THE ROYAL SOCIETY

Carotenoids, sexual signals and immune function in barn swallows from Chernobyl

Alice Camplani[1,2], Nicola Saino[1] and Anders Pape Møller[2*]

[1]*Dipartimento di Biologia, Sez. Zoologia Scienze Naturali, Università di Milano, Via Celoria 26, I-20133 Milano, Italy*
[2]*Laboratoire d'Ecologie, CNRS URA 258, Université Pierre et Marie Curie, Bât. A, 7ème étage, 7 quai St Bernard, Case 237, F-75252 Paris Cedex 5, France*

Carotenoids have been hypothesized to facilitate immune function and act as free-radical scavengers, thereby minimizing the frequency of mutations. Populations of animals exposed to higher levels of free radicals are thus expected to demonstrate reduced sexual coloration if use of carotenoids for free-radical scavenging is traded against use for sexual signals. The intensity of carotenoid-based sexual coloration was compared among three populations of barn swallows *Hirundo rustica* differing in exposure to radioactive contamination. Lymphocyte and immunoglobulin concentrations were depressed, whereas the heterophil:lymphocyte ratio, an index of stress, was enhanced in Chernobyl swallows compared to controls. Spleen size was reduced in Chernobyl compared to that of two control populations. Sexual coloration varied significantly among populations, with the size of a secondary sexual character (the length of the outermost tail feathers) being positively related to coloration in the two control populations, but not in the Chernobyl population. Thus the positive covariation between coloration and sexual signalling disappeared in the population subject to intense radioactive contamination. These findings suggest that the reliable signalling function of secondary sexual characters breaks down under extreme environmental conditions, no longer providing reliable information about the health status of males.

Keywords: *Hirundo rustica*; immunoglobulins; leucocytes; radiation; sexual signals; spleen

1. INTRODUCTION

Carotenoids are important determinants of animal coloration used in social or sexual signalling (e.g. Goodwin 1984; Gray 1996). The main sources of carotenoids are algae and plants, or secondary acquisition through ingestion of animals that have fed on carotenoids. Carotenoids are often in short supply since free-living animals have carotenoid-based colour that can readily be made brighter by the addition of carotenoids to the food (e.g. Kodric-Brown 1985; Hill 1990). Females of many species of vertebrates prefer males with more bright carotenoid-based coloration (e.g. Endler 1983; Burley & Coopersmith 1987; Hill 1990; Milinski & Bakker 1990).

Carotenoids have important physiological functions associated with immunity. They enhance T- and B-lymphocyte proliferative responses, stimulate effector T-cell function, enhance macrophage and cytotoxic T-cell capacities, increase the population of specific lymphocyte subpopulations, and stimulate the production of various cytokines and interleukins in mammals (Bendich 1989; Chew 1993; Van Poppel *et al.* 1993; Jyonouchi *et al.* 1994, 1995). Carotenoids are also well known for their activity as oxygen radical scavengers, acting as free-radical traps and efficient quenchers of singlet oxygen, thus decreasing immunosuppressive peroxides and the mutagenic effect of free radicals (Bendich 1989).

The aims of this study were to investigate the hypothesized relationship between carotenoid-based signals and antioxidant effects of carotenoids, using the barn swallow *Hirundo rustica* as a model system. Previous studies of this species in Chernobyl have demonstrated increased fluctuating asymmetry in tail length in males in the period after radioactive contamination in 1986, as compared to a control area, but not before this period (Møller 1993). Furthermore, a subsequent study revealed an increased frequency of partial albinism of feathers in barn swallows in Chernobyl compared to two control areas (Ellegren *et al.* 1997). This albinism was caused by germline mutations and associated with reduced viability (Ellegren *et al.* 1997). Finally, microsatellite genetic markers demonstrated significantly elevated mutation rates in Chernobyl compared to two control sites, with a two- to tenfold increase (Ellegren *et al.* 1997). Previous studies of barn swallows have demonstrated a strong positive correlation between the red plumage colour of the throat badge, which is produced in the African winter quarters, and plasma lutein concentration during the breeding season (Saino *et al.* 1999).

We specifically investigated one population of barn swallows subject to radioactive contamination (Chernobyl) and two control populations (Kanev, Ukraine, and Kraghede, Denmark) to determine whether (i) radioactive contamination was associated with reduced condition (leucocyte counts, heterophil: lymphocyte ratio, buffy coat), (ii) carotenoid-based coloration was reduced in the area with radioactive

* Author for correspondence (amoller@hall.snv.jussieu.fr).

Received 12 January 1999 *Accepted* 25 February 1999

contamination, and (iii) carotenoid-based coloration was related to immune response variables.

The barn swallow is a semicolonial, socially monogamous, aerially insectivorous, small (*ca.* 20 g) passerine. Sexual size dimorphism is small except for the length of the outermost tail feathers, which are longer in males. Males with relatively long outermost tail feathers are preferred by females both as social mates and extra-pair fathers of their offspring, and there is intense directional sexual selection for long tail feathers (Møller 1994; Møller *et al.* 1998). Both sexes have an orange-brown to chestnut forehead moulted at the African wintering grounds. Natal dispersal is on average 0.7 km in males and 2.5 km in females, with breeding dispersal being shorter (Cramp 1988).

2. METHODS

We studied barn swallows in three different populations: (i) Chernobyl (51° 17′ N, 30° 13′ E), (ii) Kanev (49° 42′ N, 31° 25′ E), Ukraine, and (iii) Kraghede (57° 12′ N, 10° 00′ E), Denmark. The populations at the two Ukrainian sites were studied by A.P.M. during June 1996 while the Danish population was studied May–August 1996. The Chernobyl area had an atmospheric level of radiation of 300–500 μR, considerably above the natural background level (A. A. Tokar, personal communication). Barn swallow colonies were located in stables on farms. Individual adults were captured at sunrise in mist-nets put up in front of windows and doors used by the birds for entering the indoor breeding sites.

At first capture we measured length of the left and right outermost tail feathers, innermost tail feathers, left and right wing chord, and bill, keel and right tarsus. Tail and wing length were the mean of the left and right character. From each individual we took a blood sample in capillary tubes after puncture of the ulnar vein and made a blood smear. Blood was stored in a cool bag in the field. Blood samples were then centrifuged for 10 min at 11 500 rpm and haematocrit measured with a digital calliper to the nearest 0.1%. Plasma was subsequently stored at −30 °C.

An experienced person counted 100 leucocytes, classified as lymphocytes, monocytes, eosinophils, heterophils and basophils, on blood smears that had been air-dried and stained by the May–Grünwald–Giemsa method. The proportion of each leucocyte type was multiplied with the relative size of the buffy coat, i.e. the layer of white blood cells and thrombocytes at the interface between plasma and red blood cells after the centrifugation of blood (volume of the buffy coat/blood volume) (Coles 1997) to obtain an index of concentration of leucocyte types.

Assessment of immunoglobulins was done by densitometric analysis after electrophoretic separation of plasma proteins on agarose gels (Paragon SPE Kit, Beckman). Five microlitres of plasma were applied to agarose gels and proteins allowed to migrate for 25 min at constant voltage (100 V). After electrophoresis, gels were air-dried and subsequently analysed using the procedure 'densitometric analysis of 1D gels' of the image analysis software NIH Image 1.54. The relative concentration of immunoglobulins and other proteins that co-migrate during electrophoresis was expressed as the ratio between the area of the densitometric profile corresponding to immunoglobulins and the total area of the densitometric profile (Saino *et al.* 1997).

The wet mass of the spleen was measured to the nearest 0.1 mg on a precision balance for a small number of adults collected at the three sites during a separate study in 1991 (Møller 1993).

The abundance of three species of ectoparasites (the mite *Ornithonyssus bursa*, two Mallophaga *Hirundoecus malleus* and *Myrsidea rustica*) was estimated according to standard sampling procedures (Møller 1990, 1991, 1994).

Lutein is a carotenoid component of plumage colour in barn swallows (Saino *et al.* 1999). Analysis of lutein-based plumage colour was performed on two feathers, plucked from a standard position in the centre of the throat, using a portable spectroradiometer (Ocean Optics Europe). Colour was measured in an area of the visible surface of the feather, of approximately 1 mm^2 and situated at 1 mm from the distal end of the feather. Light from a halogen light source (DH 2000) was transferred to the feather through a quartz optic fibre (Ocean Optics), reaching the feather at 90°. The sampling optic was placed at 45° to the surface of the sample and connected to a spectrometer (S2000) by a second quartz fibre optic cable. Data from the spectrometer were converted into digital information by DAQ Card 700 and passed into a computer with appropriate software (Spectrawin 3.1). The measurements were relative and referred to a standard white reference tile (WS-2) and to the dark. Each feather provided a measure of transmittance for each 1 nm interval in the interval 300–800 nm, and values were averaged between the two feathers. Since lutein absorbance peaks at 450 nm (Stradi *et al.* 1995), absorbance due to this carotenoid was calculated from mean transmittance in the interval 445–455 nm, as $-\log_{10}$(transmittance value), and used for subsequent analyses.

We tested for consistency of our colour measurements (absorbance in the lutein band) in a number of different ways by means of repeatability analyses (Falconer & Mackay 1996) and found statistically significant repeatabilities of 0.56 to 0.92 (Saino *et al.* 1999).

Values are means (s.e.). All tests are two-tailed.

3. RESULTS

(a) *Sexual and geographical variation of condition variables*

Mean concentration of lymphocytes was significantly smaller in swallows from Chernobyl than in those from Kanev (two-way ANOVA with population and sex as factors; population: $F_{1,77} = 4.33$, $p = 0.04$), while there was no significant effect of sex or sex by population interaction (table 1). We found no significant effect of sex or population on concentration of the other leucocyte types (F from two-way ANOVAs always associated with $p > 0.05$). The heterophil:lymphocyte ratio (an index of general stress (Gross & Siegel 1983; Maxwell 1993)) was significantly larger in Chernobyl than in Kanev (tables 1 and 2).

The size of the buffy coat demonstrated significant variation among populations (tables 1 and 2). However, the largest differences were between the Danish and the two Ukrainian populations, respectively, while swallows from Kanev only had slightly larger mean buffy coat values than those from Chernobyl (table 1). Sex had no effect on buffy coat values, but sexual differences varied among populations (table 2). Indeed, in the Danish population males had larger mean buffy coat values than females whereas in the two Ukrainian populations the ranking of sexes was reversed (table 1).

Table 1. *Mean (s.e.) within-sex and population concentration of leucocyte types, buffy coat relative volume and heterophil:lymphocyte ratio*

(See §2 for measurement procedures and §3 for statistical analyses.)

	Denmark	Chernobyl	Kanev
males			
lymphocytes	—	0.15(0.02)	0.17(0.03)
	—	$n=21$	$n=17$
monocytes	—	0.007(0.001)	0.006(0.002)
	—	$n=21$	$n=17$
heterophils	—	0.047(0.007)	0.048(0.01)
	—	$n=21$	$n=17$
eosinophils	—	0.01(0.002)	0.006(0.002)
	—	$n=21$	$n=17$
basophils	—	0.002(0.0006)	0.001(0.0005)
	—	$n=21$	$n=17$
heterophil:	—	0.376(0.058)	0.264(0.02)
lymphocyte ratio	—	$n=21$	$n=17$
buffy coat	0.61(0.07)	0.215(0.024)	0.242(0.04)
	$n=34$	$n=33$	$n=24$
females			
lymphocytes	—	0.16(0.002)	0.256(0.004)
	—	$n=20$	$n=20$
monocytes	—	0.01(0.002)	0.006(0.001)
	—	$n=20$	$n=20$
heterophils	—	0.05(0.007)	0.061(0.009)
	—	$n=20$	$n=20$
eosinophils	—	0.01(0.002)	0.01(0.004)
	—	$n=20$	$n=20$
basophils	—	0.001(0.0003)	0.002(0.001)
	—	$n=20$	$n=20$
heterophil:	—	0.36(0.05)	0.268(0.023)
lymphocyte ratio	—	$n=20$	$n=20$
buffy coat	0.461(0.03)	0.264(0.025)	0.35(0.05)
	$n=37$	$n=36$	$n=24$

The mass of the spleen was significantly reduced in barn swallows from Chernobyl compared to that of the two control populations (table 2; Chernobyl: 20.0 mg (3.2), $n=17$; Denmark: 33.6 mg (4.7), $n=16$; Kanev: 29.9 mg (3.9), $n=15$). Similarly, the concentration of immunoglobulins differed significantly among sites with values significantly depressed in Chernobyl compared to the two other sites (table 2; Chernobyl: 12.3% (0.5), $n=17$; Denmark: 15.2% (0.8), $n=16$; Kanev: 16.8% (0.8), $n=15$).

(b) *Coloration of red head feathers and morphology of swallows*

All colour variables, except ultraviolet (UV) absorbance, showed highly significant variation among populations (effect of population: $F_{2,201} > 6.1$ for all significant variables, $p < 0.003$; table 3). However, no significant effect of sex or sex by population interaction were observed (results not shown).

We investigated the covariation between red feather colour and morphology in analyses of covariance with sex and population as classification factors, morphological variables as covariates and the interactions. All colour features covaried significantly with tail length ($F_{1,189}=3.90$–7.36, $p \leqslant 0.05$). In addition, for all colour variables except hue, a significant interaction term between tail length and population was observed, suggesting that the slopes varied among populations (figure 1; $F_{2,189}=3.44$–7.41, $p < 0.05$). Among the other morphological variables, we detected significant covariation only between lutein absorbance, chroma and hue, respectively, and wing length ($F_{1,198}=4.37$–7.13, $p < 0.05$). Since wing and tail length in barn swallows are positively correlated (Møller 1994), this was not a surprising result.

Table 2. *Variation in immunological variables in relation to sex and population in two-way ANOVAs with population and sex as factors*

(Data for heterophil:lymphocyte ratio were only available for Chernobyl and Kanev. See table 1 for sample sizes. MS, mean square.)

	MS	d.f.	F	p
heterophil:lymphocyte ratio				
sex	0.001	1,74	0.03	0.86
population	0.195	1,74	5.58	0.02
sex × population	0.003	1,74	0.08	0.79
buffy coat				
sex	0.001	1,182	0.01	0.92
population	1.679	2,182	31.51	0.001
sex × population	0.280	2,182	5.25	0.006
immunoglobulins				
sex	26.32	1,42	2.95	0.09
population	88.70	2,42	9.93	0.003
sex × population	27.35	2,42	3.06	0.06
spleen				
sex	0.07	1,42	1.35	0.25
population	0.33	2,42	6.23	0.004
sex × population	0.02	2,42	0.44	0.64

No colour variable was significantly correlated with female morphology in any population ($p > 0.08$). For males, the pattern was different (table 4). In Denmark and Kanev, a significant positive correlation of tail length with lutein absorbance existed while that was not the case in Chernobyl (table 4). In Kanev, this relationship was still positive but not statistically significant when excluding an individual with very large tail length and absorbance values (figure 1; $F_{1,19}=2.46$, $p=0.13$). The correlation coefficients were significantly different ($\chi^2=6.02$, d.f. $=2$, $p < 0.05$ (Zar 1996, p. 384)). Similar differences were recorded for total absorbance, UV absorbance and chroma (table 3; total absorbance: $\chi^2=6.92$, d.f. $=2$, $p < 0.05$; UV absorbance: $\chi^2=7.07$, d.f. $=2$, $p < 0.05$; chroma: $\chi^2=17.22$, d.f. $=2$, $p < 0.05$), but not for hue ($\chi^2=2.61$, d.f. $=2$, n.s.).

Partial correlations between colour and tail length, while controlling for wing length and vice versa, confirmed the results of the correlation analyses. In Denmark, male tail length significantly correlated with lutein absorbance ($r_{\text{partial}}=0.42$, d.f. $=39$, $p=0.007$) and total absorbance in the 'visible' spectrum ($r_{\text{partial}}=0.34$, d.f. $=39$, $p=0.028$), but no significant partial correlations between colour and wing length existed while controlling for tail length ($r_{\text{partial}}=0.20$ to 0.08, d.f. $=39$, $p > 0.15$). In Chernobyl, the correlations of tail length with total absorbance and UV absorbance, respectively, while

Table 3. *Mean (s.e.) within-sex and population absorbance in the lutein peak absorbance band, total 'visible' spectrum, and UV band, chroma and hue*

(See § 2 for measurement procedure and § 3 for statistical analyses.)

	Denmark	Chernobyl	Kanev
males			
lutein absorbance	−1.048(0.016)	−1.159(0.025)	−1.111(0.033)
'visible' absorbance	−3.792(0.012)	−3.889(0.016)	−3.838(0.024)
UV absorbance	−3.028(0.018)	−3.116(0.024)	−3.063(0.041)
chroma	0.286(0.006)	0.255(0.006)	0.267(0.009)
hue	33.767(0.402)	35.776(0.378)	35.407(0.598)
n	43	33	24
females			
lutein absorbance	−1.08(0.016)	−1.148(0.022)	−1.172(0.027)
'visible' absorbance	−3.809(0.012)	−3.857(0.015)	−3.879(0.216)
UV absorbance	−3.078(0.015)	−3.082(0.02)	−3.110(0.030)
chroma	0.27(0.005)	0.261(0.006)	0.257(0.008)
hue	34.626(0.325)	35.290(0.445)	36.141(0.511)
n	47	36	24

Table 4. *Pearson correlation coefficients between tail length and coloration of males from three populations of barn swallows*

population	lutein absorbance	'visible' absorbance	UV absorbance	chroma	hue	*n*
Denmark	0.36	0.29	0.22	0.22	−0.01	42
	$p=0.02$	n.s.	n.s.	n.s.	n.s.	
Chernobyl	0.02	−0.18	−0.21	0.05	−0.12	32
	n.s.	n.s.	n.s.	n.s.	n.s.	
Kanev	0.56	0.50	0.50	0.61	−0.43	22
	$p=0.007$	$p=0.02$	$p=0.02$	$p=0.003$	$p=0.04$	

controlling for wing length, were significantly negative (total absorbance: $r_{\text{partial}}=-0.37$, d.f. $=29$, $p=0.04$; UV absorbance: $r_{\text{partial}}=-0.40$, d.f. $=29$, $p=0.02$). Finally, in Kanev, no significant correlations existed between colour and tail length while controlling for wing length ($r_{\text{partial}}=0.17$–0.39, d.f. $=19$, $p>0.07$).

(c) *Coloration and male immunological condition*

We ascertained whether red feathers reflected the immunological condition of swallows from the three populations in analyses of covariance of each immunological variable (concentration of each leucocyte type, heterophil:lymphocyte ratio, buffy coat) with sex and population as factors and colour variables as covariates. We found no statistically significant evidence for red feather colour reflecting immunological condition (results not shown).

(d) *Parasite load of adult barn swallows*

The prevalence and intensity of three species of ectoparasites were very similar in the three study sites (table 5).

Table 5. *Prevalence and intensity (s.e.) of three species of ectoparasites of barn swallows from the three populations*

parasite	Denmark	Chernobyl	Kanev
Ornithonyssus bursa			
prevalence (%)	24.5	4.2	4.1
intensity	0.15(0.12)	0.73(0.68)	0.16(0.11)
Hirundoecus malleus			
prevalence (%)	83.6	85.6	83.3
intensity	15.5(1.7)	14.4(0.8)	15.3(0.9)
Myrsidea rustica			
prevalence (%)	97.3	97.3	96.6
intensity	64.2(5.4)	65.6(5.8)	62.8(6.0)
n	110	75	58

4. DISCUSSION

(a) *Carotenoid-based signals and radiation*

Carotenoids have been hypothesized to (i) facilitate immune function, and (ii) act as free-radical scavengers. Increased radiation should imply an increased need for carotenoids for immune function to control the proliferation of cancer cells and for free-radical scavenging. Hence, we predicted that carotenoids should be less available for carotenoid-based signals in the radioactively contaminated Chernobyl area compared to two control areas.

The sociosexual function of the chestnut forehead and throat patch has not been experimentally tested, although correlational evidence shows that females mate preferentially with males with greater hue and absorbance at the wavelength of lutein (P. Ninni, N. Saino and A. P. Møller, unpublished results). If multiple sexual signals independently reflect the same underlying phenotypic quality of individuals, we should expect the expression of different signals to be positively correlated (Møller & Pomiankowski 1993), while that should not be the case when carotenoids otherwise used for signalling are allocated to counter the detrimental effects of radiation. In other words, we would expect a break-up of the relationship between coloration and tail length in male barn swallows in the Chernobyl area. We discuss the results in the light of these predictions in the following paragraphs, but first emphasize that the comparison strictly speaking consists of only one area with radioactive contamination and two control areas; in other words a single contrast between contaminated and uncontaminated areas.

Coloration differed significantly among study areas with less intense, red and saturated coloration in male barn swallows from the Chernobyl area, but not for females (table 3). Furthermore, the positive correlation between tail length and carotenoid-based coloration differed significantly among study sites (figure 1), with positive correlations in two control areas, but weak and non-significant correlations in Chernobyl. The differences in carotenoid-based coloration among areas are most readily reconciled with the hypothesis that carotenoids play an important function as free-radical scavengers (Bendich 1989; Chew 1993). Since barn swallows from Chernobyl demonstrated decreased levels of immune function compared to conspecifics from the control areas, we can conclude that increased immune function cannot account for a reduction in carotenoid-based coloration. The significant reduction in carotenoid-based colour in males, but not in females, from Chernobyl may have arisen from males being more susceptible to free radicals, perhaps because the higher level of male activity increases the baseline level of free radicals in males compared to females. Mutation rates are often higher in males than in females (Miyata *et al.* 1987), and that is also the case in birds (Ellegren & Fridolfsson 1997), including the barn swallow (Ellegren *et al.* 1997).

We have previously documented an increased frequency of partial albinism caused by germline mutations in Chernobyl barn swallows (Ellegren *et al.* 1997). Interestingly, 23 out of the 174 barn swallows in Chernobyl had white feathers in the red facial region, while only two had white feathers in the blue dorsal coloration. This differs dramatically from random expectation, since the red facial feathers account for < 10% of the feathered surface. This observation suggests that the physiological mechanism responsible for metabolism and deposition of carotenoids in the facial feathers partially breaks down under elevated radiation as in Chernobyl.

(b) *Radiation and health indicators*

Previous studies of the effects of radiation on health parameters of free-living organisms under natural conditions are few and scattered (Zakharov & Krysanov 1996). Chernobyl barn swallows had reduced concentrations of lymphocytes compared to barn swallows from the control population in Kanev, with a larger difference between females than males. A reduction of lymphocyte concentration was also found in bank voles (*Clethrionomys glareolus*) from a radioactive contaminated study area compared to two control areas in Russia (table 1 in Isaeva & Vyazov 1996). The difference in total leucocyte concentration, as estimated by relative buffy coat volume, also significantly varied among populations, with the pattern of geographical variation being dependent on sex.

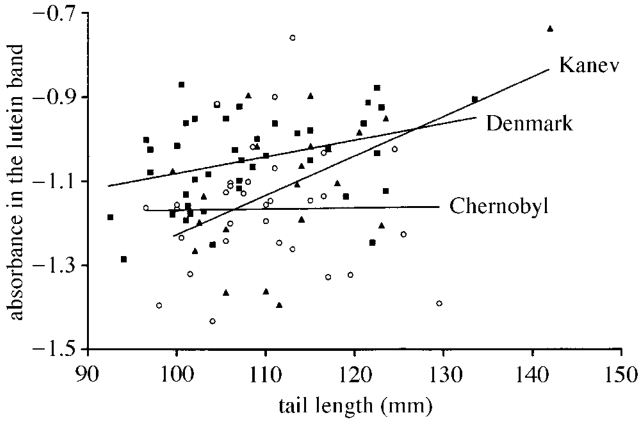

Figure 1. Relationship between absorbance in the lutein absorption band of red throat feathers and tail length in male barn swallows from Denmark (filled squares), Chernobyl (open circles) and Kanev (filled triangles). There was a significant positive correlation between tail length and feather coloration in Denmark and Kanev ($p < 0.05$), but not in Chernobyl. Regression lines for the three populations have the following equations: Denmark: absorbance = 0.0040 (0.0016 s.e.) × tail length − 1.48 (0.18), $F_{1,40} = 5.91$, $p = 0.02$, $r^2 = 0.13$; Chernobyl: absorbance = 0.00035 (0.0030 s.e.) × tail length − 1.20 (0.37), $F_{1,32} = 0.01$, $p = 0.92$, $r^2 = 0.0004$; Kanev: absorbance = 0.0094 (0.0030 s.e.) × tail length − 2.17 (0.36), $F_{1,20} = 8.93$, $p = 0.007$, $r^2 = 0.31$.

We found significant reduction in immunoglobulins in Chernobyl compared to the two control areas (table 2), matching a similar reduction in immunoglobulins in bank voles from contaminated areas compared to control sites in Russia (Isaeva & Vyazov 1996).

Finally, the ultimate sites of synthesis of products of the immune system are the immune defence organs (Roitt *et al.* 1995). Chernobyl barn swallows had smaller spleens compared to conspecifics from Kanev and Denmark (table 1). This reduction in spleen size parallels a reduction in thymus size in bank voles from radioactively contaminated areas in Russia compared to voles from control areas (Pronin *et al.* 1996).

The reduction in measures of immune function in Chernobyl could be interpreted in two different ways: (i) a reduction in the abundance of parasites is associated with reduced investment in immune function; or (ii) a deterioration of immune function caused by radiation. We believe that the latter explanation is most likely since parasite abundance was not reduced in Chernobyl (table 4). Pelgunov (1996) found similar prevalences of infections with coccidia, nematodes and cestodes in bank voles from heavily and weakly contaminated areas.

Carotenoids, in particular β-carotenoids, have been hypothesized to enhance and facilitate immune function. However, we found no evidence of increased immune function among barn swallows from the Chernobyl area. On the contrary, most measures of immune function were significantly lower in Chernobyl compared to the two control areas.

In conclusion, Chernobyl barn swallows had reduced intensity of carotenoid-based coloration compared to conspecifics from two control areas, and the positive relationship between coloration and the size of a secondary sexual character (tail length) found in control populations was absent in the Chernobyl area. The reduced amount of carotenoid coloration in Chernobyl is consistent with the hypothesis that carotenoids for signalling are traded against carotenoids used for free-radical scavenging.

We thank P. Ninni for assessment of repeatability of the colour measurements, C. Haussy for counting leucocytes and estimating the concentration of immunoglobulins and A. A. Tokar for logistic support. A.P.M. was supported by an ATIPE BLANCHE from CNRS.

REFERENCES

Bendich, A. 1989 Carotenoids and the immune response. *J. Nutr.* **119**, 112–115.

Burley, N. & Coopersmith, C. B. 1987 Bill colour preferences of zebra finches. *Ethology* **76**, 133–151.

Chew, B. P. 1993 Role of carotenoids in the immune response. *J. Dairy Sci.* **76**, 2804–2811.

Coles, B. H. 1997 *Avian medicine and surgery*, 2nd edn. Oxford: Blackwell.

Cramp, S. (ed.) 1988 *Handbook of the birds of Europe, the Middle East and North Africa*, vol. 5. Oxford University Press.

Ellegren, H. & Fridolfsson, A. K. 1997 Male-driven evolution of DNA sequences in birds. *Nature Genet.* **17**, 182–184.

Ellegren, H., Lindgren, G., Primmer, C. R. & Møller, A. P. 1997 Fitness loss and germline mutations in barn swallows breeding in Chernobyl. *Nature* **389**, 593–596.

Endler, J. A. 1983 Natural and sexual selection on colour pattern in poeciliid fishes. *Environ. Biol. Fishes* **9**, 173–190.

Falconer, D. S. & Mackay, T. F. C. 1996 *Introduction to quantitative genetics*, 4th edn. New York: Longman.

Goodwin, T. W. 1984 *The biochemistry of the carotenoids*. II. *Animals*. London: Chapman & Hall.

Gray, D. A. 1996 Carotenoids and sexual dichromatism in North American passerine birds. *Am. Nat.* **148**, 453–480.

Gross, W. B. & Siegel, H. S. 1983 Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis.* **27**, 972–979.

Hill, G. E. 1990 Female house finches prefer colorful males: sexual selection for a condition-dependent trait. *Anim. Behav.* **40**, 563–572.

Isayeva, E. I. & Vyazov, S. O. 1996 General assessment of immune status. In *Consequences of the Chernobyl catastrophe: environmental health* (ed. V. M. Zakharov & E. Y. Krysanov), pp. 80–84. Moscow: Center for Russian Environmental Policy.

Jyonouchi, H., Zhang, L., Gross, M. & Tomita, Y. 1994 Immunomodulating actions of carotenoids: enhancement of *in vivo* and *in vitro* antibody production to T-dependent antigens. *Nutr. Cancer* **21**, 47–58.

Jyonouchi, H., Sun, S. & Gross, M. 1995 Effect of carotenoids on *in vitro* immunoglobulin production by human peripheral blood mononuclear cells: astaxanthin, a carotenoid without vitamin A activity, enhances in vitro immunoglobulin production in response to a T-dependent stimulant and antigen. *Nutr. Cancer* **23**, 171–183.

Kodric-Brown, A. 1985 Female preferences and sexual selection for male coloration in the guppy. *Behav. Ecol. Sociobiol.* **17**, 199–205.

Maxwell, M. H. 1993 Avian blood leucocyte responses to stress. *World's Poultry Sci. J.* **49**, 34–43.

Milinski, M. & Bakker, T. C. M. 1990 Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344**, 330–333.

Miyata, T., Hayashida, H., Kuma, K., Mitsuyasa, K. & Yasunaga, T. 1987 Male-driven molecular evolution: a model and nucleotide sequence analysis. *Cold Spring Harb. Symp. Quant. Biol.* **52**, 863–867.

Møller, A. P. 1990 Effects of parasitism by the hematophagous mite *Ornithonyssus bursa* on reproduction in the barn swallow *Hirundo rustica*. *Ecology* **71**, 2345–2357.

Møller, A. P. 1991 Parasites, sexual ornaments and mate choice in the barn swallow *Hirundo rustica*. In *Ecology, behavior, and evolution of bird–parasite interactions* (ed. J. E. Loye & M. Zuk), pp. 328–343. Oxford University Press.

Møller, A. P. 1993 Morphology and sexual selection in the barn swallow *Hirundo rustica* in Chernobyl, Ukraine. *Proc. R. Soc. Lond.* B **252**, 51–57.

Møller, A. P. 1994 *Sexual selection and the barn swallow.* Oxford University Press.

Møller, A. P. & Pomiankowski, A. 1993 Why have birds got multiple sexual ornaments? *Behav. Ecol. Sociobiol.* **32**, 167–176.

Møller, A. P., Barbosa, A., Cuervo, J. J., de Lope, F., Merino, S. & Saino, N. 1998 Sexual selection and tail streamers in the barn swallow. *Proc. R. Soc. Lond.* B **265**, 409–414.

Pelgunov, A. N. 1996 Parasitological study of rodents. In *Consequences of the Chernobyl catastrophe: environmental health* (ed. V. M. Zakharov & E. Y. Krysanov), pp. 136–143. Moscow: Center for Russian Environmental Policy.

Pronin, A. V., Deyeva, A. V., Nikolaeva, T. N., Zaitseva, L. G., Kirillicheva, G. B., Baturina, I. G. & Solev-eva, M. S. 1996 Immune system. In *Consequences of the Chernobyl catastrophe: environmental health* (ed. V. M. Zakharov & E. Y. Krysanov), pp. 86–94. Moscow: Center for Russian Environmental Policy.

Roitt, I., Brostoff, J. & Male, D. 1995 *Immunology*, 4th edn. London: Mosby.

Saino, N., Bolzern, A. M. & Møller, A. P. 1997 Immunocompetence, ornamentation and viability of male barn swallows (*Hirundo rustica*). *Proc. Natl Acad. Sci. USA* **97**, 579–585.

Saino, N., Stradi, R., Ninni, P. & Møller, A. P. 1999 Carotenoid plasma concentration, immune profile and plumage ornamentation of male barn swallows (*Hirundo rustica*). *Am. Nat.* (In the press.)

Stradi, R., Celentano, G., Rossi, E., Rovati, G. & Pastore, M. 1995 Carotenoids in bird plumage. I. The carotenoid pattern in a series of Palearctic Carduelinae. *Comp. Biochem. Physiol.* **110B**, 131–143.

Van Poppel, G., Spanhaak, S. & Ockhuizen, T. 1993 Effect of β-carotene on immunological indexes in healthy male smokers. *Am. J. Clin. Nutr.* **57**, 402–407.

Zakharov, V. M. & Krysanov, E. Y. (ed.) 1996 *Consequences of the Chernobyl catastrophe: environmental health.* Moscow: Center for Russian Environmental Policy.

Zar, J. H. 1996 *Biostatistical analysis*, 3rd edn. Upper Saddle River, NJ: Prentice Hall.