# Different roles of natural and sexual selection on senescence of plumage colour in the barn swallow

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## Summary

**1.** Colour may show effects of senescence because the pigment or structures involved in production of colouration deteriorate with age.

**2.** We tested this hypothesis by investigating age-related changes in plumage colour for two feather tracts coloured by eumelanin or pheomelanin in a longitudinal study of a cohort of barn swallows *Hirundo rustica* that reached very old age (at least 5 years).

**3.** The level of melanization of the throat increased with age in both sexes, but particularly in females. In contrast, the black colour of the plumage of the back was unrelated to age in both sexes.

**4.** These age-dependent patterns of colouration of different feather tracts of male and female barn swallows suggest that effects of senescence are trait-specific depending on their importance in sexual signalling. The red throat colour based on pheomelanin is involved in sexual selection, with a strong effect in males, but not in females. In contrast, the black colour of the back based on eumelanin is unrelated to sexual selection, but is under natural selection due to intense abrasion of this feather tract.

**5.** These findings suggest that the relative importance of natural and sexual selection are important determinants of the pattern and rate of senescence of colour.

**Key-words:** age, barn swallow, colouration, melanin, senescence, sexual selection

## Introduction

Senescence is the decrease in survival and fecundity that organisms experience with age, and it is usually accompanied by a deterioration of the phenotype (Rose 1991; Finch & Kirkwood 2000). Various mechanisms have been hypothesized to cause senescence. They can be grouped in those related to evolutionary theories of aging (accumulation of mutations with age or pleiotropic alleles with deleterious effects late in life; Williams 1957; Hamilton 1966) and those that explain rates of senescence on the basis of differences in the performance of physiological processes (trade-offs between investment in somatic maintenance and reproduction and free radical production with subsequent accumulation of their effects; Finch & Kirkwood 2000; Finkel & Holbrook 2000). All these hypotheses are non-exclusive and are probably complementary explanations for the same phenomenon.

Like most vertebrates, the aging pattern of birds consists of gradual senescence with life span. The slow aging rates observed in this group are somewhat unexpected given their high metabolic rates, body temperatures and blood glucose levels (Holmes, Flückiger & Austad 2001). This fact has turned birds into an important model system for investigating the mechanisms involved in senescence, which in turn has permitted evaluation of evolutionary scenarios based on physiological mechanisms that could have favoured delay of aging effects for example (Holmes *et al.* 2001; Møller, de Lope & Saino 2005a; Møller 2007), probably in relation to more effective mitochondrial electron transfer and slow accumulation rates of advanced glycosylation end-products (Holmes *et al.* 2001).

Studies on senescence in vertebrates have shown increases in survival and reproductive value during the first years of life, probably as a consequence of an improvement in condition, learning, selection and a decrease in residual reproductive value, and then a decrease due to senescence effects (Clutton-Brock 1988; Newton 1989; Møller & de Lope 1999; Newton & Rothery 1997). Phenotypic traits that reveal individual quality and physiological parameters such as resistance to oxidative stress and immune response also mirror senescence effects by following a similar pattern of variation with age (Møller & de Lope 1999; Alonso-Alvarez *et al.* 2006) or a

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linear decline (Saino *et al*. 2003). However, only certain phenotypic traits are associated with an ability to survive many years (Møller *et al*. 2005a). The case of vertebrate integument colouration is intriguing because there is evidence for a decrease in pigment-based colour intensity or the occurrence of coloured traits with age (Weimerskirch, Lequette & Jouventin 1989; Allainé & Lebreton 1990; Potti & Montalvo 1991; Prince *et al*. 1997; Arck *et al*. 2006; Krištín *et al*. 2007), although other studies report an opposite tendency (Dale *et al*. 1999, 2002; West & Packer 2002; Alonso *et al*. 2006). Neither mechanisms nor adaptive function of such variation have been investigated. Because coloured traits are costly to produce and/or maintain, and thus reflect individual quality (see Hill & McGraw 2006a,b for reviews), they should be indicators of the progress of senescence like other phenotypic traits (e.g. Møller & de Lope 1999). Furthermore, they should potentially reveal information about the proposed molecular adaptations used by animals to cope with the effects of senescence (Holmes *et al*. 2001; Møller *et al*. 2005a; Møller 2007).

The aim of the present study was to explore age-related variation in intensity of melanin-based plumage colouration in the barn swallow *Hirundo rustica* in order to assess whether senescence affects this trait. The barn swallow is a *c.* 20 g migratory, insectivorous bird that breeds in large parts of temperate and subtropical zones of the northern hemisphere. Males and females are similar in morphology with the exception of the outermost tail feathers that are longer in males due to a directional female mate preference (review in Møller 1994). The mating system is social monogamy with frequent extra-pair paternity. Migration, reproduction and ability to resist parasites are all associated with long tails in male barn swallows (Møller 1994). The barn swallow has one annual moult in the African winter quarters (Møller 1994). Adult survival rate is around 30% (Møller & Szép 2002), implying that only 0·8% of all adults ever reach the age of at least 5 years.

Melanin-based colouration reflects the melanin content of feathers (McGraw, Safran & Wakamatsu 2005), and may be physiologically costly to produce or environmentally constrained in vertebrates (West & Packer 2002; Poston *et al*. 2005; McGraw 2006, 2007; Moreno & Møller 2006; Fargallo *et al*. 2007; Margalida, Negro & Galván 2008; Galván & Alonso-Alvarez 2008), as in insects (e.g. Windig 1999; Talloen, Van Dyck & Lens 2004). Both increases (Dale *et al*. 1999, 2002; West & Packer 2002; Alonso *et al*. 2006) and decreases (Weimerskirch *et al*. 1989; Allainé & Lebreton 1990; Potti & Montalvo 1991; Prince *et al*. 1997; Krištín *et al*. 2007) in the intensity of melanin-based colour with age have been reported in different species. Therefore, we predicted that senescence effects in barn swallows could either be detected as older individuals exhibiting paler or darker plumage. Decrease in colour can occur because melanocytes, cells from which feather pigments derive, are very sensitive to oxidative stress (Bowers *et al*. 1994, 1999). As free radical effects accumulate with age as a consequence of normal metabolic activity, thus constituting a major determinant factor of

senescence (Finch & Kirkwood 2000; Finkel & Holbrook 2000), and new melanocytes are required for colouring the next feather that replaces feathers that are lost during moult or loss (Prota 1992), the capacity to produce melanocytes and thus the intensity of melanin-based colour can decrease with age. Indeed, a loss of melanosomes (melanin-containing granules produced by melanocytes) and variation in their colour with age in non-integumentary mammalian tissues have been reported due to a decrease in the photo-ionization thresholds caused by lipofuscin adhered to their surface and in the metal-binding capacity (Hong & Simon 2005; Liu *et al*. 2005; Hong *et al*. 2006). Human hair greying is known to be caused particularly by oxidative stress experienced at the pigmentary unit of the hair follicles (Arck *et al*. 2006).

We measured the colouration of two melanin-based plumage patches (back and throat-breast) in the barn swallow. Feathers from the back patch are iridescent black and their major pigment is eumelanin (J.J. Negro pers. comm.), while the feathers of the throat and breast region (hereafter breast region) are reddish and mainly contain pheomelanin (McGraw *et al*. 2005). Apart from these differences in chemical composition and appearance, back and breast plumage patches differ in their signalling roles. While there is no evidence that sexual selection acts on the colour of back feathers (Perrier *et al*. 2002), the reddish breast patch is a quality-revealing signal related to reproductive performance that predicts the level of extra-pair paternity, at least in the Nearctic subspecies *Hirundo rustica erythrogaster* (Safran & McGraw 2004; Safran *et al*. 2005), and mating success in the nominate subspecies (Ninni 2003). Although decreases in the intensity of melanin-based colour with age have been reported, the opposite has also been observed in some animals (see above). Therefore, we took into account these differences in the signalling roles of plumage patches in order to explore the possibility that an age-dependent increment in melanization would only occur in the case of colour patches that signal genotypic or phenotypic quality (as these reported cases correspond to secondary sexual characters; see references above).

Finally, we explored the effect of sex on the consequences of senescence on plumage colour. Senescence effects in the barn swallow are greater for females than for males in relation to breeding performance (Møller *et al*. 2005a), immune response (Saino *et al*. 2003) and phenotypic quality of offspring (Saino *et al*. 2002), possibly due to the larger costs of reproduction in females. Therefore, we predicted that senescence effects on melanin-based colour, if expressed as a decrease in colour intensity with age, would be more marked in females than in males. These predictions were tested in a cohort of barn swallows from which feathers were collected when yearlings, 2–3 years-old, and again when 4–6 years-old.

## Methods

### STUDY AREA AND FEATHER SAMPLES

APM studied barn swallows at Kraghede, Denmark between 1996 and 2007. Adults breed indoors, facilitating capture with mist nets.

We caught adults in mist nets at least weekly during each breeding season, and provided all individuals with an individually numbered aluminium ring for individual identification. Throughout we assumed that all birds without a ring were yearlings. This assumption is supported by 414 out of 415 local recruits from Denmark, Spain and Italy, ringed as nestlings being found breeding for the first time at an age of 1 year. The single exception bred for the first time as a 2-year-old. Extensive analyses using capture-mark-recapture analyses of Danish, Spanish and Ukrainian data sets revealed recapture probabilities of adults above 98% (Møller & Szép 2002; Møller *et al.* 2005b; F. de Lope, A.P. Møller & T. Szép, unpublished data), suggesting that only a very small fraction of adult breeders avoided capture.

Upon capture we recorded a number of morphological measurements, and simultaneously we collected a small sample of feathers from the centre of the red breast patch and the middle of the back, placing these in a small plastic bag for later study. All samples were kept in complete darkness until measurements were made.

## COLOUR MEASUREMENTS

Plumage colour was measured in the laboratory using an Ocean Optics USB2000 spectrophotometer (range 250–800 nm) with ultraviolet (deuterium) and visible (tungsten-halogen) lamps and a bifurcated 400 micrometer fibre-optic probe (Dunedin, FL). The fibre optic probe both provided illumination and obtained light reflected from the sample, and it had a reading area of *c.* 1 mm$^2$. For each bird, 10–20 feathers were mounted on a black cardboard, such that they resembled the natural appearance of the feather patch. The measurements were taken at a 90° angle to the sample. Although the back feathers of barn swallows show little iridescence and in such cases spectral measurements are more accurate if taken at a 45° angle (Andersson & Prager 2006), we were interested in detecting differences in reflectance properties among individuals rather than precisely estimating the appearance of the colour, so that illumination and detection conditions were not caveats to take into account because measurements were taken under constant conditions (Fleishman, Leal & Sheehan 2006).

All measurements were relative to a white 'Spectralon' tablet (WS-1-SS, Ocean Optics, Dunedin, FL), and reference measurements were frequently made. An average spectrum of three readings on the same point of the distal part of feathers was obtained for each bird and plumage patch, removing the probe after each measurement. Reflectance curves were determined by calculating the median of the percent reflectance in 10 nm intervals beginning at 300 nm and ending at 700 nm to cover the full spectral range that can be detected by birds (Cuthill *et al.* 2000). All measurements of colouration were done blindly with respect to the age of birds.

Spectral data were assumed to represent a measure of total brightness, as this is the best predictor of melanin content in feathers of both sexes in barn swallows, with lower values (i.e. darker colours) denoting higher total melanin content (McGraw *et al.* 2005). Brightness was defined as the summed reflectance across the entire spectral range ($R_{300-700}$; Montgomerie 2006). Feathers from the back patch showed high reflectance values in the UV region (Fig. 1), so we also calculated UV chroma ($R_{300-400}/R_{300-700}$) in order to search for possible senescence effects on this colour parameter, as this parameter contains information on sex and individual quality in other birds species with similar UV-reflecting melanin-based plumage (Hegyi *et al.* 2007). Colour measurements were repeatable for red (brightness: $r = 0.82$, $F_{110,366} = 14.78$, $P < 0.0001$) and black feathers (brightness: $r = 0.55$, $F_{104,345} = 4.66$, $P < 0.0001$; UV chroma: $r = 0.65$, $F_{104,345} = 6.62$, $P < 0.0001$), but, as shown by Perrier *et al.* (2002), repeatability
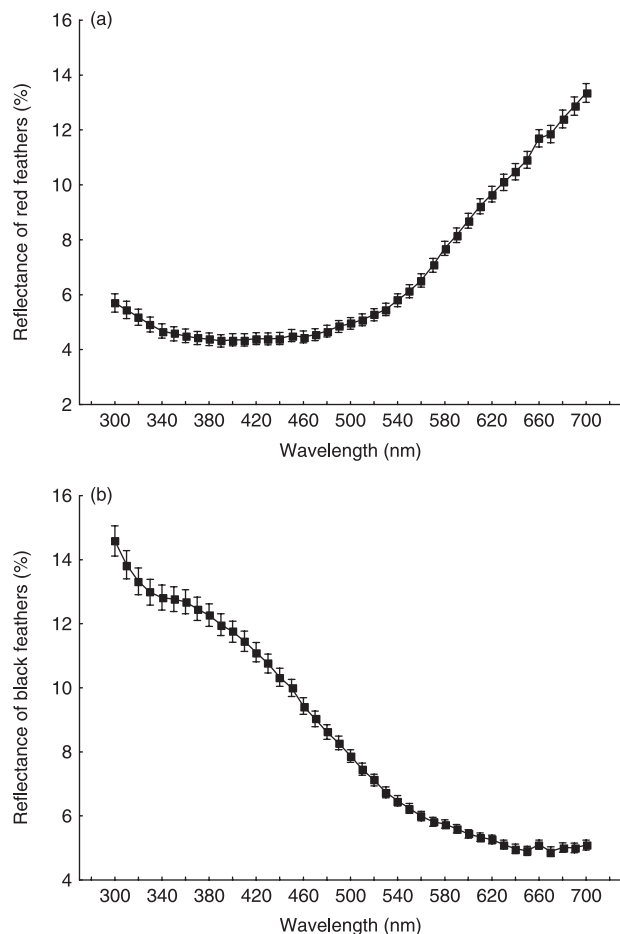


**Fig. 1.** Spectral reflectance (± SE) of the colour of plumage patches in barn swallows. (a) Red breast feathers and (b) black back feathers.

values for plumage colour variables would be higher if more than three measurements per sample were made.

Average reflectance spectra of the two plumage patches from which colour measurements were taken are shown in Fig. 1. The red breast feathers show a steady increase in reflectance from short to long wavelengths (Fig. 1a), which is the typical melanin reflectance spectrum (McGraw 2006). A different spectral shape was obtained for black back feathers, as the iridescent effect, which is caused by the interaction between keratin and melanin in feather barbules (Prum 2006), creates high reflectance values in the UV spectral region (Fig. 1b). However, as the only pigment found in these feathers is eumelanin (see above) and the spectral shape is almost uniform and lacks defined peaks, total brightness might be an indicator of melanin content (McGraw *et al.* 2005).

## STATISTICAL ANALYSES

In order to explore the effects of age on plumage colouration, we performed general linear models with brightness of the plumage patch as the dependent variable and age (in years) as a covariate. Bird individual identity was introduced as a random factor to control for inter-individual variability, and its interaction with age was also included to determine whether the effects of age on colour depended on identity or constituted general effects. The quadratic term of age was also considered in the models in order to determine whether there was an optimal value of plumage colouration.
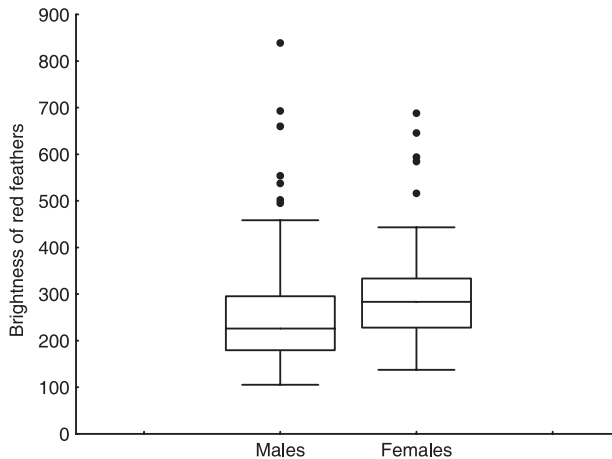
**Fig. 2.** Brightness of red breast feathers of male and female barn swallows, indicating lighter colours and lower melanin contents in females. Median, interquartilic range and non-outlier range are shown.

We made separate models for the two sexes. Non-significant terms were removed from the models on the basis of their significance, beginning with the interactions. The linear effect of age was never removed from the models before the quadratic effect. Inspections of residuals confirmed that normality assumptions were fulfilled.

## Results

In total, we obtained plumage colour measurements for the breast and back feather patches from 30 males and 18 females. This allowed us to obtain 159 measurements of red breast colour and 150 measurements of black back feathers.

Three males had two consecutive feather collections (between the ages 1–3, 1–4 and 1–5, respectively), 17 had three feather collections [between the ages 1–3 (four individuals), 1–4 (seven individuals), 1–5 (two individuals), 1–7 (one individual), 2–5 (two individuals) and 3–5 (one individual)], nine had four feather collections [between the ages 1–4 (six individuals), 1–5 (two individuals) and 2–6 (one individual)], and one had five feather collections (between the ages 1–6). 13 females had three consecutive feather collections [between the ages 1–3 (five individuals), 1–4 (five individuals), 1–5 (two individuals) and 2–5 (one individual)], four had four feather collections [between the ages 1–4 (one individual), 1–5 (two individuals) and 1–6 (one individual)] and one had five feather collections (between the ages 1–6).

Considering all individual ages, there were significant differences between sexes in the brightness of the breast plumage patch, with females having lighter coloured feathers than males (general linear model with sex as a fixed factor and its interaction with individual identity as a random factor: sex: $F_{1,156} = 5 \cdot 41$, $P = 0 \cdot 021$, identity × sex: $F_{46,110} = 1 \cdot 28$, $P = 0 \cdot 152$, Fig. 2a), although the explained variance was small (adjusted $R^2 = 0 \cdot 03$). In contrast, there were no significant differences in the case of both colour variables measured on black back feathers (brightness: sex: $F_{1,147} = 0 \cdot 25$, $P = 0 \cdot 621$, identity × sex:
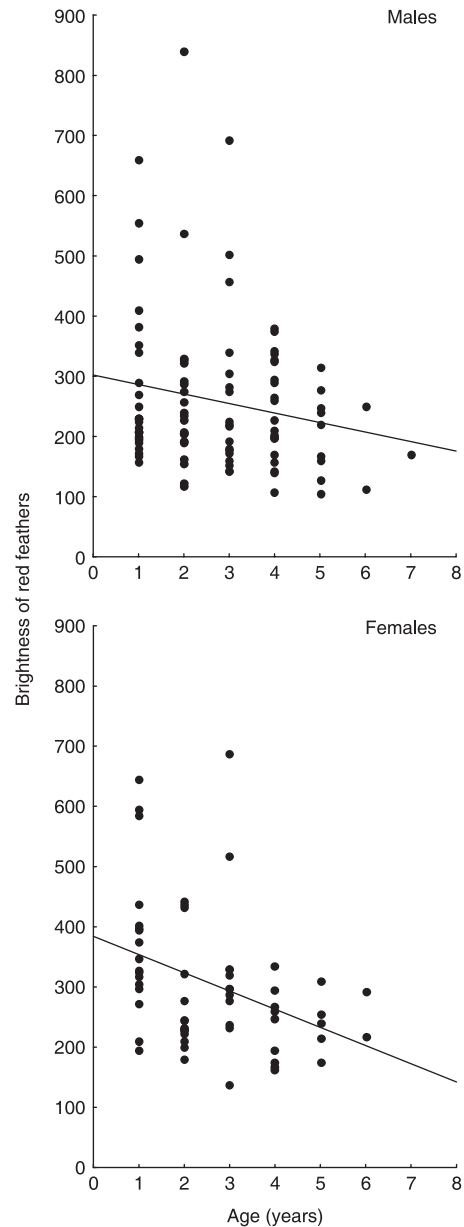


**Fig. 3.** Brightness of red breast feathers of male and female barn swallows in relation to age. The lines are the regression lines.

$F_{46,101} = 1 \cdot 15$, $P = 0 \cdot 272$; UV chroma: sex: $F_{1,101} = 0 \cdot 28$, $P = 0 \cdot 597$, identity × sex: $F_{46,101} = 1 \cdot 93$, $P = 0 \cdot 003$).

Brightness of the red breast feathers decreased (i.e. their melanin content increased) with age in females ($\beta = -0 \cdot 36$, $t = -2 \cdot 99$, $P = 0 \cdot 004$; Table 1, Fig. 3), and this effect explained 12% of the variance. A similar tendency was also observed in males (Fig. 3), although the effect was not significant (Table 2).

There was no evidence of significant age-dependent variation in brightness or UV chroma for the black back feathers in either sex (Tables 1 and 2).

The interaction term between individual identity and age was not significant in any of the models (Tables 1 and 2), indicating that individual variation was negligible when accounting for the effects of age.

| Plumage patch – variable | Effect | df | MS | *F* | *P* |
|---|---|---|---|---|---|
| Breast – total brightness | Identity | 17 | 10980·4 | 0·85 | 0·629 |
| | **Age\*** | **1** | **110277** | **8·96** | **0·004** |
| | Age$^2$ | 1 | 11608·6 | 0·94 | 0·336 |
| | Identity × Age | 17 | 8566·1 | 0·53 | 0·907 |
| | Identity × Age$^2$ | 17 | 11742·4 | 0·41 | 0·929 |
| | Error | 58 | 12313 | | |
| Back – total brightness | Identity | 17 | 10402 | 1·38 | 0·200 |
| | Age | 1 | 9645 | 0·92 | 0·342 |
| | Age$^2$ | 1 | 96·1 | 0·01 | 0·925 |
| | Identity × Age | 16 | 9969·9 | 0·89 | 0·592 |
| | Identity × Age$^{2}$\*\* | – | – | – | – |
| | Error | 39 | 14337 | | |
| Back – UV chroma | **Identity** | **17** | **0·230** | **1·95** | **0·044** |
| | **Age** | **1** | **0·366** | **3·10** | **0·086** |
| | Age$^2$ | 1 | 0·275 | 2·41 | 0·129 |
| | Identity × Age | 16 | 0·120 | 1·10 | 0·411 |
| | Identity × Age$^{2}$\*\* | – | – | – | – |
| | Error | 38 | 0·118 | | |

**Table 1.** Results of general linear models (GLM) exploring the effects of age on colour of two different plumage patches in female barn swallows. Parameter values for the different terms correspond to the step at which they were removed from the models or to the final models in case they were retained (marked in bold). Error parameters are those corresponding to the final model or to the step at which the last term was removed in case no terms were retained in the models

\*Model: adjusted $R^2 = 0·12$, $F_{1,58} = 8·96$, $P = 0·004$.
\*\*Sample size did not allow inclusion of the term in the model.

| Plumage patch – variable | Effect | df | MS | *F* | *P* |
|---|---|---|---|---|---|
| Breast – total brightness | **Identity** | **29** | **22053** | **1·67** | **0·042** |
| | Age | 1 | 33039 | 2·57 | 0·114 |
| | Age$^2$ | 1 | 23963·9 | 1·89 | 0·174 |
| | Identity × Age | 29 | 12454·9 | 0·97 | 0·533 |
| | Identity × Age$^{2}$\* | – | – | – | – |
| | Error | 68 | 13163 | | |
| Back – total brightness | Identity | 29 | 12616 | 1·04 | 0·438 |
| | Age | 1 | 2 | 0·00 | 0·989 |
| | Age$^2$ | 1 | 8932·3 | 0·72 | 0·399 |
| | Identity × Age\* | – | – | – | – |
| | Identity × Age$^{2}$\* | – | – | – | – |
| | Error | 62 | 12152 | | |
| Back – UV chroma | **Identity** | **29** | **0·285** | **2·04** | **0·010** |
| | Age | 1 | 0·184 | 1·33 | 0·254 |
| | Age$^2$ | 1 | 0·176 | 1·27 | 0·263 |
| | Identity × Age\* | – | – | – | – |
| | Identity × Age$^{2}$\* | – | – | – | – |
| | Error | 62 | 0·140 | | |

**Table 2.** Results of general linear models (GLM) exploring the effects of age on colour of two different plumage patches in male barn swallows. Parameter values for the different terms correspond to the step at which they were removed from the models or to the final models in case they were retained (marked in bold). Error parameters are those corresponding to the final model or to the step at which the last term was removed in case where no terms were retained in the models

\*Sample size did not allow inclusion of the term in the model.

## Discussion

Our study of the barn swallow showed that the intensity of the colour of the melanin-based breast patch increased with age, indicating an increment in melanin content of feathers as birds became older. This is the first longitudinal study of individuals tracked during consecutive years showing a positive relationship between age and melanization levels, and, as far as we know, it constitutes the first study on senescence effects on bird colouration.

As barn swallows do not only avoid the effects of senescence on their melanin-based plumage, but even deposit larger amounts of melanin in feathers as they age, it is possible that this pattern constitutes an adaptive strategy. The effect was only documented for the red breast patch, while the colour of black back feathers did not vary with age. The red feathers of the breast mainly contain pheomelanin, while the major pigment of back feathers is eumelanin (see Introduction). The production of pheomelanin is considered to be the default pathway during melanogenesis because it takes place without α-MSH stimulation (Wolff 2003). By contrast, eumelanogenesis requires the binding of this hormone to the receptor MC1R in the membrane of melanocytes, which led Jawor & Breitwisch (2003) to suggest that eumelanogenesis is more costly than pheomelanogenesis. Such differential costs of the two pigments could explain our results. If melanization of the plumage

progresses with age and is adaptive, but entails costs to birds, barn swallows may only have been able to avoid senescence effects on the colour of back feathers. An increase in the intensity of black colour would imply costs that are traded against other vital processes. The adaptive value of avoiding senescence effects on the colour of feathers is probably given by the increase in the stiffness and resistance to abrasion conferred by melanin (McGraw 2006; Schreiber *et al.* 2006), especially in the case of the back, where feathers are exposed to high levels of abrasion (Burtt 1986).

An alternative, but non-exclusive hypothesis for the difference in aging effects between breast and back colouration could be related to the different signalling roles in which these plumage patches are involved. While the colour of back feathers does not reveal individual quality and thus is not a sexually selected trait in the barn swallow (Perrier *et al.* 2002), the darkness of breast feathers predicts reproductive performance and paternity in the subspecies *erythrogaster* (Safran & McGraw 2004; Safran *et al.* 2005) and mating success in the nominate subspecies (Ninni 2003). Therefore, the colour of red breast feathers probably is more closely related to individual fitness than that of back feathers, as red colour is affected by both natural and sexual selection. Although this scenario must be taken with caution because populations may differ in the signalling roles of plumage colouration and other secondary sexual characters (Ninni 2003; Safran & McGraw 2004), we are aware of other reported cases of increments in the intensity of melanin-based colour with age in traits known to be involved in mate choice: mane colour of the African lion *Panthera leo*, a trait mostly pigmented by eumelanin (West & Packer 2002), and the chestnut colouration of the neck plumage of great bustards *Otis tarda* (Alonso *et al.* 2006), a melanic trait mostly pigmented by pheomelanin (J.J. Negro, pers. comm.). In addition, the black eumelanic plumage colour of pied flycatchers *Ficedula hypoleuca* increases with age in the populations where it acts as a sexually selected trait (Dale *et al.* 1999, 2002), but the opposite is observed in areas where it is not used in mate choice (Potti & Montalvo 1991). Other authors found that the intensity or presence of melanin-based colouration decreased with age in traits that, to our knowledge, are not sexually selected in wandering albatrosses *Diomedea exulans* (Weimerskirch *et al.* 1989; Prince *et al.* 1997), black-headed gulls *Larus ridibundus* (Allainé & Lebreton 1990) and lesser grey shrikes *Lanius minor* (Krištín *et al.* 2007). Hair greying and skin fading with age in humans are other examples of this latter category (Arck *et al.* 2006; Jablonski 2006). In fact, sexual selection is believed to increase ageing rate, but this may be reversed if male sexual competition selects strongly on whole-organism performance (Bonduriansky *et al.* 2008).

Interestingly, and in agreement with this idea of differential senescence effects on sexually and non-sexually selected traits, variation of colour with age was only reported for the plumage patch that exhibited sexual dichromatism (i.e. breast plumage colour), a common measure of the intensity of sexual selection (Owens 2006). An interesting aim for future studies may be to explore whether senescence differentially affects any type of sexually and non-sexually selected traits, not only those based on integument colouration. If this would be a widespread phenomenon, it may be of particular importance for the study of the evolution of senescence.

We propose that vertebrates progressively melanize their integumentary tissues with age only if they are part of quality-revealing signals that have evolved under sexual selection, but do not invest in melanizing other traits that are therefore exposed to the effects of senescence. When the intensity of melanin-based colouration increases with age, animals have the capacity of acquiring high levels of melanization late in life. This raises the question why they postpone melanization until reaching old age instead of reaching a peak of melanization when acquiring sexual maturity. The answer may be related to the cost of melanization: if melanin deposition is costly, individuals should trade resources needed for melanization against that of other costly processes. That is what happens in other secondary sexual characters, such as the length of the outermost tail feathers of barn swallows, before experiencing deteriorating senescence effects late in life (Møller & de Lope 1999). By contrast, age-dependent variation in colour intensity of breast feathers did not show the U-shape observed for tail length, indicating a lack of senescence in breast colouration. This result is surprising given that the accumulative effects of free radicals with age should prevent birds from increasing production of melanocytes and/or melanosomes. Our results on melanin-based colouration of barn swallows may thus reflect the existence of the proposed molecular adaptations in birds to attenuate the effects of senescence (Holmes *et al.* 2001).

In view of our results, it may be possible that cases of delayed plumage maturation (i.e. the retention of immature plumage characteristics after the post-juvenile moult), which are observed in species showing melanin-based colouration like *Ficedula* flycatchers (see Maynard Smith & Harper 2003 for a review) correspond to individuals unable to increase the melanization of sexually selected plumage traits with age. This mechanism could explain why these birds are unattractive to females (Maynard Smith & Harper 2003), and may represent an alternative explanation for delayed plumage maturation, which until now is mostly interpreted as a signal of poor competitive ability (Maynard Smith & Harper 2003).

Finally, the age-related increase in red breast colour of barn swallows was only significant in females. This sex difference is expected if the benefits of having intensely coloured breast patches are greater for males than for females (Ninni 2003; Safran *et al.* 2005). Following the antagonistic pleiotropy theory of senescence (Williams 1957), if the level of melanization can be considered a measure of lifetime 'repair' against the effects of senescence on melanocytes and/or melanosomes, and females are better able to melanize their plumage with increasing age than males, this could suggest greater reproductive effort in females during their first years of life and as a consequence a higher 'wear-and-tear' effect.

In conclusion, barn swallows cope with senescence by avoiding a brightening of melanin-based plumage colouration with age, supporting the hypothesis that certain molecular adaptations have evolved in birds to avoid senescence effects.

In the red patch of the breast melanization levels even increased with age in a longitudinal study of a cohort of individuals, while that was not the case for back feathers. We propose that this difference could be due to the lower costs of producing pheomelanin compared with eumelanin, and/or selection promoting an age-dependent increase in melanization only in plumage under sexual selection.

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