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Correlations between melanin pigmentation and element concentration in feathers of White-tailed Eagles (*Haliaeetus albicilla*)

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Summary

The element concentration of moult feathers of White-tailed Eagles was investigated. Using the 2- MeV Hamburg proton microprobe we tried to differentiate between elements incorporated into the feather via the food chain and those which are deposited externally onto the feather vane. Regarding incorporated elements, special attention has been given to a possible correlation between element concentration and feather pigmentation. Concerning the elements detected in this work (S, K, Ca, Ti, Mn, Fe, Cu, Zn, Hg, Pb), calcium, manganese and zinc show a considerable enhancement within the pigmented feather as compared with pigment-free feather sections. On the other hand, no differences were found in concentrations for sulfur, titanium, iron, copper, mercury and lead. Our findings therefore imply a special enrichment of Ca, Mn and Zn within melanin, the source of the feather's pigmentation. The possible role of these elements with regard to melanin formation is discussed.

Key words: melanin, heavy-metal-concentration, pigmentation

Zusammenfassung

Melaninpigmentation und Elementkonzentration in Federn des Seeadlers (*Haliaeetus albicilla*)

Es wurden die Elementkonzentrationen in Mauserfedern von Seeadlern untersucht. Mit Hilfe der Hamburger 2-MeV-Protonenmikrosonde, die die Analyse von Probendetails im Mikrometerbereich ermöglicht, versuchen wir zwischen Elementen zu unterscheiden, die vom Adler mit der Nahrung inkorporiert, verstoffwechselt und während der Mauser in die Feder eingelagert wurden und solchen, die während der etwa einjährigen Verweilzeit der Feder im Gefieder extern aus der Atmosphäre aufgelagert wurden.

Im Hinblick auf die eingelagerten Elemente untersuchen wir mögliche Zusammenhänge zwischen der Elementkonzentration und der Federpigmentierung. Von den nachgewiesenen Elementen (Schwefel, Kalium, Calcium, Titan, Mangan, Eisen, Kupfer, Zink, Quecksilber und Blei) zeigen Calcium, Mangan und Zink eine erhebliche Anreicherung in der pigmentierten Feder im Vergleich zu pigmentfreien Federausschnitten. Dagegen wurden keine signifikanten Unterschiede der Elementkonzentrationen von Schwefel, Titan, Eisen, Kupfer, Quecksilber und Blei festgestellt. Die gefundenen Ergebnisse bedeuten eine erhebliche Anreicherung von Calcium, Mangan und Zink im Melanin, das für die Federpigmentierung verantwortlich ist. Die mögliche Funktion dieser Elemente im Hinblick auf die Melaninbildung wird diskutiert.

Introduction

Several investigations have shown that feathers of certain (non migratory) bird species (such as Magpie *Pica pica*, Goshawk *Accipiter gentilis* or White-tailed Eagle) are suitable bioindicators for regional contamination (Ellenberg et al. 1986, Hahn et al. 1993; Burger 1994; Burger & Gochfeld 1995; Niecke et al. 1998). Heavy metals externally deposited on feathers in the course of a one year period of a bird's flight reflect (when measured after the moult of the feather) the integral aerosol load during that one year in the bird's habitat. On the other hand, heavy metals that are incorporated via the food chain are an index of pollutional stress on the bird during the short period of feather growth. Organisms at the top of the food chains such as eagles are particularly useful bioindicators because of bioamplification (Burger et al. 1994).

Obviously, discrimination between the two kinds of contamination is necessary in the interpretation of the measured heavy metal load (Hahn et al. 1989, Niecke et al. 1990, Niecke & Krüger 1997). Furthermore, the question arises whether any other parameters (as for example the position of the feather within the plumage, the choice of the analysed feather section, the age and sex of the bird or the pigmentation of the feather) may influence the findings. With regard to the black-brown pigmentation of feathers, which is caused in many cases by melanins, a high concentration of metal ions (Fe, Cu, Zn, Ca) has been found by several authors (Scanlon et al. 1979, Scanlon et al. 1980, Goede 1985, Hartner et al. 1992). A reliable determination of the correlation between pigmentation and incorporated elements, however, seems to be impossible without special attention to externally deposited elements.

It is the intention of this work to determine the differences in concentration of incorporated elements in melanised and melanin free sections of feathers of White-tailed Eagles. On top of this, consideration shall also be given to the question why melanin tends to concentrate several elements as frequently described in the literature (Stein 1955, Potts and Au 1976, Samuelson et al. 1993, Zecca et al. 1992). In our opinion, exact knowledge of the incorporated elements and the extent of accumulation is a prerequisite to the investigation of this question.

Materials and Methods

The samples analysed in this work were taken from moulted feathers of adult White-tailed Eagles (*Haliaeetus albicilla*), collected in the German provinces of Mecklenburg-Vorpommern and Schleswig-Holstein. Only tail feathers were used. Sites and dates of finds are shown in Table 1. Feathers with nos. 262–266 stem from the same animal but were collected on different dates. The numbering is in keeping with that used in our analysis of mercury (Niecke et al. 1998). Feathers with the greatest difference in colour between light and dark feather sections were selected for analysis.

Earlier investigations by several authors (Weyers et al. 1988, Niecke et al. 1990, Hahn 1991) have shown – in contrast to the findings of Burger (1994) – that washing achieves only an imperfect reduction of the externally deposited and strongly adhering particles. Nevertheless, cleaning is necessary in order to improve the results of the measurements of the incorporated elements.

The feathers were first brushed along the rami, starting at the keal, in order to remove as many adhering particles as possible. Then the feathers were washed for 10 minutes in an ultrasonic bath with detergent (2% Extran in aqua bidest.), cleaned twice with aqua bidest. for 5 minutes and dried at room temperature and in clean room conditions.

Samples of 3 mm diameter were taken from the feathers prior to and after cleaning in order to determine changes induced by this procedure. Two samples, one black, one white, were taken from washed feathers and used for the determination of pigment-dependent differences in element concentrations. (In this work the terms black and white are used to differentiate between feather sections that are heavily pigmented with melanin and those with a lack of melanin respectively.) The samples were taken from locations as close to each other as possible (Fig. 1) in order to minimize the influence of externally deposited elements.

The element analysis was done by proton induced X-ray emission (PIXE). Using the Hamburg 2 MeV

Table 1. Element concentrations (in ppm) of black (melanic) and white (melanin-free) samples.
Tab. 1. Elementkonzentrationen (in ppm) von schwarzen (melaninhaltigen) und weißen (melaninfreien Proben).

feather no.	district	date	pigmentation	S	K	Ca	Ti	Mn	Fe	Zn	Cu	Hg	Pb
36	Parchim	1989	black	29803	333	978	19.2	51.3	407	229	7.1	24.4	3.5
36			white	25485	248	367	28.1	17.0	523	88	8.6	25.8	14.9
81	Niedersachsen	1995	black	26197	238	842	13.9	15.2	194	219	7.4	31.6	5.9
81			white	25197	198	142	26.6	8.5	362	55	5.7	38.0	6.6
97	Demmin	1991	black	26870	468	257	47.8	181.5	936	94	0.0	70.2	11.0
97			white	25893	717	92	106.1	70.6	1702	10	n.d.	111.6	n.d.
224	Nordvorpommern	1980	black	27449	212	522	6.2	10.6	283	213	7.3	15.0	5.9
224			white	27759	168	111	8.5	4.4	294	57	6.1	17.0	6.0
262	NWMecklenburg	1959	black	26375	203	487	11.6	38.2	275	249	6.0	11.5	25.9
262			white	21759	117	21	10.0	3.6	202	21	5.9	12.0	18.3
263	NWMecklenburg	1961	black	30186	355	868	9.7	21.6	234	222	14.0	24.4	22.1
263			white	27377	243	188	18.9	10.0	287	106	10.2	15.2	14.2
264	NWMecklenburg	1962	black	27639	120	377	6.2	3.4	146	229	5.4	23.6	8.9
264			white	28555	193	126	6.3	5.2	237	85	13.4	36.4	28.4
265	NWMecklenburg	1965	black	27173	n.d.	1212	46.5	13.0	244	259	n.d.	21.3	n.d.
265			white	23542	n.d.	381	11.8	5.3	205	95	15.7	24.0	17.1
266	NWMecklenburg	1968	black	27421	94	343	2.3	5.3	71	185	6.6	31.7	11.6
266			white	22155	128	58	3.1	1.7	72	28	7.6	27.1	22.1

proton microprobe, not only the concentration, but also the distribution of elements can be measured by scanning the samples' surface. This way, externally deposited particles which were not removed by the cleaning procedure can be identified and may therefore be excluded from the analysis. Through a comparison of the microscopical image of the sample with the concentration of incorporated elements, existing correlations with feather pigmentation can easily be detected. Details of the experimental setup have been described in previous papers by members of our group (Großmann et al. 1990).

Results

The first indication of a correlation between element concentration and feather pigmentation emerged from our analysis of mercury in White-tailed Eagles' feathers (Niecke et al. 1998), which was done with PIXE as described above. In this work, we analysed about 250 unwashed feathers without any special attention to feather pigmentation or elements besides mercury. A crude classification of feather colour (white=1, black=5) yielded an interrelation between zinc and colour as depicted in Figure 2.

Owing to external deposits, a wide variation in zinc concentrations can be seen. Nevertheless, a correlation with the pigmentation is

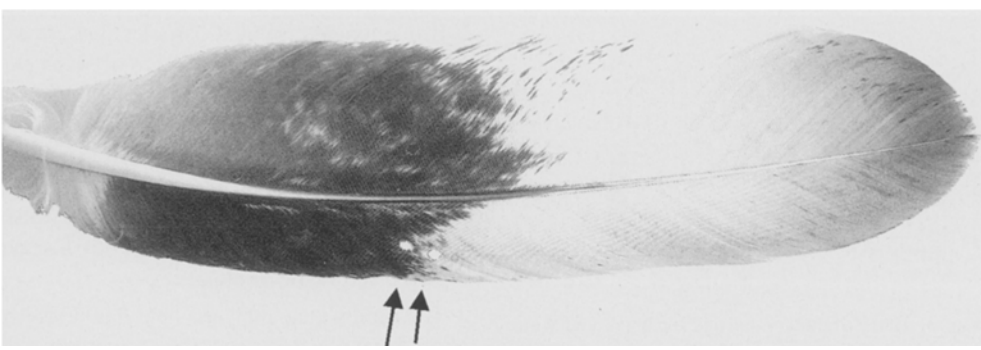

Fig. 1. White-tailed Eagle feather showing the source locations of the samples.
Abb. 1. Seeadlerfeder mit Darstellung der analysierten Federausschnitte.

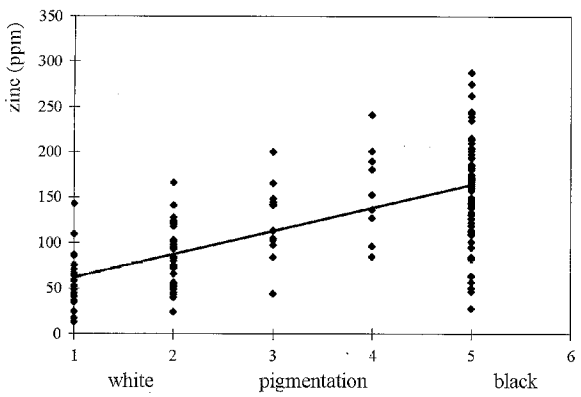

Fig. 2. Variation of zinc concentration in relation to feather pigmentation in a sample of about 200 White-tailed Eagle feathers.
Abb. 2. Abhängigkeit der Zinkkonzentration von der Federpigmentierung bei etwa 200 Proben von Seeadlerfedern.

clearly recognizable. In the present systematic work, the feathers were pretreated and sampled as described above, in order to reduce the influence of externally deposited particles. The concentrations of detected elements (S, K, Ca, Ti, Mn, Fe, Cu, Zn, Hg and Pb) for black and white feather sections are shown in Table 1. The size of the area analysed by the proton microbeam was 0.8 mm × 1.6 mm.

Sulfur is part of the keratin of feathers; titanium and lead, which are believed to be mainly externally deposited on the feather (Niecke & Krüger 1997) are shown for completeness.

A considerable variation of concentration can be seen for all elements owing to corresponding variations of these elements in the various White-tailed Eagles' diets and external deposits, which could not be completely removed by washing. This holds true also for feathers stemming from the same animal in different years (nos. 262–266).

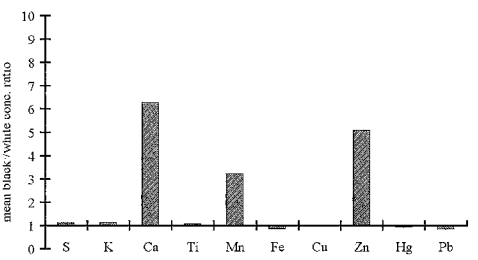

Fig. 3. Ratio of concentrations for black and white samples.
Abb. 3. Quotient der Elementkonzentrationen von schwarzen und weißen Federproben.

However, there is a significant difference in concentrations of calcium, manganese and zinc between black and white samples of the same feather. (The error probability given by the Wilcoxon signed rank test of matched pairs is less than 1%.) This can best be seen by considering the ratios between concentrations. The mean values are displayed in Figure 3.

The concentrations of calcium, manganese and zinc in black samples are higher by factors of 6.28 ± 2.22 (standard error of mean), 3.22 ± 0.97, 5.09 ± 1.17 resp., as compared to an unpigmented section of the same feather. These results can be illustrated by matching the distribution of these elements with the microscopic image of a vane with varying colour (Fig. 4).

If one ignores isolated particles that are externally deposited, the correlation between element distribution and optical image is clearly visible. This can be shown quantitatively by photographing a ramus showing a colour change from black to white and converting the (r,g,b)-values of the digitized image to values of brightness normalized onto a 0 to 1 scale. Figure 5 shows the results.

The ramus area analysed here had a size of about 1.6 mm × 0.05 mm. The increase of concentration from white to black areas is clearly visible. The larger variation as compared to ta-

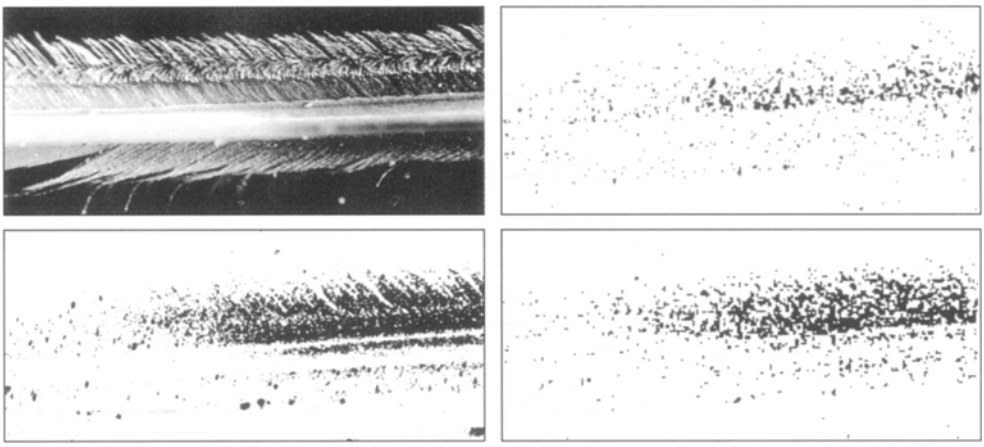

Fig. 4. Microscopic image (a), distribution of calcium (b), manganese (c) and zinc (d). In figs. 4b, c and d the secondary electron image is shown in light grey in order to guide the eye.
Abb. 4. Mikroskopisches Bild einer Seeadlerfeder im Ausschnitt (a), Verteilung der Elemente Calcium (b), Mangan (c) und Zink (d). In den Abbildungen 4b, c, d ist zur zusätzlichen Orientierung ein Sekundärelektronenbild der Feder gezeigt (hellgrau).

ble 1 may be due to the fact that a greater area (1.6 mm × 0.8 mm) is analysed and the data are average values between the concentrations of elements on overlapping proximal and distal barbules. Proximal barbules however are less pigmented and contain smaller concentrations of calcium, manganese and zinc.

As for the elements titanium, iron, copper, mercury and lead, no greater accumulation in black as compared with white feather samples could be detected (Fig. 3).

Discussion

It seems to be established that the black to black-brown colouring of White-tailed Eagles' feathers investigated in this work is caused by eumelanin (Lucas & Stettenheim 1971, Fitzpatrick & Breathnach 1963), as it is in other bird species, wool or human hair. Owing to amino acids that are found after acid hydrolysis following alcalic extraction, eumelanin is considered as being a melanin-protein complex (Jimbow et al. 1982, Greenstein 1948). Giesen (1981) determined the melanin content of hair and wool after enzymatic decomposition of keratin at about 3.5% and 7% resp. Assuming a melanin concentration of about 5% as being a rough estimate for feathers we obtain astonishingly high factors of 60 to 100 for accumulation

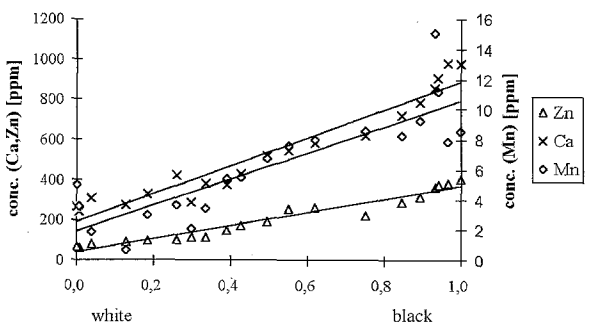

Fig. 5. Concentration of calcium, zinc and manganese versus pigmentation of one ramus.
Abb. 5. Konzentration der Elemente Calcium, Mangan und Zink in einem Federast in Abhängigkeit von dessen Pigmentierung.

of calcium, manganese and zinc in melanin granula as compared with melanin free keratins.

In contrast, no enrichment was observed in titanium, iron, copper and mercury. It is known (Potts & Au 1976, Sarna et al. 1981) that eumelanin has a large capacity for binding metal ions. High concentrations of copper, zinc, iron and manganese have been found in a variety of melanised structures (Lyden et al. 1984, Bowness et al., 1952; Flesch 1968; Dorea & Pereia 1983; Horcicko et al. 1973; Shibata et al. 1990). The findings in this work give rise to the question of why this element dependent enrichment occurs. Several hypotheses are discussed in literature in connection with the melanin granula of human tissues (e.g. iris, inner ear, substantia nigra, retinal pigment epithel, leptomeninx):

1. Melanin serves as a sink for foreign substances keeping them bound for a long time (Horcicko et al. 1973, Lindquist et al. 1987, Ulfshafer et al. 1990). With regard to hairs or feathers it plays a role in their excretion (Borovansky et al. 1976, Rorsman 1972).
2. Melanin (melanoprotein) serves as an intracellular buffering system or as a reservoir for essential trace elements (Lyttkens et al. 1979, Panessa & Zadunaisky 1981, Pfeiffer & Mailloux 1988).
3. The metal ions (Mn, Zn) are bound by a self-regulative process: they catalyse the formation of DHICA (5,6-dihydroxyindole-2-carboxylic acid) from dopachrome; the melanin formed by polymerization immobilizes these metal ions, which are cytotoxic at higher concentrations. This would be in keeping with the findings of Bogacz et al. (1989) that, at neutral pH, the melanin binding of metal ions involves mainly carboxylate groups.
4. Metal ions, especially calcium, induce an aggregation of melanin (Okazaki et al. 1985). This may results in a higher stability of the feather against mechanical stress (Bonser 1997).

On the basis of our findings concerning melanin in White-tailed Eagles' feathers, the following conclusions can be drawn:

1. The hypothesis of an enhanced excretion of toxic elements is not supported. Mercury concentrations vary widely between different feathers (Table 1), but there is no significant difference between black and white sections of the same feather. That is to say the mercury content of melanin is not increased as compared with that in feather keratin.
2. Melanin might be considered as an intracellular reservoir of essential elements needed during the short period of moult, involving a high cellular proliferation rate. After incorporation into the feather, however, it is immobilized and consequently the metals are no longer available to the organism. Compared to the quantity of metal ions needed in enzymatic processes, the measured concentrations are exceedingly high. On the other hand, it is precisely calcium, zinc and manganese that play an essential role during cell proliferation (Prasad 1993).
3. The high variation of calcium, manganese and zinc found in black feather sections even if they are taken from the same White-tailed Eagle in different years, is considered to represent the varying concentration in the bird's diet (Hanson and Jones 1968). It may therefore support the idea of a regulative process causing *homeostasis* of these essential elements within the melanocytes. This does not influence the feather pigmentation, because the metal catalysed formation of DHICA is considered to be an alternative pathway leading to melanin as compared with DHI (5,6-dihydroxyindole; Palumbo et al. 1987, Palumbo et al. 1988). On the basis of this interpretation, the question arises as to why no enrichment in copper and iron has been found in the pigmented samples, since copper in particular causes a considerable enhancement of this metabolic process (Prota 1988). Differences in the transmission probability through the melanocyte membrane are one

possible reason for a modified melanosomal metal ion concentration.

4. The high concentrations of calcium support the hypothesis of its function in feather strength.

Further work will be necessary on a variety of melanised structures in order to verify these findings and to get more insight into the interrelation between melanin and metal ions.

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