Carotenoid-Based Masks in the European Goldfinch Carduelis carduelis Reflect Different Information in Males and Females

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Carotenoid-based masks in the European Goldfinch *Carduelis carduelis* reflect different information in males and females

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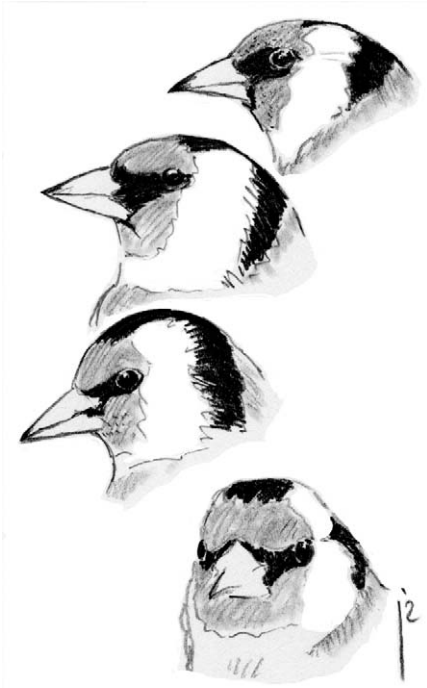

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Sexual selection may play an important role in the evolution of carotenoid-based signals. According to the parasite-mediated sexual selection hypothesis, organism health, parasite resistance and the expression of ornaments are linked. While some studies have analysed the expression of male carotenoid-based ornaments in relation to parasites and immune system capacities, few studies have focused on carotenoid-derived colour patches expressed in both sexes. We analysed the relationships between endoparasite (blood and systemic parasites) loads, haematological values and the components of red mask colour in wild European Goldfinches *Carduelis carduelis*, a species with a carotenoid-based facial mask in both sexes. Both, males and females were assessed for immune quality and face mask expression. Face mask coloration was sexually dichromatic, males have less orange masks than females. The yellow component of the mask showed less intensity in females infected with *Haemoproteus* blood parasites. The total leukocyte count was inversely correlated to the yellow component of the mask in females, suggesting that mask colour reflects the immune status of females during the breeding season. Isospora infection appeared to limit the UV reflection of the red mask of females.

Key words: sexual selection, Goldfinch, bird, carotenoid, ornament, parasites, *Coccidia, Haemoproteus*

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INTRODUCTION

Carotenoid pigments are responsible for many of the brightest colours and most conspicuous signals in birds (Hill 1999, Hill 2002). However, birds are not capable of synthesizing carotenoids from basic biological precursors (Goodwin 1984), and therefore, the expression of carotenoid pigments as colour signals requires the ingestion of carotenoids with food, as well as its absorption, transport, sometimes metabolism and incorporation into the feathers, all processes that are generally considered costly to individuals (Hill 2002, McGraw *et al.* 2005, McGraw *et al.* 2006). Scarcity of carotenoids in the food, or even the energetic cost of enzyme production and manipulation of carotenoids,

remain unclear. Carotenoids are also involved in metabolic pathways related to host immunity and a trade-off between the ornamental and health functions of carotenoids has been proposed (Hõrak *et al.* 2004a, Møller *et al.* 2004). According to this hypothesis, signals based on carotenoid pigments are costly and therefore act as honest indicators of an individual's quality.

The features that each carotenoid-based ornament reflect have been mainly studied in males of very dimorphic species. Carotenoid-based ornaments have been shown to be related to body condition (McGraw *et al.* 2002, Saks *et al.* 2003, Jawor & Breitwisch 2004, Jawor *et al.* 2004), sexual selection (Collias *et al.* 1979, Hill 1991, Drachman 1997) and even parasite loads (Figuerola *et al.* 2003, Hill *et al.* 2004, Hõrak *et al.* 2004). In the case of parasites, studies have reported variously negative (Thompson *et al.* 1997, Brawner *et al.* 2000, Figuerola *et al.* 2003, Hõrak *et al.* 2004b), positive (Burley *et al.* 1991, VanHoort & Dawson 2005) and non-significant (Seutin 1994) relationships between parasites and the expression of a carotenoid-based ornament. The potential causes of these contradictory results could stem from varying methods of study (e.g. correlational vs. experimental, ranges or doses of experimental treatments used), different impacts on parasites on their hosts and environmental-dependent effects of parasites on host condition (Figuerola *et al.* 2003). Work published up to now has generally focused on a single type of parasite, above all blood parasites, and analysis of the relative and combined impact of different types of parasites on ornament expression is still lacking.

Haematozoan parasites are blood-cell parasite protozoa transmitted by blood-sucking arthropods that are quite prevalent in wild passerines (Deviche *et al.* 2001). Coccidian protozoa are widespread intestinal epithelium parasites with a direct biological cycle; transmission results from the ingestion of oocysts liberated in the faeces of an infected individual. Passerines are mainly infected by genus *Isospora* (Hill 2002, Hõrak *et al.* 2006). Both haematozoan and coccidian parasites affect their hosts in a condition-dependent way (Merino *et al.* 2000): they have little impact when resources are abundant (Weatherhead & Bennett 1992, Friend & Franson 2001), but affect negatively the host when resources are scarce (Ots & Hõrak 1998, Ilmonen *et al.* 1999). The mechanisms leading Coccidia to limit the expression of carotenoid-based ornaments may work in at least two different direct ways. First, they may reduce the absorption of carotenoids through the intestine (Tyczkowski *et al.* 1991, Allen 1992) and, second, they may reduce the release of high-density lipoproteins (Allen 1987), which are responsible for the transport of carotenoids in the bloodstream to the tissues. Moreover, a third indirect mechanism related to body condition – to which both carotenoid ornamentation and immunity have shown to be linked – may also be at work (see Smith *et al.* 2007). The mechanisms through which Haematozoa may limit the expression of carotenoid-based ornaments are still unknown.

Most of the relationships described up to now between parasites and the expression of ornaments have been made focusing on conspicuous male ornaments, but little attention have been traditionally paid to such a relation in female ornaments. Roulin (2001), for instance, conducted an experimental study by comparing the degree of female Barn Owl *Tyto alba* ornamentation (eumelanin-based spottiness) with parasite resistance in their offspring raised by foster parents. He found that in females ornamentation positively reflects parasite resistance ability. Some observational studies have also demonstrated that the expression of ornaments is negatively related to the parasite load in female birds (Hõrak *et al.* 2001, Piersma *et al.* 2001).

The aim of this study was to explore the relationship between the colour of the carotenoid-based red mask of European Goldfinches, and a number of indices of condition (haematological parameters and different parasite loads) in both sexes, paying attention to possible inter-sex differences. The study was carried out with free-living birds during the breeding season, just after mate choice had occurred, when individuals were going through the costly task of raising young, because

under these conditions we expected the effect of parasites on their hosts to be maximal. To our knowledge, this is the first study done analysing the relationship between plumage coloration in both sexes and several groups of parasites at a time.

METHODS

The European Goldfinch is a 12-cm long, seed-eating finch that has a unique colour pattern on its head. The front of the face has a conspicuous crimson patch, which is known to be composed of four carotenoid pigments (Stradi *et al.* 1995): a) ε,ε-carotene-3,3'-dione, b) 3-hydroxy-ε,ε-carotene-3'-one, c) 4,4'-dihydroxy-ε,ε-carotene-3,3'-dione (isoastaxanthin), and d) 4-hydroxy-ε,ε-carotene-3,3'-dione.

Although ε,ε-carotene-3,3'-dione and 3-hydroxy-ε,ε-carotene-3'-one are very common yellow pigments in cardueline finches, isoastaxanthin and 4-hydroxy-ε,ε-carotene-3,3'-dione have not yet been found in any other species studied to date. These two pigments provide the red colour in the mask together with the keratin bond arrangement the pigments have in the feathers (Stradi *et al.* 1995). Although both sexes are superficially similar (Cramp & Perrins 1994), small differences in the size of the patch exist, being larger on average in males (Svensson 1996). To our knowledge, differences between the sexes in mask colour have never been investigated.

Fieldwork

In the springs of 2004 and 2005, we trapped 13 adult female and 44 adult male goldfinches in a tree nursery in the Spanish city of Seville (37°23' N, 5°57'W) where these finches are common resident breeders. Birds were captured between sunrise and sunset in 20 twelve-metre long mist-nets. Individuals were marked with numbered aluminium rings. Sex was determined by the presence of a brood patch (only present in females) or a cloacal protuberance (only present in males), and by the colour of the lesser wing-coverts (see Svensson 1996). We also measured body mass (to the nearest 0.1 g) and wing length (maximum chord). Birds were kept individually in clean ringing bags for 20 minutes to collect faecal samples. Faeces were immediately placed in individually marked vials containing 5% formol, and the time of collection was recorded for each sample. To control the mass of faecal samples, we avoided taking the urine-based part of the excretion and only collected the intestinal-based portion. We drew 0.1 ml of blood from the jugular vein using 29 G sterile insulin syringes and prepared smears on a microscopy slide as per Bennett (1970), which were air-dried, fixed and stained using Diff-Quick solution. To confirm field sexing an analysis of the cellular fraction of a drop of blood was performed. Sex was determined from blood cell DNA via a polymerase chain reaction (PCR) amplification of the CHD genes (Ellegren 1996, Griffiths *et al.* 1998). After blood extraction, we took two colour measurements of the frontal area of the red mask in the 57 trapped birds using a MINOLTA CM-2600d spectrometer, which measures the characteristics of reflected light by illuminating the feather surface under standard light conditions. We obtained the reflectance curve of the mask, that is, the light reflection from the UVA (360 nm) to the end of the visible spectrum (740 nm), measured at 10 nm intervals (39 intervals). The UVA reflection is visible to birds and has important implications in sexual selection in some passerine species (Saetre 1994, Siitari *et al.* 2002, Pearn *et al.* 2003). Although 700 nm has been shown as a maximum wavelength for avian visual sensitivity, we included 700–740 nm interval within the analysis because birds indeed present variability in visual spectrum among different species (Bowmaker *et al.* 1997) and, to our knowledge, this spectrum has never been studied in the Eurasian Goldfinch.

Blood smear analysis

For each blood smear, we estimated the total leukocyte count (TLC) by counting the number of leukocytes on twenty 400x light microscope fields of similar density and multiplying this value by

100 (Wiskott 2002). The differential leukocyte count was made by identifying (according to Campbell 1995) the cellular type (heterophils, eosinophils, basophiles, lymphocytes or monocytes) of 100 leukocytes at 1000x magnification. The heterophil-lymphocyte ratio (H/L) was calculated as the percentage of heterophils divided by the percentage of lymphocytes. A total of 15 000 erythrocytes were scanned for blood parasites at low (400x) and high (oil 1000x) magnification (Godfrey *et al.* 1987) and in infected individuals, the blood parasite load was estimated as the percentage of infected erythrocytes. Prevalence was calculated on the basis of the percentage of infected individuals. Only *Haemoproteus* spp. (prevalence: 23.7%) and *Plasmodium* spp. (7.9%) were found in the 38 samples analysed. The repeatability of all variables was estimated by counting twice the smears of ten individuals and calculated as the intra-class correlation (Lessells & Boag 1987). Repeatabilities were high for TLC (95%), H/L (90%), and blood parasite infection (92%).

Coprology

In the laboratory, faecal samples were passed through a double lint filter and mixed to obtain a homogeneous dilution, which was then analysed for coccidian oocysts and other endoparasite eggs using a McMaster chamber. This method only provides an estimation of the real parasite load, although it has been described as the only possible non-invasive method of research on the intestinal parasite of wild animals (Watve & Sukumar 1995). Samples were scored as positive, when coccidian oocysts were observed, and negative, when not. Only protozoan coccidia were found in the sample. Based on size and the number of sporocysts, the oocysts were identified as *Isospora*-like (Baker *et al.* 1972, Grulet *et al.* 1982). Repeatability of coccidian infection estimated from samples of 10 individuals scored twice was very high (97%), giving confidence in the accuracy of oocyst counts.

Colour characteristics

Reflectance curves were analysed by a Principal Component Analysis of reflectances at the 39 different wavelength intervals. Four relevant components (Eigenvalues > 1) were obtained that together summarised 99.97% of variance (Fig. 1). The first component (PC1) summarised reflection in the visible spectrum, mainly between 400 and 530 nanometers (within violets and blues). PC2 was more positive for individuals with more reflection at 650–740 nanometers (reds) and less reflection at lower wave lengths. PC3 was negatively related to reflection in the 550–590 nanometers intervals (yellows), so this component represents the yellow carotenoids reflection. PC4 was mainly influenced by reflection in the non-visible-to-humans portion of the spectra, between 360 and 390 nanometers (UVA radiation). The repeatability of the colour measurements was calculated as the intra-class correlation of the principal components from ten individuals measured twice (Lessells & Boag 1987). The repeatability was very high for all components (PC1: 98%; PC2: 96%; PC3: 97%; PC4: 96%) because of the great accuracy of the spectrometer method (see Figuerola *et al.* 1999).

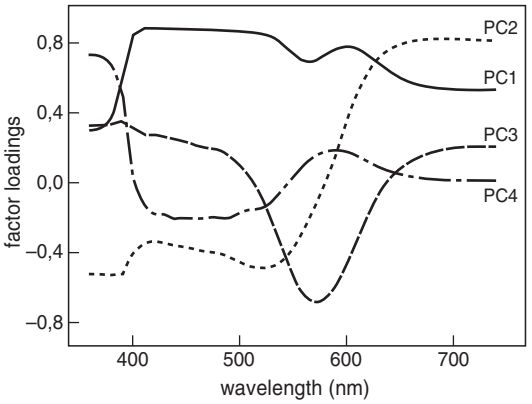

Figure 1. Factor loading of the four principal components calculated from light reflectance at 10 nm intervals between 360 and 740 nm. PC1 represents the blue-violet component, PC2 the red one, PC3 includes the yellows, and PC4 represents the UVA reflection.

Statistical analyses

TLC was log-transformed to fit a normal distribution. H/L did not fit normality by any common

transformation so ranked values were used in the analyses (Conover & Iman 1981). We analysed sexual dimorphism in colouration (PC1, PC2, PC3 and PC4), haematological values (TLC and H/L), and parasite (*Haemoproteus* and *Isospora*) load with ANOVAs including sex as a factor. We analysed the effects of *Haemoproteus* and *Isospora* infections (presence/absence) as factors, and the effects of TLC and ranked H/L on PC1, PC2, PC3, PC4 as dependent variables in two MANOVAs. Due to circadian rhythms affecting coccidian prevalence in passerines (López *et al.* 2007), morning/ afternoon factor was included in the model including *Isospora* infection. All the two-way interactions among covariates and sex and morning/ afternoon factor were included in the models, and stepwise backwards selection procedure was followed until all the independent variables remaining in the model increased significantly the fit of the model.

RESULTS

The coloration of the red mask in the European Goldfinch was sexually dichromatic. Sexes differed in reflectance along the whole visible spectrum (PC1, PC2 and PC3), especially within the yellow region (PC3), but not in the UV (PC4) (Table 1).

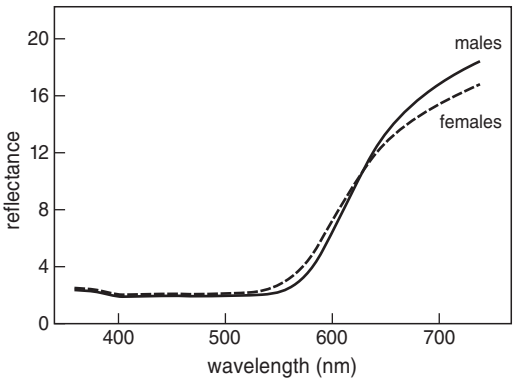

Figure 2. Mean reflection curves of the red mask of the Goldfinch along the UV and the whole visible spectrum by sex.

Reflectance curves showed that males were on average much more red and less yellow than females (Fig. 2). No differences in haematological values or parasite infection were found between sexes (Table 1). None of these variables were related to PC1 or PC2 (Tables 2 and 3). *Haemoproteus* infection, TLC, and their interaction with sex were related to PC3 (Tables 2 and 3, Fig. 3A and B). *Isospora* infection, its interaction with sex, and the interaction between sex and *Haemoproteus* infection were related to PC4 (Tables 2 and 3, Fig. 3C).

Table 1. Mean, SE and sample size for males and females for the colour, parasites and haematological variables analysed. Differences between sexes were tested by one-way ANOVA.

	Males			Females				
	mean	SE	*n*	mean	SE	*n*	*F*	*P*
PC1	–0.174	0.173	33	0.573	0.261	10	4.64	0.05
PC2	0.178	0.172	33	–0.588	0.260	10	4.93	0.03
PC3	0.248	0.132	33	–0.820	0.407	10	10.80	<0.01
PC4	0.016	0.156	33	–0.051	0.426	10	0.03	0.86
Isospora	1.300	0.231	37	1.020	0.282	12	2.37	0.13
Haemoproteus	0.030	0.013	31	0.002	0.002	7	0.92	0.35
TLC	3.771	0.03	31	8.838	0.080	7	0.87	0.36
H/L	0.856	0.040	31	0.704	0.033	7	3.25	0.08

Table 2. Results of stepwise backwards selection procedure MANOVA analysing parasite infection over colour components of the red mask of the Eurasian Goldfinch.

Source	Dependent variable	$F_{1, 32}$	*P*
Sex	PC1	7.21	0.014
	PC2	3.09	0.092
	PC3	0.03	0.872
	PC4	32.91	<0.001
Morning/afternooon	PC1	0.18	0.675
	PC2	1.64	0.214
	PC3	0.45	0.508
	PC4	1.81	0.192
Haemoproteus	PC1	0.47	0.499
	PC2	0.88	0.358
	PC3	9.80	0.005
	PC4	1.72	0.204
Isospora	PC1	1.85	0.187
	PC2	2.64	0.118
	PC3	1.37	0.254
	PC4	20.38	<0.001
Sex x *Haemoproteus*	PC1	0.60	0.446
	PC2	0.26	0.614
	PC3	8.91	0.007
	PC4	4.89	0.037
Sex x *Isospora*	PC1	2.00	0.171
	PC2	1.38	0.253
	PC3	0.26	0.616
	PC4	28.54	<0.001

Table 3. Results of stepwise backwards selection procedure MANOVA analysing haematological values over colour components of the red mask of the Eurasian Goldfinch.

Source	Dependent variable	*Df*	*F*	*P*
Sex	PC1	32	0.81	0.551
	PC2	32	0.58	0.783
	PC3	32	10.38	0.002
	PC4	32	8.24	0.498
Log (TLC+1)	PC1	32	0.49	0.616
	PC2	32	0.01	0.201
	PC3	32	6.36	0.017
	PC4	32	9.69	0.807
Sex x Log (TLC+1)	PC1	32	0.83	0.633
	PC2	32	0.63	0.741
	PC3	32	11.84	0.001
	PC4	32	8.30	0.546
Ranked H/L	PC1	31	0.21	0.651
	PC2	31	1.28	0.266
	PC3	31	2.46	0.127
	PC4	31	0.02	0.885

DISCUSSION

Colour dichromatism has not been reported before in the masks of the European Goldfinch. Our results show that hues differ between sexes: males reflect reds more strongly than females, but reflect yellows and oranges with lower intensity than females. The carotenoids expressed in the mask, are qualitatively the same in both sexes (Stradi 1995). McGraw *et al.* (2002) also found that male American Goldfinches *Carduelis tristis* artificially-fed with *ad libitum* canthaxantin were more colourful than females, due to a higher carotenoid concentration in the feathers. The larger accumulation of red pigments in males than in females seems thus to be the most plausible option for explaining colour differences in European Goldfinches. Testosterone, by means of its capacity to upregulate lipoprotein status, has been proposed as the responsible agent for such differences in the American Goldfinch (McGraw *et al.* 2006), but there are also studies showing opposite outcomes in House Finches *Carpodacus mexicanus* (Stoehr & Hill 2001). Diet differences between sexes, health variations or the effect of other hormones should not be discarded to explain this sexual dichromatism. Even a differential selection of ornaments between sexes could also be an underlying factor regarding the dichromatism. Unfortunately, no information is available on any of these aspects in the Eurasian goldfinch.

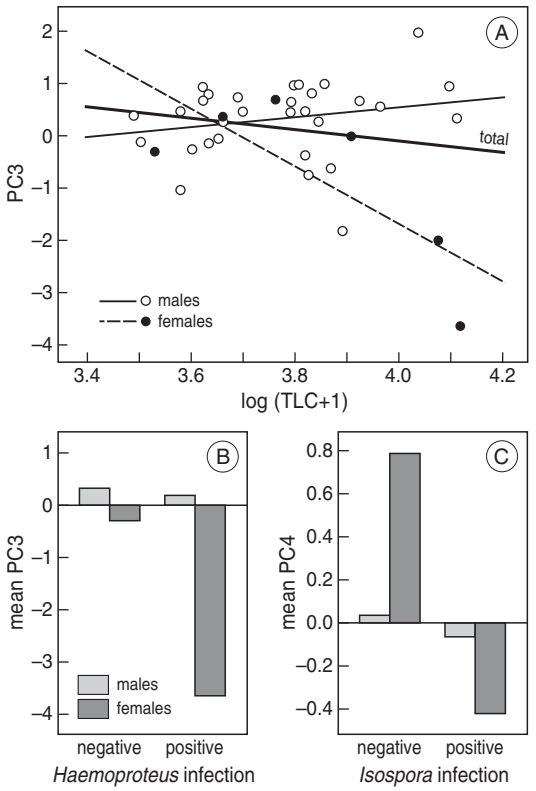

Figure 3. A) Scatter graph of log-transformed TLC over the PC3 values, by sex. B) Mean PC3 in relation to *Haemoproteus* infection state by sex. C) Mean PC4 in relation to *Isospora* infection state by sex.

When interpreting our results relating coloration to health it is important to consider that the study was carried out in the spring. European Goldfinches moult their masks in late summer (Jenni & Winkler 1994), around six months before reproduction takes place. The health status of individuals is expected to change during this time, although the signals involved in sexual selection act in the early spring at the time of mate choice (Cramp *et al.* 1994). The red of the mask is not fully developed at the time of the moult and is only completed during the spring due to the abrasion of melanin derived feather tips (Svensson 1996). We sampled birds at the time of the year when the expression of the ornament is at its fullest, just when the indicator function of a sexual selection signal should be at work.

We found a relationship between presence of different parasites and different colour components of red masks. However, these effects were sex-dependent and only significant for females. Female European Goldfinches infected with *Haemoproteus* blood parasites and those with higher TLC values were more orange, that is with a higher yellow component, than those uninfected or with lower values. A higher intensity in yellow component may be due to 1) a decrease in the intensity of red pigments, or 2) an increase in the intensity of yellow ones. Because red pigments are predominant in the red mask, we think that the first option is more plausible than the second one. In this way, the red of the mask may reflect *Haemoproteus* parasitemia or infection resistance in females. Also, female European Goldfinches with higher TLC values were more orange (with a higher yellow component) than those with lower values. Since high TLC values are linked to chronic or acute infections (Campbell 1995), the red of the mask may reflect immune levels or infection resistance in females. In this way, the most infected females would have less red ornaments, a finding that suggests that red masks act as an honest indicator of general infection in female European Goldfinches. This relationship is not significant in males, probably due to the different reproductive role of each sex, since egg-laying and incubation, a very expensive process, is carried out only by females (Cramp *et al.* 1994). UV reflection was higher in *Isospora* non-infected females than in infected ones in our study. This result seems to indicate that UV reflection acts as an honest indicator of *Isospora* infection in females. Finally, our results suggest that double-infected animals (with *Haemoproteus* and *Isospora*) reflect violets with a higher intensity that those non-infected. This fact could be due to the lack of red and yellow pigments (carotenoids) that those individuals have in their feathers. Although H/L has shown to be related with some aspects of stress and condition in passerines (Ots *et al.* 1998, Groombridge *et al.* 2004), it was not related to any colour variables in our study.

How is it possible that breeding roles had an effect on the relationship between coloration and parasites if plumage was developed several months before breeding? We suggest that under the stress derived from breeding activities females in worst condition or with a less active immune system are less able to control already present infections and/or exclude new infections when exposed to pathogens. A similar process was experimentally demonstrated to work in male Greenfinches *Carduelis chloris* experimentally infected with Sindbis virus (Lindström & Lundstrom 2000).

In conclusion, our study shows that (1) sexual dichromatism exists in the colour of the mask of the European Goldfinch, and (2) the red colour of the mask reflects different signals in both sexes and may be a reliable indicator of parasite infection during the breeding season, at least in females.

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SAMENVATTING

Verondersteld kan worden dat de kleurenpracht van het verenkleed bij vogels afhangt van de gezondheidstoestand van het individu. Of dat zo is werd onderzocht aan de hand van de markante koptekening van Putters *Carduelis carduelis*. Bij vrouwtjes – niet bij mannetjes – werden duidelijke verbanden gevonden tussen de kleur van het rood op de kop en bloedwaarden en de aanwezigheid van parasieten in het lichaam. Individuen die geïnfecteerd waren met de bloedparasiet *Haemoproteus* hadden een minder intensief gekleurde kop (vooral in het gele deel van het spectrum). Daarnaast was de UV-reflectie van de rode koptekening minder wanneer de vogels geïnfecteerd waren door de coccidiose veroorzakende protozoë *Isospora*. Bovendien bleek er een verband te bestaan tussen de kleuring van de kop en de dichtheid aan witte bloedlichaampjes, hetgeen een aanwijzing vormt dat de kopkleur een indicatie is voor de aktiviteit van het immuunsysteem. (BIT)

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