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Blood parasites, leucocytes and plumage brightness in the Cirl Bunting, *Emberiza cirlus*

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Summary

1. Although a female preference for pairing with brightly plumaged males has been reported in many species, the reasons for this choice are not fully understood.
2. Parasites have been proposed as playing an important role in shaping these preferences because, by pairing with brightly coloured individuals, females can obtain parasite-free and/or more healthy mates.
3. In this paper one of the predictions of this hypothesis is tested, namely a higher health level in brightly coloured individuals, by analysing the relationships among blood parasites, leucocyte levels and plumage brightness in the Cirl Bunting, *Emberiza cirlus*.
4. Two species of blood parasites were detected. Whereas a lower body condition was detected in individuals infected by *Leucocytozoon cambournaci*, no such differences were associated with *Plasmodium relictum* infections. Infected individuals showed higher total leucocyte counts than non-infected individuals.
5. Colour intensity of carotenoid derived colorations was negatively correlated to the relative proportion of lymphocytes and positively correlated to the relative presence of heterophils. Furthermore, the size of yellow feathered areas was positively correlated with the absolute number of leucocytes and the relative presence of heterophils. Only some of these relationships were found for non-carotenoid derived traits.
6. These results suggest that male plumage yellow coloration in the Cirl Bunting is a reliable indicator of health status and supports the hypothesis that females obtain more parasite-free mates if they pair with brightly coloured individuals.

Key-words: Hamilton–Zuk hypothesis, mate choice, parasite-mediated selection, plumage carotenoids, secondary sexual characters

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Introduction

Plumage coloration is commonly thought to be a sexually selected indicator of mate quality, and female preferences for mating with colourful males have been recently documented in an increasing number of species (Hill 1991; Palokangas *et al*. 1994; Sundberg & Larsson 1994). To explain the evolution of sexually dimorphic colour ornaments, Hamilton & Zuk (1982) proposed that plumage coloration has evolved as an honest signal of parasite and disease resistance. This and other models of parasite-mediated selection predict that by mating with brightly coloured males, females may benefit from a lower risk of disease transmission (Clayton 1990; Hillgarth 1996), more or better quality resources (Hoelzer 1989) and/or genetic benefits for their offspring in the form of increased disease resistance (Hamilton & Zuk 1982; Andersson 1986; Møller 1990; Clayton 1991). At the interspecific level, Hamilton & Zuk's hypothesis predicts a greater plumage brightness in males of more parasitized species, because selection for reliable indicators of parasite resistance should be stronger in these species where parasites could play a important role in determining individual fitness. At the intraspecific level, the hypothesis predicts a negative relationship between pathogenic parasite levels and plumage brightness, because only the healthiest individuals will be able to afford the costs of fully developing the costly sexually selected traits.

The attempts to test Hamilton & Zuk's hypothesis among different species have given contradictory results mainly because of the subjectivity of the

methods used to score plumage brightness, the difficulty in obtaining standardized estimates of the number or prevalence of parasites, and the occasional failure to use suitable statistical methods to deal with the lack of independence of the data from different species owing to their phylogenetic relationships (see Pruett-Jones, Pruett-Jones & Jones 1991 and references therein). Within-species tests of Hamilton & Zuk's hypothesis have not been more definitive in their results, because both positive, negative or non-significant correlations between parasite burden and plumage brightness have been reported (e.g. Borgia & Collis 1989; Ressel & Schall 1989; Milinski & Bakker 1990; Weatherhead *et al*. 1993; Seutin 1994). These contradictory results could be due to the current ignorance of the effects of most parasites on the condition of wild hosts or to the different pathogenicity of the different parasite species (Atkinson & van Riper 1991). This lack of knowledge makes it difficult to identify the species of parasites that must be included in the analyses of parasite-mediated selection, because the predicted effects on sexually selected characteristics will only be apparent in those parasites that reduce host fitness (Clayton 1991). Additionally, intensity of infestation change during the season, and intensity of infestation at any random sampling occasion may not be representative of the period of the most important cost of parasites (see Allander & Sundberg 1997).

The proposed origins of the relationship between plumage coloration and parasite loads make alternative tests of parasite-mediated selection hypotheses possible, since the parasites and diseases affecting each individual should influence the levels of their current immune response. In this case we have not examined the correlation of plumage brightness with the loads of any, usually randomly chosen, species of parasites, but have used a more general estimator of health status, in this case leucocyte counts. In birds, cellular immunity is usually mediated by heterophilic granulocites and lymphocytes, and the number and proportion of the different types of leucocytes reflect the levels of stress and health (Rose, Hesketh & Ogilvie 1979; Davis 1981; Hawkey *et al*. 1983; Fudge 1989; Averbeck 1992; Ots, Murumägi & Hõrak 1998). Dramatic increases in the number of leucocytes (leucocytosis) is most commonly due to infectious disease, and leucocytes quickly react to a great variety of diseases including bacterial infections and blood parasites (Fudge 1989). Lymphocytes are highly specific immune cells, involved in immune regulation, antigen elimination and the synthesis and secretion of immunoglobulins. Their action causes little or no damage to the host cells (Siegel 1985). Heterophils are non-specific immune phagocytosing cells. They enter the tissues during the inflammatory response and their lysis could be harmful to the host tissues (Parslow 1994). Relative increases in lymphocytes are usually associated with the presence of blood parasites, while relative heterophilias are commonly seen in bacterial infections moving from low-grade towards clinical infections (see Fudge 1989). Relative increases in basophil number are characteristic of respiratory infections and relative increases in monocytes characterize granulomatous diseases (Fudge 1989). Intestinal parasites usually produce increases in the relative frequency of eosinophils, although this response sometimes does not occur in birds (Dieterlen-Liévre 1988; Fudge 1989).

The Cirl Bunting, *Emberiza cirlus*, is a small passerine (155 mm in body length), widely distributed throughout southern Europe. Male plumage has highly variable breast, belly and facial yellow parts, a black bib under the bill and a grey breast band, whereas the plumage of females and especially of juveniles is less conspicuous, with mainly light-grey and brown underparts, with some light-yellow parts in females (Gutiérrez 1997). No data on female preferences are available for this species, although in the similar and closely related Yellowhammer, *Emberiza cintrinella*, females prefer to pair with the brightly yellow plumaged individuals (Sundberg 1995a) that also obtain more extrapair copulations and are less parasitized than drab-coloured individuals (Sundberg 1995b; Sundberg & Dixon 1996). This suggests a possible sexually related role for yellow coloration in mate choice in the Cirl Bunting as well.

In this paper we examine the possible pathogenic effects of two different blood parasites on the Cirl Bunting, and test the hypothesis that more brightly coloured individuals signal better health which is estimated by the cellular components of the immune response system.

Materials and methods

Fieldwork was carried out in summer 1996 and 1997 at Juncosa (NE Spain, 41°19′N, 1°27′E). On 12 and 13 July, 16 and 20 August 1996 and 10 and 15 August 1997 Cirl Buntings were mist-netted near two water spots. Birds were ringed, and wing, bill, tarsus and body mass were measured. Individuals were classified as young, adult males or adult females according to Svensson (1996).

Each male was given a colour size score based on the absolute size of the yellow patches on the breast and belly, estimated by putting a grid (modified from Hill 1992) over the birds and counting the number of 2·5-mm^2 surface squares filled with yellow feathers. The same methodology was used to estimate the size of the characteristic black-bib and grey breast band. Intensity of plumage colour was estimated in five different areas of the bird: (1) throat, (2) eye-stripe, (3) yellow breast spot, (4) breast band and (5) ventral area, by comparison with Küppers' (1996) colour tables. The values for light, chroma and hue corresponding to each one of the colours in the tables were estimated subsequently using a Minolta DR200 colorimeter (Minolta Co. Ltd., Osaka, Japan). The colorimeter

determines these three basic characteristics of the colour by sending a standardized light flash over the sample and analysing the reflected light (see Senar, Domènech & Conroy 1998). The mean light, chroma and hue of carothenoid-derived yellow areas were calculated as the average eye-stripe, yellow breast spot and ventral scores. Hue corresponds to the wavelength of a colour, while light corresponds to the physical light intensity and chroma is positively correlated to colour monochromatism (Küppers 1996).

Blood samples were obtained by venipuncture of the brachial vein. Blood smears were made, air dried and fixed in absolute methanol immediately before being stained with Giemsa's solution. The slides were examined with a light microscope at 1000 × for 15 min, which was equivalent to the observation of 120–130 microscopic fields in each smear. When no parasites were detected after this time, the smear was considered negative. The type of 100 leucocytes was determined to estimate the relative composition of the white blood cells. Basophils were identified in a very small number of individuals and were not included in the analyses. The number of leucocytes was estimated afterwards according to Campbell & Dein (1984). The total leucocyte count (TLC) was estimated by counting the number of leucocytes in 30 fields at 1000× and dividing by 6 in order to obtain the average number of leucocytes in five fields. This number is assumed to be equivalent to the number of leucocytes per 1000 erythrocytes. The following formula was used to calculate the TLC:

$$\mathrm{TLC} = \frac{A*C}{B}, \qquad \text{eqn 1}$$

where TLC = number of leucocytes per mm^3 of blood; A = average number of white blood cells in five microscopic fields at 1000×; B = 1000 (number of red blood cells in five microscopic fields at 1000×); $C = 3{\cdot}5 \times 10^6$ (number of red blood cells per mm^3 in normal birds).

The TLC was corrected to account for individual variation in the concentration of cells in the blood. Microcapillar tubes with heparinized blood were centrifuged at 16 000 r.p.m. for 2 min in a centrifuge to obtain the PCV values (packed cell volume or hematocrit). The TLCs were corrected according to Campbell & Dein's (1984) formula, considering a normal PCV in birds of 47·7%:

$$\mathrm{TLC}_{Corrected} = \mathrm{TLC}* \frac{\mathrm{PCV}_{Observed}}{\mathrm{PCV}_{Normal}}. \qquad \text{eqn 2}$$

Blood was not obtained from all individuals, and in some cases enough blood was obtained for slide examination but not for PCV determination. Consequently, the number of individuals included in each analysis differs, although the sample size is presented for each group of analyses.

The coloration of 9 males and the blood smears of 15 individuals were analysed twice to test the reliability of the measurements. Both plumage coloration (r = 0·57–0·92), total leucocyte count (r = 0·93) and the relative composition of leucocytes (r = 0·78–0·93) proved highly repeatable variables.

The effect of body size on body mass was controlled by standardizing body mass to a wing length of 80 mm using the SIZESTD program (Senar, Lleonart & Metcalfe 1994). Hour of capture was included as a covariate in the analyses of body condition because body mass increases from the morning to the afternoon (Blem 1990), and this daily fluctuation should be controlled in the analysis of body condition. Absolute leucocyte numbers were log-transformed to attain normality. Proportions of the different leucocyte types and colour characteristics were not normalized by commonly used transformations and were analysed with non-parametric methods. An ANOVA for these variables was performed on ranked values (see Conover & Iman 1981; Marden & Muyot 1995). Spearman rank and Pearson correlations were used to analyse relationships of total leucocyte count with non-normally and normally distributed variables, respectively. The relative presence of each leucocyte type was analysed with Gamma Correlation owing to the high frequency of ties (Zar 1984). Since juveniles cannot be sexed (Svensson 1996) and adult males and females did not differ for any of the analysed variables, data for both sexes were analysed together, except in the case of the relationships between plumage brightness and health status that were tested only for adult males.

Results

PARASITE IDENTIFICATION AND PREVALENCE

Two species of haematozoa, *Plasmodium relictum* Celli & Sanfelice (1891) and *Leucocytozoon cambournaci* França 1912 were identified in the examined smears. *Plasmodium relictum* was identified by its spherical or slightly egg-shaped gametocytes, which displace the nucleus of the infected erythrocyte, and by its black pigment granules (see Garnham 1966). According to Bennett, Bishop & Peirce (1993), *P. relictum* has been recorded in 26 different species of Emberizidae, which means that it has the broadest distribution of all *Plasmodium* in this family. The genus *Plasmodium*, however, comprises several close-related species and, according to Garnham (1966) it is not possible to make a completely exact identification without experimental studies. Similarly, Bennett *et al*. (1993) affirm that it is difficult to identify *Plasmodium* to species level from blood stages alone. Hence we cannot be completely certain of the species of *Plasmodium* observed in this study, and it is referred to as *Plasmodium* cf. *relictum*. *Leucocytozoon cambournaci* França 1912 was identified by the presence of the rounded forms described by this author and the absence of fusiform prolongations in

the cytoplasms of the host cells. The characteristic young parasite forms described by França (1912) were also observed.

In 48·6% of 111 birds included in the blood examination study at least one of these two parasites was found. The respective prevalences for *L. cambournaci* and *P. relictum* were 19·8% and 36·0%, including seven individuals that were infected by both parasites. These seven birds were excluded from subsequent analyses. The proportion of infected young and adults was similar ($\chi^2 = 1{\cdot}70$, 1 df, $P = 0{\cdot}19$). No differences in parasite prevalence were detected when data for adult males and females and *Leucocytozoon* and *Plasmodium* were analysed separately ($\chi^2 = 6{\cdot}46$, 4 df, $P = 0{\cdot}17$).

THE IMPACT OF PARASITES ON BODY CONDITION

The possible effect of parasites on body condition was analysed with two-way ANCOVA on body condition, using the hour of capture as a covariate and two factors: parasites (no parasites, *Plasmodium* or *Leucocytozoon*), and age (yearling or adult). Individuals infected by *Leucocytozoon* presented a poorer body condition than both non-infected and *Plasmodium*-infected individuals ($F_{2,100} = 5{\cdot}03$, $P = 0{\cdot}008$; *a posteriori* Newman–Keuls test, $P < 0{\cdot}01$; Table 1). No effects of age were detected on body condition ($F_{1,100} = 0{\cdot}001$, $P = 0{\cdot}98$), although a significant interaction within the presence of parasites and age was detected ($F_{2,100} = 5{\cdot}70$, $P = 0{\cdot}005$). This interaction between factors occurred because the effects of parasites were only significant in adults (one-way ANCOVA, $F_{2,24} = 8{\cdot}80$, $P = 0{\cdot}001$), but not in young (one-way ANCOVA, $F_{2,75} = 1{\cdot}31$, $P = 0{\cdot}28$).

LEUCOCYTES AND PARASITES

Individuals infected by blood parasites presented higher total leucocyte counts than non-infected ones ($F_{2,69} = 4{\cdot}88$, $P = 0{\cdot}01$), although the differences were only significant for *Plasmodium*-infected individuals (*a posteriori* Newman–Keuls test, $P < 0{\cdot}03$); see Table 2. No significant effect of age ($F_{1,69} = 0{\cdot}93$, $P = 0{\cdot}34$, Table 2) or interaction between the factors of age and parasites was detected ($F_{2,69} = 1{\cdot}25$, $P = 0{\cdot}29$). Young individuals presented a lower percentage of heterophils than adults ($F_{1,86} = 4{\cdot}16$, $P = 0{\cdot}04$) but a higher percentage of eosinophils ($F_{1,86} = 4{\cdot}89$, $P = 0{\cdot}03$). No differences in the proportion of lymphocytes ($F_{1,86} = 1{\cdot}31$, $P = 0{\cdot}25$) or monocytes ($F_{1,86} = 1{\cdot}24$, $P = 0{\cdot}27$) was found between young and adults. Parasitized individuals (by *Plasmodium* or *Leucocytozoon*) showed a higher proportion of lymphocytes ($F_{2,86} = 3{\cdot}68$, $P = 0{\cdot}03$) and a lower proportion of heterophils ($F_{2,86} = 3{\cdot}23$, $P = 0{\cdot}04$) than parasite-free individuals. No differences in the relative frequency of eosinophils ($F_{2,86} = 1{\cdot}36$, $P = 0{\cdot}26$) or monocytes ($F_{2,86} = 1{\cdot}87$, $P = 0{\cdot}16$) were found between parasitized and parasite-free individuals. The interaction between age and parasites was not significant for any of the types of leucocyte ($F_{2,86} < 1{\cdot}37$, $P > 0{\cdot}26$ in all four cases).

LEUCOCYTES AND PLUMAGE COLOUR

Mean light, chroma and hue coloration of the yellow body tracks of males were negatively related to the relative presence of lymphocytes and positively related to heterophils (Table 3). Relative presence of eosinophils and monocytes with yellow colour intensity were not analysed because no effect of blood parasites on these variables was previously detected. The chroma of the black bib, characteristic of the male plumage in Cirl Buntings, was related positively to the proportion of lymphocytes and negatively to the proportion of heterophils, exactly the opposite to the correlations found with the chroma of the yellow areas, whereas the rest of correlations were not significant (Table 4). The colour parameters of the grey breast band were largely unrelated to the immune system characteristics (Table 5). The total leucocyte count was unrelated to the colour intensity characteristics of the three areas considered (Tables 3–5).

Males with larger yellow areas in their plumage presented a higher proportion of heterophils

Table 1. Mean body mass, standard deviations and sample size for *Plasmodium* and *Leucocytozoon* infected and uninfected Cirl Buntings in relation to age

	Uninfected	*Plasmodium*	*Leucocytozoon*
Young	22·75 ± 1·20 (37)	23·24 ± 1·21 (27)	23·14 ± 1·29 (15)
Adult	23·62 ± 1·07 (18)	23·92 ± 1·10 (5)	21·61 ± 0·59 (5)

Table 2. Total leucocyte count (TLC) and relative presence of different leucocyte cells in the studied sample

	Young			Adults		
	Parasite free	*Leucocytozoon*	*Plasmodium*	Parasite free	*Leucocytozoon*	*Plasmodium*
TLC	8146 ± 5832 (25)	10838 ± 5971 (6)	15922 ± 8781 (19)	7931 ± 3234 (16)	4771 ± 2446 (3)	17223 ± 13480 (6)
Lymphocytes	70·38 ± 18·85 (34)	80·75 ± 12·28 (8)	77·79 ± 18·65 (24)	56·29 ± 23·29 (17)	74·00 ± 25·53 (3)	72·50 ± 25·97 (6)
Heterophils	22·32 ± 19·59	9·62 ± 9·78	16·88 ± 18·29	37·41 ± 21·33	24·33 ± 27·06	23·67 ± 23·86
Eosinophils	6·35 ± 8·15	5·75 ± 4·65	4·00 ± 4·42	5·59 ± 7·48	1·00 ± 1·73	1·50 ± 1·97
Monocytes	0·94 ± 1·39	3·88 ± 4·61	1·33 ± 1·52	0·59 ± 1·23	0·67 ± 1·15	2·33 ± 3·20

Table 3. Relationship of the colour characteristics of the yellow areas in adult male Cirl Buntings and total leucocyte count and composition, $n = 20$. r_s and t correpond to the parameters of Spearman Rank Correlation and G and Z are the equivalents for Gamma Correlation

Colour	TLC			Lymphocytes			Heterophils		
	r_s	t	P	G	Z	P	G	Z	P
Light	0·27	1·20	0·25	–0·53	–2·92	0·003	0·53	2·94	0·003
Chroma	0·31	1·41	0·18	–0·39	–2·15	0·03	0·44	2·42	0·02
Hue	0·22	0·96	0·35	–0·44	–2·44	0·01	0·44	2·42	0·02

($G = 0{\cdot}44$, $Z = 2{\cdot}66$, $P = 0{\cdot}008$, $n = 20$), whereas no significant relationship occurred with any other leucocyte type (Table 6). The size of the black bib was unrelated to the relative presence of leucocyte cells. Breast band size was positively related to relative lymphocyte presence ($G = 0{\cdot}40, Z = 2{\cdot}39, P = 0{\cdot}02$) and negatively related to heterophil presence ($G = -0{\cdot}53$, $Z = -3{\cdot}18, P = 0{\cdot}001$).

The total leucocyte count was negatively related to the size of the yellow areas of the body ($r_p = -0{\cdot}51$, $F_{1,18} = 6{\cdot}40$, $P = 0{\cdot}02$). However, the absolute leucocyte count was unrelated to the size of the black bib ($r_p = -0{\cdot}03, F_{1,18} = 0{\cdot}01, P = 0{\cdot}91$), or the size of the grey breast band ($r_p = -0{\cdot}01, F_{1,18} = 0{\cdot}01, P = 0{\cdot}96$).

Discussion

In this study we report a significantly lower body mass in birds infected by *Leucocytozoon* but not in those infected by *Plasmodium*. The former has been previously reported as the cause of severe diseases in poultry and waterfowl, but to our knowledge this is the first study to deal with its effects in free-living passerines (see Atkinson & van Riper 1991). No detrimental effects have been reported in the body condition of birds infected by *Plasmodium*. Detrimental effects of this species have been reported in laboratory studies with canaries, chickens, ducks and turkeys, but data from free-living birds are lacking. In free-living animals, *Plasmodium* seems to affect survival and future reproduction of Great Tits, *Parus major* (Richner, Christe & Oppliger 1995), and is responsible for multiple pathologies in other groups such as lizards (see Schall 1996). However, the detection of the subtle effects on body condition needs a larger sample size than those used in our study. The exclusion of the negative effects of any parasite is not possible without an experimental approach (Clayton 1990). Additionally, the poorer body condition of birds infected with *Leucocytozoon* is not necessarily the result of parasite infection, because, alternatively, the lower body condition could also occur if parasite infections tend to occur in individuals in a poorer body condition previous to infection. The poorer body condition of adults infected by *Leucocytozoon* and the lack of effects on young could be due to the short time occurring between infection of the young and blood sampling. Additionally, other factors such as food shortage or stress during breeding may alter parasite–host interactions, leading to detrimental effects emerging in the host in some groups (see, for example, Korpimäki, Hakkarainen & Bennett 1993; Bosch

Table 4 Relationship of colour characteristics of the black bib of adult male Cirl Buntings and total leucocyte count and composition, $n = 20$

Colour	TLC			Lymphocytes			Heterophils		
	r_s	t	P	G	Z	P	G	Z	P
Light	–0·06	–0·26	0·79	0·17	1·02	0·31	–0·09	–0·54	0·59
Chroma	–0·03	–0·14	0·89	0·34	2·04	0·04	–0·36	–2·16	0·03
Hue	–0·25	–1·10	0·28	0·24	1·43	0·15	–0·28	–1·69	0·09

Table 5. Relationship of the colour characteristics of the grey breast band of adult male Cirl Buntings and total leucocyte count and composition, $n = 20$

Colour	TLC			Lymphocytes			Heterophils		
	r_s	t	P	G	Z	P	G	Z	P
Light	0·25	1·11	0·28	–0·27	–1·54	0·12	0·28	1·64	0·10
Chroma	0·24	1·03	0·32	0·09	0·49	0·62	–0·02	–0·10	0·92
Hue	–0·38	–1·73	0·10	–0·04	–0·21	0·83	–0·03	–0·17	0·86

et al. 1997). Nevertheless, our results at least suggest that the magnitude of the effects produced by the two species of blood parasites could widely differ. This is one of the potential problems suggested as having the most effect on comparative tests of Hamilton & Zuk's hypothesis, and illustrates the need for an alternative approach for testing this hypothesis. Additionally, infection intensities peaked during the breeding season, and comparatively low levels occur during the rest of the year (see Allander & Sundberg 1997). This could invalidate any analyses relating infestation intensities to plumage brightness using data collected outside the breeding season (such as data analysed in this paper). Nevertheless, by using an independent estimate of health status such as leucocyte number and composition (see Ots *et al*. 1998), our results could not be significantly affected by fluctuations in infestation intensity.

The age-related differences in the proportions of different leucocyte cells reported in this study closely match those predicted by previous studies (see review in Campbell & Coles 1986). The reasons for these differences are not clear, although they apparently could be explained by a higher incidence of endoparasites in young individuals (see Fudge 1989), or a higher eosinophil activity in response to recently acquired infections in young.

We have reported a relationship between different variables related to the cellular immune response and male plumage characteristics. Given the high number of correlations calculated, we would expect to find two or less spurious correlations by chance (Rice 1989; Chandler 1995). However, the 11 significant correlations reported suggest that the analyses performed reflect a true relationship between plumage brightness and the immune system.

A significant correlation was found between the proportion of lymphocytes and heterophils and the three components of plumage coloration (light, chroma and hue). Interestingly, these relationships occur more clearly in the carotenoid-derived colorations (yellow parts of the plumage, see Hudon & Brush 1992), whereas less clear results are obtained for the probably melanin-derived black bib and grey breast band. The relationship for the coloration of the black bib was in the opposite direction to those found for carotenoid-derived colorations. Birds are unable to synthesize carotenoids and have to incorporate them through their diet (Goodwin 1950; Brush 1978, 1990). In addition, parasites seem to interfere with physiological mechanisms involved in carotenoid absorption, transport, metabolism and deposition (Zuk *et al*. 1990; Burley, Price & Zann 1992; Hamilton 1992). On the other hand, melanin seems to be synthesized at a very low energetic and metabolic cost and so apparently its handicapping potential could be lower than that of carotenoid-derived signals (Gray 1996). Gray's (1996) analyses suggest that sexual dimorphism in plumage colour is more common in the carotenoid-coloured areas of male plumage. The fact that the relationships between colour intensity and immune responses were higher in the carotenoid-derived coloration gives some support to Gray's hypothesis.

Both environmental and physiological stress could increase the heterophil : lymphocyte ratio (Maxwell 1993). Hence, the relationship found between plumage coloration and current immune response could have at least two possible explanations. Firstly, the increased proportion of heterophils could reflect a higher presence of bacterial infections and low immunocompetence. In this case, brightly coloured individuals would be the most infected individuals, unlike in Hamilton & Zuk's predictions. However, in our case infection by blood parasites was associated with a reduction in heterophil relative presence. Additionally, according to this explanation plumage brightness should be positively correlated to a higher total leucocyte count (see Averbeck 1992), contrary to our results. Alternatively, high heterophil relative presence in relation to lymphocytes and low total leucocyte counts in brighter males would indicate the absence of parasites and infectious diseases and correspondingly better overall health (see also Dufva & Allander 1995). Individuals with larger yellow areas also presented lower total leucocyte counts, an immunological characteristic that was associated with the absence of parasites (see Results) and the absence of other infectious diseases (i.e. Averbeck 1992). According to these results, male plumage brightness is a reliable signal of superior health and the absence of parasites and infectious diseases. In this case by mating with brightly coloured males, females can select mates that show better health.

In conclusion, our results support the parasite-mediated hypotheses on the evolution of plumage brightness (Freeland 1976; Hamilton & Zuk 1982; Borgia & Collis 1989, 1990; Read 1990; Kirkpatrick & Ryan 1991; Zuk 1992; Saino, Møller & Bolzern 1995), while not discounting other unknown factors not observed in the analyses presented here.

Table 6. Relationship of leucocyte composition to the size of the three coloured areas studied in adult male Cirl Buntings, $n = 20$

Coloured area	Lymphocytes			Heterophils		
	G	*Z*	*P*	*G*	*Z*	*P*
Black bib	–0·04	–0·24	0·81	–0·01	–0·07	0·95
Breast band	0·40	2·39	0·02	–0·53	–3·18	0·001
Yellow area	–0·26	–1·59	0·11	0·44	2·66	0·008

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