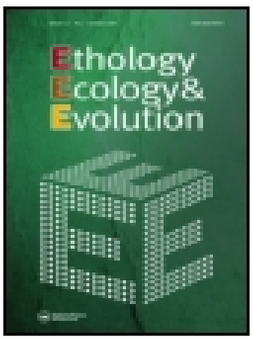

Ethology Ecology & Evolution

ISSN: 0394-9370 (Print) 1828-7131 (Online) Journal homepage: http://www.tandfonline.com/loi/teee20

Plumage coloration, body condition and immunological status in Yellow-billed Cardinals (Paroaria capitata)

R.I. Dias, L.T. Manica, D. Gressler, J.A. Bell & A. Fecchio

To cite this article: R.I. Dias, L.T. Manica, D. Gressler, J.A. Bell & A. Fecchio (2015): Plumage coloration, body condition and immunological status in Yellow-billed Cardinals (Paroaria capitata), Ethology Ecology & Evolution, DOI: 10.1080/03949370.2015.1077892

To link to this article: http://dx.doi.org/10.1080/03949370.2015.1077892

Published online: 18 Sep 2015.

Ethology Ecology & Evolution, 2015
http://dx.doi.org/10.1080/03949370.2015.1077892

Plumage coloration, body condition and immunological status in Yellow-billed Cardinals (*Paroaria capitata*)

R.I. Dias [1,2,7], L.T. Manica [1,3], D. Gressler [1], J.A. Bell [4] and A. Fecchio [5,6]

[1] *Programa de Pós-Graduação em Ecologia, Universidade de Brasília, Brasília, 70910-900, Brazil*
[2] *Faculdade de Ciências da Educação e Saúde, Centro Universitário de Brasília, Brasília, 70790-075, Brazil*
[3] *Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, 81531-980, Brazil*
[4] *Department of Biology, University of North Dakota, Grand Forks, ND 58202, USA*
[5] *Programa de Pós-Graduação em Biologia Animal, Universidade de Brasília, Brasília, 70910-900, Brazil*
[6] *Ornithology Department, Academy of Natural Sciences of Drexel University, Philadelphia, PA 19103, USA*

Received 18 March 2015, accepted 19 July 2015

Plumage coloration deriving from carotenoid and melanin pigments can be a quality signal in birds, and can be under conspecific inspection in social interactions. For example, parasite load and immune system status can be inferred through plumage color intensity, and can influence the choice of sexual partners. Here, we evaluated two plumage ornaments in the Yellow-billed Cardinal [*Paroaria capitata* (d'Orbigny & Lafresnaye 1837)]: the carotenoid-based coloration of the cap and the melanin-based coloration of the bib. We evaluated whether these ornaments were related to blood parasite burden, immunological status and body condition, and whether they could reveal individual sex and age. Cardinals were mist-netted in a Brazilian wetland, and 12 individuals were infected with malaria parasites. Both carotenoid and melanin colorations were related to age, but only carotenoids reflected immunological status. Adult cardinals presented redder caps and darker bibs in comparison to juveniles, and redder caps were associated with low values of heterophil to lymphocyte ratio (H/L, indicating lower stress level). Plumage coloration did not indicate individual sex or parasite infection. Taken together, these results demonstrated that both melanin- and carotenoid-based coloration in cardinals can potentially reflect significant information for social interactions, such as individual age and experience, but apparently only carotenoid coloration is condition-dependent and could reliably indicate quality.

KEY WORDS: neotropics, ornaments, Pantanal, sexual signaling, WBC, avian malaria, *Plasmodium*, *Haemoproteus*.

[7] Corresponding author: Raphael I. Dias, Centro Universitário de Brasília, Faculdade de Ciências da Educação e Saúde, Brasília, DF, 70790-075, Brazil (E-mail: raphael.dias@uniceub.br).

INTRODUCTION

Elaborate and colorful feathers in birds are usually the result of increased intrasexual competition and careful mate choice (Andersson 1994). It is often assumed that sexual traits, such as plumage ornaments, reflect individual health status that could be under conspecific inspection in social interactions (Zahavi 1975; Andersson 1994). For example, rivals can predict their opponent's health condition, dominance status and fighting ability by the plumage coloration conspicuity (e.g. Greene et al. 2000; Pryke et al. 2001), or females may prefer brightly colored males for copulations (Hill 1990; Sætre et al. 1994). Besides the biased interest in male ornaments, a significant amount of evidence has revealed that males can also be selective based on females' ornaments (Clutton-Brock 2009), especially regarding coloration patterns (Griggio et al. 2005, 2009).

Colorations deriving from carotenoid and melanin pigments are considered important plumage quality signals. Carotenoids are responsible for most of the yellow, orange and red colorations (Brush 1990), and melanin produces the black and brown color of feathers (McGraw 2006). Because the former cannot be synthesized metabolically and are obtained exclusively through the diet, condition-dependency of carotenoid-based ornaments is predictable since they may co-vary with individual access to pigment resources (Hill 1992). In addition, carotenoids may have multiple physiological functions, such as antioxidants and immune-stimulators (Lozano 1994; Møller et al. 2000), affecting individual health status.

Melanin pigment can in turn be synthesized metabolically; thus, the potential for honesty as a quality signal is less evident (Badyaev & Hill 2000; Senar et al. 2003; McGraw 2006; but see 2003 for the role of metals in physiological processes and melanin synthesis). However, recent evidence reinforces the idea that melanin-based ornamentation may be associated with condition, predicting both reproductive output and levels of oxidative stress (Grunst et al. 2014; Wiebe & Vitousek 2015). Moreover, different studies have demonstrated that testosterone levels were also associated with melanin ornaments, suggesting that more extensively ornamented individuals were more affected by immunosuppressive effects (reviewed in McGraw 2008). Examples of the importance of melanin-based ornaments in social or sexual contexts have been accumulating (Rohwer 1975; Griffith 2000; McGraw 2006; Guindre-Parker & Love 2014).

Plumage coloration based on melanin and carotenoids may reflect individual parasite load and immune system status (Hõrak et al. 2001; Doucet & Montgomerie 2003; del Cerro et al. 2010; Guindre-Parker et al. 2013). The additional function of carotenoids as immune-stimulators (e.g. in the production of lymphocytes, neutrophils and macrophages) suggests that a mutual investment in coloration and immune response can serve as a trade-off (Møller et al. 2000). If more investment is necessary for physiological functions that prevent or aid in recovery from infections, fewer resources are available to allocate in feather coloration. As a consequence, mate choice favoring birds with colorful feathers may guarantee bonding with sexual partners in good health condition (e.g. Lozano 1994; Garamszegi 2005; Aguilar et al. 2007) and transfer of "good genes" to the offspring (Hamilton & Zuk 1982). Similarly, melanin synthesis can be condition-dependent (see Guindre-Parker & Love 2014 and references therein), and its use in ornaments may also function as a trade-off against other physiological activities, even though the cost of melanin production is still unclear.

Here, we studied plumage coloration, body condition and immunological status in the Yellow-billed Cardinal [*Paroaria capitata* (d'Orbigny & Lafresnaye 1837)]. Biochemical analysis performed on the genus demonstrated a higher proportion of

α-doradexanthin and canthaxanthin in the feathers (Thomas et al. 2014). Yellow-billed Cardinals are distributed throughout southwestern Brazil, Paraguay and northern Argentina (Ridgely & Tudor 1989). Males and females are alike, presenting a bright red cap, a black pointed bib, darker wings and white underparts (Ridgely & Tudor 1989). The bill has a characteristic pinkish-yellow coloration (Sick 1997), which is responsible for the common name. Juvenile individuals are not as brightly colored as adults, and have generally slightly paler red bibs. Despite being commonly found throughout its distribution, information regarding social and sexual behavior of this species is rare. Yellow-billed Cardinals are single-brooded and socially monogamous, and breed from September to March in Argentina (Di Giacomo 2005). Within this context, our goal was to determine whether the expression of two plumage ornaments in the Yellow-billed Cardinals, the carotenoid-based coloration of the red cap and the melanin-based coloration of the bib, reflect individual quality. We hypothesized that ornaments would be negatively correlated to blood parasite burden, and positively correlated to immunological and body condition, measured as feather growth rate and mass-to-size ratio. We also investigated whether plumage coloration in Yellow-billed Cardinals can indicate individual sex and age.

METHODS

Study area

This work was conducted in the dry season of 2009 at a study station of the Universidade Federal de Mato Grosso do Sul (19°34'37"S, 57°00'42"W), in southwestern Brazil. The study site is within Pantanal, a low-altitude Brazilian wetland with well-defined dry and rainy seasons (Pott & Pott 1994). Extensive areas of Pantanal become flooded during January to June, and yearly mean temperatures vary from 20 to 27 °C (Pott & Pott 1994). The area is composed by typical Brazilian wetland vegetation, including gallery forests, aquatic and open habitats, and human-modified areas.

General procedures

Yellow-billed Cardinals were mist-netted (license issued by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA, and Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio, no. 12322–3). All individuals were identified with a numbered metal band supplied by the Brazilian birding agency (CEMAVE/ICMBio). At the time of capture, individuals were weighed (to the nearest gram) with a Pesola® spring scale and the tarsus was measured with a caliper (to the nearest 0.02 mm). We recorded the age (adult or juvenile) by assessing the presence of a colored gape flange, and whether the individual was molting or not. From each captured individual, we collected the following: the right outermost rectrix for ptilochronology analysis, a blood sample for immunological and parasitological analysis and a sample of body feathers for the plumage coloration analysis (see details below). Birds were molecularly sexed following the protocol of Griffiths et al. (1998).

Ptilochronology and body condition index

To estimate body condition during molt, we calculated the feather growth rate using a ptilochronology technique (Grubb 2006). Under direct illumination, we measured with a caliper

(to the nearest 0.02 mm) the total width of 10 growth bars centered on a point two thirds of the way from the feather's distal end. The total width was divided by 10 to calculate the average growth bar width (Grubb 2006). This measurement can honestly signal health quality (Carlson 1998; Takaki et al. 2001), considering that nutritional condition during molt may constrain ornament production (Hill & Montgomerie 1994; Hegyi et al. 2007). For example, a faster growth rate of flight feathers may be important for survival because they determine maneuvering capacity; however, a trade-off may exist against other energetic costs depending on bird nutritional condition during molt (Grubb 2006). Another condition measurement, the body condition index, was calculated as the residuals of a linear regression of body mass (power-transformed) on tarsus length (fourth power-transformed; Brown 1996).

Ornament coloration analysis

We measured the spectral reflectance of red cap (Fig. 1) and black bib (Fig. 2) feathers from all individuals using a USB4000 spectrometer with a PX-2 pulsed xenon light source (range 250–750 nm) (Ocean Optics, Dunedin, FL). We removed 5–6 feathers with forceps and arranged them in an overlapping disposition to simulate the bird body. We took three readings for each sample using a bifurcated fiber-optic probe (Ocean Optics, Dunedin, FL), mounted on a holder at 90° that excluded all ambient light, at 2 mm from the feather. All measurements were taken relative to a WS-1-SS diffuse reflectance white standard (Ocean Optics). Reflectance data were used to calculate brightness and hue for the two body parts, and red and ultraviolet (UV) chroma for both cap and bib, respectively (Montgomerie 2006). Brightness (R320-700), or total light reflected, was calculated as the sum of the percentage reflectance values from 320 to 700 nm. Hue (λRmax), which is the main color reflected by the feather, was calculated as the wavelength of maximum reflectance. Chroma, a measure of spectral purity, was calculated as the ratio between total reflectance in the range of interest and total reflectance across the entire spectrum. Thus, red chroma was calculated as the ratio between red reflectance (R625–700) and total reflectance (R320–700), and UV chroma as the ratio between UV reflectance (R320–400) and total reflectance (R320–700). Repeatability among measurements (intra-class correlation) from the same body region was high (all $r > 0.75$; all $P < 0.001$; Lessells & Boag 1987).

To avoid redundant variables in statistical analysis, we ran a principal component analysis (PCA) for the cap and the bib spectral measurements separately. We used the first two principal components (PC), because they explained more than 80% of the variation in both PCAs. The first

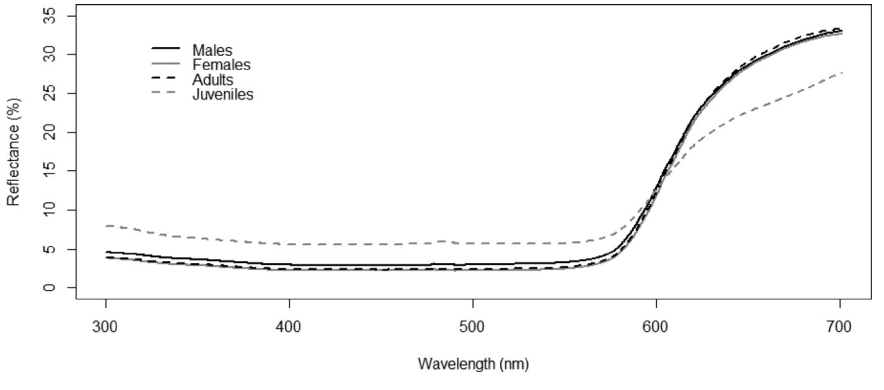

Fig. 1. — Spectral reflectance curves for the red cap of males (black solid line), females (gray solid line), adults (black dashed line) and juveniles (gray dashed line) of Yellow-billed Cardinals (*Paroaria capitata*).

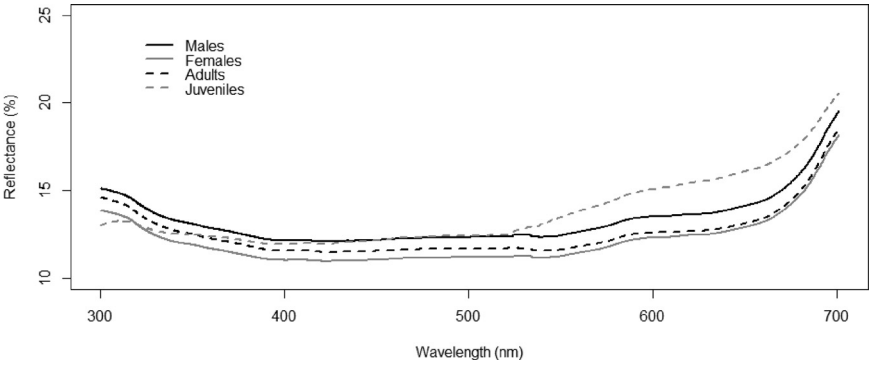

Fig. 2. — Spectral reflectance curves for the black bib of males (black solid line), females (gray solid line), adults (black dashed line) and juveniles (gray dashed line) of Yellow-billed Cardinals (*Paroaria capitata*).

Table 1.

Loadings of the principal component (PC) analysis on colorimetric variables from the cap carotenoid-based coloration and bib melanin-based coloration of Yellow-billed Cardinals (*Paroaria capitata*).

Ornament	PC1	PC2
Carotenoid-based coloration		
Brightness	– 0.759	0.050
Hue	– 0.391	0.759
Red chroma	0.519	0.648
Proportion of variance explained (%)	43.7	37.2
Melanin-based coloration		
Brightness	– 0.492	0.799
Hue	0.656	0.080
Ultraviolet (UV) chroma	0.571	0.595
Proportion of variance explained (%)	58.0	27.4

component for the red cap ($PC_{cap}1$) loaded negatively with brightness. The second component for the red cap ($PC_{cap}2$) loaded positively with hue and red chroma. For the black bib, the first component ($PC_{bib}1$) loaded negatively with brightness and positively with hue and UV chroma. The second component for the black bib ($PC_{bib}2$) was strongly loaded with brightness and UV chroma (Table 1).

Immunological and parasitological analysis

Blood samples were collected by puncturing the brachial vein with a sterile needle to prepare blood smears for microscopic observation and molecular analysis. Smears were air-dried, fixed in

absolute methanol and stained with Giemsa solution (Sigma Chemical Co.). The remainder of the blood was stored in 95% ethanol until DNA extraction.

Traditional and molecular methods were used to evaluate infection status by blood parasites. Slides were examined by light microscopy (Olympus, Japan). To detect *Trypanosoma* and microfilaria, 100 fields were examined at a magnification of 400 ×, and to detect *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, 200 fields were examined at 1000 ×. The microscopy of each sample took approximately 25 min, and only slides in good condition (i.e. visually uniform in consistency, and well stained) and with homogenous distribution of blood cells were analyzed.

DNA from blood samples was extracted using conventional phenol–chloroform extraction followed by ethanol precipitation. DNA extractions were screened by real time polymerase chain reaction (PCR) to detect haemosporidian DNA, following the protocol of Bell et al. (2015). Positive and negative controls were included in all real-time PCR runs. For the positive control, a synthetic double-stranded DNA product (G-Block- IDT DNA, Coralville, IA) produced from the published sequence of *Plasmodium relictum* (accession no. NC012426) was used. All positives determined by real-time analysis were amplified by nested PCR to amplify a 477-bp region of the cytochrome b gene (Bell et al. 2015). All nested PCRs were run using OneTaq Master Mix (New England Biolabs, Ipswich, MA) following the manufacturer's protocols. Due to the high sensitivity of nested PCR, negative controls were included in runs to check against possible contamination, although none was found in any PCR runs.

Products from PCR amplifications were run on 1.25% agarose gels, stained with ethidium bromide and visualized under UV light. Positive PCR products were purified using ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye terminator v. 3.1 cycle sequencing kit (Applied Bio systems, Foster City, CA) with the internal primers FIFI and R2 (Ishtiaq et al. 2007). DNA was cleaned up by ethanol precipitation, dried by vacufuge, resuspended with 10 µL of molecular grade water, and run on an ABI 3700 DNA sequencer (Applied Bio systems, Foster City, CA).

Forward and reverse sequences were visualized, edited and assembled using Sequencher v. 5.0.1 (Gene Codes Corp., Ann Arbor, MI). Chromatograms that showed the presence of multiple infections were scored as co-infections. Co-infections were separated using the program PHASE 2.1.1 (Stephens et al. 2001; Stephens & Donnelly 2003) following the protocol of Harrigan et al. (2014).

Assembled sequences for haemosporidians were aligned using BioEdit v. 7.2.0 (Hall 1999). Aligned sequences were collapsed to unique haplotypes using the FaBox haplotype collapse and converter tool (Villesen 2007). A local BLAST (Basic Local Alignment Search Tool) against the MalAvi database (Bensch et al. 2009) using BioEdit v. 7.2.0 (Hall 1999) was conducted for all unique haplotypes to identify lineages. Haplotypes with 100% pairwise identity to known lineage sequences were named accordingly, and new lineages with less than 100% pairwise identity were identified as new lineages.

Leukocyte counts were determined after counting 100 fields at a magnification of 400× in the same slide used for blood parasite diagnosis. The cells were identified as lymphocytes, heterophils, eosinophils, basophils or monocytes. However, we decided to focus the analysis on the heterophil to lymphocyte ratio (H/L) because this ratio is traditionally considered to indicate stress in poultry, with lower values indicating less stressed individuals (Gross & Siegel 1983). Additionally, variation among individuals regarding leukocyte count was very low (eosinophils, basophils and monocytes: mean 0.55, 0.20 and 1.68; range 0–3, 0–1 and 0–8, respectively).

Statistical analysis

We used linear models in the program R (v. 2.15.2, R Development Core Team 2012) to test whether condition parameters were affected by parasitism and if coloration (both carotenoid- and melanin-based) was associated with condition parameters, age, sex or molting occurrence. Data were visually inspected to check for distribution, homogeneity of variance, and outliers. Possible outliers were detected and removed. We used PCs obtained from coloration data as the response variable and H/L ratio (log-transformed), feather growth rate, body condition index, sex, age (adult or juvenile), parasitism (yes or no) and molting (yes or no) as explanatory variables. The

most parsimonious models were obtained by sequentially removing the variables, starting with interactions. We used likelihood ratio tests (LRT) to evaluate differences in the model fit, preferring the simpler model whenever no significant difference in model fit was detected. All variables were initially included, and then dropped until the model contained only significant terms.

RESULTS

We mist-netted and sampled 54 Yellow-billed Cardinals. Molecular analysis revealed that 18 of them were females and 35 were males. One individual was not sexed due to problems in blood sampling, and it was removed from the analysis. Approximately 60% of the individuals were molting and 22% were infected by haemosporidian parasites (n = 12). Most individuals were infected by *Plasmodium* (n = 11), two of which represented dual infections by two different *Plasmodium* lineages. Only one individual was infected by *Haemoproteus*. However, no parasites were visualized on blood slides. Parasitized individuals were mainly males (75%; n = 9). Parasitism did not explain variation in body condition, feather growth rate or H/L ratio (Table 2).

$PC_{cap}1$ (cap brightness) was not related to any explanatory variables (Table 2). On the other hand, $PC_{cap}2$ (cap hue and red chroma) was explained by age (Fig. 3), with adults presenting higher hue and red chroma, and thus a redder cap. $PC_{cap}2$ was also explained by H/L (Fig. 4). Higher hue and red chroma of the cap were associated with lower H/L, indicating that birds in better body condition presented a redder cap. $PC_{bib}1$ was also explained by age (Fig. 5); older individuals had lower brightness but higher hue and UV chroma. The other variables did not significantly explain variation in $PC_{bib}1$. Similarly, $PC_{bib}2$ (bib brightness and UV chroma) was not explained by any explanatory variables (Table 2).

DISCUSSION

We found evidence of a condition dependency for the carotenoid-based coloration on the cardinal's caps. The results suggest that this ornament may have an important role in honestly signaling individual quality. Physiologically stressed individuals (i.e. those presenting high values of H/L ratio) exhibited duller cap coloration with inferior

Table 2.

Linear model results from the effects of parasitism on condition parameters (heterophil to lymphocyte [H/L] ratio, feather growth rate, body condition index) and of condition, sex, age, parasitism and molting on coloration parameters of Yellow-billed Cardinals (*Paroaria capitata*).

	Estimate (β ± SE)	F	df	*P*
H/L ratio				
Parasitism	– 0.07 (0.10)	0.56	1,51	0.45
Feather growth rate				
Parasitism	0.00 (0.04)	0.00	1,51	0.92

(*Continued*)

Table 2.

(*Continued*)

	Estimate (β ± SE)	F	df	*P*
Body condition index				
Parasitism	0.05 (0.06)	0.72	1,51	0.39
$PC_{cap}1$				
H/L ratio	– 0.10 (0.18)	0.32	1,44	0.57
Feather growth rate	– 0.82 (1.38)	0.42	1,44	0.51
Body condition index	0.01 (0.01)	0.66	1,44	0.41
Sex [a]	– 0.16 (0.35)	0.32	1,44	0.57
Age [b]	– 0.62 (0.54)	1.54	1,44	0.22
Parasitism	– 0.11 (0.40)	0.20	1,44	0.65
Molting	0.24 (0.34)	0.21	1,44	0.64
$PC_{cap}2$				
H/L ratio	– 0.59 (0.14)	15.26	1,44	< 0.01
Feather growth rate	– 0.88 (1.27)	3.88	1,44	0.07
Body condition index	0.02 (0.01)	2.78	1,44	0.10
Sex [a]	0.30 (0.32)	0.46	1,44	0.49
Age [b]	– 1.21 (0.47)	7.71	1,44	< 0.01
Parasitism	0.54 (0.36)	1.64	1,44	0.20
Molting	0.16 (0.31)	0.18	1,44	0.66
$PC_{bib}1$				
H/L ratio	0.00 (0.19)	0.00	1,50	0.94
Feather growth rate	1.72 (1.48)	0.05	1,50	0.81
Body condition index	– 0.18 (1.20)	0.20	1,50	0.65
Sex [a]	0.38 (0.38)	1.05	1,50	0.30
Age [b]	– 2.06 (0.42)	20.64	1,50	< 0.01
Parasitism	– 0.08 (0.45)	0.46	1,50	0.49
Molting	– 0.09 (0.37)	0.00	1,50	0.98
$PC_{bib}2$				
H/L ratio	0.05 (0.13)	0.17	1,50	0.67
Feather growth rate	0.43 (1.03)	0.19	1,50	0.66
Body condition index	– 0.55 (0.82)	0.49	1,50	0.48
Sex [a]	0.23 (0.26)	0.78	1,50	0.37
Age [b]	– 0.34 (0.34)	0.74	1,50	0.39
Parasitism	– 0.45 (0.30)	1.79	1,50	0.18
Molting	– 0.51 (0.24)	3.48	1,50	0.07

[a] Estimate is relative to males.
[b] Estimate is relative to juveniles.
df: degrees of freedom; PC: principal component.

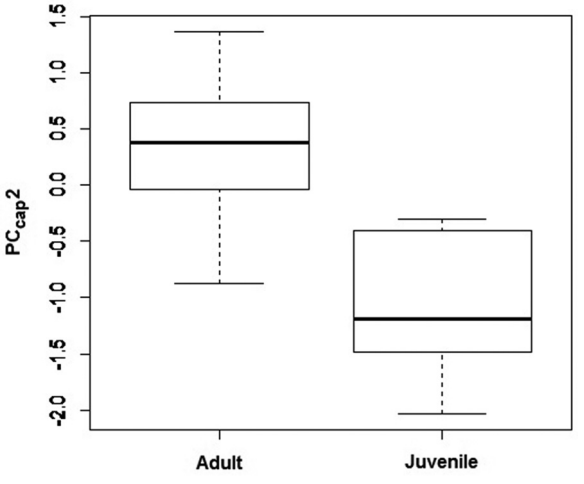

Fig. 3. — Differences in $PC_{cap}2$ scores (representing hue and red chroma) for carotenoid-based ornament in relation to age of Yellow-billed Cardinals (*Paroaria capitata*).

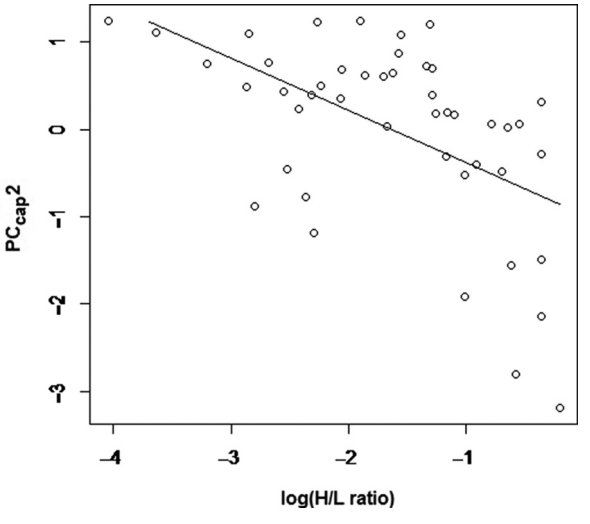

Fig. 4. — Relationship between heterophil/lymphocyte ratio (H/L) and $PC_{cap}2$ scores (representing hue and red chroma) of carotenoid-based ornament in Yellow-billed Cardinals (*Paroaria capitata*).

purity of the color. High levels of H/L ratio have been associated with lower survival in the field (reviewed in Sepp et al. 2010), besides being traditionally related to chronic stress (Gross & Siegel 1983; Davis et al. 2008; Krams et al. 2011). In American Goldfinches [*Spinus tristis* (Linnaeus 1758)], there was a negative relationship between hue and H/L ratio, indicating that individuals bearing colorful bills presented low stress levels (Kelly et al. 2012). This result reinforced the evidence that carotenoid-based ornaments may signal immunological capacity. Similar results were also found in the

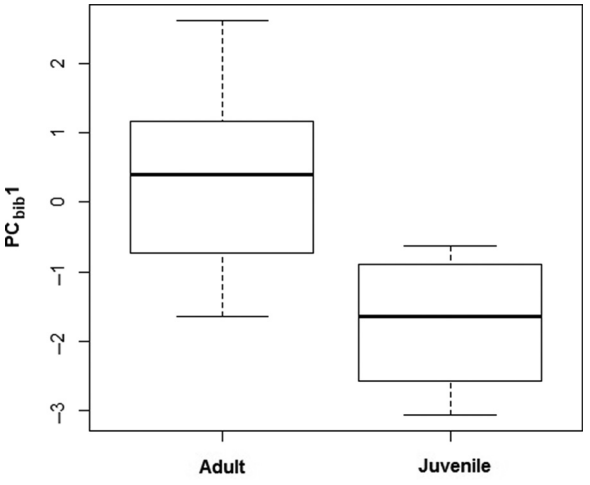

Fig. 5. — Differences in $PC_{bib}1$ scores (representing brightness, hue and ultraviolet [UV] chroma) for melanin-based ornament in relation to age of Yellow-billed Cardinals (*Paroaria capitata*).

Red-legged Partridge (*Alectoris rufa* Linnaeus 1758), where ornamentation has been suggested to have evolved through a process of quality assessment between potential mates (Pérez-Rodríguez & Viñuela 2008).

Distinct ornaments in plumage can predict age in several species (Siefferman et al. 2005; Krištín et al. 2007). Our analysis of cardinals' plumage demonstrated that both carotenoid- and melanin-based ornaments are affected by age independently of sex. In birds, plumage coloration is assumed to become more elaborate as individuals get older. For most species, juveniles present plumage ornamentation similar to that of the adults, although incomplete or duller (Hill & McGraw 2006). This is the case for the yellow ventral coloration of Great Tits (*Parus major* Linnaeus 1758), in which older individuals presented higher values for chroma and hue (del Val et al. 2010). Similarly, here we showed that older Yellow-billed Cardinals presented redder cap coloration. Because carotenoids must be obtained from the diet (Olson & Owens 1998), a possible explanation could be that Yellow-billed Cardinals' foraging ability for items with a high density of carotenoids are enhanced with time, as has been found in Great Tits (Heise & Moore 2003).

Despite being expected to convey less information than carotenoid ornaments, melanin signals may also reveal quality or condition. Black or brown coloration is important in the establishment of dominance hierarchies and during mate choice in black-capped chickadees [*Poecile atricapillus* (Linnaeus 1766)] and collared flycatchers [*Ficedula albicollis* (Temminck 1815)], for example (Mennill et al. 2003; Török et al. 2003). Age-dependency of melanin-based ornaments has also been demonstrated in several species (Hegyi et al. 2007; Krištín et al. 2007). Here we showed that, similarly to the red cap, the bib coloration of Yellow-billed Cardinals was affected by age: older individuals showed darker bibs, higher hues and a greater reflectance in the UV range. A study of Eastern Bluebirds [*Sialia sialis* (Linnaeus 1758)] that investigated variation of the melanin-based ornamentation among age classes also found that older birds were brighter, and thus less ornamented (Siefferman et al. 2005).

Bib characteristics can work as a "badge of status" in contexts of same-sex competition and mate choice (Dawkins & Krebs 1978), and in fact are considered honest signals of quality in several species (Nakagawa et al. 2007; Hoi & Griggio 2008; for the black beard). Although we did not evaluate bib size, bib coloration in cardinals might convey relevant information about their age. A possible hypothesis to explain differences in this ornament between age classes relates to physiological and behavioral changes throughout developmental stages. In House Sparrows (*Passer domesticus* Linnaeus 1758), for example, black bib size is affected by circulating testosterone during molt (Gonzalez et al. 1999). Alternative explanations rely on natural feather degradation through time, enhanced in adult birds, or on relationships with survival (e.g. birds with less investment in color survive longer). Nonetheless, age-dependency signaling allows conspecific identification, especially for more experienced individuals, and may be important for sexual selection, because it may directly affect individual fitness (e.g. Greene et al. 2000).

Given that immune responses are costly in resources, one would expect a negative relationship between carotenoid-based feather ornaments and haemosporidian infection in a bird population for two reasons: (1) parasites consume resources from the host, which cannot therefore be allocated to ornament (Møller 1994); (2) individuals that are forced to fight infections during the formation of carotenoid-based ornaments have less carotenoid available for deposition into plumage, since activation and maintenance of the immune system demands a cost (i.e. energy, protein) and those resources are limited (Norris & Evans 2000). However, this relationship was not found in Yellow-billed Cardinals in Pantanal prior to the breeding season. In fact, few studies have shown empirical evidence of negative associations between male ornamentation and parasite loads, as well as a female preference for ornamented and parasite-free males (but see Milinski & Bakker 1990; Møller 1990).

The low parasitemia of malaria parasites found during the non-breeding season can be explained by the interplay between the reproductive strategy of haemosporidian parasites and the immune system of hosts. Adult birds should harbor higher parasite burdens during their reproductive state, when birds are more susceptible to infection due to the effects of stress and hormones on immune function or due the heavy workload of reproduction, such as egg production, parental care, courtship and territory defense (Sheldon & Verhulst 1996; Zuk & McKean 1996; Saino et al. 2002; Greenman et al. 2005). Conversely, outside of the breeding season, when this study was carried out, there is an expected reduction in the abundance of parasites in the peripheral bloodstream (Valkiūnas 2005). During the initial acute phase of the disease (high parasitemia), in which hosts are anemic, are less active and lose their appetite, the parasites could have an effect on birds' body condition. If the hosts survive this acute phase, they will remain infected with low levels of parasites in their blood, only showing relapses of high parasitemia during the breeding season (Valkiūnas 2005). This is supported by the inability to identify blood stages in blood films from birds identified by PCR as infected. Chronically infected Yellow-billed Cardinals seemed unaffected by these detrimental effects of malaria parasites, and managed to invest in feather grow and coloration as uninfected individuals did.

In conclusion, our data show the condition-dependency of the carotenoid-based coloration of the red cap and suggest that this trait may have an important role as an honest signal during social interactions. However, this still needs to be assessed experimentally. We also revealed the age-dependent effect on both melanin and carotenoid ornamentation, which could facilitate conspecific recognition based on individual

experience. Our data do not support the Hamilton and Zuk hypothesis on parasites and sexual selection since the expression of secondary sexual characters in Yellow-billed Cardinals is not affected by intensity of blood parasite infection. Although they are chronically infected with malaria parasites, their body condition, immune response and feather coloration seem unaffected, and they might reproduce just as well as non-infected birds. Future studies should seek to understand the role of feather coloration in intrasexual competition and mate choice, in order to determine how they may influence reproductive output.

ACKNOWLEDGEMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for support through student scholarships. We are grateful to V.V. Tkach who kindly allowed access to his lab for molecular diagnosis of parasites (supported by NSF project numbers [DEB-150525] and [DEB-1503804]), M.Â. Marini and R.H. Macedo for material support, P.R. Sicsú for field assistance and R. Caparroz for molecular sexing. We are grateful to Universidade Federal de Mato Grosso do Sul which allowed our study at Base de Estudos do Pantanal, and CEMAVE/ICMBio for the banding permits and metal bands provided to M.Â Marini. A. Fecchio is currently maintained by a Postdoctoral Fellowship from CNPq [Process number 201275/2014-7].

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

Aguilar TM, Maia R, Santos ESA, Macedo RH. 2007. Parasite levels in blue-black grassquits correlate with male displays but not female mate preference. Behav Ecol. 19:292–301.

Andersson M. 1994. Sexual selection. Princeton: Princeton University Press.

Badyaev AV, Hill GE. 2000. Evolution of sexual dichromatism in birds: contribution of carotenoid- versus melanin-based plumage coloration. Biol J Linn Soc Lond. 69:153–172.

Bell JA, Weckstein JD, Fecchio A, Tkach VT. 2015. A new real-time PCR protocol for detection of avian haemosporidians. Parasit Vectors. 8:383.

Bensch S, Hellgren O, Pérez-Tris J. 2009. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. Mol Ecol Resour. 9:1353–1358.

Brown ME. 1996. Assessing body condition in birds. Curr Ornithol. 13:67–135.

Brush AH. 1990. Metabolism of carotenoid pigments in birds. FASEB J. 4:2969–2977.

Carlson A. 1998. Territory quality and feather growth in the White-backed Woodpecker *Dendrocopos leucotos*. J Avian Biol. 29:205–207.

Clutton-Brock T. 2009. Sexual selection in females. Anim Behav. 75:3–11.

Davis AK, Maney DL, Maerz JC. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct Ecol. 22:760–772.

Dawkins R, Krebs JR. 1978. Animal signals: information or manipulation. In: Krebs JR, Davies NB, editors. Behavioural ecology: an evolutionary approach. Oxford: Blackwell; p. 282–309.

del Cerro S, Merino S, Martínez-de la Puente J, Lobato E, Ruiz-de-Castañeda R, Aguilar JR, Martínez J, Morales J, Tomás G, Moreno J. 2010. Carotenoid-based plumage colouration is associated with blood parasite richness and stress protein levels in blue tits (*Cyanistes caeruleus*). Oecologia. 162:825–835.

del Val E, Quesada J, Senar JC. 2010. Age-related differences in a carotenoid-based coloration trait are due to within-individual changes in Great Tits *Parus major*. Ardea. 98:179–184.
Di Giacomo AG. 2005. Aves de la Reserva El Bagual [Birds of El Bagual]. In: Di Giacomo AG, Krapovickas SF, editors. Historia natural y paisaje de la Reserva El Bagual, Provincia de Formosa, Argentina [Natural history and landscape of El Bagual, Formosa State, Argentina]. Buenos Aires: Asociación Ornitológica del Plata; p. 201–465.
Doucet SM, Montgomerie R. 2003. Multiple sexual ornaments in satin bowerbirds: ultraviolet plumage and bowers signal different aspects of male quality. Behav Ecol. 14:503–509.
Garamszegi LZ. 2005. Bird song and parasites. Behav Ecol Sociobiol. 59:167–180.
Gonzalez G, Sorci G, de Lope F. 1999. Seasonal variation in the relationship between cellular immune response and badge size in male House Sparrows (*Passer domesticus*). Behav Ecol Sociobiol. 46:117–122.
Greene E, Lyon BE, Muehter VR, Ratcliffe L, Oliver SJ, Boag PT. 2000. Disruptive sexual selection for plumage coloration in a passerine bird. Nature. 407:1000–1003.
Greenman CG, Martin LB, Hau M. 2005. Reproductive state, but not testosterone, reduces immune function in male house sparrows (*Passer domesticus*). Physiol Biochem Zool. 78:60–68.
Griffith SC. 2000. A trade-off between reproduction and a condition-dependent sexually selected ornament in the house sparrow *Passer domesticus*. Proc R Soc Lond B Biol Sci. 267:1115–1119.
Griffiths R, Double MC, Orr K, Dawson RJG. 1998. A DNA test to sex most birds. Mol Ecol. 7:1071–1075.
Griggio M, Devigili A, Hoi H, Pilastro A. 2009. Female ornamentation and directional male mate preference in the rock sparrow. Behav Ecol. 20:1072–1078.
Griggio M, Valera F, Casas A, Pilastro A. 2005. Males prefer ornamented females: a field experiment of male choice in the rock sparrow. Anim Behav. 69:1243–1250.
Gross WB, Siegel HS. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 27:972–979.
Grubb TC. 2006. Ptilochronology: feather time and the biology of birds. Oxford: Oxford University Press.
Grunst AS, Salgado-Ortiz J, Rotenberry JT, Grunst ML. 2014. Phaeomelanin- and carotenoid-based pigmentation reflect oxidative status in two populations of the yellow warbler (*Setophaga petechia*). Behav Ecol Sociobiol. 68:669–680.
Guindre-Parker S, Gilchrist HG, Baldo S, Doucet SM, Love OP. 2013. Multiple achromatic plumage ornaments signal to multiple receivers. Behav Ecol. 24:672–682.
Guindre-Parker S, Love OP. 2014. Revisiting the condition-dependence of melanin-based plumage. J Avian Biol. 45:29–33.
Hall TA. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95–98.
Hamilton WD, Zuk M. 1982. Heritable true fitness and bright birds: a role for parasites? Science. 218:384–387.
Harrigan RJ, Sedano R, Chasar AC, Chaves JA, Nguyen JT, Whitaker A, Smith TB. 2014. New host and lineage diversity of avian haemosporidia in the northern Andes. Evol Appl. 7:799–811.
Hegyi G, Szigeti B, Török J, Eens M. 2007. Melanin, carotenoid and structural plumage ornaments: information content and role in great tits *Parus major*. J Avian Biol. 38:698–708.
Heise CD, Moore FR. 2003. Age-related differences in foraging efficiency, molt, and fat deposition of Grey Catbirds prior to autumn migration. Condor. 105:496–504.
Hill GE. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. Anim Behav. 40:563–572.
Hill GE. 1992. Proximate basis of variation in carotenoid pigmentation in male house finches. Auk. 109:1–12.
Hill GE, McGraw KJ. 2006. Bird coloration. II. Function and evolution. Cambridge: Harvard University Press.
Hill GE, Montgomerie R. 1994. Plumage colour signals nutritional condition in the house finch. Proc R Soc Lond B Biol Sci. 258:47–52.

Hoi H, Griggio M. 2008. Dual utility of a melanin-based ornament in Bearded Tits. Ethology. 114:1094–1100.
Hõrak P, Ots I, Vellau H, Spottiswoode C, Møller AP. 2001. Carotenoid-based plumage coloration reflects hemoparasite infection and local survival in breeding great tits. Oecologia. 126:166–173.
Ishtiaq F, Gering E, Rappole JH, Rahmani AR, Jhala YV, Dove CJ, Milensky C, Olson SL, Peirce MA, Fleischer RC. 2007. Prevalence and diversity of avian hematozoan parasites in Asia: a regional survey. J Wildl Dis. 43:382–398.
Kelly RJ, Murphy TG, Tarvin KA, Burness G. 2012. Carotenoid-based ornaments of female and male American goldfinches (*Spinus tristis*) show sex-specific correlations with immune function and metabolic rate. Physiol Biochem Zool. 85:348–363.
Krams I, Cirule D, Krama T, Vrublevska J. 2011. Extremely low ambient temperature affects haematological parameters and body condition in wintering Great Tits (*Parus major*). J Ornithol. 152:889–895.
Krištín A, Valera F, Hoi C, Hoi H. 2007. Do melanin-based tail patterns predict individual quality and sex in Lesser Grey Shrikes *Lanius minor*? J Ornithol. 148:1–8.
Lessells CM, Boag PT. 1987. Unrepeatable repeatabilities: a common mistake. Auk. 104:116–121.
Lozano GA. 1994. Carotenoids, parasites, and sexual selection. Oikos. 70:309–311.
McGraw KJ. 2003. Melanins, metals, and mate quality. Oikos. 102:402–406.
McGraw KJ. 2006. The mechanics of melanin coloration in birds. In:Hill GE, McGraw KJ, editors. Bird coloration. Vol. 1. Mechanisms and measurements. Cambridge: Harvard University Press; p. 243–294.
McGraw KJ. 2008. An update of the honesty of melanin-based color signals in birds. Pigment Cell Melanoma Res. 21:133–138.
Mennill DJ, Doucet SM, Montgomerie R, Ratcliffe LM. 2003. Achromatic color variation in black-capped chickadees, *Poecile atricapilla*: black and white signals of sex and rank. Behav Ecol Sociobiol. 53:350–357.
Milinski M, Bakker TCM. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. Nature. 344:330–333.
Møller AP. 1990. Effects of haematophagous mite on the barn swallow (*Hirundo rustica*): a test of the Hamilton and Zuk hypothesis. Evolution. 44:771–784.
Møller AP. 1994. Sexual selection and the Barn Swallow. Oxford: Oxford University Press.
Møller AP, Biard C, Blount JD, Houston DC, Ninni P, Saino N, Surai PF. 2000. Carotenoid-dependent signals: indicators of foraging efficiency, immunocompetence or detoxification ability?. Avian Biol Res. 11:137–159.
Montgomerie R. 2006. Analyzing colors. In:Hill GE, McGraw KJ, editors. Bird coloration. Vol. 1. Mechanisms and measurements. Cambridge: Harvard University Press; p. 90–147.
Nakagawa S, Ockendon N, Gillespie DOS, Hatchwell BJ, Burke T. 2007. Assessing the function of house sparrows' bib size using a flexible meta-analysis method. Behav Ecol. 18:831–840.
Norris K, Evans MR. 2000. Ecological immunology: life history trade-offs and immune defense in birds. Behav Ecol. 11:19–26.
Olson VA, Owens IPF. 1998. Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol. 13:510–514.
Pérez-Rodríguez L, Viñuela J. 2008. Carotenoid-based bill and eye ring coloration as honest signals of condition: an experimental test in the red-legged partridge (*Alectoris rufa*). Naturwissenschaften. 95:821–830.
Pott A, Pott VJ. 1994. Plantas do Pantanal [Plants of Pantanal]. Brasília: Embrapa.
Pryke SR, Lawes MJ, Andersson S. 2001. Agonistic carotenoid signalling in male red-collared widowbirds: aggression related to the colour signal of both the territory owner and model intruder. Anim Behav. 62:695–704.
R Development Core Team. 2012. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Available from: http://www.R-project.org
Ridgely RS, Tudor G. 1989. The birds of South America. Austin: University of Texas Press.
Rohwer S. 1975. The social significance of avian winter plumage variability. Evolution. 29:593–610.

Sætre G, Dale S, Slagsvold T. 1994. Female pied flycatchers prefer brightly coloured males. Anim Behav. 48:1407–1416.
Saino N, Incagli M, Martinelli R, Møller AP. 2002. Immune response of male barn swallows in relation to parental effort, corticosterone plasma levels, and sexual ornamentation. Behav Ecol. 13:169–174.
Senar JC, Figuerola J, Domenech J. 2003. Plumage coloration and nutritional condition in the great tit *Parus major*: the roles of carotenoids and melanins differ. Naturwissenschaften. 90:234–237.
Sepp T, Sild E, Hõrak P. 2010. Hematological condition indexes in greenfinches: effects of captivity and diurnal variation. Physiol Biochem Zool. 83:276–282.
Sheldon BC, Verhulst S. 1996. Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology. Trends Ecol Evol. 11:317–321.
Sick H. 1997. Ornitologia Brasileira [Brazilian Ornithology]. Rio de Janeiro: Editora Nova Fronteira.
Siefferman L, Hill GE, Dobson FS. 2005. Ornamental plumage coloration and condition are dependent on age in eastern bluebirds *Sialia sialis*. J Avian Biol. 36:428–435.
Stephens M, Donnelly P. 2003. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 73:1162–1169.
Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. Am J Hum Genet. 68:978–989.
Takaki Y, Eguchi K, Nagata H. 2001. The growth bars on tail feathers in the male Styan's Grasshopper Warbler may indicate quality. J Avian Biol. 32:319–325.
Thomas DB, McGraw KJ, James HF, Madden O. 2014. Non-destructive descriptions of carotenoids in feathers using Raman spectroscopy. Anal Methods. 6:1301–1308.
Török J, Hegyi G, Garamszegi LZ. 2003. Depigmented wing patch size is a condition-dependent indicator of viability in male collared flycatchers. Behav Ecol. 14:382–388.
Valkiūnas G. 2005. Avian malaria parasites and other haemosporidia. Boca Raton: CRC Press.
Villesen P. 2007. FaBox: an online toolbox for fasta sequences. Mol Ecol Notes. 7:965–968.
Wiebe KL, Vitousek MN. 2015. Melanin plumage ornaments in both sexes of Northern Flicker are associated with body condition and predict reproductive output independent of age. Auk. 132:507–517.
Zahavi A. 1975. Mate selection – A selection for a handicap. J Theor Biol. 53:205–214.
Zuk M, McKean KA. 1996. Sex differences in parasite infections: patterns and processes. Int J Parasitol. 26:1009–1023.