

SHORT REPORT

Bill colour and body condition in the Moorhen *Gallinula chloropus*

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Capsule We found yellow to be an honest signal of health status.

There is increasing evidence that birds communicate their health status and reproductive quality through signals. These can be as song rate (Saino *et al.* 1997) or morphological, such as wattles (Zuk *et al.* 1990) or plumage coloration. According to Zahavi's (1975) handicap hypothesis, it is assumed that the production of these signals is costly for the organism. Hamilton & Zuk (1982) suggested that plumage coloration has evolved as an honest signal of parasite and disease resistance, and so indirectly signals the individual's quality as a mate. This and other models of parasite-mediated selection predict that by mating with brightly coloured males, females may benefit by a lower risk of disease transmission (Hillgarth 1996) and gain genetic benefits for their offspring in the form of disease resistance. The hypothesis predicts a negative relationship between pathogenic parasite levels (and thus indirectly immune status) and colour brightness because the healthiest individuals will be able to afford the costs of fully developing the sexually selected traits. However, this hypothesis has led to conflicting results in birds. It has not been verified in Redpoll Finches *Carduelis* spp. (Seutin 1994) nor Barn Swallows *Hirundo rustica* (Camplani *et al.* 1999). In contrast, there is evidence of a relationship between immune response and plumage brightness in other passerine birds (Thompson *et al.* 1997, Dufva & Allander 1995, Figuerola *et al.* 1999, Faivre *et al.* 2000). Apart from a study on the Pheasant *Phasianus colchicus* (Hillgarth 1996), few studies have dealt with this problem in non-passerine birds.

Carotenoids play an important role in animal coloration through reflecting general quality influencing mate choice, resolving aggressive interactions and revealing parasite infestations. In birds, colours such as

the yellow, orange and red in feathers, bill and head ornaments (wattles, combs, shields) are produced mostly by carotenoids. Carotenoids also play important roles in different aspects of immune function and free radical removal (Bendich 1989). These terpenic pigments must be consumed in the diet; often they are in short supply in nature and it is likely that birds prefer pigment-rich food. Once assimilated, carotenoids can be modified and used in many ways that are under genetic and physiological control (Hill 1991). Recent studies have revealed correlation between carotenoid-dependent coloration and individual quality in birds. Hōrak *et al.* (2001) found a negative relationship between carotenoid-based coloration and the haemoparasite infection level in Great Tits *Parus major*. Hill (1994, 1996) has shown that the red colour of male House Finches *Carpodacus mexicanus* reflects their diet and also that females prefer more brightly coloured males. Furthermore, bright red males survive winter better than dull males and thus are available for more than one breeding season. In Northern Cardinals *Cardinalis cardinalis*, redness is a dominance and male status signal, used to defend territories and in intraspecific communication in the breeding season (Wolfenbarger 1999).

We studied the Moorhen *Gallinula chloropus*, a monomorphic bird with a bright red frontal shield and yellow-tipped bill in adults and a green-brownish shield and bill in juveniles. To test the hypothesis that these bill characteristics are honest signals of general health conditions, we looked for correlations between bill colours and body condition indices. We performed observations in winter when food is less available, at a time when Moorhens lose mass (Acquarone *et al.* 1998) and face more difficulty in utilizing the pigments in food for yellow-red colours. Moreover, this season is

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also the pre-reproductive period, in which pairs are formed and animals pass from a gregarious stage to one with strong intraspecific aggression. We set out to verify whether or not bill colours are correlated to health status in the period before breeding.

The study was conducted at Novi and Racconigi in Piedmont, northwest Italy. In total, 38 individuals were captured by funnel traps in January and February of 1998 and 1999. We measured the structural size, bill and eye coloration, mass, lean mass and some haematic indices for all birds. Wing and tail lengths (to the nearest 0.5 mm), tarsus and bill lengths (to the nearest 0.1 mm) were all measured. These were then compacted into an index of structural size calculated as the first factor from a principal components analysis (PCA), separately for adults and young.^a An index of body fat was calculated from mass as the residuals of a regression of mass on PCA factor 1.

We measured colours in the field using a Pantone code and converted them to colour ranks using their respective coordinates in the L*a*b CIE method (Figuerola *et al.* 1999). The ranks (from the most opaque to the brightest) ranged from 1 to 21 for the bill redness, from 1 to 17 for the yellow bill-tip, and from 1 to 11 for the red of the eyes.

Birds were weighed (to 0.1 g accuracy) and then body composition was evaluated using electroconductivity with the TOBEC (total body electrical conductivity) system. This device gives an indication of the lean body mass, since the contribution of lipid tissue to conductivity is negligible (Castro *et al.* 1990). Each individual was placed in the detection chamber (76 mm diameter) of an SA-3000 Small Animal Body Composition Analyzer (EM-SCAN Inc., Springfield, Illinois) for 5 to 6 seconds and the electrical conductivity index was recorded as: $TOBEC\ index = (S - E)/R$, where S = measurement with the specimen, E = empty measurement, and R = reference number (EM-SCAN operator's manual). For each individual, the measurement was carried out three times and the average value was employed in statistical analyses.

Blood was collected from the brachial vein into 75-mm heparinized capillary tubes. These were placed in a vertical position in a refrigerator for four hours at 4°C, after which the erythro sedimentation rate (ES rate) was measured as the ratio between the length of the capillary tube with blood cells and the total length (Saino & Møller 1996). We also collected blood smears: they were air-dried, fixed with absolute ethanol and stained with the May-Grünwald Giemsa method. Smears were scanned at 630× magnification

until 100 white blood cells were counted. Heterophils, eosinophils, basophils, lymphocytes and monocytes were differentiated and the erythrocytes were counted. We then calculated the relative frequency of the leukocytes of each family with respect to the total population of leukocytes (relative counts) and the number of leukocytes of the different families per 10 000 red blood cells (absolute counts). This method has been shown to give significantly repeatable relative and absolute leukocyte counts (Saino & Møller 1996). Absolute values were \log_{10} transformed for statistical analysis.

Young and adults differed in the red and yellow bill colour intensities (brighter in adults; Mann-Whitney tests $U = 310.5$, $P < 0.001$ and $U = 334.5$, $P < 0.01$ respectively). Therefore, all statistics will be presented separately for the two age-classes. In young birds, the brightness of the yellow bill-tip and of the red frontal shield were not correlated with any index (Table 1). In adults the bill-tip was more intensely yellow-coloured in the heaviest individuals [regression line equation: $yellow = (0.040 \times mass) - 5.07$], with better haematic conditions [regression line equation: $yellow = (2.46 \times heterophil/lymphocyte\ ratio) - 8.67$]. No other relationships reached statistical significance after Bonferroni correction (Table 1).

The Moorhen is a species with high intraspecific aggressiveness, and in conflictual interactions the coloured frontal shield is exhibited prominently against black head feathers and half-closed wings. The shield has been considered as a display structure that is used in competitor assessment and a valid signal of competitive ability (Petrie 1988). It is likely that the colour intensity in young should be minimized, and not be selected as an honest indirect signal of health condition, because young are not involved in sexual competition.

In adults, the yellow colour index (but not the red) was positively correlated to body mass and heterophil/lymphocyte ratio. Interestingly, our correlational data do not fit the proposal by Hill (1996) that red displays but not orange or yellow are closely correlated with individual quality, perhaps suggesting the existence of differences between species (Hill & McGraw 2000). Our results confirm that yellow can be an honest signal of health status, as found in the Cirl Bunting *Emberiza cirius* (Figuerola *et al.* 1999), Great Tit *Parus major* (Dufva & Allander 1995) and Blackbird *Turdus merula* (Faivre *et al.* 2000). In these studies, high heterophil and low lymphocyte percentages were correlated with yellow colour intensity, and both indicated the absence of parasites and infectious diseases. In order to clarify the relationship between

Table 1. Relationships between bill colour intensity, fat index and some body and health condition indices (Spearman's correlation tests¹) in young and adult Moorhens.

	Yellow intensity of bill-tip		Red intensity of bill and shield		Fat index (mass/factor 1 residuals)	
	Young	Adults	Young	Adults	Young	Adults
Mass	0.07	0.71***	0.43*	0.41*	0.67**	0.69***
Size (PCA factor 1)	0.19	0.39	0.28	-0.08	0.12	-0.07
Lean mass (TOBEC)	0.24	0.34	0.16	0.02	0.30	0.23
Fat index (residuals)	0.03	0.48*	0.41	0.55*	1.00	-
Bill length	-0.08	-0.31	-0.49*	-0.50*	-0.49*	0.47*
Heterophil (%)	-0.11	0.68***	0.14	0.23	0.12	0.31*
Eosinophil (%)	0.15	0.23	-0.15	-0.05	0.26*	0.19
Lymphocyte (%)	0.12	-0.61**	-0.03	-0.15	-0.04	0.31
Heterophil (N/10 000 rbc)	0.16	0.65***	0.41	0.24	0.39	0.58**
Eosinophil (N/10 000 rbc)	0.08	0.28	0.01	0.03	0.26	0.26
Lymphocyte (N/10 000 rbc)	0.20	0.32	0.33	0.35	0.35	0.59**
Heterophil/lymphocyte rate	0.08	0.67***	0.14	0.23	0.09	0.32
Leukocyte abundance	0.23	0.49*	0.25	0.35	0.28	0.77***
ES rate	-0.28	0.11	0.12	-0.01	-0.19	0.09
Eye colour	0.45*	-0.10	0.58**	0.18	0.21	-0.20
Red intensity of bill	0.32	0.45*	1.00	-	0.41	0.55*
Yellow intensity of bill-tip	1.00	-	0.32	0.45*	0.03	0.48*

¹Because 48 multiple non-independent tests were conducted, Bonferroni correction was applied, and only correlations where $P < 0.001$ (i.e. $0.05/48$) were considered significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. rbc, red blood cells.

carotenoid-dependent coloration and individual health status, the time lag between developing the colour and testing body conditions should also be considered. In the case of plumage coloration, pigments are concentrated in growing feathers at the time of the moult. In House Finches, Hill (1994) showed that individuals cannot use carotenoids stored in advance of the moult and can only use those eaten while they are replacing their feathers. In the case of bill and shield coloration, additional research is most needed to elucidate the absorption and metabolism of carotenoids.

Bill coloration seems to be an important element in sexual selection in some bird species. In dimorphic and polygamous species, such as the Mallard *Anas platyrhynchos*, the reproductive success of males is influenced by the intensity of bill colour (Omland 1996). The Moorhen is a monogamous species, in which males play an important role in brooding: up to 72% of the incubation is performed by the male (Petrie 1983). There are no appreciable differences in bill and shield coloration between males and females. This monomorphism is probably related to the fact that both parents participate in brooding and parental care; thus sexual selection has operated equally in both sexes, giving rise to the same valid honest signal for males and females. Our results suggest that bill coloration is an honest signal of health status in adults. Further studies will test

the hypothesis that the combination of bill colour and shield size works as an effective signal of health condition and reproductive quality.

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ENDNOTE

a. In adults, factor 1 explained 67.9 % of the variance in the original variables and was highly related to them (loadings: wing = 0.89, tail = 0.87, tarsus = 0.70), while in the young, factor 1 explained 73.1 % of the variance and the loadings were: wing = 0.91, tail = 0.76 and tarsus = 0.89.

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