of the frills of 18 lizards (7 from Kununurra and 11 from Cairns) using an Ocean Optics (Dunedin, FL, USA) Jaz portable spectrometer (see electronic supplementary material, Methods).

(b) Pteridine and carotenoid pigment identification

To identify the specific metabolites responsible for red–yellow coloration in *C. kingii*, we extracted pigments from five samples of each of west form red skin, west form dull skin, east form yellow skin and east form dull skin (electronic supplementary material, table S1). We used a sequential carotenoid and pteridine pigment extraction procedure which was previously developed for lizard skin [30]. In brief, samples were weighed and homogenized and the resulting carotenoid extract was collected following centrifugation, before pteridines were extracted from the tissue pellet. The two pigment classes were semi-quantified in separate LC-MS analyses on an Agilent 6490 triple quadrupole MS system coupled to an Agilent 1290 series LC system (Agilent Technologies Inc., Santa Clara, CA, USA). Data analysis was conducted using the Agilent MassHunter Workstation software (v. B.07.00) and peak assignments were matched against commercial or purified standards (canthaxanthin, β-carotene, β-cryptoxanthin,

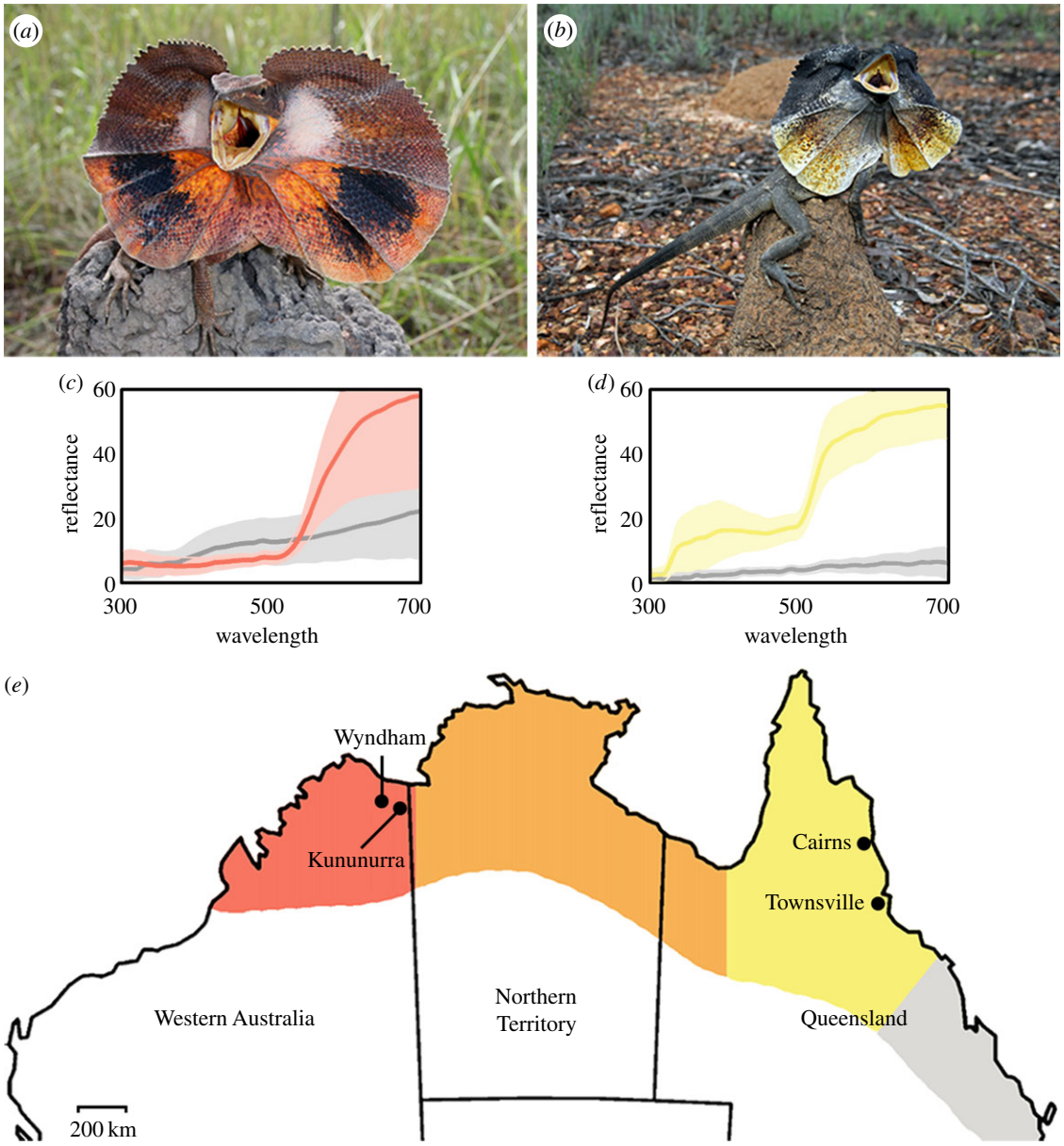

Figure 1. Colour variation in the frillneck lizard, *C. kingii*. (*e*) Frill colour varies from red in the west, through orange to yellow and white in the east. We sampled (*a*) western red-frilled lizards from populations around Wyndham and Kununurra in Western Australia and (*b*) eastern yellow-frilled lizards from populations around Cairns and Townsville in Queensland. (*c*,*d*) We also measured spectral reflectance of red/yellow and dull (grey–brown) frill colour patches in the two forms (data are mean ± s.d. reflectance; west: $n = 14$, east: $n = 21$).

3′-dehydrolutein, astaxanthin, lutein and zeaxanthin, xanthopterin, isoxanthopterin, pterin, pterine-6-carboxylic acid, 6-biopterin, sepiapterin and drosopterin; see electronic supplementary material, Methods).

Peak signals obtained from the LC-MS analysis were normalized to the weight (as a proxy for biomass) of the skin tissue extracted. We compared these relative responses of individual pigments and the relative proportions of pigment groups (all carotenoids, coloured pteridines, ketocarotenoids, provitamin A carotenoids) among skin colours and sexes using two-way ANOVAs and Tukey's *post hoc* tests performed in the stats R package [41]. Data were log-transformed prior to analysis to meet model assumptions.

(c) Transcriptome sequencing, filtering and assembly

RNA was extracted from red/yellow and dull skin for three west form and three east form males (12 samples total; electronic supplementary material, table S1) and sequenced on the Illumina NextSeq 500 sequencing platform (Illumina, San Diego, CA, USA). Following quality filtering and contaminant read removal, transcriptomes were de novo assembled using Trinity v. 2.0.6 [42,43] (electronic supplementary material, table S8), and assessed for completeness using BUSCO v. 1.1b [44] (electronic supplementary material, table S9).

(d) Abundance estimation, differential expression and functional annotation

We combined the assembled transcriptomes of one east form (T39) and one west form (W83) individual and compared them to a non-redundant protein database of the central bearded dragon, *Pogona vitticeps*, the closest con-familial species for which a genome is currently available [45]. This produced a reference for the subsequent differential expression analysis consisting of 15 074 *C. kingii* sequences corresponding to annotated *P. vitticeps* protein coding regions.

To calculate the relative expression levels of genes (in transcripts per million reads; TPM), we used RSEM [46] and Bowtie 2 v. 2.2.2 [47] to map the trimmed and filtered reads for

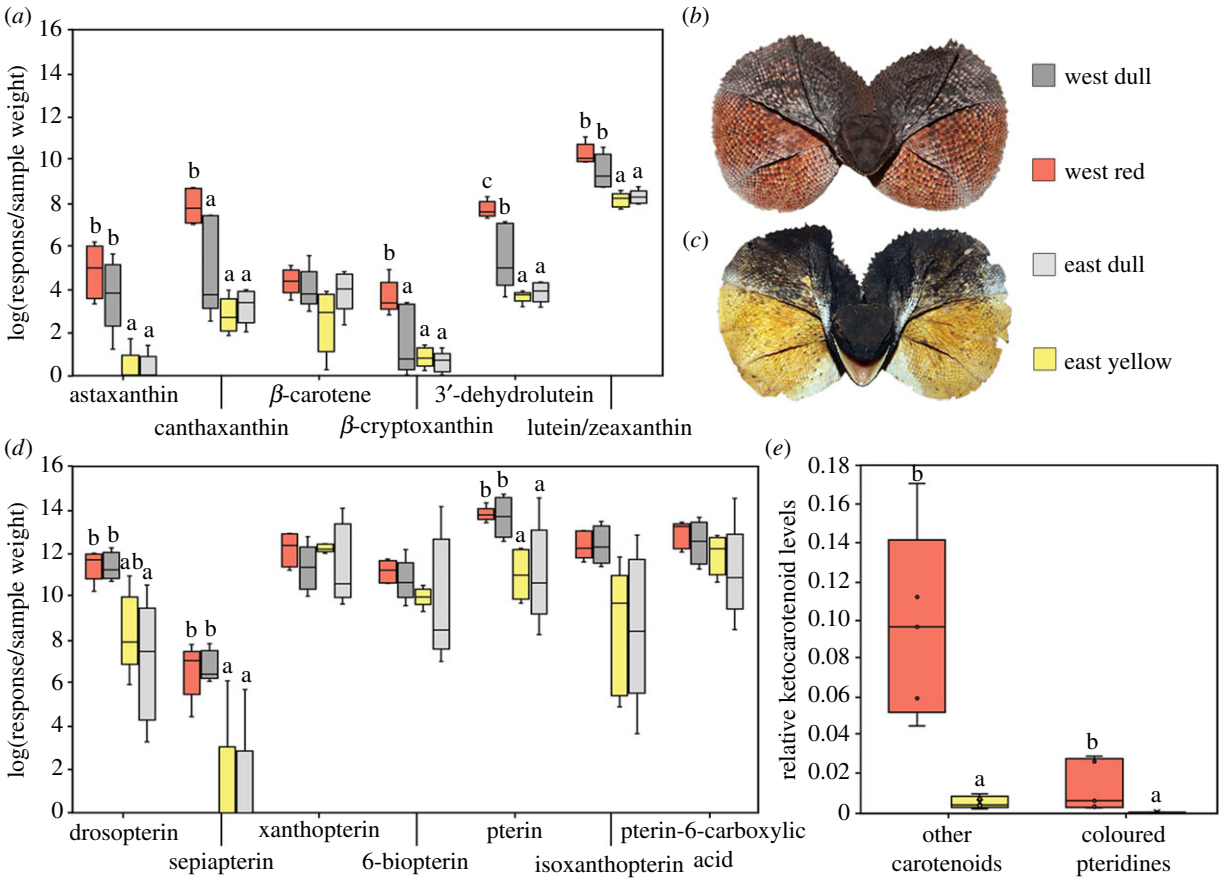

Figure 2. Variation in pigment levels between forms and skin colours. (*a*) Carotenoid and (*d*) pteridine pigment levels in red/yellow and dull skin in the (*b*) western red-frilled and (*c*) eastern yellow-frilled forms. (*e*) Red and yellow skin also differ in the levels of red ketocarotenoids relative to other carotenoids and coloured pteridines. Letters indicate significant differences between skin colours for a given pigment identified from Tukey's pairwise comparisons.

each transcriptome to the reference. Gene expression was analysed using edgeR [48]. We conservatively considered genes to be differentially expressed when the false discovery rate corrected *p*-value was less than 0.05 and expression values did not overlap between the two groups compared. Genes differentially expressed between west form red and east form yellow skin were further functionally annotated using Blast2Go v. 5.0.22 [49]. Detailed molecular methods are provided in electronic supplementary material.

3. Results

(a) Carotenoid and pteridine pigment levels

We found that both carotenoid and pteridine pigments were present in both the western red-frilled and eastern yellow-frilled form of *C. kingii* (figures 1 and 2; electronic supplementary material, figure S1 and table S2). Males and females did not differ in pigment levels (all $p > 0.05$; electronic supplementary material, tables S2 and S4). However, when compared with east form skin, west form red skin had significantly higher levels of the ketocarotenoids astaxanthin and canthaxanthin, the provitamin A carotenoid β-cryptoxanthin and the yellow carotenoids 3′-dehydrolutein and lutein/zeaxanthin (all $p < 0.05$; electronic supplementary material, table S3). West form red skin also had higher levels of the pteridines sepiapterin (yellow; all $p < 0.05$), pterin (colourless; all $p < 0.05$) and drosopterin (red; all $p < 0.05$, but red skin versus yellow skin, $p = 0.057$; electronic supplementary material, table S3) than east form skin. In addition to absolute levels, the ratio of red ketocarotenoids (astaxanthin and canthaxanthin) relative to other carotenoids ($p = 0.001$) and ketocarotenoids to coloured pteridines ($p = 0.001$; figure 2) was higher in west form red than east form yellow skin, while the ratio of total carotenoids to total coloured pteridines did not significantly differ (electronic supplementary material, tables S4 and S5).

Overall, there was little difference in carotenoid and pteridine pigment levels between red/yellow and dull skin within populations (electronic supplementary material, figure S1). Colour differences between frill colour patches are instead likely driven by variation in melanin pigment levels and/or structural components in the skin, which were not quantified in this study. However, within the western population, red skin had higher levels of canthaxanthin, 3′-dehydrolutein and β-cryptoxanthin than dull skin (all $p < 0.05$; electronic supplementary material, table S3), while there were no differences in carotenoid or pteridine pigment levels between yellow and dull skin in the eastern population (electronic supplementary material, table S3).

(b) Differential gene expression between populations

Our gene expression analysis revealed 471 genes that were differentially expressed between geographical colour forms and/or skin colours (table 1; electronic supplementary material, figure S1 and table S6). Of these, 117 genes were differentially expressed when comparing west form red skin to east form yellow skin, and thus may play a role in red versus yellow colour production in *C. kingii*. We investigated these genes for previous links to chromatophore cells [50],

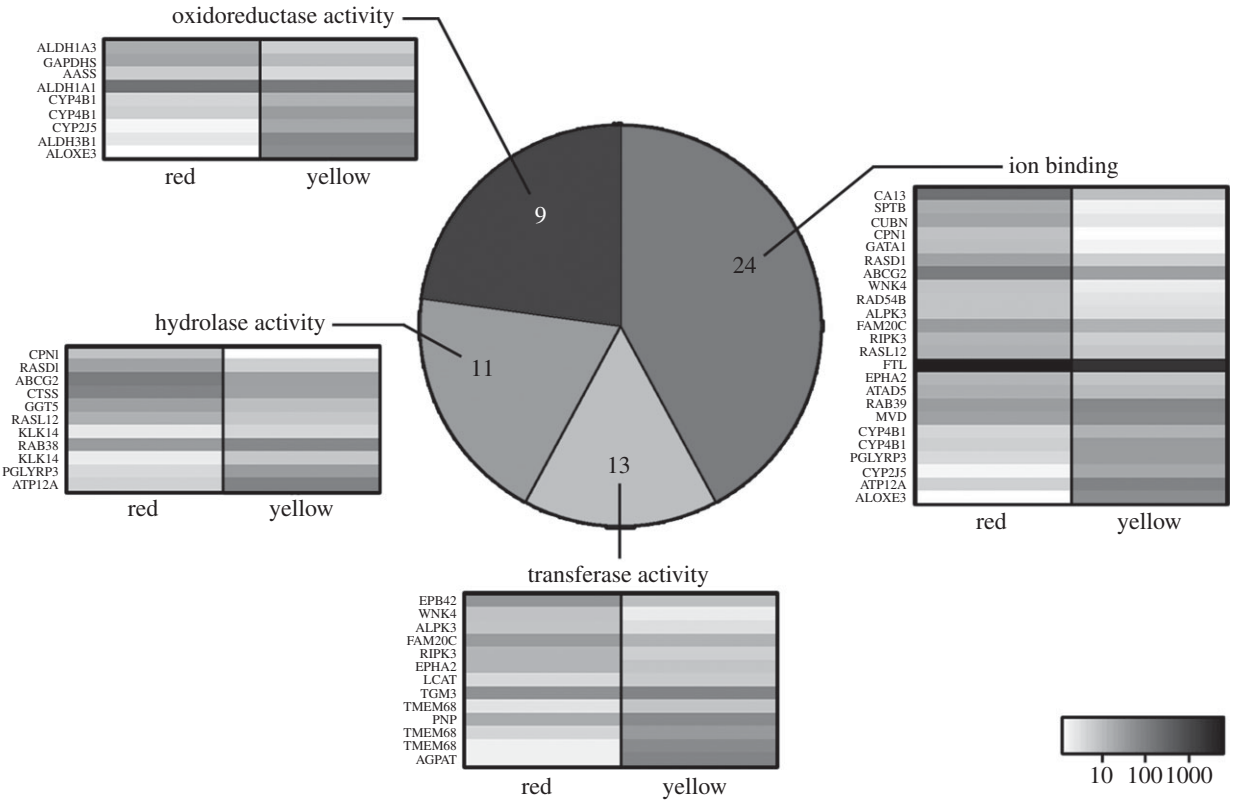

Figure 3. Molecular function annotation of genes differentially expressed between west form red and east form yellow skin. Results of Blast2Go analysis showing the number of sequences annotated for the molecular function gene ontology (GO) domain. Heatmaps show gene expression in TPM for each of the annotated sequences for red and yellow skin.

melanin and pteridine pigment synthesis pathways [51–53], and/or carotenoid pigment metabolism, transport and storage (electronic supplementary material, figure S2; [10,54–56]). Functional annotation analysis classified 104 of these genes (88.9%) into the three main gene ontology (GO) domains (biological processes, molecular function, cellular component; figure 3; electronic supplementary material, figure S3).

Sixty-five genes were more highly expressed in red skin than yellow skin. These included two genes, ALDH1A1 and ALDH1A3, involved in provitamin A carotenoid metabolism, and a PTS-like gene, involved in the pteridine synthesis pathway (table 1; electronic supplementary material, figure S2). Conversely, 52 genes were more highly expressed in yellow skin relative to red skin. One of these genes (RDH7) may also be involved in provitamin A carotenoid metabolism. RDH7 converts retinol (vitamin A) into retinal (the precursor to retinoic acid; electronic supplementary material, figure S2); however, this gene is also known to be involved in androgen metabolism [57]. In fact, yellow skin exhibited higher expression for a number of genes associated with steroid hormones, including RDH7, CYP2J5, MVD, PLA2G4E and SULT2B1 (table 1).

As well as carotenoid- and pteridine-linked genes, we identified a number of differentially expressed genes previously associated with iridophore and/or melanophore cells (higher in red: ABCG2, AGTR1, CTSS, FAM20C and MLANA; higher in yellow: FZD8 and RAB38 [50]), as well as genes linked to the pteridine precursor purine (higher in red: ALPK3; higher in yellow: ACPP, ATP12A and PNP) and the melanin precursor tyrosine (higher in red: EPHA2 and GATA1; higher in yellow: ALDH3B1; table 1). Six of these colour- or steroid-hormone-associated genes have previously been found to be differentially expressed between skin colours of the con-familial tawny dragon, *C. decresii* (ALDH1A3, ALPK3, ATP12A, MLANA, PLA2G4E, SULT2B1; electronic supplementary material, table S6 [30]), in addition to nine genes (BTC, CYP4B1, FTL, GJA5, HEPACAM, MR1, RAD54B, TMEM45B, ZIC1; electronic supplementary material, table S6) with no known role in colour production and which may be promising candidate genes for future study.

(c) Differential gene expression between red or yellow and dull skin

Five genes were more highly expressed in both west form red and east form yellow skin relative to dull skin (BARX1, GPR37 L, PTS, SIM2, SRD5A2; electronic supplementary material, table S6). Most notably, these included a second PTS-like gene associated with the pteridine synthesis pathway. This was unexpected given that the levels of sepiapterin and drosopterin did not differ between red/yellow and dull skin (figure 2), indicating that red/yellow may be producing higher levels of 6-pyruvoyltetrahydropterin, but not the subsequent coloured pigments. Alternatively, this PTS-like gene may mediate a different step in the pteridine synthesis pathway. For example, in cyanobacteria, a PTS orthologue is involved in a salvage pathway in which sepiapterin is converted to 7,8-dihydrobiopterin [58]. Salvaging sepiapterin facilitates greater production of tetrahydrobiopterin, which is essential for the synthesis of neurotransmitters and tyrosine (the precursor to melanin; electronic supplementary material, figure S2). Two other genes with higher expression in red/yellow than dull skin were SRD5A2, which catalyses the conversion of testosterone to dihydrotestosterone in certain tissues, including skin [59], and BARX1, which has no known role in colour

Table 1. Genes differentially expressed between frillneck lizard skin colours with a previous link to colour production or steroid hormone pathways.

gene name	full name	association	gene expression pattern
ABCG2	ATP-binding cassette subfamily G member 2-like	melanophore	red > yellow
AGTR1	type-1 angiotensin II receptor	iridophore	red > yellow
ALDH1A1	aldehyde dehydrogenase family 1 member A1	provitamin A carotenoid metabolism	red > yellow
ALDH1A3	aldehyde dehydrogenase family 1 member A3	provitamin A carotenoid metabolism	red > yellow
ALPK3	α-protein kinase 3-like	purine	red > yellow
CTSS	cathepsin S	iridophore	red > yellow
EPHA2	ephrin type-A receptor 2	tyrosine	red > yellow
FAM20C	extracellular serine/threonine protein kinase FAM20C	iridophore, melanophore	red > yellow
GATA1	erythroid transcription factor	tyrosine	red > yellow
MLANA	melanoma antigen recognized by T-cells 1	melanin synthesis	red > yellow
PTS	6-pyruvoyl tetrahydrobiopterin synthase-like	pteridine synthesis	red > yellow
ACPP	prostatic acid phosphatase-like	purine	yellow > red
ALDH3B1	aldehyde dehydrogenase family 3 member B1	tyrosine	yellow > red
ATP12A	potassium-transporting ATPase α chain 2	purine	yellow > red
CYP2J5	cytochrome P450 2J5-like	steroid hormone	yellow > red
FZD8	frizzled-8	melanin synthesis	yellow > red
MVD	diphosphomevalonate decarboxylase	steroid hormone	yellow > red
PLA2G4E	cytosolic phospholipase A2 epsilon-like	steroid hormone	yellow > red
PNP	purine nucleoside phosphorylase-like	purine	yellow > red
RAB38	ras-related protein Rab-38	iridophore	yellow > red
RDH7	retinol dehydrogenase 7-like	provitamin A carotenoid metabolism	yellow > red
SULT2B1	sulfotransferase family cytosolic 2B member 1	steroid hormone	yellow > red
PTS	6-pyruvoyl tetrahydrobiopterin synthase-like	pteridine synthesis	yellow/red > dull[a]
SRD5A2	3-oxo-5-α-steroid 4-dehydrogenase 2	steroid hormone	yellow/red > dull[a]
AHRR	aryl hydrocarbon receptor repressor	melanin synthesis	dull > yellow/red[a]

[a]Within-population differences between colour patches.

production; however, both of these genes have been previously associated with skin coloration in *C. decresii* [30].

4. Discussion

In squamate reptiles studied to date, red–orange skin coloration is produced by pteridines alone (xanthopterin and/or drosopterin [25,26,29]) or by the combination of drosopterin and dietary yellow carotenoids [22–24,28,31,60,61]. The one exception is the European common lizard, *L. vivipara*, in which yellow–orange coloration appears to be produced exclusively by carotenoids [32,62]. Here, we show that both the red skin and dull skin of western populations have higher levels of both carotenoid and pteridine pigments than eastern skin, consistent with previous work [39]. However, unlike other lizards, the redder hues of western frills are owing to a greater proportion of ketocarotenoids relative to either dietary yellow carotenoids or coloured pteridines, including drosopterin. A high proportion of ketocarotenoids, despite their lower dietary availability in terrestrial environments, could suggest bioconversion by carotenoid ketolation. This, in turn, would indicate the existence of an alternative ketolation enzyme to the carotenoid ketolase identified in birds and turtles encoded by the CYP2J19 gene, which is absent in squamates [34–36]. Our results indicate that ketolation (which often occurs in the liver rather than, or in addition to, the integument [63–65]) warrants further investigation in reptiles, although a dietary source of ketocarotenoids remains a possibility.

The higher levels of carotenoid and pteridine pigments in western populations were underscored by differences in the expression of a number of colour-associated genes. Most notably, western frills had higher levels of drosopterin (red) and sepiapterin (yellow), and higher expression of 65 genes, including a PTS-like gene. PTS converts 7,8-dihydroneopterin triphosphate to 6-pyruvoyltetrahydropterin (the precursor to sepiapterin and drosopterin; electronic supplementary material, figure S2), potentially indicating that this is a rate-limiting step in the pteridine synthesis pathway. This gene has also been implicated in colour variation in the common wall lizard, *Podarcis muralis*, in which the presence or absence of yellow coloration is associated with differential expression of PTS [66]. In comparison to synthesized pigments (melanins and pteridines), relatively little is known about the genetics of carotenoid-based coloration; however, the majority of research has focused on the conversion of provitamin A carotenoids to vitamin A (retinol) [10,11]. This

pathway is of particular interest because vitamin A regulates multiple physiological processes, including growth, development and immune function. Two genes more highly expressed in red skin relative to other skin colours were ALDH1A1 and ALDH1A3, which convert retinal to retinoic acid (electronic supplementary material, figure S2), a metabolite of retinol (vitamin A) that mediates the function of vitamin A for growth and development [67]. Therefore, differential expression of these genes may indicate that the yellow and red colour forms of *C. kingii* differ in retinoid levels and activity.

The higher carotenoid levels (both red and yellow) in western than eastern populations could be owing to geographical variation in carotenoid availability and/or diet across the species's range. Carotenoids are produced by plants and obtained indirectly by *C. kingii* through a diet consisting primarily of insects such as grasshoppers and termites, and occasionally small vertebrates [40]. The most dominant carotenoid in angiosperms is lutein (yellow), whereas ketocarotenoids (red) are rare or absent (primarily produced by microalgae and yeast), and insect herbivores generally sequester carotenoids in proportion to the concentration found in the diet [68]. The higher relative proportion of ketocarotenoids to lutein/zeaxanthin in the western population suggests that selection on signal colour and underlying pigment metabolism may differ between the two forms. Western individuals may allocate more carotenoids to the integument and/or assimilate carotenoids more efficiently. Therefore, selection may lead to better allocation of carotenoids to signalling function, as in kokanee salmon (*Oncorhynchus nerka*), where strong sexual selection for red breeding coloration has led to the repeated evolution of more efficient carotenoid assimilation to compensate for low environmental carotenoid availability in oligotrophic lakes [69]. Similarly, higher carotenoid levels in western frillneck populations could reflect selection for improved carotenoid assimilation efficiency rather than environmental availability.

We found higher expression of genes involved in provitamin A carotenoid metabolism in the western red-frilled form, but higher expression of genes associated with steroid hormones in the eastern yellow-frilled form. Colour polymorphic populations of another agamid lizard, the tawny dragon (*C. decresii*), similarly show higher expression of genes associated with steroid hormones in yellow than orange skin [30], suggesting that yellow coloration in particular may be hormonally regulated. We also found higher expression of the SRD5A2 gene (which catalyses the conversion of testosterone to dihydrotestosterone [59]) in red/yellow than in dull frill skin in both populations. This is consistent with sex steroid-mediated colour expression more generally, which is well documented in reptiles [70–72].

The differences in pigment levels and gene expression between populations lead us to hypothesize that the information signalled by frill colour differs between the western and eastern forms. Current evidence indicates that red coloration is a more reliable indicator of various measures of individual quality than yellow coloration [4], probably owing to limited availability and/or metabolic costs of bioconversion. We hypothesize that in frillneck lizards, red frill coloration is more likely to convey information on individual health than yellow coloration owing to higher levels of both ketocarotenoids and yellow dietary carotenoids, together with upregulated provitamin A carotenoid metabolism genes. However, yellow frill coloration could convey information on other aspects of individual quality, such as dominance and aggression, given the links with genes associated with steroid hormones. Testing this hypothesis requires physiological and behavioural experiments. Nevertheless, data presented here provide a basis for further understanding the genetic control, physiological costs, information content and evolution of colour signals in frillneck lizards and squamate reptiles more generally.

Ethics. Fieldwork and procedures were conducted with approval from The Australian National University Ethics Committee (A2011/65), the Western Australian Department of Parks and Wildlife (SF010097) and the Queensland Department of Heritage Protection (WISP12200212).

Data accessibility. Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.3v3m004 [73]. RNA sequence data are available at the National Center for Biotechnology Information Sequence Read Archive: PRJNA542730, SAMN11637615-26.

Authors' contributions. C.A.M., A.M. and D.S.-F. designed the study. C.A.M. performed the transcriptomic analysis, analysed and interpreted the data and wrote the manuscript. A.L. and K.J.R. designed and performed the metabolomic analysis. A.E. collected skin samples, spectral reflectance measurements and photographs. A.M. and D.S.-F. conceived of the study and critically revised the manuscript. All authors read, edited and approved the final manuscript.

Competing interests. The authors declare that they have no competing interests.

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