SEX- AND AGE-RELATED VARIATION IN PLASMA CAROTENOIDS DESPITE A CONSTANT DIET IN THE RED-LEGGED PARTRIDGE *ALECTORIS RUFA*

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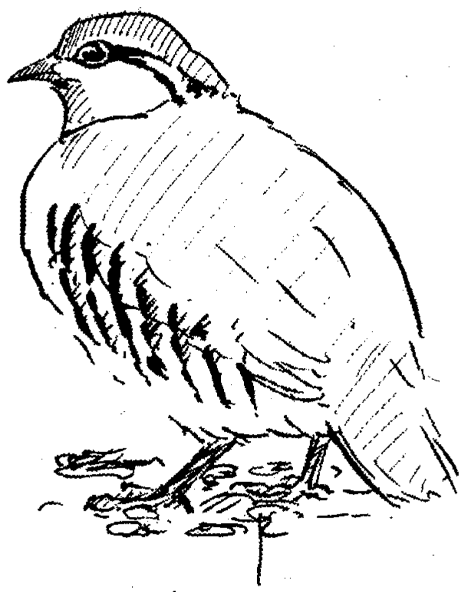

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We studied seasonal variation in plasma carotenoid levels of captive Red-legged Partridges *Alectoris rufa* in relation to sex, age and condition. The legs, bill and eye lores in this species are bright red due to carotenoid pigments. Carotenoid levels in the plasma varied significantly among sampling periods. Males presented higher values than females of their same age group, except in the last sampling period in summer. Adult partridges (2-year old) presented significantly higher carotenoid levels than first-year birds at the beginning of the study, but this age effect was not constant throughout the season. The sex-specific variation in plasma carotenoids cannot be attributed to diet, as this was the same for all individuals across the study. Body mass, and thus condition, did not change significantly across the study, and was therefore not related to seasonal variation in plasma carotenoids. These results imply physiological regulation of carotenoids, opposite to a direct relationship among carotenoid content in the diet and colour expression in the integument, as suggested in some previous studies.

Key words: *Alectoris rufa* – carotenoid - bird colour - sexual selection

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INTRODUCTION

The evolution of bird colouration is the subject of much research and debate (Gray 1996; Olson & Owens 1998). Carotenoids are currently at the centre of this debate (Møller *et al.* 2000). These pigments, obtained from the diet in the case of birds (Goodwin 1950; Brush 1990), are responsible for some of their brightest colours and are very prevalent in body parts used as sexual signals. Carotenoids are important antioxidants, and a trade-off between their ornamental and health functions has been hypothesised (Shykoff & Widmer 1996; Saino *et al.* 1999; but see Hill 1999). According to this hypothesis, birds whose immune system has been challenged should divert more carotenoids into health maintenance and less would remain for ornamentation. Only the healthiest individuals would thus express maximum coloration.

Within avian species, the carotenoid-based coloration may vary between sexes, age classes, or even among populations (Hill *et al.* 1994; Figuerola & Gutiérrez 1998). Some of these differences may be genetically fixed (i.e., sexual dimorphisms), but others have been attributed to different access to carotenoid sources (Endler 1980; Hill 1991; Bortolotti *et al.* 2000), or to the condition of the individual (Hudon 1994; Bortolotti *et al.* 1996; Shykoff 1997; Hill 1998). However, Bortolotti *et al.* (1996) and Negro *et al.* (1998) reported age-, sex-, and seasonal variations in carotenoid-dependent colorations of American Kestrels *Falco sparverius* independent of the diet. Levels of carotenoids in the blood also changed significantly, suggesting that there is not a simple relationship between ingestion of carotenoids and external coloration, and that there is a physiological regulation of carotenoids.

It is still unknown whether interindividual differences in plasma carotenoid unrelated to diet occur in other avian species. The question is not trivial, as individual characteristics (including sex, age and body condition), rather than dietary differences, may determine to some extent the absorption of carotenoids. In this study we report plasma carotenoid levels of captive Red-legged Partridges *Alectoris rufa* in males and females of two different cohorts (one and two-year old) during the breeding season. The pattern of change in body mass was studied concurrently to calculate an index of condition and to determine whether it correlated with carotenoid concentration.

The Red-legged Partridge is a medium-sized galliform showing crimson or coral-red coloration in the exposed integuments of legs, bill and eye lores. The pigments involved are probably carotenoids, which have been found in other galliforms (Marusich & Bauernfeind 1981; Egeland *et al.* 1993). Recently, a subtle sexual dichromatism has been reported, with the males showing redder bills (Villafuerte & Negro 1998). Higher plasma levels of carotenoids in males can thus be predicted, as a positive correlation between levels of circulating carotenoids and colour brightness of carotenoid-dependent traits has been reported in the species studies so far (Hill *et al.* 1994; Bortolotti *et al.* 1996; Saino *et al.* 1999).

METHODS

This study was carried out at a game farm in southern Spain. We started with 64 non-breeding individuals placed in 4 pens that contained, respectively: eight 2-year old males and ten 1-year old males; eight 2-year males and nine 1-year-old males; eight 2-year old females and seven 1-year old females; eight two-year old females and six one-year old females. In the course of the experiment eleven birds died, seven of them during the first two weeks, due to a coccidiosis outbreak. Each pen comprised an indoor (6.5 m^2) and an outdoor enclosure (12 m^2). All birds were exposed to natural photoperiod and temperature. Partridges were given a commercial feed (Purina España, S.A.) daily *ad libitum* at around 09.00 H. The feed, which was bought all at once before the study began, incorporated an anticoccidian agent (Amprol) two weeks after the beginning of the experiment.

The birds were blood sampled on four occasions: 27 February, 4 April, 6 May and 7 July, in 1997, spanning the whole breeding season for partridges, both captive and free-ranging, in the area (Calderón 1983, authors unpub. data). Each sampling time the birds were weighed with a Pesola spring scale, and 0.25 ml of blood was drawn from the brachial vein. Blood was extracted at 10-12 h, transported in a cooler and centrifuged within 24 h of collection. Resulting plasma was frozen at –20°C until analysis. Total plasma carotenoids were measured spectrophotometrically, using acetone as a solvent and reading absorbance at 476 nm; carotenoid concentration was estimated as μg/ml of plasma, using a standard curve of lutein (Tella *et al.* 1998). Tarsus length of each individual was measured at the beginning of the study.

Plasma carotenoid levels were normally distributed. Pen effects were tested with one-way ANOVA's. Differences were non-significant ($P > 0.05$) for all variables and data were pooled in subsequent analyses. Individual differences in both levels of blood carotenoids and body mass were analysed by repeated measures ANOVA, with sampling period as repeated measurement, and sex and age as independent factors. Body condition in each period was estimated as the residual value obtained in a reduced major axis regression (Sokal & Rohlf 1995) of log mass on log tarsus. Given that males were significantly heavier, males and females were analysed separately.

RESULTS

Plasma carotenoids

According to the repeated measures ANOVA, plasma carotenoid levels (Fig. 1) varied significantly due to the effect of sex ($F_{1,48}$ = 14.9, $P <$ 0.001) and sampling period ($F_{3,144}$ = 15.9, $P = <$ 0.0001). The age factor had no significant effect ($F_{1,44}$ = 0.03, P = 0.85). As for the interaction effects, significant ones were 'sex x season' ($F_{3,144}$ = 3.5, $P < 0.05$) and 'age x season' ($F_{3,144}$ = 5.9, $P < 0.001$). The fact that those two interaction effects were significant indicate that both sex and age differences were not constant throughout the season. Plasma levels were higher for 2-year old birds in the first sampling in February ($F_{1,60}$ = 6.42, $P < 0.05$), but the opposite was true for the last sampling in July ($F_{1,51}$ = 5.24, P = 0.02) (Fig. 1).

Body mass and condition

Males were heavier than females (Fig. 2) in every sampling period. According to the repeated measures ANOVA, body mass varied significantly due to the effect of sex ($F_{1,45}$= 47.1, $P <$ 0.0001), but age ($F_{1,45}$ = 0.4, P = 0.51) and sampling period ($F_{3,135}$ = 2.3, $P > 0.05$) had no significant effect. As for the interaction effects, the only significant one was 'age x season' ($F_{3,135}$ = 6.4, P < 0.001); this means that the significance of the age effect depended on the sampling period. In fact, a significant difference only occurred in the last sampling date, when 2-year old birds of both sexes lost mass in relation to the preceding periods. We examined how carotenoid levels may be related to condition within each time period by correlating the residuals of the log mass on log tarsus regression with carotenoid concentrations. Correlations were not significant ($P > 0.05$) in all cases for adults of both sexes. Correlations were positive and significant for 1-yr old males in the first three sampling dates (February: r = 0.47, P =

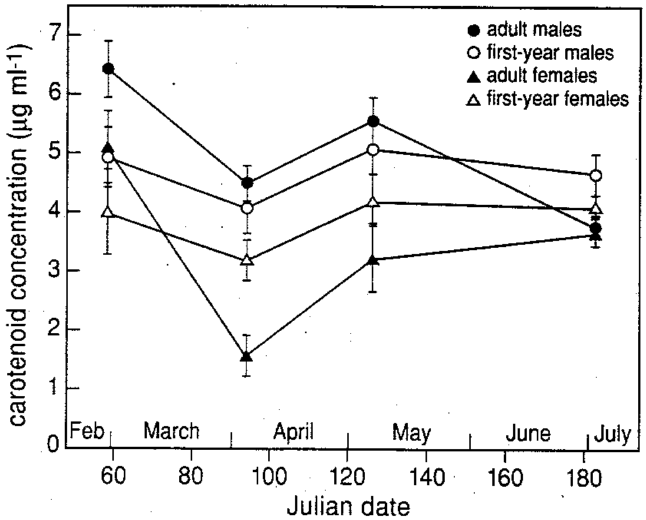

Fig. 1. Mean ± SE of concentrations of plasma carotenoids at four sampling dates in springtime, for male and female Red-legged Partridges. Adult (2-year-old) and first-year birds are treated separately.

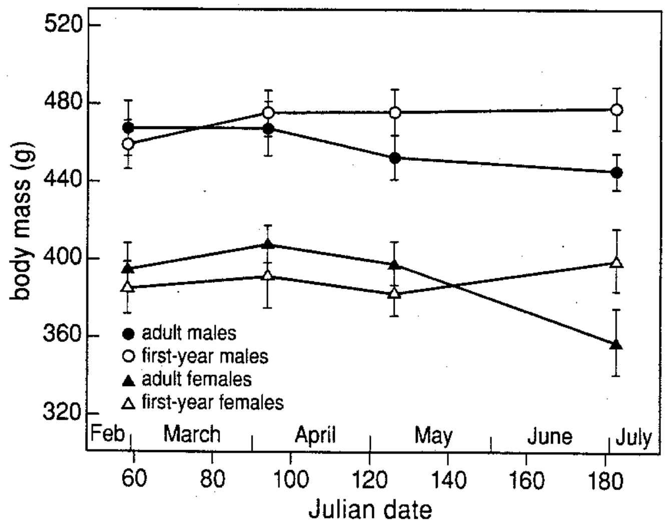

Fig. 2. Mean ± SE of body masses at four sampling dates in springtime, for male and female Red-legged Partridges. Adult (2-year-old) and first-year birds are treated separately.

0.04, n = 18; April: r = 0.62, P = 0.01, n = 16; May: r = 0.62, P = 0.01, n = 15), and marginally significant for 1-year old females (r = 0.54, P = 0.06, n = 12) in February.

DISCUSSION

Plasma carotenoid levels of the partridges presented significant differences among sampling periods, depending on the sex and age of the individuals. In addition, males – as predicted – showed significantly higher levels than females. These differences took place despite the fact that the diet, and thus access to carotenoids, was kept constant throughout the study. Changes in condition, at least measured as an index of mass scaled to body size, did not seem to play a very important role, as mass did not change significantly throughout the study in most age and sex groups, and individuals had reached adult sizes when the study began (i.e. body measurements did not change). We only found significant positive correlations between the condition index and carotenoid levels in first-year birds, and we suspect that mass may be a poor indicator of condition in these captive birds with unlimited access to food.

Significant seasonal and sex-related differences were also observed in the only previous study (Bortolotti *et al.* 1996) where the diet was held constant (for captive American Kestrels and Loggerhead Shrikes *Lanius ludovicianus*, see also Negro *et al.* 1998). Overall, these works indicate that dietary variation is not the only factor that may produce intraspecific differences in plasma carotenoid levels. If intrinsic characteristics of the individual, including its sex and age, explain a significant part of its capacity to absorb carotenoids from the diet, sex- and age-related dichromatisms may be a reflection of those capacities, without a need to invoke different foraging abilities or different dietary preferences (see, e.g. Linville & Breitwisch 1997).

Negro *et al.* (1998) suggested that the patterns of change observed in the coloration and plasma levels of American kestrels were consistent with sexual selection. This was so because brighter colours and higher carotenoid concentrations declined from beginning to end of the breeding season –Darwin (1871) predicted maximum expression during the mating period for sexually-selected traits-. In our partridge study, where external colour was not evaluated, plasma levels were higher in February (first sampling) compared to July (last sampling). However, the decline was not smooth, and was perhaps obscured by an early coccidiosis outbreak and the subsequent treatment with an anticcocidian agent. This is so because coccidiosis typically inhibit absorption of carotenoids in the gut and deplete plasma carotenoid levels (Allen 1992). Under the circumstances, we cannot argue whether the observed patterns were consistent with sexual selection.

We have not yet identified the physiological control mechanisms responsible for both the sex and age-specific variation. Further research is also needed to validate these results in species with different extent of carotenoid-based ornaments. So far data are only available for two species with carotenoids in restricted skin areas (i.e., kestrels and partridges), and one species with no external display of carotenoids (i.e., the loggerhead shrike). There is a difference, however, depending on whether carotenoids are displayed in skin areas, from which they may be rapidly reabsorbed (Lozano 1994; Shykoff 1997), or in feathers, where the pigments stay unavailable for other bodily functions. The next logical step would be to experiment with birds showing carotenoids in their feathers. The reason is that different predictions are made for skin and feathers, respectively, on the timing when plasma carotenoids should reach a peak if sexual selection was operating (Hill 1995). For skin, maximum plasma levels should be found on the mating season, as they have an immediate effect on the external colour. For feathers, higher levels should be observed on the moult period –typically at the end of the summer and early fall for birds in temperate regions- because it is the only time when individuals can allocate pigments into ornamentation.

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SAMENVATTING

De Rode Patrijs *Alectoris rufa* ontleent zijn naam aan de felrode kleur van snavel, poten en oogring. De kleur ontstaat door caroteenpigmenten die opgenomen worden met het voedsel. Ook bij andere vogelsoorten zijn caroteenpigmenten vaak verantwoordelijk voor de felle kleuren, waarvan gedacht wordt dat ze een belangrijke rol spelen als seksueel signaal. De belangrijke vraag is in hoeverre een individu middels de intensiteit van zijn kleuren andere dieren iets laat weten over zijn genetische kwaliteit, dan wel zijn lichamelijke conditie, of de mate waarin het individu toegang heeft tot voedsel met veel caroteenpigmenten. Een interessante hypothese is dat zieke dieren hun carotenen moeten inzetten ter bestrijding van de ziekte, terwijl gezonde dieren met hun carotenen kunnen pronken in 'nutteloze' ornamenten.

Volgens de auteurs is tot nu toe alleen bij de Amerikaanse Torenvalk *Falco sparverius* vastgesteld dat caroteenkleuring onafhankelijk van het menu varieerde met leeftijd, geslacht en seizoen. In deze studie kregen verschillende groepen in gevangenschap gehouden Rode Patrijzen van verschillend geslacht en leeftijd hetzelfde voedsel. Aan het begin van de studie hadden volwassen dieren de hoogste concentratie caroteen in het bloed, maar de verschillen tussen de groepen waren niet constant in de loop van het seizoen. Verder was het zo dat mannetjes vrijwel steeds een hogere concentratie caroteen in hun bloed hadden dan vrouwtjes, ondanks het gelijke menu. Mannetjes zijn zwaarder dan vrouwtjes, maar binnen een sekse waren de oudere dieren eerst zwaarder en later lichter dan de jonge dieren. Het gewicht werd bepaald om te kijken of caroteenopname samenhing met de conditie van de vogels. Alleen voor jonge vogels was er in een aantal gevallen sprake van een positief verband tussen de conditiemaat en de opname van caroteen in het bloed, maar de auteurs hechten hier weinig waarde aan. Een probleem bij het onderzoek was dat een aantal dieren stierf aan het begin van de studie als gevolg van een uitbraak van coccidiosis, waarna er een antibioticum aan het voedsel werd toegevoegd. Deze studie bewijst in ieder geval dat de kleuring als gevolg van caroteenpigmenten fysiologisch wordt gereguleerd. Er is dus geen simpele en directe relatie met het voedsel, zoals eerder is beweerd. (BJE)

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