Synchronizing feather-based measures of corticosterone and carotenoid-dependent signals: what relationships do we expect?

Graham D. Fairhurst · Russell D. Dawson · Harry van Oort · Gary R. Bortolotti

Abstract Carotenoids produce many of the red, orange and yellow signal traits of birds, and individuals must trade off utilizing carotenoids for physiological processes versus ornamentation. Proximate mechanisms regulating this trade-off are poorly understood, despite their importance for expression of color signals. Corticosterone (CORT) may play a significant mechanistic role in signal expression because it mobilizes energy substrates and influences foraging behavior. We used a unique feather-based approach to test whether CORT mediates expression of carotenoid-based coloration. First, we investigated relationships between levels of CORT from feathers ($CORT_f$) and carotenoid-based plumage signals in common redpolls (*Acanthis flammea*). Then, we determined how the width of growth bars and probability of having fault bars on feathers varied with $CORT_f$, specifically whether these metrics reflected developmental costs of elevated CORT ("stress" hypothesis) or represented an individual's quality ("quality" hypothesis). $CORT_f$ correlated positively with the strength of carotenoid signals, but only in adult males. However, also in adult males, $CORT_f$ was positively related to width of feather growth bars and negatively with probability of having fault bars, providing support for the quality hypothesis. Overall, $CORT_f$ was lower in adult males than in females or young males, possibly due to dominance patterns. Our results indicate that CORT may indirectly benefit feather quality, potentially by mediating the expression of carotenoid signals. We place our sex-specific findings into a novel framework that proposes that the influences of CORT in mediating carotenoid-based plumage traits will depend on the extent to which carotenoids are traded off between competing functions.

Keywords *Acanthis flammea* · Carotenoid-dependent plumage color · Common redpoll · Corticosterone · Feather quality

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G. R. Bortolotti: deceased.

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Introduction

Conditional handicap models of sexual selection suggest that elaborate ornaments, such as brightly colored plumage or skin, signal individual quality because they are costly to produce and maintain (Andersson 1994). Carotenoids are responsible for many of the red, orange and yellow colors expressed by animals, and play a key role in vital physiological processes, including immune function (Goodwin 1984; Bendich 1989; Olson and Owens 1998; McGraw and Ardia 2003; McGraw et al. 2011a). Producing carotenoid-dependent colors may be expensive because of energetic costs of acquiring carotenoids in the diet and metabolizing them into pigments suitable for deposition in ornaments (Britton 1976; Brush 1990; Pérez-Rodríguez 2008). Individuals may also face a trade-off between utilizing

carotenoids for physiological processes versus deposition in ornaments (Olson and Owens 1998; Møller et al. 2000). Proximate mechanisms that regulate how individuals resolve this trade-off are poorly understood, despite the importance of such mechanisms to the expression of color signals.

Evidence is accumulating that the hormone corticosterone (CORT) plays a significant mechanistic role in the production of carotenoid-based plumage. CORT helps vertebrates adjust their energy balance and respond appropriately to environmental variation, including stressors (Sapolsky et al. 2000; Wingfield and Kitaysky 2002; Romero 2004). Importantly, CORT can influence carotenoid-based coloration in a condition-dependent manner (Loiseau et al. 2008; Cote et al. 2010). This apparently occurs through a combination of the acquisition, absorption, transport, conversion, and deposition of carotenoids and influences on feather quality. For example, elevated CORT levels can increase locomotion and food intake that would facilitate carotenoid acquisition (Astheimer et al. 1992; Breuner et al. 1998; Cash and Holberton 1999; Cote et al. 2008), and its involvement in regulating energy and mobilizing carotenoid-rich fat stores (Dallman and Bhatnagar 2001; Negro et al. 2001; McGraw and Toomey 2010; McGraw et al. 2011b) implicates CORT in the energetically expensive process of carotenoid transport and utilization (Hill 2000). CORT can also alter feather quality (DesRochers et al. 2009), which may affect the brightness of carotenoid-based feather traits (Kennedy et al. 2013). Finally, CORT can influence the balance between pro- and antioxidants (Costantini et al. 2008), and mediate how parasites and testosterone interact to determine ornament color (Bortolotti et al. 2009; Mougeot et al. 2010). In addition to influencing carotenoid-dependent color, CORT can also produce significant negative effects. When elevated for sustained periods, CORT can suppress the immune response (Sapolsky et al. 2000; Saino et al. 2003; Martin et al. 2005), increase oxidative stress (Lin et al. 2004), and alter testosterone secretion (Deviche et al. 2001; see also Johnsen 1998). Thus, the actions of CORT may link environmental variation to the expression of carotenoid-based signals via effects on individual physiological state, making a CORT-based mechanism attractive from the perspective of honest signaling. However, our understanding of these mechanisms is limited.

In birds, both positive (McGraw et al. 2011b; Kennedy et al. 2013) and negative (Loiseau et al. 2008) relationships have been observed between CORT and attributes of carotenoid-based plumage, suggesting both benefits and costs of increased CORT. Increased CORT may facilitate the expression of high-quality phenotypes, as only the best-quality birds would be able to tolerate the high levels of CORT required to produce the best-quality plumage signals ("quality" hypothesis). This is similar to positive relationships between CORT and measures of performance (e.g., Love et al. 2004; Kouwenberg et al. 2013; Bonier et al. 2011; Ouyang et al. 2011), where increased metabolic demands required of high-quality individuals are presumably facilitated by higher CORT levels (Bonier et al. 2009). Alternatively, if elevated CORT levels reflect an individual's inability to cope with energetic demands during feather growth [homeostatic overload (Romero et al. 2009)], then decreased phenotypic quality would be expected in feathers ("stress" hypothesis).

One way to assess support for these hypotheses is to quantify multiple markers of phenotypic quality. The period during which feathers grow should be particularly important for the expression of carotenoid-based plumage traits, so costs or benefits of CORT may be evident in feathers themselves. Thus, we analyzed for the first time relationships among four synchronized feather-based markers to test the hypothesis that CORT mediates the expression of carotenoid-based coloration. First, we analyzed the relationship between an integrated measure of CORT physiology from feathers ($CORT_f$) (Bortolotti et al. 2008) and a carotenoid-based plumage signal in common redpolls (*Acanthis flammea*) (van Oort and Dawson 2005). We predicted this relationship would be positive if CORT promotes plumage color (quality hypothesis), but negative if CORT inhibits color (stress hypothesis). Then, to provide context to the CORT–signal relationship, we related $CORT_f$ to measures of feather growth bars (Grubb 1995) and fault bars (Bortolotti et al. 2002) formed over the same period as CORT deposition (i.e., during moult). We predicted that $CORT_f$ and the width of growth bars would positively covary if higher CORT represents individual quality, but would negatively covary if elevated CORT causes a developmental cost. Similarly, we predicted a negative relationship between $CORT_f$ and the probability of having fault bars if higher CORT indicates an individual's quality, but expected a positive relationship if higher CORT represents a cost (Bortolotti et al. 2002). If $CORT_f$ reflects neither quality nor a cost of signal production, then we expected no relationships with phenotypic measures of feather quality. Thus, by synchronizing a measure of CORT physiology with phenotypic traits that can reflect quality (e.g., Grubb 1995; Bortolotti et al. 2002; Griffith and Pryke 2006), we address the relationship between individual physiological responses and the costs of a carotenoid-based plumage signal at the time of ornament development. We additionally related $CORT_f$ to body condition measured during the winter following feather growth (van Oort and Dawson 2005) to provide a longer-term perspective on the CORT–carotenoid relationship.

Materials and methods

We studied common redpolls in the vicinity of Prince George, British Columbia, Canada (53°N, 122°W) during the winter of 2002. Birds were captured ($n = 57$) using walk-in traps baited with black oil sunflower seeds, and leg-banded so that recaptures could be identified and omitted from the analysis. Concurrent with our trapping sessions, a severe salmonellosis outbreak also occurred in the interior of north-central British Columbia (van Oort and Dawson 2005), and we also collected dead redpolls during this time ($n = 79$). The weather was seasonally cold with temperatures consistently below freezing (average hourly temperature $\pm$ SE $= -7.3 \pm 0.32$ °C), and so dead redpolls were found in a frozen state, usually lying in feeders or on the snow pack nearby. Specimens were stored in air-tight plastic bags at -20 °C, and later thawed for processing.

For both dead and living redpolls, we measured mass (nearest 0.25 g) using a spring balance, and lengths of wing chord, ninth primary feather, and tail (nearest 0.5 mm) using a ruler. Furcular fat was scored using a six-point scale (after Gosler 1996). We inspected all remiges and rectrices for the presence of fault bars, and categorized birds having either no fault bars or at least one fault bar on these feathers. Fault bars are conspicuous irregularities in feathers, seen as streaks of frayed or missing vane running approximately perpendicular to the rachis. They are formed on the growing feather and are the result of abnormally formed or missing barbules, thought to result from a variety of stresses that occur during feather growth (see Bortolotti et al. 2002). The fifth right rectrix was collected to assess nutritional status of the birds during their last moult using ptilochronology (Grubb 1995) as well as levels of CORT (Bortolotti et al. 2008); the length of this feather was also measured using digital calipers (nearest 0.1 mm). We took two photographs, one of the breast and one of the rump, using a digital camera (Sony Cybershot DSC-F505V) mounted on a tripod with the lens positioned 60 cm above the bird to quantify the area of carotenoid pigmentation on each bird, and collected a single representative feather from the center of the breast, and another from the center of the rump to objectively quantify color (details below). Adult male common redpolls have extensive red carotenoid plumage ornaments on their breast and rump (Troy and Brush 1983) that are variable both in size and color (Seutin 1994). These red plumage patches are absent or highly reduced in females and juvenile males (Knox and Lowther 2000). Moreover, it can be difficult in the field to accurately distinguish between female common redpolls and young males (Pyle 1997). Birds were therefore categorized into two groups: adult males or females/young males.

Carotenoid signal size

Carotenoid plumage signals vary in both size and color, so we measured the area of carotenoid plumage patches for each bird from the high-resolution photographs (2,580,000 pixels/photograph), and color from the feather samples, for both the breast and the rump. Area of red pigmentation was determined from photographs using the polygon tool in ImageJ software, version 1.3 (US National Institutes of Health). As we only collected a single feather from the breast and rump of each bird, it was difficult to use spectrometry to quantify color due to the relatively plumaceous nature of these feathers. Instead, breast and rump feathers were mounted on white paper and digitized by scanning an image at 2,400 dots per inch. Scanned images were of sufficient resolution to sample color on individual feather barbs. Using the tools in Adobe Photoshop 6.0, we recorded values of color components red, green and blue (RGB system). RGB measures give equal scores to each component color when sampling white (high scores) or black (low scores) colors. The hue of a color is determined by the relative difference in component colors (red, green, or blue). For example, highly saturated red colors score high in the red component relative to either the green or blue, whereas red colors that are less saturated will have a larger green and/or blue component, relative to the red component. We calculated "redness" of the color by subtracting the average of green and blue scores from the red score. We took four measurements of color from each feather, and then used the averages in further analyses. By using this relative difference, we were essentially measuring saturation, while also capturing variation in hue.

To confirm that our method of quantifying redness gave data comparable to measures obtained using a spectrometer, we collected a large sample of dead redpolls that succumbed to salmonellosis during an outbreak in March 2013. From this sample, we selected 30 individual birds that represented the entire range of variation in carotenoid pigmentation, and measured the spectral characteristics of the breast area from 400 to 700 nm using a spectrometer (USB2000; Ocean Optics, Dunedin, FL) with a deuterium tungsten halogen light source (Avantes, Broomfield, CO). Reflectance at each wavelength was determined as the proportion of light reflected relative to a white standard (WS-1; Ocean Optics). We used a bifurcated probe fixed in a cylindrical sheath that excluded ambient light and maintained the probe perpendicular to the feather surface. Three measurements were taken from a representative area of each bird's breast, and we used the average of these values to generate individual spectral curves. From each spectral curve we calculated the hue and red chroma using CLR version 1.05 (Montgomerie 2008). Hue represented the wavelength of maximal light reflectance, while red chroma
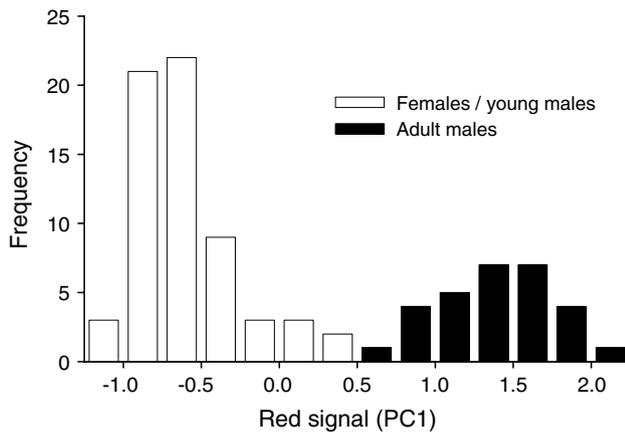
Fig. 1 Frequency distribution of carotenoid signals in common redpolls. Red signal is the first principal component (*PC1*) of a principal components analysis of size, hue and saturation of the red breast and rump feather patches; *larger values* indicate greater signal strength. See "Materials and methods" for further details

represented the proportion of light reflected within the red wavelength range (605–700 nm) relative to the entire 400–700 nm spectrum analyzed. For each bird we also plucked a single feather from the area measured with the spectrometer, which was mounted on white paper and digitized, and redness was calculated as described above. We found that our measure of redness was strongly correlated with both red chroma ($r = 0.87$, $n = 30$, $P < 0.0001$) and hue ($r = 0.61$, $n = 30$, $P < 0.0001$), and so we are confident that our methodology adequately described the color of the feathers (see van Oort and Dawson 2005 for additional details).

We entered the four distinct measures of carotenoid signals (breast patch area, breast redness, rump patch area, and rump redness) into a principal components (PC) analysis, and used the first component (PC1) as our measure of carotenoid signal strength (Fig. 1). The four input variables all loaded strongly and positively in PC1 (factor loadings were 0.96, 0.93, 0.98, and 0.86, respectively), which had an eigenvalue of 3.28, and explained 82 % of the total variance. Data from a random subset of nine birds, with signal area re-measured from photographs and redness scores measured from a second set of feathers, showed that these composite signal scores were highly repeatable (repeatability $r = 0.97$) (see Lessells and Boag 1987).

Feather growth, ptilochronology and CORT assays

Adult redpolls undergo complete moult at the end of summer, and juveniles moult their body feathers during this same period, but retain tail feathers grown in the nest (Knox and Lowther 2000). Greater widths of alternating dark and light bands (growth bars) on tail feathers can

positively reflect nutritional status during the period of feather growth (Grubb 1995). In adults, this corresponds to the time of year when carotenoid signals were produced; in juveniles, this reflects conditions experienced in the nest. Variation within the female/young male group in when feathers were grown may have prevented us from detecting significant CORT–color relationships within those sex/age classes, had they been present (see "Results"), but our primary focus was in examining relationships within adult males. Tail feathers were mounted on black construction paper, and holes were inserted through the card at the leading edge of each growth bar. Seven to ten growth bars centered on the distal two-thirds of the feather shaft were measured, and average growth bar size was calculated. To ensure reliability, we analyzed data only from cases with distinct growth bars. A sample of these feathers ($n = 35$ and 62 from live and dead redpolls, respectively) were subsequently analyzed for $CORT_f$ using a methanol-based extraction technique and the methods of Bortolotti et al. (2008). Compared to CORT from plasma, $CORT_f$ provides a longer-term perspective on physiology that incorporates both the amplitude and duration of hormone secretion over the period of feather growth. $CORT_f$ can reflect instantaneous levels of the hormone (Fairhurst et al. 2013) and avoids the known effects on plasma CORT levels of capture and handling. Feathers were prepared by first removing the calamus and the length of the remaining portion was measured and weighed. Samples were not washed prior to hormone analysis (Bortolotti et al. 2008). The feather minus the calamus was then cut into pieces smaller than 5×5 mm with scissors. We added 10 ml of high performance liquid chromatography-grade methanol (VWR International, Mississauga, ON) to the samples which were then placed in a sonicating water bath at room temperature for 30 min, followed by incubation at 50 °C overnight in a shaking water bath. Vacuum filtration using a plug of synthetic polyester fibre in the filtration funnel was used to separate the methanol from the feather material. We washed the feather remnants, original sample vial and filtration material twice with approximately 2.5 ml of additional methanol and this was added to the original methanol extract. The methanol extract was placed in a 50 °C water bath and subsequently evaporated in a fume hood. Evaporation of the samples was completed within a few hours. Samples were extracted in a single batch. Recovery efficiency of the methanol extraction was assessed by including feather samples spiked with approximately 5,000 CPM of ^{3}H-labeled CORT, and 86 % of the radioactivity was recoverable in the reconstituted samples. Extract residues were reconstituted in a small volume of phosphate buffer (0.05 mol/l, pH 7.6) and analyzed by radioimmunoassay in duplicate (Blas et al. 2005). Serial dilutions of sample extracts were parallel to the standard curve. Samples were randomly distributed throughout three

assays and run blind with regards to individual identity. Average ($\pm$SD) intra-assay variability was 7.2 ($\pm$2.03) % and inter-assay variability was 21.9 %. All data were at or above the limit of detection (ED80; average $\pm$ SD 9.70 $\pm$ 2.08 pg/μl).

Statistical analysis

We compared levels of $CORT_f$ of adult males to females/young males using ANOVA. We used analysis of covariance to test for the influence of $CORT_f$ and sex/age (adult male, females/young males) on body size (length of wing), mass, body condition, fat score, size of feather growth bars, and size of red signals, and logistic regression was used to test whether $CORT_f$ or sex/age was related to the probability of redpolls having one or more fault bars on their wing and tail feathers. As an index of body condition, we used the residuals from a linear regression of body mass on wing length (live birds only; $F_{1,33} = 7.53$, $P < 0.01$). For body mass and condition, as well as fat scores, we did not include data in these analyses from birds that died of salmonellosis, as emaciation and lack of fat are normal clinical signs associated with salmonellosis (van Oort and Dawson 2005). Finally, the small sample size of males surviving the outbreak of salmonellosis (see van Oort and Dawson 2005) prevented us from analyzing relationships between CORT and survival; results for the size of red signals and susceptibility to *Salmonella* of adult males have been presented elsewhere (van Oort and Dawson 2005), so we do not consider salmonellosis or survival further here. Samples sizes vary among analyses due to missing data for some variables.

Results

Adult male redpolls had lower levels of $CORT_f$ than females/young males ($F_{1,91} = 8.14$, $P < 0.01$; Fig. 2). Adult males also had longer wings than females/young males ($F_{1,90} = 27.31$, $P < 0.001$), and among all birds, levels of $CORT_f$ increased with wing length ($F_{1,90} = 4.42$, $P = 0.04$). Within the sample of live birds (i.e., those that did not succumb to salmonellosis), adult males were heavier than females/young males ($F_{1,29} = 7.45$, $P = 0.01$), but $CORT_f$ was not related to body mass ($F_{1,29} = 1.26$, $P = 0.27$). Similarly, body condition index ($F_{1,30} = 0.07$, $P = 0.79$) and fat score ($F_{1,30} = 0.23$, $P = 0.64$) also were unrelated to $CORT_f$.

We detected an interaction between sex/age and $CORT_f$ on the width of feather growth bars ($F_{1,54} = 3.90$, $P = 0.05$); to explore the nature of this interaction we analyzed data separately for adult males and for females/young males. For adult males, width of growth bars increased with
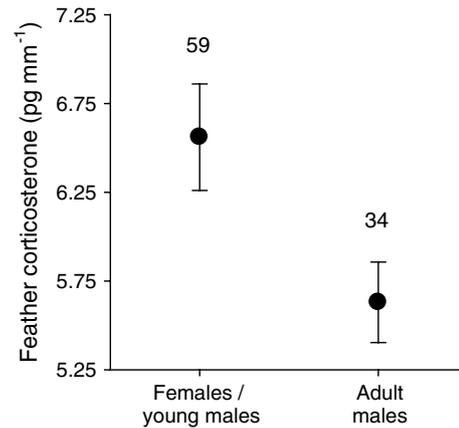

Fig. 2 Mean ($\pm$SE) level of corticosterone in feathers of common redpolls. Adult males redpolls had significantly lower levels than females/young males ($P < 0.01$)
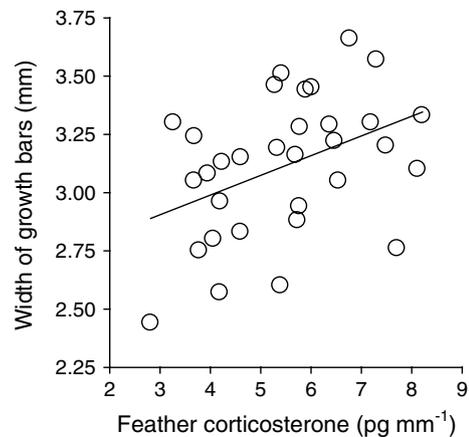

Fig. 3 Increasing width of growth bars on feathers of adult male common redpolls is associated with higher levels of corticosterone in feathers ($P = 0.02$)

the level of $CORT_f$ ($r = 0.42$, $n = 32$, $P = 0.02$; Fig. 3), while there was no relationship between width of growth bars and $CORT_f$ for females/young males ($r = -0.17$, $n = 26$, $P = 0.42$). Logistic regression showed that adult males had a lower probability than females/young males of having fault bars present on their wing and tail feathers ($\chi^2 = 4.51$, $df = 1$, $P = 0.03$), and that the probability of having fault bars present decreased with increasing levels of $CORT_f$, although these latter results only approached significance ($\chi^2 = 3.29$, $df = 1$, $P = 0.07$; Fig. 4).

When we analyzed data for strength of red signals, we found an interaction between sex/age and $CORT_f$ ($F_{1,89} = 10.35$, $P < 0.01$). Separate analyses by sex/age showed that there was no relationship between level of $CORT_f$ and strength of red signals among females/young males ($r = -0.04$, $n = 59$, $P = 0.79$), but $CORT_f$ was
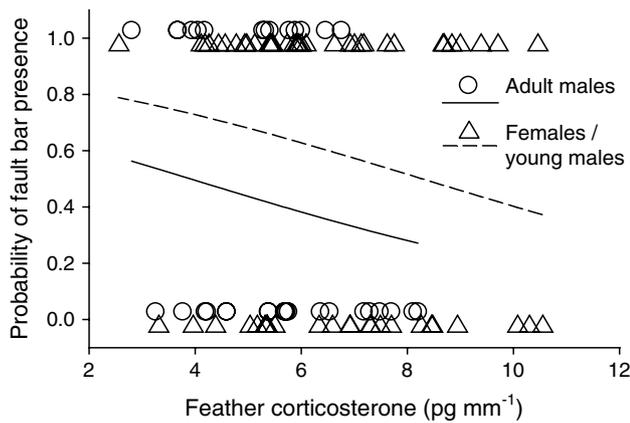
Fig. 4 The probability of wing and tail feathers of common redpolls having fault bars in relation to levels of corticosterone in feathers. To illustrate the distribution of data for presence or absence of fault bars of adult males and females/young males, data points have been offset slightly from 0 and 1
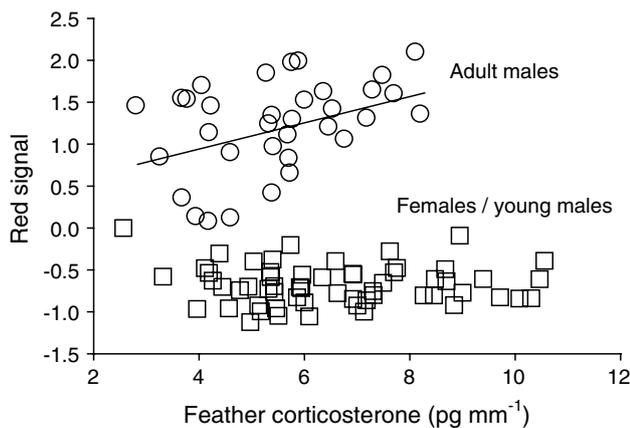

Fig. 5 Relationship between elaboration of the red carotenoid signals of redpolls and level of corticosterone in feathers for adult males ($P < 0.01$) and females/young males (NS). Red signal is first component (*PC1*) of a principal components analysis of size, hue and saturation of the red breast and rump feather patches; *larger values* indicate greater signal strength. See "Materials and methods" for further details

positively related to strength of red signals for adult males ($r = 0.41$, $n = 34$, $P = 0.02$; Fig. 5).

Discussion

We used synchronized measures of feather-based markers to distinguish between two hypotheses that explain how CORT relates to the expression of a carotenoid-based plumage trait. Examining phenotypic traits other than carotenoid coloration that are evident during moult provided insight into the CORT–carotenoid relationship. Our

results support the quality hypothesis, as we found that adult male redpolls with stronger red plumage ornamentation had more CORT in their feathers (Fig. 5). Work with closely related *Carduelis* species has shown positive relationships between carotenoid color and immune response (Kelly et al. 2012), condition (Peters et al. 2008; Rosenthal et al. 2012), and foraging ability (Mateos-Gonzalez et al. 2011). Inter-relationships among these variables are complex (Hõrak et al. 2006), but they all have the potential to contribute to individual quality. Although some evidence suggests that birds with elevated CORT levels produce feathers that are lighter and weaker (DesRochers et al. 2009), and grow more slowly (Romero et al. 2005), we found that adult males with higher CORT$_f$ levels produced better quality feathers that had wider growth bars (Fig. 3) and tended to have fewer fault bars (Fig. 4). These results suggest an additional benefit of increased availability of energy substrates as a consequence of higher levels of CORT. The ability to capitalize on this benefit is likely related to individual quality, as only high-quality birds are predicted to tolerate high CORT levels (Bonier et al. 2009). Thus, if the patterns seen in these feather markers are the consequences of increased CORT, these variables may have indicated male quality. Nonetheless, if this were true, the effect did not carry over to winter mass, body condition, or fat scores. Although this may be explained by the temporal separation of CORT deposition from the measurement of these variables, a previous study of these redpolls (van Oort and Dawson 2005) showed a similar lack of relationship between these variables and carotenoid plumage color. Future experiments will be essential in substantiating this claim and determining which physical traits best quantify downstream effects of quality.

Mechanisms explaining how CORT relates to increased carotenoid coloration could also account for the variation in feather growth bars and fault bars. For example, elevated CORT levels can increase locomotion and food intake (Astheimer et al. 1992; Breuner et al. 1998; Cash and Holberton 1999; Cote et al. 2008). As a consequence of these CORT-mediated changes in behavior, adult male redpolls may have consumed more carotenoid-rich food, were thus able to produce stronger signals, and would have been in better nutritional condition (i.e., wider growth bars and fewer fault bars). Recent work suggests that acquisition ability is not always an important source of variation for color traits in some species (e.g., McGraw et al. 2002; Hadfield and Owens 2006) and other processes such as mobilization, transport, and metabolism of carotenoids after ingestion have a greater effect on color variation. For example, if carotenoids that are used to color feathers are mobilized from body stores such as adipose tissue (McGraw and Toomey 2010; Negro et al. 2001) then increased metabolic activity required for this process could

account for the higher CORT levels that we observed in males with stronger red signals (Dallman and Bhatnagar 2001; McGraw et al. 2011b). Mobilization of body stores could also have increased the substrates required for better quality feather growth (i.e., wider growth bars and fewer fault bars). Our study was not designed to test specific mechanisms. Nevertheless, synchronized relationships between $CORT_f$ and feather color, width of growth bars, and presence of fault bars suggest that CORT may have mediated all three.

While the hypotheses described above suggest a direct causal role of CORT in mediating carotenoid-dependent plumage expression in adult male redpolls, it is also possible that higher CORT levels in more elaborately ornamented males are an indirect consequence of dominance hierarchies. During the non-breeding season, there is a clear linear dominance hierarchy with male redpolls being dominant over females (Dilger 1960). Although it is unclear whether plumage ornaments play a role in dominance relationships in redpolls (Romero et al. 1997), plumage-based hierarchies have been found in other species (e.g., Marra and Holberton 1998; Marra 2000; Reudink et al. 2009; Germain et al. 2010; but see Belthoff et al. 1994; McGraw and Hill 2000; McGraw et al. 2007). Among those species where the relative costs of being subordinate exceed those of being dominant, such as in cases where dominance is maintained by aggression and intimidation, it is expected that subordinate individuals will have higher levels of circulating glucocorticoids than dominants (Goymann and Wingfield 2004). This was the case in our study (Fig. 2), although we cannot rule out the possibility that grouping females and young males together may have biased our average measure of CORT, or that adult males likely had preferential access to food (van Oort and Dawson 2005), which may have alleviated nutritional stressors and reduced levels of CORT relative to subordinates. Regardless, in many systems dominance is maintained by individuals being challenged by opponents with higher status (e.g., Maynard Smith and Harper 1988; Parker and Ligon 2002). Although on average adult male redpolls had lower CORT levels than females/young males, the positive correlation between CORT and strength of red signals within adult males could arise if higher quality individuals (more colorful) are more likely to challenge other individuals or be challenged themselves (higher CORT). Clearly, more experimental work is required to fully understand relationships among CORT, dominance, and red signals in this species.

Why did we find a relationship between CORT and carotenoid-based ornament color in adult males only, particularly if they had, on average, the lowest feather CORT levels? It is possible that an interaction of CORT with testosterone could explain our results: individuals experiencing higher levels of testosterone (such as adult males in our study) may have been more sensitive to the actions of CORT (Bortolotti et al. 2009; Mougeot et al. 2010; Blas et al. 2006). However, it is unlikely that testosterone levels would have been high during post-breeding moult. It is also possible that the variation within females/young males in when feathers were grown may have contributed to the sex difference we report. However, there was no overlap in red signal strength between adult males and females/young males, and similar strong sex differences in color have been reported for other closely related finch species (e.g., McGraw et al. 2002). Thus, we suggest that the extent to which CORT influences the expression of carotenoid-based ornaments may depend on the strength of the trade-off between ornament expression and other demands competing for carotenoids (Lozano 1994). In groups that have a strong trade-off (e.g., adult male redpolls in our study), the physiological actions of CORT would be expected to shift the outcome of the trade-off and thus relate to trait expression; the direction of this relationship will depend on how well the individual can cope with demands of producing carotenoid-based ornaments (Fig. 6). Conversely, in groups where such trade-offs are weak or absent, the effects of CORT would be less pronounced or absent, as was likely the case with female/young male redpolls in our study. Although our sex-specific results appear to support this framework, we did not measure trade-offs directly, so we can only speculate that between-group differences in the strength of trade-offs existed in redpolls. However, sex-specific expression of carotenoid-based plumage in the closely related American goldfinch (*Carduelis trista*) appears to be due to physiological differences in the sexes (McGraw et al. 2002), suggesting sex-specific trade-offs may indeed be present in redpolls. Clearly, future tests of any hypothesis within this framework will require direct evidence of the strength of trade-offs involving carotenoids.

This framework unifies hypothesized mechanisms that underpin the CORT–carotenoid–color relationship (e.g., Loiseau et al. 2008; McGraw et al. 2011b; Cote et al. 2010). Numerous factors (e.g., life history stage, sex, season, quality, health, environment) will set the stage for trade-offs between carotenoid-based ornamentation and other biological functions on which CORT-mediated mechanisms can act (e.g., Bortolotti et al. 2009; Cote et al. 2010; McGraw et al. 2011b). Similarly, the range of CORT values that individuals express is determined by numerous environmental influences and physiological regulators (Romero et al. 2009), dominance rank among them. We reason that it is the interaction of CORT with the physiology of carotenoid-based trade-offs that should determine trait expression. Our hypothesis suggests that the influence of CORT (positive or negative) would be expected to increase with
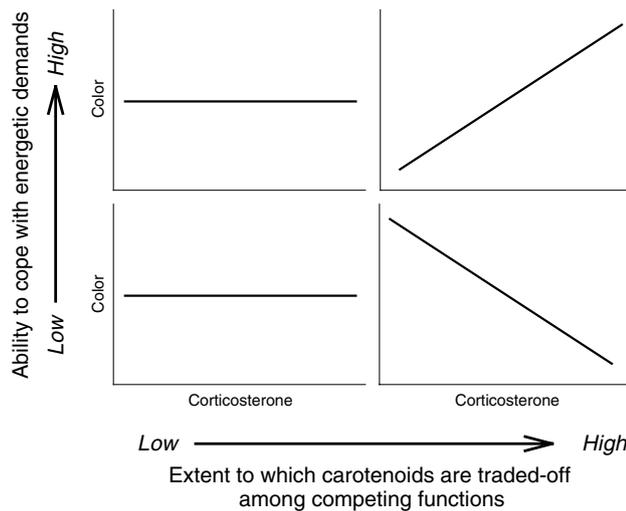
Fig. 6 Whether corticosterone (CORT) will correlate with expression of carotenoid-dependent ornaments will depend on the extent to which carotenoids are traded off among competing functions at the time of ornament production (*x*-axis). If carotenoids are only traded off weakly, such as when ornament expression is not a priority (e.g., in female and young male redpolls), then CORT may be expected to have little or no influence on carotenoid ornament expression (*left quadrants*). However, if ornament expression is important (e.g., for attracting a mate, maintaining dominance, or signaling to parents) and carotenoids are traded off strongly, then CORT-based mechanisms may influence the outcome of the trade-off. The direction of the CORT-carotenoid relationship will depend on the physiological ability of the individual to cope with the energetic demands of producing the ornament (*y*-axis). If the individual possesses the energetic resources to tolerate elevated CORT levels, then a positive relationship with phenotype is expected ["quality" hypothesis, *upper right quadrant*; e.g., Cote et al. (2010); McGraw et al. (2011b)]. If the individual cannot cope with elevated levels of CORT, then a negative relationship with phenotype is expected ["stress" hypothesis, *lower right quadrant*; e.g., Loiseau et al. (2008)]. Relationships between CORT and phenotype are depicted here as categories for simplicity, but likely represent a continuum based on numerous factors such as life history stage, health, and environment

the need to express ornaments and, thus, the strength of the trade-off.

Synchronizing feather-based measures of CORT physiology with phenotypic traits related to plumage quality provided insight into sex-specific CORT–carotenoid–color relationships. Our work suggests that rather than reflecting stress, $CORT_f$ may have revealed adult male quality. That $CORT_f$ was significantly related to both signal strength and two markers of feather quality suggests both primary and secondary effects of CORT on trait expression. The factor(s) responsible for variation in CORT in redpolls remain unknown. However, if CORT does mediate the physiology of carotenoid allocation, any factor that influences expression of the hypothalamo–pituitary–adrenal axis (e.g., predators, contaminants) has the potential to influence the expression of carotenoid-based signaling.

Our proposed theoretical framework suggests that this will depend on the extent to which carotenoids are traded off between competing functions and how well individuals can cope with elevated levels of CORT (Fig. 6).

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