The adaptive function of melanin-based plumage coloration to trace metals

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Trace metals produced by anthropogenic activities are of major importance in urban areas and might constitute a new evolutionary force selecting for the ability to cope with their deleterious effects. Interestingly, melanin pigments are known to bind metal ions, thereby potentially sequestering them in inert body parts such as coat and feathers, and facilitating body detoxification. Thus, a more melanic plumage or coat coloration could bring a selective advantage for animals living in polluted areas. We tested this hypothesis by investigating the link between melanin-based coloration and zinc and lead concentrations in feathers of urban feral pigeons, both at capture time and after one year of captivity in standardized conditions. Results show that differently coloured pigeons had similar metal concentrations at capture time. Metal concentrations strongly decreased after one year in standardized conditions, and more melanic pigeons had higher concentrations of zinc (but not lead) in their feathers. This suggests that more melanic pigeons have a higher ability to store some metals in their feathers compared with their paler counterparts, which could explain their higher success in urbanized areas. Overall, this work suggests that trace metal pollution may exert new selective forces favouring more melanic phenotypes in polluted environments.

1. Introduction

Trace metals are bioavailable chemicals found in very small concentrations in the environment. They are emitted by natural processes [1,2] and by anthropogenic activities [3]. Consequently, their concentrations are much higher in urban environments and urban wildlife populations than in their rural counterparts [3–5]. Recent correlative and experimental studies have shown that some trace metals affect individual fitness by impairing reproductive success and survival [6–13]. For example, bird populations exposed to higher trace metal levels have a reduced clutch size [12] and impaired male fertility [9]. Because of this, trace metals are likely to exert new selective pressures on wildlife in urban areas.

Such evolutionary pressures may select for individuals able to detoxify their body or tolerate large amounts of trace metals. Because the property of melanin to bind metal ions may allow some body detoxification, we hypothesized that more melanic plumage may be selected in urban environments. Indeed, melanin is composed of several polymers with negatively charged free carboxyl, hydroxyl and amine functions which bind metal ions in vitro [14–16] explaining the relation found between some metals and melanic pigmentation [17]. Therefore, highly melanic birds could transfer more metals to their growing feathers during moult, thereby detoxifying their blood at a higher rate in comparison with less melanic individuals. First, this hypothesis predicts that an environment with a high level of trace metals, such as urban habitat, would select for highly melanic individuals. In agreement with this, the degree of melanin-based coloration correlates with the degree of urbanization in feral pigeons from European cities [18,19].
Secondly, this hypothesis predicts that, in a standardized environment, the concentration of trace metals in the feathers should be positively related to the degree of individual melanin-based coloration. To test this prediction, we investigated the link between zinc and lead concentrations and the degree of melanin-based coloration in feral pigeon’s feathers in metropolitan Paris, France, where zinc and lead are two of the most widespread environmental metals contaminants [3].

2. Material and methods
A total of 97 (53 male and 44 female) eumelanic adult free-living feral pigeons (Columba livia) were caught in February 2009 at two locations of the Parisian suburbs (Courbevoie and Gennevilliers) and randomly distributed with respect to origin, gender and coloration in 10 outdoor aviaries (2.20 m × 2.20 m) at the CEREEP field station (Centre d’Ecologie Expérimentale et Prédictive-Ecotron Ile-de-France, UMS 3194, Ecole Normale Supérieure, Saint-Pierre-lès-Nemours, France) in standardized conditions. Birds were fed ad libitum with a mix of maize, wheat and peas. The aviaries were enriched with a bowl of water used for bathing and with branches as perches. At capture time, birds were individually identified with a numbered colour plastic ring and photographed to measure the degree of coloration: we calculated a continuous coloration score as the percentage of dark surface on the wing which is a reliable and repeatable index [20]. Two innermost greater secondary coverts were collected on each wing and stored in metal-free polyethylene bags until metal concentration measurements. The two same new-grown feathers were collected one year later (February 2010).

Zinc (Zn) and lead feather concentrations were measured for all individuals at capture and after one year of captivity. Feathers were mineralized as described in Frantz et al. [21]. Feathers were washed vigorously with 0.25 M NaOH solution, rinsed energetically three times in ultrapure water (Milli-Q purified) to remove external contamination [5], left for 1 h in ultrapure water, dried for 12 h at 80°C to dry mass, crushed to powder and weighed to the nearest 0.1 mg. Feathers were then digested twice in 1 ml nitric acid (67%), followed by a final digestion in 1 ml hydrogen peroxide (30%).

Total Zn concentrations were determined using flame atomic absorption spectrometry (FAAS, Unicam AA Series Spectrometer, Thermo Electron Corporation) and total Pb concentrations using graphite furnace spectrometry (ETAS, Unicam 989 QZ AA Spectrometer, Zeeman SOLAAR). For each sample, three values measured for each metal were averaged when the relative standard deviation was less than 5%.

Statistical analyses were performed using R (R v. 2.12.0). To test for a relationship between the degree of coloration and the concentrations of trace metals in the feathers, we used two independent mixed linear models with zinc or lead as fixed factors. Capture site and individual identity were included as random effects in the models. Capture time (Pearson’s correlation test: \( r = 0.62, t_{44} = 7.52, p < 0.001 \); figure 1). This positive relation disappeared one year later (Pearson’s correlation test: \( r = 0.12, t_{44} = 0.87, p = 0.297 \)).

Lead concentration in the feathers decreased after one year of captivity (table 1; respectively, 24.340 ppm ± 18.078 and 0.706 ppm ± 0.421), but was not related to coloration score (table 1).

Zinc concentration in the feathers also decreased after one year of captivity (table 1; respectively, 328 ppm ± 238 and 89 ppm ± 30). Moreover, zinc concentration was significantly affected by the interaction between coloration score and collection time (table 1 and figure 2): while differently coloured pigeons had similar zinc concentration in their feathers at capture time (ANOVA: \( \chi^2 = 0.36, d.f. = 86, p = 0.551 \)), more melanic pigeons had higher zinc concentration in their feathers than paler ones after one year of captivity (ANOVA: \( \chi^2 = 24.94, d.f. = 84, p < 0.001 \)).

4. Discussion
Results showed that darker individuals had a higher zinc burden in their feathers compared with paler ones when kept in standardized conditions. This result is consistent with the hypothesis that more pigmented individuals can store higher amounts of metal ions.

This relation was only found after one year of captivity. Differently coloured birds were exposed to the same environment, and metal availability was consequently similar, suggesting that the difference between morphs was not due to different uptake rates. Some environmental factors may have hidden the correlation between coloration and metal concentrations at capture time. Although birds were captured from closely located sites, we cannot exclude local differences in pollution levels, known to change even at a small scale [1,21]. Moreover, habitat use may vary among differently coloured pigeons, as is often the case in polymorphic species [22]. For instance, darker pigeons might exploit less polluted areas but have a greater ability to store metals in their feathers than paler pigeons, which could result in comparable levels of metals in dark and pale pigeons at capture time.

In contrast to zinc, we did not find any correlation between feather lead concentration and melanin-based coloration score. It is likely that the very small amount and low variation of...
Table 1. Final mixed linear model ANOVAs with log-transformed zinc or lead concentration as the dependant variable in separate models, coloration score and time of collection as covariates, and capture site and ring name as random effects. Lead concentration was neither explained by the interaction between coloration score and time of collection ($\chi^2 = 0.33$, d.f. = 81, $p = 0.568$) nor by coloration score as simple effect ($\chi^2 = 0.42$, d.f. = 90, $p = 0.519$) and were removed from the model. *$p$-value < 0.05; **$p$-value < 0.01; ***$p$-value < 0.001.

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<tr>
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<th>Zinc concentration</th>
<th>Lead concentration</th>
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<td></td>
<td>$\chi^2$</td>
<td>d.f.</td>
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<tr>
<td>coloration score</td>
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<tr>
<td>time of collection</td>
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<td>coloration score $\times$ time</td>
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<td>85</td>
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Figure 2. Relationship between the degree of melanin-based coloration and concentration of zinc in the feathers (ppm) at the time of capture (open circles) and after 1 year of captivity (filled circles).

lead concentrations in feathers after one year of captivity do not allow detection of any relationship. However, the elevated values of lead concentrations in feathers collected at capture time confirm that feathers do sequester lead [5,21]. Thus, we cannot exclude the possibility that feathers constitute a way to detoxify vital organs of lead as well as zinc. In conclusion, this correlative study sheds light on the link between melanin-based coloration and metal concentrations in feathers. Obviously, melanin could have evolved for several reasons such as parasite resistance [20], camouflage [23] or thermoregulation [24]. Nevertheless, this study suggests that trace metals could represent a current selective force favouring melanin phenotypes among urban organisms. Such selection could explain the higher frequency of more melanic birds in urban habitats [18,19]. Experimental manipulations of trace metal exposure are now required, to first test whether melanism enables maintenance of reduced metal concentrations in blood, then to compare fitness traits between differently coloured individuals. In particular, the effectiveness of detoxification via melanin feathers may depend on moulting intensity and level of metal intake.

All experiments were carried out in strict accordance with the recommendations of the ‘European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes’ and were conducted under the authorization of the ‘Direction Départementale des Services Vétérinaires de Seine et Marne’ (permit no. 77-05).

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Data accessibility. Raw data are provided in the electronic supplementary material.

References


