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Early Oxidative Stress May Prevent a Red Ornament From Signaling Longevity

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ABSTRACT

Harsh early environmental conditions can exert delayed, long-lasting effects on phenotypes, including reproductive traits such as sexual signals. Indeed, adverse early conditions can accelerate development, increasing oxidative stress that may, in turn, impact adult sexual signals. Among signals, colorations produced by red ketocarotenoids seem to depend on mitochondrial functioning. Hence, they could reveal individual cell respiration efficiency. It has been hypothesized that these traits are unfalsifiable “index” signals of condition due to their deep connection to individual metabolism. Since mitochondrial dysfunction is frequently linked to aging, red ketocarotenoid-based ornaments could also be good signals of a critical fitness component: longevity. We tested this red color per longevity correlation in captive zebra finches. In addition, we experimentally decreased the synthesis of glutathione (a critical intracellular antioxidant) during the first days of the birds' life to resemble harsh early environmental conditions (e.g., undernutrition). Longevity was recorded until the death of the last bird (almost 9 years). Males, but not females, exhibiting a redder bill in early adulthood lived longer than males with paler bills, which agrees with some precedent studies. However, such bill redness—longevity connection was absent among males with inhibited glutathione synthesis. These findings may suggest that environmental factors can alter the reliability of red ketocarotenoid-based sexual signals, making them less unfalsifiable than believed.

1 | Introduction

Harsh environmental conditions during early development are expected to cause stronger, long-lasting effects on phenotypes than conditions impacting later in life (Metcalfe and Monaghan 2001; Yearsley, Kyriazakis, and Gordon 2004). These early environmental conditions can trigger metabolic responses leading to particular phenotypes more or less adapted to future environments (“environmental matching,” “predictive adaptive response,” or “thrifty phenotype” hypotheses; Wells 2007; Monaghan 2008; Bateson, Gluckman, and Hanson 2014). In this context, limited food intake or extreme environmental conditions (e.g., low temperatures, hyperoxia) during early life may increase metabolic rates to accelerate growth (i.e., catch-up

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Summary

- Red colorations produced by carotenoids are supposedly be linked to mitochondria function, and probably longevity.
- We found that the positive link between such coloration and longevity can be distorted by early oxidative stress in an avian species.

growth; e.g., Arnott et al. 2006; Criscuolo et al. 2008; Owerkowicz, Elsey, and Hicks 2009). This challenge may imbalance the production of reactive oxygen species (ROS) regarding the state of the antioxidant machinery (i.e., oxidative stress), generating oxidative damage on biomolecules (Alonso-Alvarez et al. 2007; Tarry-Adkins et al. 2009; Christensen et al. 2016; Kim, Noguera, and Velando 2019; Janssens and Stoks 2020). Thus, early oxidative stress could play a role in determining the phenotypic profiles and individual life history trajectories (Alonso-Alvarez et al. 2006; Monaghan, Metcalfe, and Torres 2009; Romero-Haro and Alonso-Alvarez 2015).

The foremost life-history traits comprise reproductive parameters (e.g., number or size of the offspring, the age and size at maturity) and longevity (e.g., Braendle, Heyland, and Flatt 2011). Those traits allowing individuals to obtain mates are considered reproductive parameters, and, among them, those conveying information to potential partners and benefiting both transmitter and receptor are considered sexual signals (Maynard Smith and Harper 2003). The conspicuous colorations generated by carotenoid pigments (e.g., many yellow-to-red colorations) are among the most intensely studied sexual signals (e.g., McGraw and Ardia 2003; Hartley and Kennedy 2004; Delhey et al. 2023). Carotenoids are acquired with food, allocated to colorations, or used as antioxidants or provitamin molecules for homeostatic functions (Britton, Pfander, and Liaaen-Jensen 2009; Pérez-Rodríguez, 2009). The foraging effort needed for acquiring carotenoids and metabolic demands led to the hypothesis that coloration intensity is traded against self-maintenance (von Schantz et al. 1999; Blount 2004; Alonso-Alvarez et al. 2008; but see Koch and Hill 2018). Resolving such a trade-off by investing more in coloration would imply proportionally higher costs for low-quality individuals (e.g., Weaver et al. 2018; Penn and Számadó 2020). Low-quality animals should avoid a high investment in coloration, thus converting colored traits into reliable signals of the individual condition.

In addition, carotenoids include many types of compounds. Yellow carotenoids (xanthophylls) are commonly acquired with the diet and directly deposited on ornament tissues (McGraw 2006). In contrast, red carotenoids (ketocarotenoids) are obtained from enzymatic transformations of dietary yellow carotenoids (McGraw 2006; Hill and Johnson 2012). This transformation seems to be made into the inner mitochondrial membrane (Johnson and Hill 2013; Hill et al. 2019). This scenario would add complexity to signal production. Thus, it has been hypothesized that the intensity of red ketocarotenoid-based coloration reveals individual cell respiration efficiency (Johnson and Hill 2013; Cantarero and Alonso-Alvarez 2017; Cantarero et al. 2020). Such a deep link to cell function would imply that red ketocarotenoid-based ornaments could be fully unfalsifiable, resource-independent, and cost-free (i.e., the shared-pathway hypothesis; Hill 2011). This type of unfalsifiable signal is usually called an "index" or "index signal" (Maynard Smith and Harper 2003; Weaver et al. 2018).

Interestingly, given that mitochondrial dysfunction is commonly associated with oxidative stress, and since the latter is linked to aging and aging-related diseases (Barja 2014; Sun, Youle, and Finkel 2016), the intensity of ketocarotenoid-based colorations should predict individual lifespan as an ultimate fitness index. This is supported by studies in sticklebacks (*Gasterosteus aculeatus*; Pike et al. 2007; Simons et al. 2021), zebra finches (*Taeniopygia guttata*: Simons et al. 2012; Simons, Briga, and Verhulst 2016) and red-legged partridges (*Alectoris rufa*; Cantarero et al. 2019).

However, fluctuating environmental conditions throughout development could make sexual signals less reliable as a consequence of individual phenotypic plasticity acting on the trait (signal) expression level (e.g., Higginson and Reader 2009; Ingleby, Hunt, and Hosken 2013; Candolin and Wong 2019). In other words, early development conditions can be so diverse (heterogeneous) that they can deviate the phenotype (within its reaction norm) in such a way that the expression level of the signal may not correlate with individual quality. Thus, when environmental conditions became harder during growth (e.g., by reduced food intake or increased thermal stress), sexual signals in adulthood could transmit dishonest information. In that case, their expression level should not be well correlated to individual fitness traits (i.e., the ultimate indices of individual quality: longevity and fecundity). Here, we test this hypothesis on a red-ketocarotenoid-based ornament that could signal individual longevity, that is, the red bill of zebra finches (see Simons et al. 2012; Simons, Briga, and Verhulst 2016). In particular, we hypothesize that early oxidative stress, potentially influenced by environmental changes, may disrupt the information transmission from sexual signals. In a long-term study, we artificially decreased the blood levels of a key intracellular antioxidant (glutathione) in captive birds during their growth by administering an inhibitor of glutathione synthesis (buthionine sulfoximine; BSO) from 6 to 14 days old (i.e., Romero-Haro and Alonso-Alvarez 2015). Glutathione is synthesized from amino acids, and a low amino acid intake decreases glutathione synthesis in mammals and birds (Gould and Pazdro 2019; Kachungwa Lugata, Ortega, and Szabó 2022). Similarly, in avian species, birds raised in artificially enlarged broods (i.e., probably receiving less food per capita; e.g., Saino, Calza, and pape Moller 1997; Westneat et al. 2017) showed lower blood glutathione levels than individuals reared in artificially reduced broods (Gil et al. 2019; Romero-Haro and Alonso-Alvarez 2020). Thus, our glutathione synthesis inhibition aims to resemble a situation in which a transient nutritional shortage disrupts the antioxidant machinery. We previously analyzed the effect of this manipulation on phenotypic and life-history traits for 9 years (until the death of the last bird). Just ending the treatment (i.e., 14 days old), nestlings endured low blood plasma antioxidant levels (Romero-Haro and Alonso-Alvarez 2015). In early adulthood (100 days old), these BSO-treated birds showed higher oxidative damage (lipid peroxidation in erythrocytes), with longevity not differing between experimental groups (Romero-Haro and Alonso-Alvarez 2015; Romero-Haro, Figuerola, and Alonso-Alvarez 2023). Here, we tested the impact of this manipulation on the capacity of a sexual signal expressed during early life to reveal the individuals' longevity potential. Sexual signaling theory would predict that zebra finches with redder bills should live longer (high-quality birds), whereas life-history theory and the associated trade-offs predict birds investing more in (early) reproduction (here sexual signaling) living shorter (Stearns 1992; Braendle, Heyland, and Flatt 2011). The latter should be expected among BSO-treated birds since color investment when enduring high oxidative stress would imply physiological costs. Hence, a color-per-treatment interaction on longevity would reveal a life-history trade-off in signal expression rather than supporting a deeply constrained index signaling mechanism. Finally, we may also predict the color per longevity association will differ among sexes considering differences in sex-specific selection pressures (e.g., carotenoid investment in egg yolk in females; Bertrand, Faivre, and Sorci 2006) and coloration (male zebra finches show redder bills due to higher bill ketocarotenoid content; McGraw 2006).

2 | Methods

This study is part of a long-term experiment that tested the influence of experimentally decreased early-life levels of a critical intracellular antioxidant (i.e., glutathione) on the expression of life-history traits in captive zebra finches (Romero-Haro and Alonso-Alvarez 2015, 2020; Romero-Haro, Canelo, and Alonso-Alvarez 2015; Romero-Haro, Sorci, and Alonso-Alvarez 2016; Romero-Haro, Figuerola, and Alonso-Alvarez 2023). Eighty randomly formed pairs (F0 birds) were placed in breeding cages (details in (Romero-Haro and Alonso-Alvarez 2015) for 5 months, and the breeding pairs produced 409 nestlings (F1). Among them, 206 randomly selected individuals were treated with the glutathione inhibitor BSO, and 203 were control-treated (experimental chronogram in Figure 1). An experimental treatment was applied to nestlings reaching 3 g minimum (mean ± SE: 4.82 g ± 0.03). Half of the nestlings in a brood were assigned to the treatment receiving DL-buthionine-S, R-sulfoximine (BSO; Sigma, ref. B2640) in saline solution (0.90% w/v of NaCl; BSO individuals). The other half of the brood was assigned to the control group and only received saline. We randomly assigned one treatment to the heaviest chick in a brood and then alternated the treatment assignation among the siblings (e.g., control, BSO, control, BSO). The BSO solution was 50 mg BSO in 1 mL of saline solution. Birds received 0.06 mL of BSO solution (BSO individuals) or 0.06 mL of saline (controls). The cited volume was subcutaneously injected into the back every other day from 6 to 12 days old (i.e., four injections). To validate the manipulation, total glutathione levels in erythrocytes were determined in 14 days old (mean age) nestlings using a spectrophotometer (A25-Autoanalyzer, Biosystems) and following Griffith, (1980) method with modifications (Romero-Haro and Alonso-Alvarez 2014). BSO nestlings reported significantly lower glutathione levels than controls (see Figure 2 in Romero-Haro and Alonso-Alvarez 2015).

Males and females (F1) were housed in different indoor aviaries (2.80 × 3 × 2.50 m each; details in Romero-Haro, Canelo, and Alonso-Alvarez 2015) at 40 days of age (average). The adult bird's bill was photographed at 99 days (average; "100d" to simplify; details in Romero-Haro and Alonso-Alvarez 2015). This age is higher than the earliest first egg-laying age reported in the field (Zann 1996) and also in outdoor aviaries in another population (median 94 days old; Alonso-Alvarez et al. 2006). Also, note that mate choice occurs before the first egg-laying date. The black surfaces present in the beaks of immature birds (Zann 1996; Boruszewska, Witkowski, and Jaszczak 2007) were fully absent in the birds assessed for red coloration. Moreover, there was no difference between treatments (or treatment × sex interaction) in the proportion of the black surface in the beak when early measured (40 days of age; all p values > 0.30; Supporting Information). The sample of birds assessed for bird coloration and

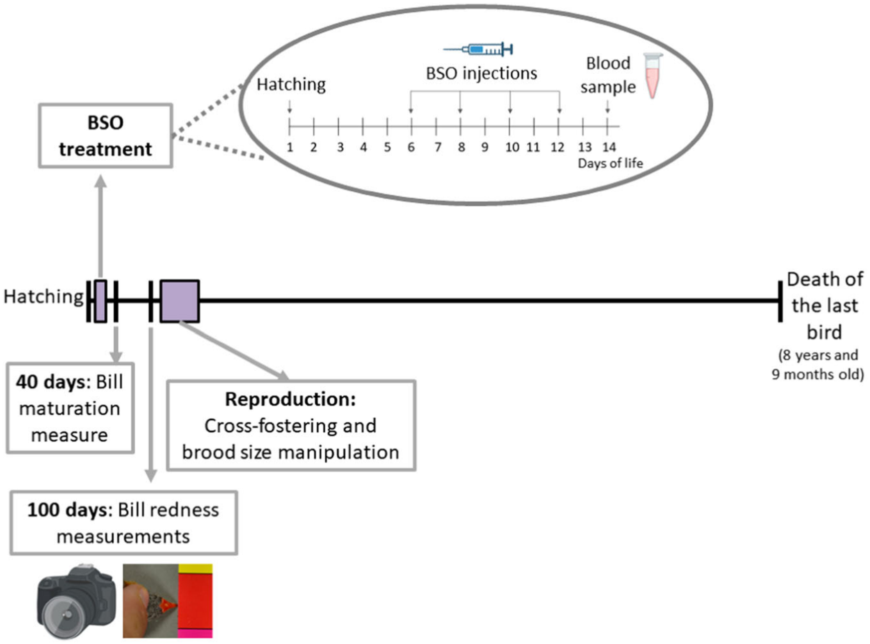

FIGURE 1 | Chronogram of the experiment. BSO, buthionine sulfoximine.

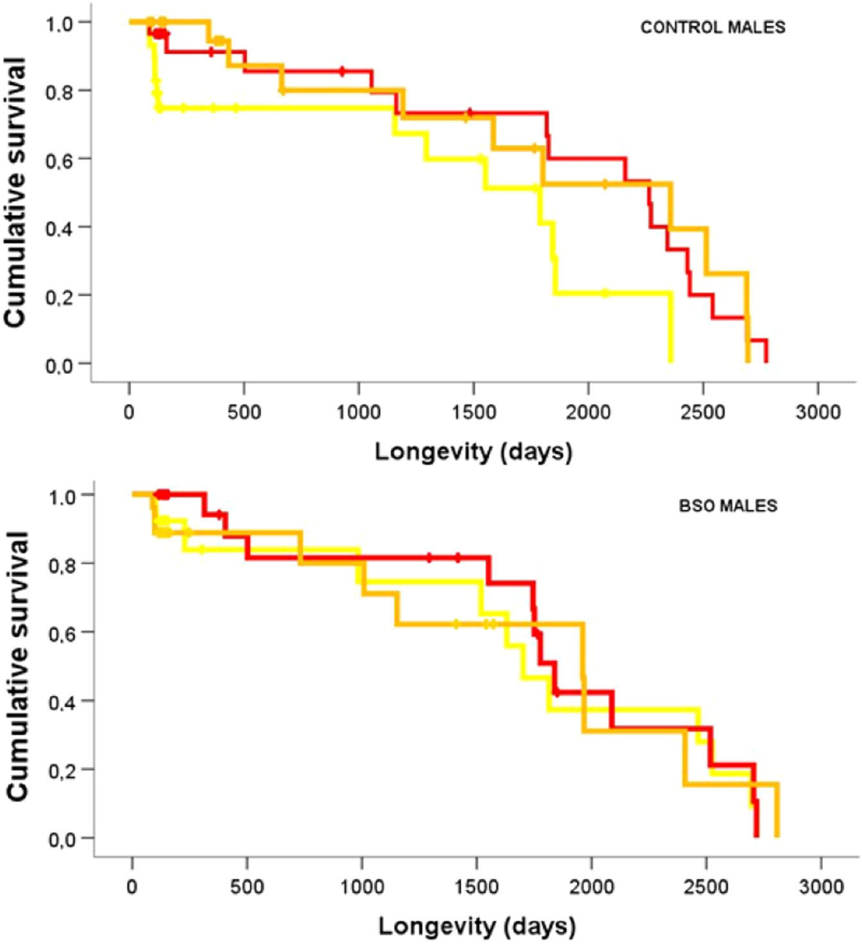

FIGURE 2 | Cumulative survival of male zebra finches depending on their bill color at early adulthood (average: 100 days old). Above: controls. Below: birds early and transitorily exposed to buthionine sulfoximine (BSO; an inhibitor of glutathione synthesis). Bill hue residuals grouped birds into tertiles (Methods). The birds with the reddest bills were included in the lowest tertile (red line). The birds with intermediate coloration were in the mid-tertile (orange line), and those with the palest bills were in the highest tertile (yellow line). Note that Table 1 models tested bill hue as a continuous covariate.

longevity was statistically balanced (control males: 86; BSO males: 79; control females: 72; BSO females: 76; $\chi^2 = 0.376$, $p = 0.540$).

To avoid physiological stress due to high social density (Poot et al. 2012), among all individuals reaching at least 110 days of life ($n = 283$; mean ± SE: 137d ± 1.52 days old), 65% were randomly chosen and released into a large outdoor aviary (223 m^3). The treatment and sex categories were balanced in this subsample ($\chi^2 = 1.26$, $p = 0.26$; Supporting Information), and BSO-treated birds also showed lower glutathione levels at 14 days of age than controls ($p < 0.001$; Romero-Haro, Figuerola, and Alonso-Alvarez 2023). These individuals were monitored throughout their entire lifetime. First, the birds mated and bred freely for 7 months after release (Romero-Haro and Alonso-Alvarez 2020; Romero-Haro, Sorci, and Alonso-Alvarez 2016). Treatment did not influence reproductive parameters (Romero-Haro, Sorci, and Alonso-Alvarez 2016; Romero-Haro and Alonso-Alvarez 2020; Romero-Haro, Figuerola, and Alonso-Alvarez 2023). After the reproductive period, males and females (F1) were separated, and each sex was placed in each half of the aviary with similar social density, with their lifespan being monitored every 2 or 3 days throughout the 8 years (2012–2020) until the death of the oldest bird (104 months).

The longevity data of animals whose date of natural death could not be well established were censored in Cox models (below). In this case, the last date a bird was found alive was used to assign its minimum lifespan. This included three situations: (1) missing birds that could only be identified when capturing all the birds during sampling events (mainly a consequence of ants making corpses quickly disappear; see Romero-Haro, Figuerola, and Alonso-Alvarez 2023), (2) birds that died due to causes unrelated to natural aging (e.g., accidents, predation by thin snakes entering the facilities), and (3) individuals not released in the outdoor aviary (see above). In the latter case, the minimum longevity was calculated until the cohort members were released. The comparison of the presence/absence of censored data between the sexes, treatments, or the four sex × treatment combinations did not provide significant biases (χ^2 tests p values > 0.27; also Supporting Information).

2.1 | Zebra Finch Bill Color Measurement

Digital images of the upper surface of the upper mandible were obtained using a Nikon D3100 under standardized conditions (see details in Supporting Information). The mean red, green, and blue (RGB) values were determined (Alonso-Alvarez et al. 2008) using Adobe Photoshop CS3. A standard red chip (Kodak, NY) was placed close to the bird's head. The bill and red chip hue and brightness were calculated using Foley and Van Dam (1984). High hue values indicate less redness than low hue values. Three pictures were not analyzed because of a lack of focus or incorrect bird position. Bill color parameters were finally obtained from 165 males and 148 females (full details in Supporting Information).

2.2 | Statistical Analyses

We tested whether bill color intensity predicted longevity. We ran a mixed-effects Cox model that included sex and early treatment as fixed factors and bill hue as a covariate, including all possible interactions between them. The brood ID nested into the cage ID where the bird was born was included as a random factor. The hue values included were residuals previously obtained from a mixed-effect linear model. In this previous model, the bill brightness and red chip hue were added as covariates to control the amount of light reflected by the bill surface or subtle illumination variability among pictures, respectively (Romero-Haro and Alonso-Alvarez 2015; Cantarero et al. 2020). Here, the random term was also the brood ID nested into the cage ID.

The analyses were performed in *R* version 4.2.3. (R Core Team 2021). The *R* package *lme4* (Bates et al. 2015) was used to perform mixed models to obtain residuals for the color covariate. The *coxme* and *survival* packages were used for mixed Cox models (Therneau, Grambsch, and Pankratz 2003). The predictors' significance was obtained using the analysis of variance (ANOVA) function from the car package to perform likelihood-ratio tests for mixed models and Wald χ^2 tests for Cox models (Fox and Weisberg 2019). Normality and homoscedasticity of residuals from mixed models were met.

3 | Results

First, it should be noted that a negative slope in Cox models indicates that the hazard (mortality) decreases with increasing levels of the covariate values. Thus, a positive slope indicates that paler individuals (higher hue values) had a higher mortality risk (or reduced longevity). We found a significant interaction between sex and bill color hue ($p = 0.012$ in Table 1, model above). To understand this result, we tested the influence of bill hue on longevity for each sex separately and excluding the treatment. Here, the bill hue predicted longevity in males, with paler individuals showing a higher mortality risk ($\beta = 0.497$, SE = 0,128, $\chi^2 = 15.01$, df = 1, $p < 0.001$, $n = 165$). This relationship was not significant when testing only females ($\beta = 0.024$, SE = 0.107, $\chi^2 = 0.051$, df = 1, $p = 0.821$, $n = 148$).

Nonetheless, even when the three-order interaction between treatment, sex, and hue values did not reach significance ($p = 0.143$ in Table 1), we explored the color and longevity correlation separately for each sex, including the treatment and its interaction with hue (Table 1). The treatment × bill hue interaction was only significant in males ($p = 0.043$, in Table 1). The cited correlation (paler individuals living shorter) was detected among control males ($\beta = 0.631$, SE = 0.141, $\chi^2 = 20.09$, df = 1, $p < 0.001$, $n = 86$) but not among BSO males ($\beta = 0.202$, SE = 0.211, $\chi^2 = 0.918$, df = 1, $p = 0.338$, $n = 79$; see Table 1 and Figure 2). The result is illustrated in Figure 2, representing cumulative survival for birds in tertiles that divided the color distribution into high, intermedia, or low values. The difference between control and BSO males could not be attributed to a sample size difference. The statistical power of a model with the same effect size that was found in male controls (0.631) but the sample size used in the BSO group (79) provided a very high power (0.998), which is only subtly different from that found in male controls (0.999; XLSTAT 2024 software, Supporting Information).

4 | Discussion

Our longitudinal study supports the hypothesis that red ketocarotenoid-based signals transmit information about the longevity potential of the trait bearer, at least among male zebra finches. However, exposure to adverse conditions during development (high levels of oxidative stress) would weaken the information transfer, making the signal unreliable.

First, we should mention that the coloration of birds was assessed early in adult life, some days later than the first reproduction reported for this species (Zann 1996). The trait assumedly subject to sexual selection (i.e., the red bill) was fully developed in all the birds (i.e., no sign of the blackish color characteristic of immature birds; see Section 2). Thus, our approach focusing on young but eligible-for-reproduction birds would have increased the sample size, allowing the detection of small but significant statistical effects. Furthermore, from a male's life history point of view, the investment in coloration at the first opportunity should be critical as the first reproduction is crucial in short-lived species where future reproductive events are subject of unpredictable mortalities (Stearns 1992; Braendle, Heyland, and Flatt 2011; Griffith 2019, 2021). Moreover, we should also consider that the reproductive potential of a younger bird would be higher than that of an older individual. Therefore, the benefit of making a good choice for a young mate should be higher than the benefit of making a good choice for an older bird. This is particularly important here since zebra finches are considered monogamous birds with lifetime pairbonds (e.g., Zann 1994; Adkins-Regan and Tomaszycki 2007; Griffith 2019).

In our longitudinal study, the males with the reddest bills lived longer. Such a result agrees with some previous works in zebra finches but differs from others, perhaps due to differences in the approaches. Price and Burley (1994) did not detect a significant correlation between bill redness and longevity in males but a negative association in females. However, they used a smaller sample size (30 birds per sex) than ours and stopped monitoring birds when > 50% of birds were still alive. Similar to our results, Simons et al. (2012) reported a positive link for males and a quadratic (first positive) association for females, with approximately 70 birds per sex and 40% remaining alive. However, their color measurements were performed at around 660 days of age,

TABLE 1 | Generalized Cox models testing the influence of bill hue at 100 days of age, sex and early treatment (glutathione synthesis inhibition; see Section 2) on zebra finch longevity.

Predictors	β	SE	χ^2	df	p
Both sexes ($n = 313$)					
Treatment (control)	0.218	0.249	0.413	1	0.521
Sex (males)	−0.056	0.268	0.216	1	0.642
Bill hue	−0.033	0.159	9.325	1	**0.002**
Treatment (control) × Sex (males)	−0.153	0.358	0.223	1	0.637
Treatment (control) × Bill hue	0.121	1.128	3.539	1	0.060
Sex (males) × Bill hue	0.078	0.275	6.302	1	**0.012**
Treatment (control) × Sex (males) × Bill hue	0.496	0.339	2.144	1	0.143
Males ($n = 165$)					
Treatment (control)	0.060	0.269	0.0003	1	0.987
Bill hue	0.129	0.229	17.656	1	**< 0.001**
Treatment (control) × Bill hue	0.547	0.270	4.112	1	**0.043**
Females ($n = 148$)					
Treatment (control)	0.184	0.249	0.592	1	0.442
Bill hue	−0.041	0.157	0.049	1	0.825
Treatment (control) × Bill hue	0.119	0.212	0.312	1	0.577

Note: Bold values indicate significant tests at $p < 0.05$.

precluding testing the entire life range. Lastly, Simons, Briga and Verhulst (2016) tested whether annual bill redness predicted yearly mortality, finding a significant positive correlation in males but not in females. The latter study was conducted on the Simons et al. (2012) population but on a larger sample size (about 180 birds per sex) and during a more extended period. Overall, a positive link between the intensity of ketocarotenoid-based coloration and longevity is supported by zebra finch studies, although females seem to show a weaker association, perhaps due to sex-specific selection pressures (e.g., carotenoid investment in egg yolk; Bertrand, Faivre, and Sorci 2006; Price 1996).

Male longevity was explained by the interaction of color and treatment (Table 1). The link between color and longevity was not detected in males exposed to early low glutathione levels. BSO birds did not show a significant negative correlation as initially predicted. Some of the reddest BSO males could have paid the cost of investing carotenoids in coloration, dying earlier and making the positive correlation fade. Also, some of the palest BSO males could live longer (see Figure 2) due to a potential mobilization of carotenoids from coloration into protection (homeostasis) under oxidative stress. Such extended longevity among the palest BSO males could be explained at the proximate level by physiological compensatory responses controlled by mitochondria (see mito-hormesis; Yun and Finkel 2014; Zhang and Hood 2016). Regarding the latter, we should highlight that astaxanthin (a red ketocarotenoid in the zebra finch beak; McGraw and Toomey 2010) seems able to penetrate the mitochondrial membrane and modulate its function (Nishida et al. 2022; see also Hill et al. 2019). In any case, the results overall suggest a life-history trade-off between early investment in sexual signaling and longevity, disagreeing with the nonplastic signaling implicit in the index concept (Maynard Smith and Harper 2003; Hill 2011) and opening the possibility of signal dishonesty (cheating) in some circumstances.

We should note that avian colorations in nonfeathered body parts are particularly dynamic (e.g., Alonso-Alvarez et al. 2022; Iverson and Karubian 2017). Thus, in zebra finches, males can intensify bill color in a few days when housed with females (Gautier et al. 2008; but see also Romero-Haro, Canelo, and Alonso-Alvarez 2015). Even the handling stress increased the red bill coloration of male zebra finches in a few weeks (McGraw, Lee, and Lewin 2011). The latter can be interpreted as birds investing in coloration when perceiving a high predation risk, trying to advance reproduction (i.e., a terminal investment strategy; Clutton-Brock 1984; Velando, Drummond, and Torres 2006). Similarly, we have previously shown that BSO birds faced oxidative stress but simultaneously increased bill redness (Romero-Haro and Alonso-Alvarez 2015), suggesting a terminal investment strategy triggered by stressful conditions. Several studies on ketocarotenoid-based ornaments support this scenario. In male sticklebacks, low food availability increased red skin coloration (Candolin 2000b). Old zebra finches exposed to bacterial antigens increased coloration, whereas the opposite was found among younger birds (Cote et al. 2010). In male red-legged partridges, exposure to a ROS generator (diquat) produced oxidative stress but intensified the red coloration by converting yellow carotenoids to ketocarotenoids (García-de Blas, Mateo, and Alonso-Alvarez 2016). In all these examples, we should note that terminal investment could have been induced by mito-hormetic responses (i.e., as a potential proximate mechanism; see above). In any event, the lack of signal reliability under early oxidative stress highlights the relevance of genotype × environment (GxE) interactions on sexual signaling evolution (Greenfield and Rodriguez 2004; Higginson and Reader 2009; Ingleby, Hunt, and Hosken 2013). Such a lack of reliability can sustain sexual selection in some circumstances (e.g., when immigrants are not well adapted to the new environment; Getty 2014; Holman and Kokko 2014).

Nevertheless, animals can evolve mechanisms to avoid reliability fluctuations. Female sticklebacks change how they assess males' sexiness by focusing on other traits instead of red coloration, such as the nest size (made by the male), when the environmental conditions change (Head, Fox, and Barber 2017). Indeed, the influence of environmental factors disrupting the link between the signal level and individual quality seems to promote the evolution of multiple signals in the same individual (Bro-Jørgensen, 2010; Candolin 2000a, 2003; Lozano 2009). The analysis of several signals would improve mating decisions. Male zebra finches exhibit different ornamental colors in plumage, songs, and displays, thus adding complexity to female choice. Unraveling the influence of harsh early conditions on potential signaling traits of individuals, as a whole, is an avenue for a better understanding of signal evolution.

Finally, our longitudinal and transgenerational studies support the role of glutathione as a connector of multiple functionally related traits (see “phenotypic integration” in, e.g., Costantini, Monaghan, and Metcalfe 2013; Cox, McGlothlin, and Bonier 2016). Individuals whose glutathione levels were experimentally decreased suffered a widespread loss of integration among physiological traits, coloration, and longevity. Erythrocyte glutathione concentrations were negatively related to blood total carotenoid levels in nestlings from another independent captive zebra finch population (i.e., Romero-Haro and Alonso-Alvarez 2014) and again in the present population (Romero-Haro and Alonso-Alvarez 2015). This link suggests that developing birds with low glutathione levels mobilized carotenoids to avoid oxidative stress or vice versa. However, this compensatory mechanism was absent when birds endured decreased glutathione synthesis due to BSO (Romero-Haro and Alonso-Alvarez 2015). Early glutathione levels were also associated with telomere length in the present population (Romero-Haro, Figuerola, and Alonso-Alvarez 2023). Telomeres are protective, highly repetitive, noncoding DNA sequences at the end of chromosomes that shorten in each cell division (Blackburn 1991; Harley et al. 1992), being considered predictors of individuals' longevity (e.g., Heidinger et al. 2012; Reichert et al., 2015; Srinivas, Rachakonda, and Kumar 2020). The erythrocyte glutathione level of nestlings was positively correlated to erythrocyte telomere length when birds became adults (Romero-Haro, Figuerola, and Alonso-Alvarez 2023), suggesting that glutathione protects telomeres from oxidative stress-mediated shortening (Stauffer, Panda, and Ilmonen 2018; Yadav and Maurya 2022). However, BSO tended to make such a link significantly negative, pointing to a telomere compensatory mechanism (e.g., via telomerase) triggered by low early glutathione levels (Romero-Haro, Figuerola, and Alonso-Alvarez 2023). Moreover, control females with low early glutathione levels but long telomeres at adulthood (perhaps better in compensating for harsh early conditions) lived longer than other phenotypic profiles (Romero-Haro, Figuerola, and Alonso-Alvarez 2023). This association between glutathione levels, telomere length, and longevity was absent among BSO females (Romero-Haro, Figuerola, and Alonso-Alvarez 2023). Here, the association between bill redness and longevity appears only among control males and not among BSO males, once again supporting the role of glutathione as a phenotypic integrator.

In conclusion, the bill redness predicts lifespan in male zebra finches, but an early harsh environment, inducing low levels of the antioxidant glutathione, would make this sexual signal less reliable. The findings support that investment in coloration would be traded against longevity, highlighting the integrative role of glutathione in shaping phenotypes. However, the correlation between the signal level and fitness should be estimated from more than longevity but also from reproductive success and offspring fitness. In this line, we have previously found that BSO birds produced smaller offspring than controls (Romero-Haro and Alonso-Alvarez 2020). Thus, in environments constraining glutathione synthesis, choosing a redder partner could be risky not only because its color does not correctly reveal longevity but also because these individuals could lead to small offspring (negative transgenerational effects).

Author Contributions

C.A.-A. and A.A.R.-H. conceived and performed the experimental study and analyzed samples. A.C. contributed to animal monitoring for some years. A.A.R.-H. performed statistical models on experimental data. C.A.-A. wrote a first draft. A.A.R.-H. and A.C. contributed to the first draft, and all the authors reviewed the final text.

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Ethics Statement

The University of Castilla La Mancha's animal experimentation committee approved this research project under licence number CEEA: 1201_08.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supplementary material of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.