The Domestic *BCO2* Allele Buffers Low-Carotenoid Diets in Chickens: Possible Fitness Increase Through Species Hybridization

Amir Fallahshahroudi,* Enrico Sorato,† Jordi Altimiras,‡ and Per Jensen‡,1

*Department of Medical Biochemistry and Microbiology, Biomedicine Centrum (BMC), Uppsala University, 752 37, Sweden, †Reneco International Wildlife Consultants, Abu Dhabi, UAE, and ‡AVIAN Behavioural Genomics and Physiology Group, IFM Biology, Linköping University, 58183, Sweden

ORCID IDs: 0000-0001-8820-0098 (A.F.); 0000-0002-5223-4496 (E.S.)

ABSTRACT Domestic animals are adapted to conditions vastly different from those of their wild ancestors, and this is particularly true for their diets. The most numerous of all domestic species, the chicken, originated from the Red Junglefowl (RJF), a native of subtropical forests in Southeast Asia. Surprisingly however, in domestic chicken breeds, a common haplotype of the β-*carotene oxygenase 2* (*BCO2*) gene, which is involved in carotenoid metabolism, is introgressed from a related species, the Gray Junglefowl, and has been under strong selective pressure during domestication. This suggests that a hybridization event may have conferred a fitness advantage on chickens carrying the derived allele. To investigate the possible biological function of the introgressed *BCO2* allele in chicken, we introgressed the ancestral *BCO2* allele into domestic *White Leghorn* chickens. We measured gene expression as well as carotenoid accumulation in skin and eggs of chickens carrying either the ancestral or the derived *BCO2* allele. The derived haplotype was associated with down-regulation of *BCO2* in skin, muscle, and adipose tissue, but not in liver or duodenum, indicating that carotenoid accumulation occurred in the tissues with reduced gene expression. Most importantly, we found that hens with the derived *BCO2* genotype were capable of allocating stored carotenoids to their eggs, suggesting a functional benefit through buffering any shortage in the diet during egg production. Nevertheless, it is of interest that loss of function mutations in *BCO2* gene are prevalent in other domesticates including cows, rabbits, and sheep, and, given the importance of carotenoids in development, reproduction, and immunity, it is possible that derived *BCO2* alleles may provide a general mechanism in multiple domestic species to deal with higher demand for carotenoids in an environment with carotenoid shortage in the diet.

KEYWORDS Animal domestication; species hybridization; carotenoid physiology; *BCO2*

ANIMAL domestication is an excellent model for studying the genetic mechanisms that underlie phenotypes—a fundamental quest in the field of evolutionary biology (Andersson 2012). The process of domestication entails the accumulation of genetic adaptations that allows domestics to flourish in a new and vastly different ecological niche from their natural habitats (Price 1999). Such adaptations include lowered fear of humans, increased resistance to disease and stress, increased cold or heat tolerance, as well as the ability to efficiently utilize new diets (Mirkena *et al.* 2010). For instance, domestic dogs are suggested to have a drastically increased ability to digest high-starch diets compared to their wolf ancestors (Axelsson *et al.* 2013).

With an estimated population of ~50 billion, the chicken (*Gallus gallus domesticus*) is the most numerous domestic animal species (Perry-Gal *et al.* 2015). Domestic chickens originated ~8–9000 years ago from the Red Junglefowl (RJF)—a member of the family *Phasianidae* that lives in tropical and subtropical forests in southeast Asia—and has since spread throughout the world, diversifying into hundreds of varieties selected for meat- and egg-production, fighting, and ornamental functions (Tixier-Boichard *et al.* 2011; Jensen 2014). An important key to the success of the chicken as a

doi: https://doi.org/10.1534/genetics.119.302258
Manuscript received March 25, 2019; accepted for publication May 29, 2019; published Early Online May 31, 2019.
Supplemental material available at FigShare: https://doi.org/10.25386/genetics.8210171.
[1]Corresponding author: AVIAN Behavioural Genomics and Physiology Group, IFM Biology, Linköping University, 58183, Sweden E-mail: perje@ifm.liu.se

domestic species has been its broad and adaptable diet, allowing subsistence on grains and human food waste. Because cereals constitute the staple of agricultural societies, chickens provided an ideal avian species for domestication and have spread in parallel with agriculture over the last few thousand years from eastern Asia into western Eurasia and the rest of the world (Tixier-Boichard *et al.* 2011).

However, major staple cereals are extremely low in carotenoids compared to fruit, grasses, and other vegetables (Zhai *et al.* 2016), on which the wild counterparts of the domestic chicken rely (Collias and Saichuae 1967). Carotenoids constitute essential nutrients that cannot be synthetized *de novo* and are involved in a range of biological functions, such as immunity and reproduction (Goodwin 1986; Chew and Park 2004; Moreno *et al.* 2016). In birds, these component are used extensively in ornamentation and enhance sexual attractiveness (Andersson and Simmons 2006). In chickens and other bird species, carotenoids are stored into the egg yolk, conferring its characteristic yellow coloration, and constitute one of the main determinants of egg quality. In turn, egg quality is the main mediator of maternal effects, which profoundly affect offspring fitness (Bernardo 1996; Mousseau and Fox 1998). Previous studies have extensively investigated the impact of maternal carotenoids on offspring fitness across a range of bird species, by mean of direct or indirect manipulation of carotenoids in the egg yolk (Surai *et al.* 2001; Blount *et al.* 2002; Saino *et al.* 2003, 2011; McGraw *et al.* 2005; Romano *et al.* 2008; Giraudeau *et al.* 2016). Egg carotenoids have important biological functions during embryonic development (Blount *et al.* 2000; Koutsos *et al.* 2003; Tyndale *et al.* 2008). In birds, maternal carotenoids are considered to affect growth (Romano *et al.* 2008; Saino *et al.* 2011), antioxidation capacity (Edge *et al.* 1997; Blount *et al.* 2000, 2002; Surai *et al.* 2001; Costantini and Møller 2008), immunocompetence (Haq *et al.* 1996; Saino *et al.* 2003; Chew and Park 2004; Berthouly *et al.* 2007), and survival of hatchlings (McGraw *et al.* 2005). In general, although the exact mechanisms by which maternal carotenoids exert their fitness benefits is debated, the hypothesis that carotenoids are an important factor for egg quality in birds is well supported.

Given the importance of carotenoids in pre and postnatal life, it is likely that carotenoid physiology has undergone strong selection during the chicken domestication process, resulting in significant changes in carotenoid absorption, metabolism, and a host of linked metabolic pathways. Remarkably, while all characterized chicken alleles can be traced back to the RJF (*Gallus gallus*), the *β-caroten dioxygenase 2* (*BCO2*) allele, involved in carotenoid metabolism, is introgressed from a related species, the Gray Junglefowl (GJF, *Gallus sonneratii*), presumably through hybridization of early domestics in SE Asia (Eriksson *et al.* 2008). This derived allele is now fixed in many village and commercial chicken breeds (Rubin *et al.* 2010; Wragg *et al.* 2012; Qanbari *et al.* 2015; Loog *et al.* 2017). Domestic chickens carrying the derived allele are easily recognizable due to accumulation of carotenoids in the skin, leading to conspicuous yellow-colored legs which are visibly different from the gray legs of the RJF (Eriksson *et al.* 2008). Various inactivating mutations in *BCO2* have been reported and linked to carotenoid accumulation in other domestic animals including cow, sheep, and rabbit (Berry *et al.* 2009; Våge and Boman 2010; Strychalski *et al.* 2016). Fixation of the derived allele in many village and commercial chicken breeds suggests that this allele may play a functionally important role conveying some specific fitness advantage in these domesticates (Qanbari *et al.* 2015). However, the function and biological consequences of these loss-of-function mutations in the *BCO2* gene are largely unknown. Two main enzymes are involved in the breakdown of carotenoids; β-carotene oxygenase 1 (BCO1) and β-carotene oxygenase 2 (BCO2). Within the β-carotene oxygenase family, the *BCO1* gene converts provitamin A carotenoids (*e.g.*, α-carotene, β-carotene, β-cryptoxanthin) to retinol (vitamin A), and is therefore involved in vision and development (Olson 1989). By contrast, BCO2 cleaves nonprovitamin A carotenoids, including zeaxanthin, lutein, and lycopene, into colorless apocarotenoids such as β-apo-100-carotenal and β-ionone, which can be further catabolized by endogenous enzymes. Therefore, BCO2 functions as a carotenoid scavenger and prevents the adverse effects of excess carotenoids in sensitive tissues such as the liver (Lobo *et al.* 2012; Wu *et al.* 2017).

In this study, we aim to unravel the role of the derived allele in relation to carotenoid biology in chicken. We hypothesized that the derived *BCO2* allele plays an important biological function by enabling hens to store carotenoids when they are plentiful in the diet, and allocate them to eggs, thereby buffering any shortage in the diet at the time of egg production. This would presumably be a highly favorable trait in domestic chickens, particularly for high productivity breeds, given the importance of carotenoids for egg production and fitness of the hatchling (Blount *et al.* 2002; Karadas *et al.* 2005; McGraw *et al.* 2005; McGraw and Toomey 2010). To test our hypothesis, we integrated the ancestral *BCO2* haplotype into the domestic *White Leghorn's* (WL) genome by mean of six generations of targeted backcrossing. Transferring the ancestral *BCO2* haplotype into the WL genome allowed us to study its functional effects in a homogenous genetic background by comparing two groups of chickens differing systematically in a single gene only. We focused on carotenoid deposition in the leg skin, beak integument, and egg yolk as well as gene expression (*BCO1* and *BCO2*) in skin, muscle, adipose, liver, and small intestine in backcrossed line chickens homozygous for ancestral or derived *BCO2* alleles.

Materials and Methods

Generating the advanced backcross line

Generating the study population started with birds from the eighth generation of an advanced intercross between *White*

Leghorn and Red Junglefowl. Briefly, the intercross was generated by crossing one RJF male and three WL females to obtain 41 F1 intercross, and then 821 F2 intercross animals. The subsequent generations were kept at population sizes of >100 chickens per generation with designed pedigrees (the complete information for the intercross has previously been described in detail; see Elfwing *et al.* 2014; Johnsson *et al.* 2016).

Using pyrosequencing to genotype the *BCO2* allele, we selected 6 (F0) males that were homozygous for the ancestral allele, and paired them with 12 pure WL females (homozygous for the derived allele) to obtain heterozygous F1 (one male was kept with two females). Six heterozygous F1 males were then bred with 15 pure WL females to generate F2, which were either heterozygous or homozygous for the derived allele. For generating the F2 to F6 backcross, we housed male and females in a single pen for 3 days; the males were then removed and the females were put in individual cages for egg collection (30–40 eggs). For each generation we selected 5–6 heterozygous males and bred them with 12–15 pure WLs to generate a F6 backcross, which therefore had >99% of its alleles derived from WL.

From the F6, we then bred six heterozygous males and 18 heterozygous females with each other (one male was paired with three females and females were later kept individually for egg collection) generating the advanced backcross line (ABL) that included both homozygous birds for the ancestral and homozygous for the derived allele in an otherwise almost pure WL-genome. F6 heterozygote ABL birds were not used in this study. Further information on SNP genotyping using pyrosequencing and related primers is provided elsewhere (Eriksson *et al.* 2008).

Ethical statement

All experimental protocols were approved by the Linköping Ethical Board of Animal Experiments (permit no 50–13). Experiments were conducted in accordance with the approved guidelines.

Study animals

We generated two homozygous ABL batches for phenotyping ($N = 40$ individuals) and gene expression ($N = 24$). Birds were kept in a 4 m^2 pen until they were 6 weeks old. Thereafter they were moved to another animal facility, with both sexes kept together in a 9 m^2 pen until sexual maturity. All birds had access to *ad libitum* food plus shredded carrots (∼50 g for each bird) and *ad libitum* dried alfalfa (as an extra source of carotene) on a daily basis. At the age of 19 weeks, all hens were moved to individual cages (0.6 × 0.6 × 0.6 m), whereas males remained in their home pens. After moving the hens to the new cages, we stopped giving carrots or alfalfa to all birds. We collected the eggs laid by each female daily for measuring yellow color intensity and carotenoid contents of yolk. The second ABL batch was used for quantifying gene expression in tissues of adult birds, and hence was not used for phenotyping. These birds were kept in a 2 × 2 m^2 pen until they were 6 weeks old, when they were killed to obtain tissue samples.

Skin colorimetry

To quantify individual differences in skin coloration (yellowness), we took leg (shank) and beak pictures when birds were 1, 2, 4, 8, 18, 23, and 26 weeks old, under similar light conditions, using a tripod-mounted NIKON D5500 camera with a 40 mm lens. We selected leg and beak for measuring color intensity because they are not covered by feathers and hence it was possible to measure skin coloration noninvasively (Supplemental Material, Figure S1).

Egg yolk yellowness and carotenoid measurement of eggs

Eggs were collected and analyzed daily from the age of 19 weeks, when hens started to laying, until 26 weeks old. We recorded laying date, weight, and yolk "yellowness" for each laid egg. We used a commercial digital YolkFan to obtain DSM YolkFan score (DSM Nutritional Products; Nix Sensor, Basel, Switzerland), a measure of yolk yellowness, (Figure S2) on an average of 27.3 egg yolks (min 16, max 30 eggs) for each hen, totaling 23 females (11 ancestral, 12 derived type; average eggs weight = 43.7 and 44.3 g for eggs laid by chicken with ancestral and derived genotype, respectively).

Yolk yellowness depends directly on carotenoid content. However, to validate the Yolkfan results, the levels of Astaxanthin, Astaxanthin esters, Lutein, Zeaxanthin, β-Carotene, and Canthaxanthine were measured in a subset of eggs using high performance liquid chromatography (HPLC). All carotenoid measurements were conducted by Eurofins Steins, Vitamin Competence Centre, Denmark. To examine how the amount of egg carotenoids varied according to laying order and genotype, we analyzed, for each female, the third to fifth laid eggs, and 15th till 17th laid eggs. In total, 12 pooled samples were sent for carotenoid analysis. Each egg yolk pool contained 2 ml yolk samples (homogenized manually) from eggs laid by three individual hens during three consecutive days (each pool contained a sample from nine yolks).

Gene expression

Animals (24 in all, 6 of each genotype and sex combinations) were killed by decapitation. We collected tissues samples from the skin pectoral area (all three layers), liver, pectoral muscle, abdominal adipose tissue, and duodenum. The tissues were immediately snap frozen in liquid nitrogen and later stored in a −80° freezer until further analysis. RNA extraction, quality control, and RT-PCR was conducted according to previously published protocols (Løtvedt *et al.* 2017). PCR primers for *BCO1* and *BCO2* were designed using NCBI primer design tool (Ye *et al.* 2012) (Table S1).

Statistical analysis

All statistical analyses were performed in R version 3.5.1, and plots were made with the ggplot2 R package (Wickham 2009).

Linear regression was used to study the effects of genotype and sex on expression of *BCO1* and *BCO2* genes in skin, muscle, adipose, liver, and duodenum. The model included gene expression as response variable and genotype and sex as predictors (glm (formula = gene expression ~ genotype + sex). The interaction between genotype and sex was not significant, and, hence, it was not included in the final model. To study the intensity of yellow color in beak and leg skin, we fitted a linear regression model with color intensity as response variable and genotype, sex, and interaction between genotype and sex as predictors. For analysis of yolk yellowness scores, we fitted linear mixed-effects model with the lme function using the nlme R package version 3.1 (Pinheiro *et al.* 2018). The model included yellowness score as response variable and laying order, genotype, and the interaction between laying order and genotype as predictors. Different random intercepts were assumed for each laying hen, to model the statistical nonindependence of eggs originating from the same female [lme (yellowness score (DSM) ~ laying-order + genotype + genotype: laying-order, random = ~1|id, method = "REML"]. For analyzing HPLC results, we used linear regression with carotenoid levels as response variable and genotype, sampling-time and their interaction as predictors (glm (formula (carotenoid levels ~ genotype + sampling time + genotype: sampling time). However, because levels of zeaxanthin and canthaxanthine were below detection limits of HPLC analysis in chickens with RJF genotype, we conducted statistical analysis only on lutein levels.

Data availability

File S1 provides the details for measuring phenotypes, primer sequences, and the statistical analysis of color intensities. The supplemental dataset contains all data used in the analyses and producing the figures, including genotypes, gene expression, color and DSM values, as well as HPLC results. Supplemental material available at FigShare: https://doi.org/10.25386/genetics.8210171.

Results

Gene expression in tissues

In chickens possessing the ancestral allele, the expression of *BCO2* was significantly higher than for chickens with the derived haplotype in skin (estimate ±SE: 2.19 ± 0.25, $P < 0.001$), muscle (0.60 ± 0.16, $P = 0.001$), and adipose tissue (0.30 ± 0.13, $P = 0.03$), but not in liver (−0.23 ± 0.13, $P = 0.11$) or duodenum (0.06 ± 0.3, $P = 0.8$) (Figure 1). *BCO2* expression did not differ between males and females. Finally, expression levels of *BCO1* were not affected by genotype or sex in any of the examined tissues (Figure S3).

Intensity of yellow color in beak and skin

In both sexes, the intensity of yellow coloration in beak and legs was higher in individuals possessing the derived allele, starting from week 1 (legs) and week 3 (beak) (Figure 2 and Table S2). Sex and genotype by sex interaction were not significant predictors of color intensity until onset of egg-laying (Table S2). However, due to the sharp decrease in yellow intensity in both beak and skin, particularly in females after the onset of egg-laying, sex and sex by genotype interaction were the main predictors of yellow intensity at the age of 26 weeks.

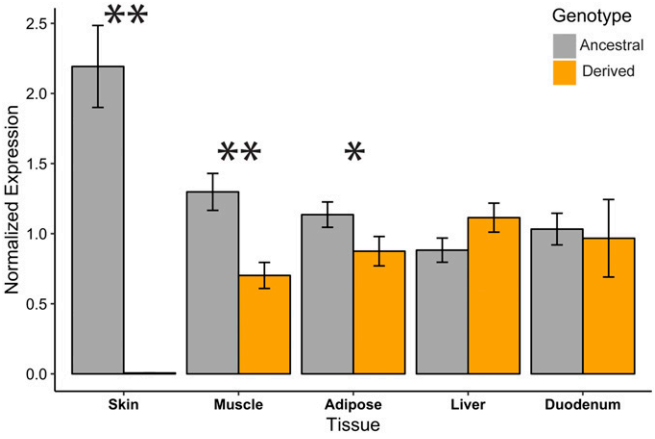

Figure 1 Relative gene expression of *BCO2* in skin, muscle, adipose, liver, and duodenum. Ancestral represent the Red Junglefowl genotype and derived represent *White Leghorn* genotype. Bars, mean ± SE. ANOVA: * $P < 0.05$, ** $P < 0.01$.

Egg yolk yellowness and carotenoids concentration in egg yolk

Egg yolk yellowness was significantly affected by genotype, and showed an interaction with laying order. Overall, eggs from hens with derived genotype had yellower yolk compared to eggs from the hens with ancestral genotype (estimate ±SE = −1.29 ± 0.20, $P < 0.001$). There was a significant interaction between egg laying-order and genotype (0.04 ± 0.01, $P < 0.001$), because yolk yellowness decreased steadily with laying order in chickens with derived genotype but not in ancestral ones. Hence, yellowness of the last-laid eggs did not differ between the two genotypes (Figure 3A). In HPLC analysis, levels of lutein—the most abundant carotenoid in the eggs—were almost three times higher in eggs from hens with the derived allele than in eggs from hens with the ancestral allele (estimate ±SE = −31.6 ± 6.43, $P < 0.001$). Lutein levels decreased with laying order (−11.6 ± 2.87, $P < 0.01$), particularly within eggs from hens with the derived allele (Figure 3B), in accordance with egg yolk yellowness patterns.

Discussion

We have found that the derived *BCO2* haplotype, an allele introgressed in domestic chickens from a different species than the ancestral RJF, is associated with down-regulation of the gene expression in skin, muscle, and adipose tissue (Figure 1), which in turn leads to gradual carotenoid

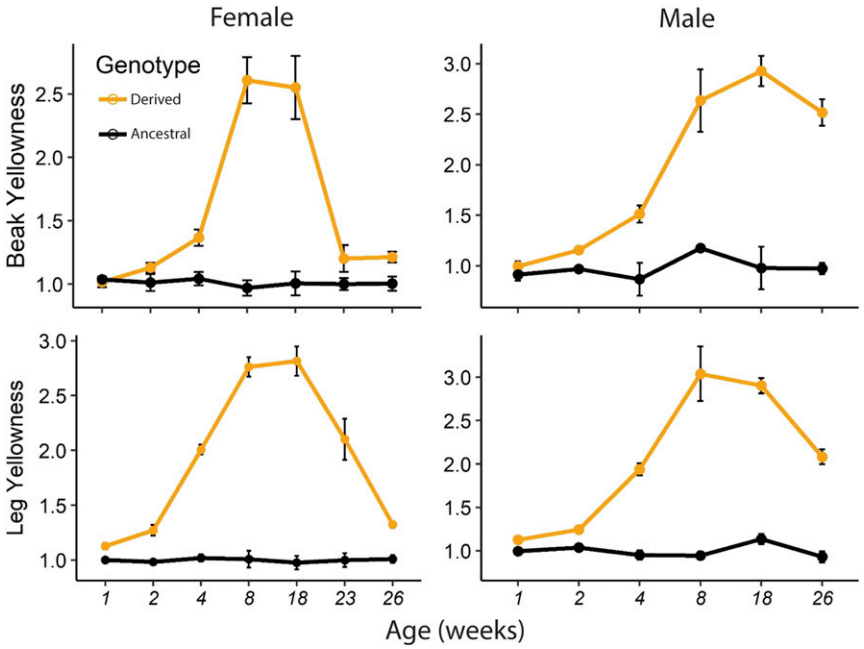

Figure 2 Relative intensity of yellow color over age in beak and leg skin, as a function of genotype and sex. Skin yellow intensity is calculated by normalizing yellow intensity measured from pictures over white scale. Derived represent *White Leghorn* genotype and ancestral represents Red Junglefowl genotype. Statistics for the intensity of yellow color in beak and leg skin are presented in Table S2. Points represent mean ± SE.

accumulation in the respective tissues. Crucially, we found that hens utilize stored carotenoids to lay eggs with higher carotenoid content. Thus, our study suggests a possible functional benefit for the introgressed *BCO2* allele, in that it may enable chickens to buffer carotenoids in their eggs under nutritionally suboptimal conditions, and thus increase reproductive investment compared to their wild ancestors.

Changes in gene expression underlie most phenotypic differences between closely related species (Albert *et al.* 2012; Fallahshahroudi *et al.* 2019). Lower expression levels of *BCO2* in skin, muscle, and adipose tissue of chickens possessing the derived allele lead to accumulation of carotenoids in these tissues, but not in the liver and other internal organs, in which expression is not lowered. Indeed, *BCO2* knockout in mice results in carotenoid accumulation in various tissues, giving rise to pathologic effects due to interference with mitochondrial function in hepatocytes (Lobo *et al.* 2012; Wu *et al.* 2017). Therefore, our findings suggest that the derived allele leads to carotenoid deposition in the tissues that are presumably less sensitive to the toxic effects of excess carotenoids such as skin and fat, but not in sensitive organs such as the liver.

In agreement with our gene expression results we showed that the derived *BCO2* allele causes gradual accumulation of carotenoids in skin and beak in both sexes. Furthermore, we showed that the intensity of carotenoid-dependent yellow coloration of skin declined sharply from the onset of sexual maturity, most strongly in females, concomitantly with egg laying progression (Figure 2). The yellower egg yolk, and higher levels of Lutein and Zeaxanthin in eggs from hens with the derived *BCO2* allele, suggest that carotenoids accumulated in the skin can be allocated into the yolk of eggs. In hens with the derived genotype, this is further supported by the observed decrease in egg yolk yellowness and carotenoid content with laying order, paralleling analogous changes in maternal skin coloration.

Across bird species, egg quality represents an important mediator of maternal effects on offspring phenotype, and may have far-reaching consequences for offspring fitness (Blount *et al.* 2002). Previous studies in domestic chickens have demonstrated that dietary carotenoids are deposited into the egg yolk, and, subsequently, into various tissues of the offspring (Karadas *et al.* 2005). Moreover, carotenoids have been shown to reduce susceptibility of embryonic tissues to free radical attack and to enhance immune function in hatchlings (Haq *et al.* 1996; Surai and Speake 1998). Similarly, maternally derived carotenoid pigments increase offspring survival in song birds (McGraw *et al.* 2005) and experimental supplementation of egg yolk with lutein enhanced T-cell-mediated immune response of barn swallow nestlings (Saino *et al.* 2003).

Shortage of carotenoids in grains, the staple diet of domestic chicken, combined with selection for carotenoid-demanding traits, such as enhanced egg production, fast-growth and increased immunity (Mirkena *et al.* 2010) may have contributed to the high frequency of the derived *BCO2* allele across many domesticated chicken breeds, most notably commercial ones selected for high productivity. Across domestic specie, loss-of-function mutations in the *BCO2* gene are present in sheep, cow, and rabbit breeds. When carotenoids are abundant in the diet, such as when animals have access to grasses during summer, these genetic-derived alleles lead to carotenoid accumulation in the adipose tissue, plasma, and milk (Nozière *et al.* 2006; Berry *et al.* 2009; Våge and Boman 2010; Strychalski *et al.* 2015). Domestic animals, particularly modern widespread breeds, have been commonly selected for increased productivity (*e.g.*, milk production), and, due to more crowded conditions and proximity to other domestic

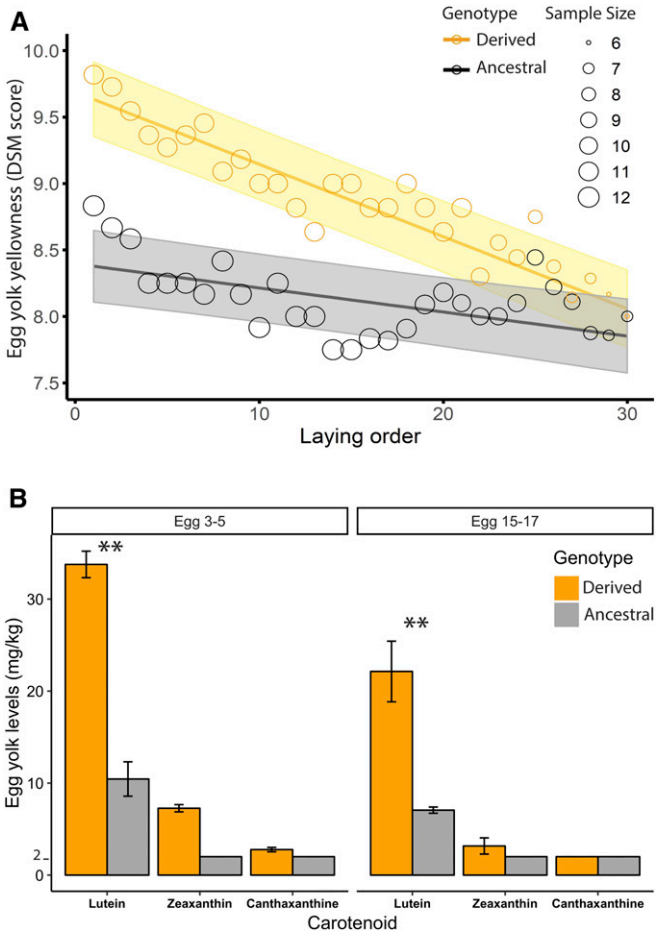

Figure 3 Egg yolk yellowness and carotenoid levels. (A) Egg yolk yellowness from the eggs laid by hens from both genotypes in the order they were laid. The circles represent the average DSM score for each sampling, and the size of the circles represent the total number of analyzed egg. The fitted regression lines and confidence intervals for the model are presented. (B) Carotenoid levels in egg yolk samples, measured by HPLC. Derived represent *White Leghorn* genotype and ancestral represents Red Junglefowl genotype. Levels of zeaxanthin and canthaxanthine were below the detection limit of HPLC in chickens with ancestral genotype and hence the minimum detection limits values are assigned. Bars represent mean ± SE.

species, have been facing increased pathogenic and parasitic challenges in their environment compared to wild conspecifics. Considering the importance of carotenoids in the modulation of immune response (Saino *et al.* 2003, 2011), reproduction (Blount *et al.* 2002), and development (Karadas *et al.* 2005), we suggest that the derived *BCO2* alleles may provide an important mechanism for buffering carotenoid shortage in the diet of domesticated animals.

Nonetheless, we cannot rule out that the high prevalence of the introgressed *BCO2* allele in modern chicken breeds may be influenced by other selective forces, independently of any putative functional benefit. For instance, the derived allele may have initially spread, through human artificial selection, into domestic breeds, due to its aesthetic value or because it increased the perceived nutritional and health status of chickens (Eriksson *et al.* 2008). For the same reason this same trait may and have come under even stronger selection with the creation of modern commercial breeds (Loog *et al.* 2017). Sexual selection (Blount *et al.* 2003; Pennisi 2003) might also have favored the spread and fixation of the derived allele. Importantly, these hypotheses are not mutually exclusive, and may in fact relate to different phases and geographical regions involved in the chicken domestication process. Evaluating the merits of different explanations for the prevalence of the derived *BCO2* allele in modern breeds is challenging, particularly since different selective mechanism may have been operating concomitantly and are therefore different to disentangle. Given that the focus of this study was to measure the biological effect of the derived *BCO2* variant in a modern chicken breed, rather than providing an experimental test of different hypothesis for spread and prevalence of the derived allele and its associated phenotype, we cannot offer a conclusive answer here. Nonetheless, regardless of the selective forces that have been involved in spread of the derived allele in the past, our study suggests current beneficial functionality for this allele.

Conclusion

Our results suggest that introgression of the *BCO2* allele from GJf into the genome of domestic chickens by means of a species-hybridization event may confer biological benefits by allowing hens to deposit more carotenoids into their eggs when dietary carotenoids are not plentiful in the diet. Mechanistically, we showed that this higher carotenoid deposition in the body of domestic chicken is achieved through down-regulation of the *BCO2* gene in skin, adipose, and muscle tissues. Future empirical research should quantify fitness of individuals possessing either the ancestral or derived *BCO2* allele, in sheep, cow, and rabbits, as well as other chicken breeds in combination with dietary treatments varying in carotenoids content.

Acknowledgments

We thank Julia Buskas and Jesper Fogelholm for their technical assistance. The project was supported by grants from Swedish Research Council (VR): 2015–05444 and 2017–06218, and the European Research Council (ERC; Advanced Grant 322206 GENEWELL).

Literature Cited

Albert, F. W., M. Somel, M. Carneiro, A. Aximu-Petri, M. Halbwax *et al.*, 2012 A comparison of brain gene expression levels in domesticated and wild animals. *PLoS Genet.* 8: e1002962. https://doi.org/10.1371/journal.pgen.1002962

Andersson, L., 2012 How selective sweeps in domestic animals provide new insight into biological mechanisms. J. Intern. Med. 271: 1–14. https://doi.org/10.1111/j.1365-2796.2011.02450.x

Andersson, M., and L. W. Simmons, 2006 Sexual selection and mate choice. Trends Ecol. Evol. 21: 296–302. https://doi.org/10.1016/j.tree.2006.03.015

Axelsson, E., A. Ratnakumar, M. L. Arendt, K. Maqbool, M. T. Webster *et al.*, 2013 The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature 495: 360–364. https://doi.org/10.1038/nature11837

Bernardo, J., 1996 The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. Am. Zool. 36: 216–236. https://doi.org/10.1093/icb/36.2.216

Berry, S. D., S. R. Davis, E. M. Beattie, N. L. Thomas, A. K. Burrett *et al.*, 2009 Mutation in bovine beta-carotene oxygenase 2 affects milk color. Genetics 182: 923–926. https://doi.org/10.1534/genetics.109.101741

Berthouly, A., F. Helfenstein, and H. Richner, 2007 Cellular immune response, stress resistance and competitiveness in nestling great tits in relation to maternally transmitted carotenoids. Funct. Ecol. 21: 335–343. https://doi.org/10.1111/j.1365-2435.2006.01236.x

Blount, J. D., D. C. Houston, and A. P. Moller, 2000 Why egg yolk is yellow. Trends Ecol. Evol. 15: 47–49. https://doi.org/10.1016/S0169-5347(99)01774-7

Blount, J. D., P. F. Surai, R. G. Nager, D. C. Houston, A. P. Møller *et al.*, 2002 Carotenoids and egg quality in the lesser black-backed gull Larus fuscus: a supplemental feeding study of maternal effects. Proc. Biol. Sci. 269: 29–36. https://doi.org/10.1098/rspb.2001.1840

Blount, J. D., N. B. Metcalfe, T. R. Birkhead, and P. F. Surai, 2003 Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300: 125–127. https://doi.org/10.1126/science.1082142

Chew, B. P., and J. S. Park, 2004 Carotenoid action on the immune response. J. Nutr. 134: 257S–261S. https://doi.org/10.1093/jn/134.1.257S

Collias, N. E., and P. Saichuae, 1967 Ecology of the red jungle fowl in Thailand and Malaya with reference to the origin of domestication. Nat. Hist. Bull. Siam Soc. 22: 189–209.

Costantini, D., and A. P. Møller, 2008 Carotenoids are minor antioxidants for birds. Funct. Ecol. 22: 367–370. https://doi.org/10.1111/j.1365-2435.2007.01366.x

Edge, R., D. J. McGarvey, and T. G. Truscott, 1997 The carotenoids as anti-oxidants — a review. J. Photochem. Photobiol. B. 41: 189–200.

Elfwing, M., A. Fallahshahroudi, I. Lindgren, P. Jensen, and J. Altimiras, 2014 The strong selective sweep candidate gene ADRA2C does not explain domestication related changes in the stress response of chickens. PLoS One 9: e103218. https://doi.org/10.1371/journal.pone.0103218

Eriksson, J., G. Larson, U. Gunnarsson, B. Bed'hom, M. Tixier-Boichard *et al.*, 2008 Identification of the yellow skin gene reveals a hybrid origin of the domestic chicken. PLoS Genet. 4: e1000010. https://doi.org/10.1371/journal.pgen.1000010

Fallahshahroudi, A., P. Løtvedt, J. Bélteky, J. Altimiras, and P. Jensen, 2019 Changes in pituitary gene expression may underlie multiple domesticated traits in chickens. Heredity 122: 195–204. https://doi.org/10.1038/s41437-018-0092-z

Giraudeau, M., A.-K. Ziegler, and B. Tschirren, 2016 Long-term effect of yolk carotenoid levels on testis size in a precocial bird. Biol. Lett. 12: 20160008. https://doi.org/10.1098/rsbl.2016.0008

Goodwin, T., 1986 Metabolism, nutrition, and function of carotenoids. Annu. Rev. Nutr. 6: 273–297. https://doi.org/10.1146/annurev.nu.06.070186.001421

Haq, A. U., C. A. Bailey, and A. Chinnah, 1996 Effect of β-Carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. Poult. Sci. 75: 1092–1097. https://doi.org/10.3382/ps.0751092

Jensen, P., 2014 Behavior genetics and the domestication of animals. Annu. Rev. Anim. Biosci. 2: 85–104. https://doi.org/10.1146/annurev-animal-022513-114135

Johnsson, M., M. J. Williams, P. Jensen, and D. Wright, 2016 Genetical genomics of behavior: a novel chicken genomic model for anxiety behavior. Genetics 202: 327–340. https://doi.org/10.1534/genetics.115.179010

Karadas, F., A. C. Pappas, P. F. Surai, and B. K. Speake, 2005 Embryonic development within carotenoid-enriched eggs influences the post-hatch carotenoid status of the chicken. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 141: 244–251.

Koutsos, E. A., A. J. Clifford, C. C. Calvert, and K. C. Klasing, 2003 Maternal carotenoid status modifies the incorporation of dietary carotenoids into immune tissues of growing chickens (Gallus gallus domesticus). J. Nutr. 133: 1132–1138. https://doi.org/10.1093/jn/133.4.1132

Lobo, G. P., A. Isken, S. Hoff, D. Babino, and J. von Lintig, 2012 BCDO2 acts as a carotenoid scavenger and gatekeeper for the mitochondrial apoptotic pathway. Development 139: 2966–2977. https://doi.org/10.1242/dev.079632

Loog, L., M. G. Thomas, R. Barnett, R. Allen, N. Sykes *et al.*, 2017 Inferring allele frequency trajectories from ancient DNA indicates that selection on a chicken gene coincided with changes in medieval husbandry practices. Mol. Biol. Evol. 34: 1981–1990. https://doi.org/10.1093/molbev/msx142

Løtvedt, P., A. Fallahshahroudi, L. Bektic, J. Altimiras, and P. Jensen, 2017 Chicken domestication changes expression of stress-related genes in brain, pituitary and adrenals. Neurobiol. Stress 7: 113–121. https://doi.org/10.1016/j.ynstr.2017.08.002

McGraw, K. J., and M. B. Toomey, 2010 Carotenoid accumulation in the tissues of Zebra Finches: predictors of integumentary pigmentation and implications for carotenoid allocation strategies. Physiol. Biochem. Zool. 83: 97–109. https://doi.org/10.1086/648396

McGraw, K. J., E. Adkins-Regan, and R. S. Parker, 2005 Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. Naturwissenschaften 92: 375–380. https://doi.org/10.1007/s00114-005-0003-z

Mirkena, T., G. Duguma, A. Haile, M. Tibbo, A. M. Okeyo *et al.*, 2010 Genetics of adaptation in domestic farm animals: a review. Livest. Sci. 132: 1–12. https://doi.org/10.1016/j.livsci.2010.05.003

Moreno, J. A., C. Nogareda, E. Angulo, G. Sandmann, M. Portero-Otin *et al.*, 2016 The distribution of carotenoids in hens fed on biofortified maize is influenced by feed composition, absorption, resource allocation and storage. Sci. Rep. 6: 35346. https://doi.org/10.1038/srep35346

Mousseau, T. A., and C. W. Fox, 1998 The adaptive significance of maternal effects. Trends Ecol. Evol. 13: 403–407. https://doi.org/10.1016/S0169-5347(98)01472-4

Nozière, P., P. Grolier, D. Durand, A. Ferlay, P. Pradel *et al.*, 2006 Variations in carotenoids, fat-soluble micronutrients, and color in cows' plasma and milk following changes in forage and feeding level. J. Dairy Sci. 89: 2634–2648. https://doi.org/10.3168/jds.S0022-0302(06)72340-2

Olson, J. A., 1989 Provitamin A function of carotenoids: the conversion of β-carotene into vitamin A. J. Nutr. 119: 105–108. https://doi.org/10.1093/jn/119.1.105

Pennisi, E., 2003 EVOLUTION: colorful males flaunt their health. Science 300: 29–31. https://doi.org/10.1126/science.300.5616.29

Perry-Gal, L., A. Erlich, A. Gilboa, and G. Bar-Oz, 2015 Earliest economic exploitation of chicken outside east Asia: evidence from the hellenistic southern levant. Proc. Natl. Acad. Sci. USA 112: 9849–9854. https://doi.org/10.1073/pnas.1504236112

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team, 2018 nlme: linear and nonlinear mixed effects models. R package version 3.1–137. https://CRAN.R-project.org/package=nlme

Price, E. O., 1999 Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. 65: 245–271. https://doi.org/10.1016/S0168-1591(99)00087-8

Qanbari, S., M. Seidel, T. M. Strom, K. F. X. Mayer, R. Preisinger *et al.*, 2015 Parallel sel2ection revealed by population sequencing in chicken. Genome Biol. Evol. 7: 3299–3306. https://doi.org/10.1093/gbe/evv222

Romano, M., M. Caprioli, R. Ambrosini, D. Rubolini, M. Fasola *et al.*, 2008 Maternal allocation strategies and differential effects of yolk carotenoids on the phenotype and viability of yellow-legged gull (Larus michahellis) chicks in relation to sex and laying order. J. Evol. Biol. 21: 1626–1640. https://doi.org/10.1111/j.1420-9101.2008.01599.x

Rubin, C.-J., M. C. Zody, J. Eriksson, J. R. S. Meadows, E. Sherwood *et al.*, 2010 Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464: 587–591. https://doi.org/10.1038/nature08832

Saino, N., R. Ferrari, M. Romano, R. Martinelli, and A. P. Møller, 2003 Experimental manipulation of egg carotenoids affects immunity of barn swallow nestlings. Proc. Biol. Sci. 270: 2485–2489. https://doi.org/10.1098/rspb.2003.2534

Saino, N., M. Romano, M. Caprioli, D. Rubolini, and R. Ambrosini, 2011 Yolk carotenoids have sex-dependent effects on redox status and influence the resolution of growth trade-offs in yellow-legged gull chicks. Behav. Ecol. 22: 411–421. https://doi.org/10.1093/beheco/arq220

Strychalski, J., P. Brym, U. Czarnik, and A. Gugołek, 2015 A novel AAT-deletion mutation in the coding sequence of the BCO2 gene in yellow-fat rabbits. J. Appl. Genet. 56: 535–537. https://doi.org/10.1007/s13353-015-0290-9

Strychalski, J., A. Gugołek, Z. Antoszkiewicz, D. Kowalska, and M. Konstantynowicz, 2016 Biologically active compounds in selected tissues of white-fat and yellow-fat rabbits and their production performance parameters. Livest. Sci. 183: 92–97. https://doi.org/10.1016/j.livsci.2015.11.024

Surai, P. F., and B. K. Speake, 1998 Distribution of carotenoids from the yolk to the tissues of the chick embryo. J. Nutr. Biochem. 9: 645–651. https://doi.org/10.1016/S0955-2863(98)00068-0

Surai, P. F., N. H. C. Sparks, B. K. Speake, and N. H. C. Sparks, 2001 Carotenoids in avian nutrition and embryonic development. 2. Antioxidant properties and discrimination in embryonic tissues. J. Poult. Sci. 38: 117–145. https://doi.org/10.2141/jpsa.38.117

Tixier-Boichard, M., B. Bed'hom, and X. Rognon, 2011 Chicken domestication: from archeology to genomics. C. R. Biol. 334: 197–204. https://doi.org/10.1016/j.crvi.2010.12.012

Tyndale, S. T., R. J. Letcher, J. W. Heath, and D. D. Heath, 2008 Why are salmon eggs red? Egg carotenoids and early life survival of Chinook salmon (Oncorhynchus tshawytscha). Evol. Ecol. Res. 10: 1187–1199.

Våge, D. I., and I. A. Boman, 2010 A nonsense mutation in the beta-carotene oxygenase 2 (BCO2) gene is tightly associated with accumulation of carotenoids in adipose tissue in sheep (Ovis aries). BMC Genet. 11: 10. https://doi.org/10.1186/1471-2156-11-10

Wickham, H., 2009 ggplot2 Elegant Graphics for Data Analysis. Springer, New York.

Wragg, D., J. M. Mwacharo, J. A. Alcalde, P. M. Hocking, and O. Hanotte, 2012 Analysis of genome-wide structure, diversity and fine mapping of Mendelian traits in traditional and village chickens. Heredity 109: 6–18. https://doi.org/10.1038/hdy.2012.9

Wu, L., X. Guo, S. D. Hartson, M. A. Davis, H. He *et al.*, 2017 Lack of β, β-carotene-9′, 10′-oxygenase 2 leads to hepatic mitochondrial dysfunction and cellular oxidative stress in mice. Mol. Nutr. Food Res. 61: 1600576. https://doi.org/10.1002/mnfr.201600576

Ye, J., G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen *et al.*, 2012 Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13: 134. https://doi.org/10.1186/1471-2105-13-134

Zhai, S., X. Xia, and Z. He, 2016 Carotenoids in staple cereals: metabolism, regulation, and genetic manipulation. Front. Plant Sci. 7: 1197. https://doi.org/10.3389/fpls.2016.01197

Communicating editor: M. Calus