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Expression of *SCARB1* in feather follicles is necessary but not sufficient for carotenoid-based feather pigmentation in Gouldian finches

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Carotenoids play fundamental roles in avian ecology and evolution. Full expression of carotenoid-based plumage relies on key molecular mechanisms that regulate the uptake, processing and deposition of dietary pigments into target tissues. The *blue* Gouldian finch (*Erythrura gouldiae*) variety is explained by an autosomal recessive locus that affects carotenoid coloration. Using whole-genome sequencing data and genetic mapping, we identified a splice-acceptor mutation in scavenger receptor B1 (*SCARB1*) that abolishes carotenoid-based pigmentation. Biochemical analyses revealed that *blue* individuals circulate extremely low concentrations of dietary carotenoids, confirming a genetic disruption of carotenoid uptake and transport in the gut. Through genotyping of the candidate causative mutation in carotenoid-containing and carotenoid-free mask feathers of a mosaic *blue* individual, we further demonstrate that somatic reversion of *blue* rescues *SCARB1* function and locally restores carotenoid deposition in feathers. Finally, by comparing the transcriptomes of carotenoid-containing and carotenoid-free feather follicles from different plumage regions of wild-type birds, we show that *SCARB1* expression alone is not sufficient to trigger carotenoid pigmentation. These findings reinforce the role of *SCARB1* as a key facilitator of carotenoid uptake and transport in the gut, while also establishing its necessity for localized uptake of carotenoids to developing feather follicles.

1. Background

Carotenoids are the second most common pigment in avian integuments and serve fundamental functions in bird vision, physiology and communication. To generate the variety of yellow-to-red hues that embellish their plumage and bare parts, birds must source carotenoids from their diet [1,2]. While some species express unmodified dietary carotenoids [3,4], multiple others have independently evolved the biochemical machinery to modify these molecules [5–8], which are then incorporated into target tissues. Thus, variation in carotenoid-based phenotypes is influenced not only by dietary sources but also by the molecular mechanisms that regulate the uptake, metabolism and deposition of these pigments.

Recent advances have contributed to understanding the origin and diversification of carotenoid coloration [9,10]. In particular, the discovery of *CYP2J19* and *BDH1L*, two crucial enzymes for carotenoid metabolism, has provided important insights into the evolution of red plumage and colour vision across multiple species [11–17] (reviewed in [9,18]). This, alongside the characterization of *TTC39B*, a ketocarotenoid conversion enhancer, established a simple metabolic route for ketocarotenoid synthesis [19]. Variation in cleavage enzyme *BCO2* has been frequently associated with pigmentation loss through increased carotenoid degradation, and has been linked to the evolution of sexual dichromatism [20,21]. Lastly, lipoprotein receptor *SCARB1* was implicated in dietary carotenoid uptake and transport in canaries [22]. Despite these breakthroughs, how dietary and metabolized carotenoids are selectively transferred to developing feathers is still poorly understood.

Carotenoid pigments contribute substantially to the remarkable colour diversity of Gouldian finches (*Erythrura gouldiae*). Extensive selective breeding has led to the emergence of multiple colour varieties, such as the Mendelian recessive *blue* mutation, which inhibits the overall expression of carotenoids. *Blue* Gouldian finches exhibit beige head and off-white belly feathers, combined with structural blue colour in the back, which would otherwise appear green in the presence of yellow carotenoids (figure 1*a*). Similar to the *white recessive* canary [22], this mutation is thought to affect carotenoid uptake in the gut. Thus, the Gouldian finch *blue* mutation offers an independent system to explore the genetic mechanisms of carotenoid uptake and feather deposition, and provides an ideal opportunity to examine the evolutionary repeatability of carotenoid-based colouration.

Here, we determine the genetic basis of the *blue* phenotype. Specifically, we compared the genomes of wild-type and *blue* individuals to identify candidate genomic regions and genes underlying the loss of carotenoid pigmentation. We then used biochemical analyses to confirm whether the *blue* phenotype is associated with a heritable defect in carotenoid uptake or transport, as described for the *white recessive* canary breed. Finally, we demonstrate that carotenoid deposition can be restored *in loco* by reverting the candidate causative mutation in restricted feather follicles. Our findings improve our understanding of carotenoid incorporation into feathers and the evolutionary constraints of carotenoid pigmentation in birds.

2. Material and methods

A full description of laboratory procedures and analyses is given in the electronic supplementary materials.

(a) Whole-genome resequencing and read mapping

Blood samples from 49 wild-type (*non-blue*) and 11 *blue* adult Gouldian finches (N_{total} = 60) were collected from licensed Portuguese breeders and stored in 96% ethanol at −20°C. Genomic DNA extraction, Illumina library preparation and sequencing were performed as described in [23]. Following quality filtering, reads were mapped to the Gouldian finch reference genome with *BWA-MEM* [24]. Technical duplicates were removed for downstream analysis.

(b) Genetic mapping of the *blue* locus and variant discovery

Prior to genetic mapping, we investigated patterns of population substructure with principal component analysis (PCA) using *ngsPopGen* [25]. Next, genetic differentiation (F_{ST}) and nucleotide diversity (π) between wild-type and *blue* individuals were summarized in sliding windows of 20 kb using *ANGSD* [26]. We also estimated the relatedness index (r_{xy}) between all possible pairs of individuals as implemented in *NgsRelate* [27]. To refine the region associated with the *blue* locus, we selected 40 random polymorphic sites (SNPs) encompassing the candidate genomic region and performed identical-by-descent (IBD) mapping using Sequenom's iPlex technology. The full sample set was genotyped (n = 60 birds). To further pinpoint candidate causative mutations, we performed SNP calling around the candidate genomic region using *Freebayes* [28]. Resulting variants were functionally annotated with the *SnpEff* [29] toolbox and screened for substitutions with potential impact on protein function.

(c) Analyses of plasma carotenoid content

Plasma was collected from four wild-type and four *blue* individuals for carotenoid content analyses. Samples were kept at −20°C away from light and under a nitrogen atmosphere to prevent degradation. Pigments were extracted using ethanol (1 : 10, v/v) and a hexane : tert-butyl methyl ether (TBME) mixture (1 : 1, v/v), followed by sonication and centrifugation. Extracts were analysed using an ultra-high performance liquid chromatography (UHPLC) instrument equipped with a photodiode array detector. Pigment identities were confirmed through high-resolution accurate-mass/mass spectrometry (HRAM/MS) and compared to analytical standards.

(d) Amplicon sequencing of the *SCARB1* candidate causative mutation

We extracted DNA from beige (carotenoid-free) and red (carotenoid-containing) feathers that co-occurred within the mask of a mosaic individual and prepared amplicon libraries for targeted Illumina sequencing. Primers were designed to target *SCARB1* exon 4 and modified to allow individual barcoding. The resulting reads were screened for wild-type and mutant alleles at the candidate splice-site mutation.

(e) Gene expression in regenerating feather follicles

Total RNA was isolated from regenerating feather follicles of carotenoid-containing (back; n = 4) and carotenoid-free (chest; n = 4) plumage regions from four wild-type individuals. Libraries were prepared with Illumina TruSeq Stranded Total RNA Library prep kit and sequenced in an Illumina NovaSeq 6000 using 150 bp paired-end reads. Reads were checked for quality and pre-processed prior to reference mapping. Gene expression analysis was conducted using *DESeq2 v1.39.3* [30].

3. Results

(a) Genetic mapping of the *blue* locus

Selective breeding for traits that deviate from the wild-type has led to the fixation of a spontaneous mutation in domesticated Gouldian finches, resulting in the *blue* phenotype (figure 1*a*). This process is known to elevate allele frequency differentiation in the genomic region underlying the loss of carotenoid pigmentation. Additionally, nucleotide diversity in this region is expected to be reduced, as *blue* individuals predominantly share a single haplotype.

To identify outlier genomic regions matching these expectations, we generated whole-genome resequencing data from 60 Gouldian finches, including wild-type and *blue* varieties (detailed in the electronic supplementary material, table S1). We sequenced approximately 2 billion reads, resulting in an effective average coverage of 2.7X (range: 0.2–13.7X; median = 0.9X).

PCA analysis revealed no signature of population substructure associated with colour phenotype (electronic supplementary material, figure S1a). This indicates that colour varieties interbreed extensively, facilitating genetic mapping. Allele frequency differentiation and nucleotide diversity were summarized across the genome using a sliding window approach (figure 1*b*,*c*). Average levels of genetic differentiation and nucleotide diversity ratio between wild-type and *blue* were very low (F_{ST} ~ 0.01 and π~ 0.005), which constitutes an ideal scenario for identifying unusually differentiated regions. The low overall genetic differentiation occurred despite the majority of individuals in our dataset being unrelated. Only 3% (out of 1771 pairwise comparisons) exhibited a relatedness higher than first cousins (r_{xy} > 0.125; electronic supplementary material, figure S1b). All *blue* individuals were unrelated, except for three half-sibling pairs (electronic supplementary material, figure S1b).

Compatible with a monogenic Mendelian inheritance, our approach uncovered a single highly differentiated genomic region underlying the *blue* phenotype (figure 1*b*). This region contained the top 23 windows above the 99.98th percentile of the empirical distribution (F_{ST} > 0.21) and spanned approximately 196 kb on scaffold QUSF01000066.1: 406 000–602 000 bp. A secondary non-significant differentiation peak was also evident. Further inspection revealed that this genomic region is likely physically linked to the primary locus, indicating that both peaks reflect a single selective sweep signature. The nucleotide diversity ratio comparing wild-type and *blue* genomes reinforced this pattern by revealing a single genome-wide outlier region with reduced diversity in *blue* individuals (figure 1*c*). This region is therefore a strong candidate to harbour the gene explaining the loss of carotenoid coloration in *blue* Gouldian finches.

(b) A splice-acceptor mutation in *SCARB1* explains the *blue* Gouldian finch variety

To refine the position of the *blue* locus within the candidate genomic region, we genotyped a set of random SNPs, blind to their allele frequency differences, and established a minimal interval for which all *blue* individuals carried a single common haplotype (figure 1*d*). Compared to wild-type individuals (n = 49), all *blue* Gouldian finches (n = 11) were homozygous for an approximately 140 kb segment (QUSF01000066.1: 569 318–708 618 bp) enclosing five protein-coding genes: *SCARB1, UBC, DHX37*, *BRIP3B* and *AACS* (figure 1*e*; further details on gene content are provided in electronic supplementary material, table S2). Assuming a single origin for *blue*, the candidate causative locus is contained within this IBD segment.

Next, we searched the IBD interval for potential causative mutations affecting protein function (i.e. non-synonymous, stop, frameshift and splice-site mutations) and detected a splice-site mutation in *SCARB1* that was unique to *blue* Gouldian finches. This variant corresponds to an AG > TG change at the 3′ splice-acceptor site immediately upstream of exon 4 (QUSF01000066.1: 595 682 bp; electronic supplementary material, figure S2). *SCARB1* is an excellent candidate for loss of carotenoid coloration in Gouldian finches since it encodes a class B scavenger receptor with affinity for lipoproteins, known to mediate dietary carotenoid uptake and pigmentation in animals [22,31–38]. Moreover, a multiple sequence alignment of genomes from >100 bird species showed that this splice mutation occurs in a genomic position that is universally conserved (electronic supplementary material, figure S2). Taken together, these results suggest that the splice-acceptor mutation identified in *SCARB1* is likely to cause the *blue* phenotype.

(c) *Blue* abrogates dietary carotenoid uptake and transport

In other taxa, disruption of scavenger receptor *SCARB1* has been linked to significant reductions in dietary carotenoid uptake and transport [22,31–38]. To understand if the *blue* mutation in Gouldian finches exerts a similar effect on carotenoid transport, we compared levels of circulating pigments and their profiles between wild-type and *blue* individuals.

Biochemical characterization by UHPLC and HRAM/MS revealed the presence of yellow dietary carotenoids, lutein and zeaxanthin, in the plasma of wild-type birds, whereas *blue* individuals circulated negligible amounts of these pigments (figure 2*a*,*b*). Lutein was the predominant carotenoid form in the bloodstream of wild-type birds (figure 2*a*,*c*), whereas in the plasma

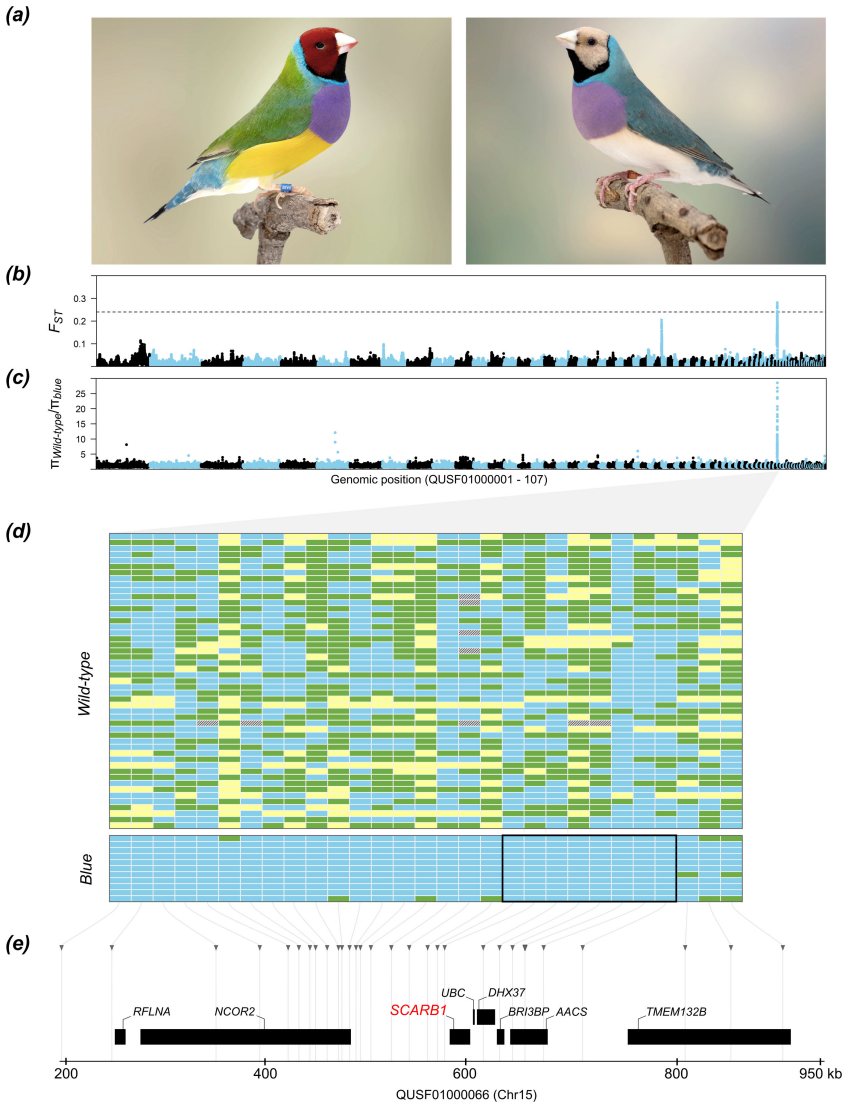

Figure 1. Genetic mapping of the *blue* Gouldian finch mutation. (*a*) A wild-type Gouldian finch showcasing remarkable diversity in carotenoid pigmentation (left), and the *blue* Gouldian finch mutation characterized by loss of plumage carotenoid pigmentation (right). Photo credits: Antonio Javier Sanz. (*b*) Genome-wide genetic differentiation between wild-type ($n = 49$) and *blue* ($n = 11$) Gouldian finches. Each dot represents the fixation index (F_{ST}) averaged in 20 kb windows. The horizontal dashed line indicates the 99.98th percentile of the empirical distribution. (*c*) Nucleotide diversity (π) ratio for wild-type and *blue* Gouldian finches. Each dot represents the ratio summarized across the genome in 20 kb windows. In (*b*) and (*c*), scaffolds <1 Mb are omitted for visual clarity. (*d*) Individual genotypes across the candidate region for the *blue* locus. Each column represents one selected variant, and rows correspond to individuals. Genotypes more common in *blue* birds are coloured light blue, alternative alleles are in yellow, heterozygous are coloured green, and missing data filled with grey patterning. For a subset of these positions (black-outlined box), all *blue* individuals carried a common haplotype. (*e*) Protein-coding genes are shown as dark blocks. Light grey lines connect the IBD genotypes in (*d*) with gene coordinates in (*e*).

of *blue* individuals, this pigment was detected at approximately 500-fold lower concentrations (mean ± s.e., *blue*: 19.86 ± 7.36 ng μl^{-1}; wild-type: 9763.68 ± 1314.59 ng μl^{-1}; $t = 7.41$, d.f. = 6, $p = 0.0003$; figure 2*c*). Zeaxanthin was also present in wild-type individuals (figure 2*b*), although contributing proportionately less to the overall carotenoid content (mean ± s.e., lutein: 85.79 ± 1.12%; zeaxanthin: 14.21 ± 1.12%; $t = 49.05$, d.f. = 6, $p = 0.0001$). We did not detect zeaxanthin in the plasma of *blue* Gouldian finches (figure 2*a*–*c*). Overall, these findings indicate that carotenoid uptake or transport is compromised by the splice-acceptor mutation identified in *SCARB1* in *blue* Gouldian finches.

(d) *SCARB1* function is restored by somatic mutation in a mosaic individual

To further investigate the functional relevance of *SCARB1* on localized carotenoid uptake, we took advantage of a very rare case of pigmentary mosaicism. In an Italian aviary, a unique *blue* individual was identified exhibiting an overall phenotype consistent with *SCARB1* impairment, except for a few feather patches where wild-type-like carotenoid pigmentation was visible (figure 3*a*). This suggests that a somatic mutation occurred during development, reverting the *blue* allele in a small population of progenitor cells and enabling localized restoration of *SCARB1* function. Genotyping of the splice-acceptor mutation in beige and red feathers from the mask area revealed that carotenoid-free beige feathers almost exclusively carried the *blue* allele (TG: $n = 94.4\%$ versus AG: $n = 0.04\%$; figure 3*b*), while feathers containing carotenoid coloration harboured both the *blue* and wild-type alleles (TG: $n = 87.2\%$ versus AG: $n = 9.4\%$; figure 3*b*). These results demonstrate that reverting the *blue* locus to its wild-type allele restores carotenoid pigmentation *in loco*, providing compelling evidence that the AG > TG splice-acceptor

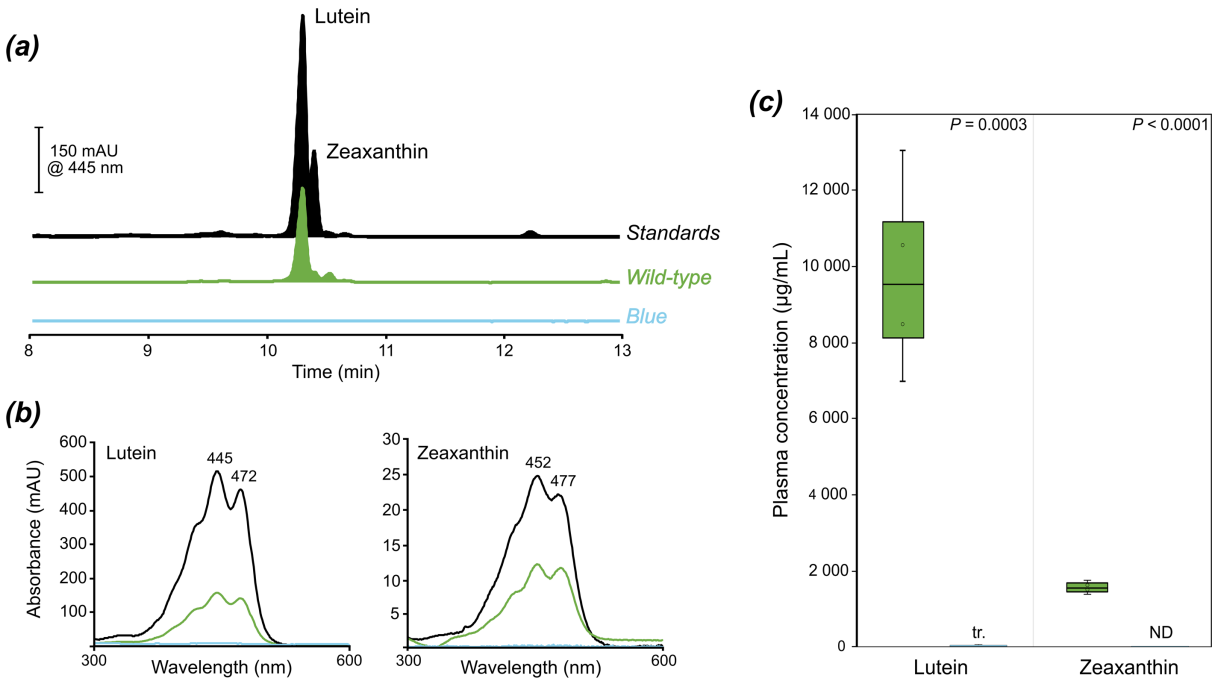

Figure 2. Biochemical analyses of plasma carotenoid content. (*a*) UHPLC UV/VIS chromatogram of carotenoids extracted from Gouldian finch plasma. (*b*) Absorbance spectra of carotenoid extracts. In (*a*) and (*b*) wild-type spectra are identical to those of lutein and zeaxanthin analytical standards (black). (*c*) Concentration of dietary carotenoids, lutein (right) and zeaxanthin (left), in the plasma of wild-type and *blue* Gouldian finches. The absolute concentration of pigments was quantified as the integral peak area from the exact mass spectrum (HRAM-QTOF). *p*-Values for Welch's *t*-tests are shown at the top right corner of each panel. tr., traces; ND, not detected.

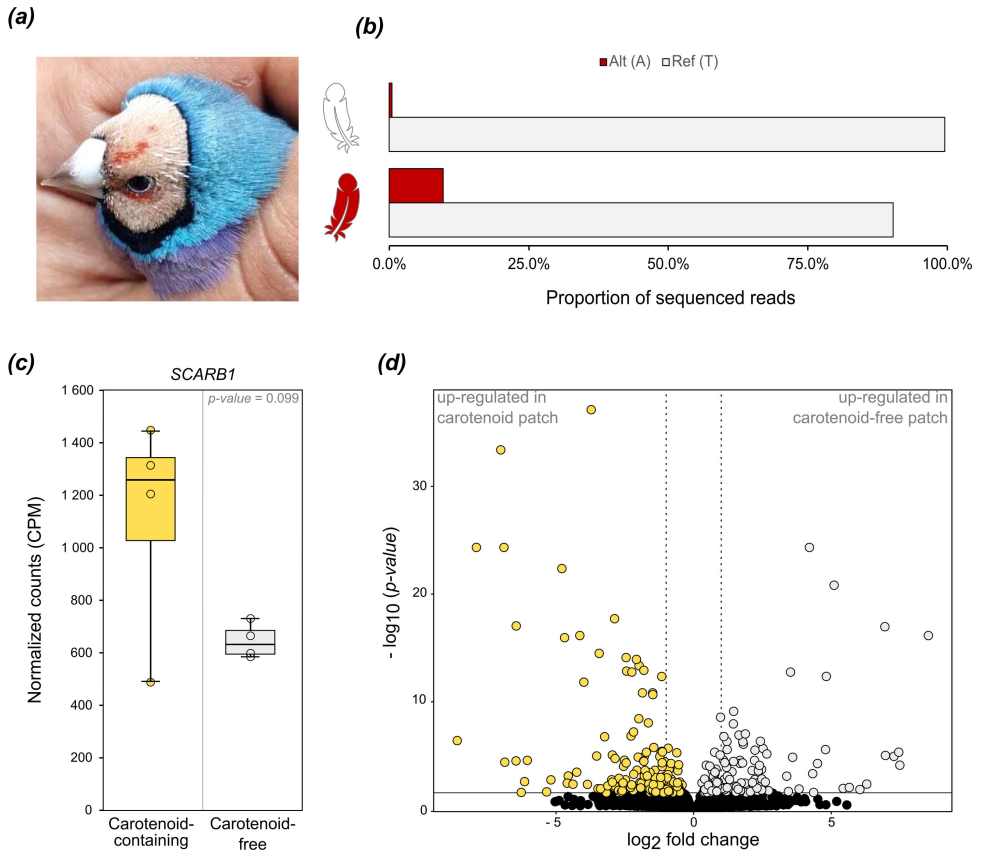

Figure 3. Somatic reversion of the *SCARB1 blue* allele and expression analysis in carotenoid-free versus carotenoid-containing plumage regions. (*a*) A *blue* Gouldian finch exhibiting pigmentary mosaicism in the mask region. Photo credits: Nicola Moccia. (*b*) Genotyping of the *SCARB1* splice-acceptor variant (*blue* locus) in beige and red feathers. Wild-type (A, red) and *blue* (T, beige) alleles are expressed as the proportion of sequenced reads supporting each allele in beige and red feathers, respectively. (*c*) Normalized expression counts showing *SCARB1* is expressed in both plumage regions regardless of carotenoid presence. Adjusted *p*-value is indicated on the top right corner of the plot. (*d*) Volcano plot depicting results of differential gene expression analysis. Contrast groups were arranged between carotenoid-containing ($n = 4$) and carotenoid-free ($n = 4$) plumage patches sampled from the same wild-type individuals (intra-individual comparison). Changes in gene expression from regenerating feather follicles are compared with FDR-adjusted *p*-values.

change in *SCARB1* causes the *blue* phenotype in Gouldian finches. Moreover, this natural rescue experiment shows that *SCARB1* is necessary for the localized uptake of carotenoids into feathers.

(e) Expression of *SCARB1* alone does not determine carotenoid-based plumage colour

To determine whether expression of *SCARB1* in feather follicles is sufficient for permanent carotenoid deposition in feathers, we compared gene expression levels in carotenoid-containing (back) and carotenoid-free (chest) plumage regions from wild-type birds. *SCARB1* was expressed in follicles of both plumage regions regardless of pigmentation (figure 3*c*). This excludes the possibility that *SCARB1* expression alone is sufficient for carotenoid deposition in feathers.

Next, to find additional factors contributing to carotenoid deposition in feather follicles, we compared the transcriptomic profiles of the two plumage regions. Differential expression analysis revealed 323 differentially expressed genes between these regions (figure 3*d*; electronic supplementary material, table S5). We found that a suite of genes involved in carotenoid transport and stabilization (*TTC39B, ELOVL4* and *ELOVL5*), lipoprotein metabolism and homeostasis (*APOH* and *CYP7A1*) and fatty acid metabolism (*FABP5* and *FABP7*) was consistently upregulated in carotenoid-containing feather follicles. In contrast, key genes linked to lipid efflux and cholesterol turnover (*ABCA2, VLDLR* and *LPL*) were upregulated in carotenoid-free follicles, suggesting a mechanism to actively prevent carotenoid accumulation. The expression of carotenoid cleavage enzyme (*BCO2*) is known to determine carotenoid retention in feathers of mosaic canaries [21]. We assessed *BCO2* expression levels and found that it was absent at the time of sampling in most of our samples. Taken together, our findings suggest that, while *SCARB1* is essential for carotenoid delivery to developing feather follicles, additional factors are required to incorporate these pigments into mature feathers and achieve full expression of carotenoid-based phenotypes.

4. Discussion

Carotenoid-based plumage coloration depends on the efficient uptake, processing and deposition of dietary (or modified) pigments into the integument. In the present study, we used genomic and biochemical analyses to reveal that a single nucleotide substitution is associated with the *blue* phenotype in domesticated Gouldian finches. This mutation alters a splice-acceptor site of the scavenger receptor *SCARB1* and disrupts dietary carotenoid uptake and transport, leading to the loss of carotenoid-based plumage coloration. By leveraging the genotyping of a spontaneous somatic variant present in a mosaic individual, we link the *blue* mutation to its functional relevance and to the essential role of *SCARB1* in avian carotenoid-based pigmentation.

Carotenoids and vitamins are hydrophobic and thus require lipoprotein-mediated transport to target tissues [39,40]. Promiscuous scavenger receptors recognize these lipoproteins and mediate the cellular trafficking of various molecules, including cholesterol, phospholipids, dietary carotenoids and vitamins [41]. The *blue* Gouldian finch mutation prevents carotenoid transport and accumulation in feathers, resulting in a profound impact on plumage coloration. As in *white recessive* canaries [22], we observed a marked reduction in circulating total carotenoid levels in *blue* Gouldian finches with complete absence of zeaxanthin and a substantial decline of lutein levels. The presence of detectable trace amounts of lutein in carotenoid-deficient birds suggests that a low-efficiency *SCARB1*-independent mechanism for lutein uptake exists in the gut. In mammals, *NPC1L1*, another lipoprotein transporter, was found to partially mediate lutein gut absorption, which might explain its incomplete abrogation [41,42].

SCARB1 is an integral component of the intestinal lumen, facilitating the uptake of provitamin A carotenoids, such as β-carotene and β-cryptoxanthin [34,43,44]. These are then enzymatically converted to vitamin A (retinol) [45,46]. Congenital retinol deficiency has been shown in *white recessive* canaries in consequence of *SCARB1* loss of function [47,48]. Thus, *blue* Gouldian finches are likely retinol-deficient as well. Despite lacking formal demonstration, breeders consistently report respiratory symptoms matching hypovitaminosis A to be associated with the *blue* variety (e.g. sinusitis). This is not observed in wild-type birds, indicating an underlying deficiency linked to the *SCARB1 blue* mutation. In line with previous studies, *SCARB1* malfunction likely reduces the bioavailability of vitamin A precursors [34,49]. Notwithstanding, this condition seems to be prevented with vitamin A supplementation, which hints at a specific mechanism for retinol uptake that bypasses *SCARB1*. Further study into these alternatives may offer broad insights into the mechanistic redundancy that allows micronutrient uptake to persist in *SCARB1* mutants.

The identification of a *blue* Gouldian finch with somatic mosaicism provided an unprecedented opportunity to study the functional role of *SCARB1* at the final stages of carotenoid uptake to feather follicles. Unlike between-group comparisons (wild-type versus *blue*), which can be influenced by genetic background, intra-individual analyses test the functional effects of a specific allele in a stable cellular environment. Genotyping of this individual revealed that beige mask feathers carried the *blue* allele exclusively, while the presence of the wild-type allele in the affected red feathers was sufficient to rescue *SCARB1* function locally and restore wild-type-like carotenoid pigmentation.

Due to limited access to the mosaic *blue* individual, we were unable to determine whether the trace levels of circulating lutein typically observed in *blue* Gouldian finches are sufficient for carotenoid deposition in feathers when *SCARB1* is functional, or whether the somatic mosaicism in this individual extended to the gut, allowing for enhanced carotenoid absorption compared to classic *blue* Gouldian finches. Despite this limitation, it is expected that both red and beige feathers of the mosaic *blue* Gouldian finch were supplied with the same circulating carotenoid levels. Thus, our findings provide unequivocal evidence for *SCARB1's* role in avian carotenoid pigmentation and demonstrate that expression of this transporter is essential not only for the uptake of carotenoids in the gut but also into the cells of developing feathers.

Consistent with this observation, developing feather follicles from carotenoid-containing plumage regions of wild-type Gouldian finches expressed *SCARB1*. Interestingly, *SCARB1* mRNA was also detected in feather follicles from the carotenoid-free chest region, indicating that although *SCARB1* expression is necessary for carotenoid uptake to feather follicles, its presence alone is not sufficient for carotenoid deposition. While the expression of *SCARB1* in carotenoid-free feather follicles may seem surprising, carotenoids play key roles in cellular processes such as signalling, metabolism and regulation of cell cycle [50], thus justifying their bioavailability even in non-pigmented tissues.

By comparing the transcriptomes of carotenoid-containing and carotenoid-free plumage regions, we identified additional factors that likely collaborate with *SCARB1* to determine feather pigmentation. Following *SCARB1*-mediated uptake, additional intracellular mechanisms are required to package, stabilize and incorporate carotenoids into keratin structures. Supporting this, we observed upregulation of genes involved in lipoprotein transport and homeostasis, intracellular lipid handling and fatty acid metabolism in carotenoid-containing follicles, possibly facilitating carotenoid transport, stabilization and deposition. In contrast, follicles from carotenoid-free plumage regions upregulated genes linked to lipid efflux and cholesterol turnover, suggesting that carotenoid pigmentation is suppressed not only due to a deposition impairment but also from active pigment removal. Further studies are needed to elucidate the specific roles of the differentially expressed genes in orchestrating regionality in carotenoid-based plumage pigmentation.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee. All samples were collected with permission from certified animal breeders in their private facilities.

Data accessibility. Electronic supplementary materials are available online [51]. Raw whole-genome resequencing data are available from the NCBI Sequence Read Archive (SRA) under BioProjects PRJNA478907 and PRJNA1322491. RNA-seq and target amplicon sequencing data are available under BioProject PRJNA1322491. Sequenom's MassARRAY iPLEX design, individual SNP genotypes and genome-wide relatedness matrix are available online from Dryad [52].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. C.I.M.: conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing—original draft, writing—review and editing; C.F.: conceptualization, methodology, writing—review and editing; S.A.: investigation, methodology, writing—review and editing; P.M.: investigation, methodology, writing—review and editing; P.M.A.: resources, writing—review and editing; M.C.: conceptualization, funding acquisition, methodology, project administration, supervision, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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