Eumelanic coloration and age interact to influence breath rate following a boldness test in urban pigeons

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Abstract

The rapid urbanization of the past decades has forced numerous species to adapt to their new environments over a very restricted time scale. Previous studies suggested that individuals living in urban areas have specific characteristics as compared to those living in rural areas. In feral pigeon populations (Columba livia), individuals living in cities are more melanic than those living in less urbanized areas. In this study, we tested whether the degree of eumelanin-based coloration in pigeons reflects certain adaptations to urban areas such as boldness and tolerance to stress. Hence, we examined the relationship between eumelanin-based coloration and three variables of boldness (arrival to a new food source, arrival latency, and flight distance) and tolerance to stress (breath rate). Our results show that the eumelanin-based coloration of individuals is not correlated with the boldness variables, but tends to be positively related to breath rate in adults. Therefore, eumelanin-based coloration could reflect the capacity of adult individuals to manage stress in an urban environment, but not the boldness. The higher frequency of eumelanic pigeons observed in cities might thus be due to urban selective forces that favor individuals with a higher response to stress than bolder individuals.

Introduction

Nowadays, urban areas constitute environments of particular interest in ecology and evolution. The rapid urbanization of the past decades as part of global change has forced numerous species to adapt to these new environments over a very restricted time scale. In the context of growing worldwide urbanization (increase of up to 1.2 million km² between 2000 and 2030, [1], it becomes crucial to understand how natural species adapt to this new environment and, more generally, how this ecosystem functions.

While few studies focus on the adaptation of populations to the urban environment, they suggest that individuals living in urban areas have specific characteristics as compared to those living in rural areas [2]. The combination of particular traits would enable individuals to successfully exploit urban environments. This urbanization syndrome or synurbization [3] may include high reproduction rates, higher levels of aggressiveness and boldness, attenuated physiological responses to stressors, and increased tameness toward humans [5]. Moreover, the degree of melanin-based coloration has been frequently reported to covary with life-history, morphological, physiological, and behavioral traits, as well as with the sign and magnitude of these covariations fluctuating in time and space [6]. These results suggest that the degree of melanin-based coloration might reflect adaptations to different environmental conditions [7]. A recent review highlighted potential candidate genes pleiotropically affecting both the degree of individual melanization and other phenotypic traits, including aggressiveness, tolerance to stress, energy homeostasis, and anti-inflammatory immune reactions [8], suggesting that melanin-based coloration could be associated with several
aspects of the urbanization syndrome. Accordingly, a recent study in urban pigeons (*Columba livia*) showed that the degree of eumelanin-based coloration was positively correlated with the urbanization rate [9]. If this hypothesis is true, we should observe a correlation between eumelanin-based coloration and certain aspects of the urbanization syndrome.

In this study, using the feral pigeon (*Columba livia*), we tested this prediction by investigating the association between the eumelanin-based coloration of pigeons and two potential aspects of the urbanization syndrome: boldness – the tendency of individuals to take risks, particularly in novel contexts – and tolerance to stress. Urban pigeons display alternative melanin-based coloration, which varies in terms of both melanin types and pigmentation patterns [10]: red coloration is due to pheomelanic pigments and black coloration to eumelanic pigments. These two types of melanic pigments can vary in intensity in feathers, ranging from a total absence of pigmentation (white coloration) to intermediate and even dark melanin coloration. In Paris (France), a preliminary observational study based on photos (*n* = 3041) revealed that pheomelanic morphs are rare (7%) as compared to eumelanic morphs (93%) (Lisa Jacquin and Julien Gasparini, personal observation). The main color polymorphism concerns the quantitative variation of eumelanin present in feathers [9]. A cross-fostering experiment also showed a strong heritability (0.88), suggesting a strong genetic control of this phenotype [11].

We used a boldness test in captivity to quantify the behavioral variables of pigeons (i.e., arrival to a new food source, latency of this arrival, and flight distance) as well as a physiological variable related to stress response (i.e., breathing rate) and then correlated these variables with eumelanin-based coloration, the main color polymorphism in pigeons.

**Material and methods**

In June 2015, we captured 39 feral pigeons (25 adults and 14 juveniles, sex undetermined) in four different locations in Paris (France): Gare Montparnasse (14th district), Eglise Saint-Sulpice (7th district), Notre-Dame de Paris (1st district), and Place Bernard Halper (5th district). The pigeons were then kept in outdoor aviaries (dimensions 310 x 200 x 240 cm) with bamboo perches, a shelter, and a bath at the CEREEP field station (Centre de recherche en Ecologie Expérimentale et Prédicitive – Ecotron Ile-de-France, UMS 3194, Saint Pierre lès Nemours, France). They were hosted in similar conditions for 10 days before the boldness test. During this acclimation period, pigeons were fed *ad libitum* with a mix of maize, wheat, and peas, as well as mineral supplements and water. Upon their arrival at the aviaries, the birds were weighed and their coloration score determined (percentage of black coloration on wings) following the method described in Jacquin, et al. [12]. This score is a good approximation of the eumelanin content of feathers [13].

The subsequent testing period lasted 8 days: 3 days of environmental acclimation and 5 days of testing. During this period, all individuals were housed separately in a new aviary and fed with a restricted diet. Individuals were given a daily diet of 10 grams of a wheat and corn mix (delivered every morning). Because of the restricted caloric intake of this diet and the stress associated with captivity, individuals’ weight was regularly monitored. Individuals whose weight decreased too much were excluded from the experiment and re-fed *ad libitum*. During the 5 days of testing, birds were submitted to two boldness tests followed by two breath rate measurements, the first in the morning and the second in the afternoon (78 trials for 39 individuals). For the boldness tests, two protagonists were considered, namely a feeder who was known to the pigeons and an unknown observer. First, the feeder placed some wheat and corn mix on the floor of the aviary. The latency (in seconds) between the setting down of the food and the beginning of its consumption was measured using a chronometer. The trial was stopped if the animal did not eat the food after a time lapse of 20 minutes. When the individual started to eat, the observer, initially located 10 meters away from the aviary, walked in straight line toward it. As soon as the pigeon moved away from the seeds, the observer stopped walking. The distance between the observer and the food source was called the flight distance. After measuring the flight distance, each individual was captured to evaluate its stress level based on the breath rate [14]. The time taken to capture the bird (latency of capture) was noted because of its possible effect on this measurement. The number of respiratory movements in 1 minute was determined by placing the pigeon on its back in the hands of the observer. In summary, the 78 trials allowed us to obtain 4 variables of interest for 39 individuals: the arrival (or not) of the birds at the food site, the latency of this arrival, the flight distance, and the breath rate (a proxy of stress during this test).

We performed four mixed models to investigate whether the coloration and age (adults/juveniles) of birds and their interaction impacted the four variables of interest. All statistical analyses were performed using SAS (version 9.4). First, the arrival was coded as a binary variable (0: the bird did not come to eat the food; 1: the bird came to eat food). We used a mixed model with binomial distribution (logit link function) with the arrival as the dependent variable and the age (juveniles vs. adults), the period of the day where the trial has been done (morning vs. afternoon) and eumelanic coloration (percentage of black coloration on wings) in addition to their interaction as the explanatory variables. We included the location of capture and the individual nested within the location as random factors to take pseudoreplication into account. Then, similar mixed models were run on the logarithm of the arrival latency, flight distance, and breath rate with normal distributions. We log-transformed the arrival latency to better satisfy the assumptions of normality. For the breath rate model, we also included the latency of capture as a covariate. Non-significant interactions were removed step-by-step to obtain the final model.

**Results**

Among the 78 trials (*n* = 39), the birds decided to go to the food on 68 occasions. This decision was not affected by the age or coloration of the pigeons (Table 1), and only one individual never went to food. However, pigeons decided to
go to the food more often in the afternoon (38 times over 39) as compared to the morning (30 over 39; Table 1). We then investigated the arrival latency, flight distance, and breath rate for these 68 trials involving 38 birds. We first found a positive relationship between arrival latency and flight distance (F_{1,28} = 6.05, P = 0.02, Figure 1). However, these two variables were not correlated with the breath rate (mixed models; arrival latency: F_{1,28} = 1.33, P = 0.26; flight distance: F_{1,28} = 0.12, P = 0.73). The arrival latency was not affected by coloration and age of the birds whether individually or in interaction (Table 1) but tended to be longer during trials done in the morning (365 seconds ± 83) than in the afternoon (134 seconds ± 42; non-significant trend, Table 1). The flight distance was longer for adults (206 cm ± 21, n = 24) than for juveniles (149 cm ± 25, n = 14; Table 1) and was longer during trials done in the morning (303 cm ± 39) than during trials done in the afternoon (185 cm ± 16; Table 1).

The breath rate was significantly affected by the arrival latency and by the interaction between age and coloration (Table 1). The longer the time necessary to catch birds, the higher their breath rate was. In adults, darker pigeons tended to have higher breath rates than paler ones (F_{1,20} = 3.66, P = 0.07; Figure 2a). This trend was not observed in juveniles (F_{1,8} = 2.02, P = 0.19; Figure 2b).

**Discussion**

In the present study, we wanted to test whether a darker eumelanin-based coloration in pigeons reflects their better adaptation to urbanization, namely in terms of greater boldness and tolerance to stress. Our experiment provided contrasting results. When considering boldness variables and contrary to previous studies in other species [15], the results did not support our hypothesis as eumelanin-based coloration was not related to arrival, arrival latency, or flight distance (Table 1). In contrast, the breath rate, as a proxy of tolerance to stress, tended to be higher in darker adults, a trend that we did not observe for juveniles. This suggests that eumelanin adult pigeons tend to have a higher response to stress than paler ones immediately after capture and therefore a lower capacity to cope with stress. In contrast with our results, breath rate was found to be negatively correlated with the width of eumelanin black tail band in kestrels (*Falco tinnunculus*) [16] and with the eumelanin black spots numbers in barn owls (*Tyto alba*) [17,18]. Overall, our results are rather contrasted with previous studies [8,19] but it is possible that the direction of the relationship between response to stress and melanin-based coloration might depend on species as observed for others physiological component [8].

In our species, darker eumelanin individuals are more prolific in highly urbanized areas [9], suggesting that they are potentially better adapted to urban areas than paler ones. From this result, we hypothesized that one of this adaptation would be a better capacity to cope with stress for darker eumelanin pigeons and we predicted that darker eumelanin pigeons would have a better capacity to cope with stress as compared to paler ones. We tested this hypothesis in the present study, but our results found a relation opposite to our prediction. Indeed, in our study, paler pigeons tend to better tolerate stress following capture, at least in adults. Our results are nevertheless consistent with a recent study in pigeons showing that corticosterone response, as a physiological measure of stress, increased with the degree of eumelanin-based coloration in a rural population [20]. Previous studies suggest that urbanization selects animals that are more tolerant to stress [21]. Yet perhaps this speculation is incorrect, as urbanization would appear to select individuals that maintain a high level of stress response. Indeed, a higher stress response may have a positive impact on fitness in certain habitats such as those with a high predation rate [22]. Urban areas may also include several environmental stressors (anthropic stressors) that select individuals with a high stress response. In our pigeon population, such individuals seem to be eumelanin, which could explain their higher frequency in highly urbanized areas. This interpretation is based on our correlative result (Figure 2a). Future studies should thus experimentally validate this scenario and identify the environmental stressor in urban areas that favors individuals with a higher stress response. Furthermore, our correlative approach has been performed on pigeons alone in an aviary, which is not the same environment than those experimented by pigeons in cities (social interactions and/or other environmental urban factors). Therefore, our results could not be representative of the reality of what it happens in cities. Even if our approach enabled to standardize environmental factors, future studies involving approach in nature are required to confirm our results.

Even if the boldness measurements (arrival, arrival latency, and flight distance) were not correlated with eumelanin-based coloration in pigeons (our main hypothesis), we found an interesting positive relationship between arrival latency and flight distance (Figure 1). This result confirms that these two variables measured the same behavioral syndrome, notably boldness. Flight distance is a common method used to estimate boldness in birds and is applied in many studies [23,24]. By contrast, arrival latency is used less often when investigating

**Table 1**: Output of the generalized mixed models explaining variations in the arrival (arrived or not, binary distribution), the logarithm of the arrival latency, the flight distance, and the breath rate (normal distribution) of birds according to their ages (juvenile or adult), the time of the day (trials done in the morning or in the afternoon), eumelanin coloration, and interaction. For the breath rate, capture latency was added to the model as a covariate and was therefore not available (NA) for the three other variables. Non-significant interactions were removed step-by-step (dash sign) to obtain the final model.

| Effects                  | Arrived or not | Arrival latency | Flight distance | Breath rate |
|-------------------------|----------------|-----------------|-----------------|-------------|
| Latency of capture      | NA             | NA              | NA              | NA          |
| Time of the day (AM/PM) | 1.38           | 5.54            | 0.02            | 1.29        |
| Age (Juv/Ad)            | 1.38           | 1.31            | 0.26            | 1.29        |
| Coloration              | 1.38           | 1.38            | 0.25            | 1.29        |
| Age * coloration        |                |                 |                 | 1.28        |

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boldness. Flight distance is a less invasive method, because its measurement does not depend on the restriction of food intake. As a result, future studies should favor this measurement when investigating boldness. However, arrival was not correlated to arrival latency or flight distance, and in our study, most pigeons decided to go to the food (68 out of 78 trials). Therefore, the low variation of this estimate did not enable us to distinguish between bold and less bold individuals. This low variation may be due to the starvation of birds during the experiment. Indeed, it has been suggested that hunger might increase risk-taking among individuals [25,26]. Hence, the food restriction of our study might explain why most of birds took the risk of going to the food. Accordingly, pigeons were more prone to go to the food in the afternoon than in the morning. Indeed, birds were daily fed in the early morning and therefore they were more starved in the afternoon than in the morning. Globally, we observed that this higher starvation increases the risk-taking: a higher frequency to go the food, a shorter latency to go the food and a shorter flying distance (Table 1). Future studies should therefore test the effect of food restriction on the different estimates of boldness to determine the most suitable, that is, the estimate for which food restriction does not alter the variation among individuals.

Conclusion

Our study shows that the eumelanin-based coloration of individuals could reflect the capacity of adults to manage stress in an urban environment, but not boldness. Therefore, the higher frequency of eumelanin pigeons observed in cities might be due to urban selective forces favoring individuals with a higher response to stress as opposed to bold individuals. Boldness is therefore not an aspect of the urbanization syndrome. By contrast, a higher response to stress could be part of the urbanization syndrome in pigeons.

Ethical note

This study was 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 (revised Appendix A). All experiments and captures were approved by local authorities and the “Direction Départementale des Services Vétérinaires de Seine-et-Marne” (permit No. 77–05).

Data sharing and data accessibility

The data will be deposited in Dryad or other equivalent archives when accepted.

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