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Age-related change in melanin-based coloration of Barn owls (*Tyto alba*): females that become more female-like and males that become more male-like perform better

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Ornament expression fluctuates with age in many organisms. Whether these changes are adaptively plastic is poorly known. In order to understand the ultimate function of melanin-based ornaments, we studied their within-individual fluctuations and their covariation with fitness-related traits. In barn owls (*Tyto alba*), individuals vary from reddish-brown pheomelanic to white, and from immaculate to marked with black eumelanic spots, with males being less reddish and less spotted than females. During the first molt, both sexes became less pheomelanic, females displayed larger spots and males fewer spots, but the extent of these changes was not associated with reproduction. At subsequent molts, intra-individual changes in melanin-based traits covaried with simultaneous reproduction changes. Adult females bred earlier in the season and laid larger eggs when they became scattered with larger spots, whereas adults of both sexes produced larger broods when they became whiter. These results suggest that the production of melanin pigments and fitness-related life-history traits are concomitantly regulated in a sex-specific way.

ADDITIONAL KEYWORDS: age – life history – ornament – phenotype plasticity.

INTRODUCTION

In many animals, individual phenotypic traits including sexual characters, morphology, and physiology change with age (e.g. Deviche, Wingfield & Sharp, 2000; Angelier *et al*., 2006). Typically, some traits develop after a maturation period, whereas others vary throughout the lifetime or from the onset of senescence (the period during which survival and fecundity decline with age; e.g. Garamszegi, Heylen & Møller, 2005; Siefferman, Hill & Dobson, 2005; Torres & Velando, 2007). At a proximal level, age-related changes in phenotypic traits can involve hormonal (e.g. Schwabl *et al*., 1988; Dawson, 2006), environmental, and genetic factors that promote a change in trait expression. Therefore, age-related changes in one phenotypic trait may be associated with similar changes in life-history traits if expression relies on the same regulatory factors. Few studies have investigated the fluctuations in ornamental traits with age, and whether these fluctuations are genetically or environmentally controlled (Badyaev & Duckworth, 2003; Hegyi *et al*., 2006; Hegyi *et al*., 2008). Analysing the lifetime patterns of ornament expression and the covariation between ornamental changes and changes in reproductive traits would help us to understand the potential adaptive value of ornaments.

Individual phenotypic characters and reproductive parameters change in concert throughout life, as shown in a few species (Potti & Montalvo, 1991; Candolin, 2000; Badyaev & Duckworth, 2003; Vanpe *et al*., 2007). It is therefore not surprising if an experimentally induced increase in reproductive effort can lead to a simultaneous decline in secondary sexual characters and reproductive success the following year (Møller, 1989; Gustafsson, Qvarnström & Sheldon, 1995; Siefferman & Hill, 2005). The covariation between phenotypic and reproductive traits can lead

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*Corresponding author. E-mail: amelie.dreiss@unil.ch to strategies that maximize reproductive success, as long as individuals can divide advertising effort through their lifetime (Kokko, 1997). For instance, in the house finch *Carpodacus mexicanus*, ornament expression might display adaptive plasticity. Unmated males in one breeding season invest more into ornaments in the subsequent moult than mated males (Badyaev & Duckworth, 2003). Hence, ornament expression could be context and condition dependent. Moreover, covariation between ornament and reproductive traits may depend upon individual age. In many passerine birds for instance, sexually mature males do not produce the typical adult plumage until the first or second year of age, a phenomenon referred to as delayed plumage maturation. The retention of drab plumage in the first years of life has been hypothesized to be related to the physiological costs of producing adult plumage (Rohwer & Butcher, 1988; Galván & Møller, 2009) or to be an adaptation to resemble females or juveniles, and thereby avoid aggression from dominant male conspecifics (Rohwer, Fretwell & Niles, 1980; Lyon & Montgomerie, 1986).

Melanin-based ornaments are widespread in the animal kingdom. Their expression has often been found to be highly heritable, and can be sensitive to environmental and body conditions (Majerus, 1998; Gantz & Fong, 2003; Horth, 2003; Roulin & Dijkstra, 2003; Fargallo *et al*., 2007). Covariation between melanin-based traits, produced either by black eumelanin or reddish-brown pheomelanin pigments, and various phenotypic characters has been suggested to stem from pleiotropic effects of the genes regulating melanin synthesis (Ducrest, Keller & Roulin, 2008). Next to melanin production, some chain components of melanogenesis indeed have other functions as well, like aggressiveness, sexual behaviour, regulation of stress responses, immune function, and energy homeostasis. For these reasons, melanin-based traits are particularly valuable to investigate whether the degree of age-related change in coloration covaries with concurrent change in life-history traits. Although the intensity of such coloration is known to change with age in many organisms (e.g. Weimerskirch, Lequette & Jouventin, 1989; West & Packer, 2002; Galván & Møller, 2009), we are aware of only one study reporting a relationship between the extent of changes between two age classes of both a melanin-based trait and reproductive parameters (Potti & Montalvo, 1991). This study found that when male pied flycatchers *Ficedula hypoleuca* expressed darker eumelanic plumage in a given year compared with the previous one, they also bred earlier in the season compared with the previous year, although this relationship was probably not causal.

In the present study, we investigate the within-individual changes in three melanin-based traits and their covariation with simultaneous change in reproductive parameters in the barn owl *Tyto alba*. In this species the ventral body face varies continuously from reddish-brown pheomelanic to white, and from immaculate to heavily marked with black spots, described as two eumelanic traits: spot diameter and spot number (for pictures, see Ducrest *et al*., 2008). Although members of the two sexes exhibit the traits in the same range of possible values, males are on average less reddish-brown and present fewer and smaller spots than females (Roulin, 2004). Colour, spot number, and spot diameter are correlated, with more reddish individuals showing more and larger spots (Roulin, 2004). Among individual *T. alba*, melanin-based plumage traits have been shown to correlate with different fitness components in a sex-specific way (Roulin, 2009; Roulin *et al*., 2010). In particular, females with larger black spots are preferred as social mates, produce higher quality young, and have a higher survival rate (Roulin, 2009), whereas less pheomelanic males have a higher probability of breeding in their first year (Roulin & Altwegg, 2007). However, it is still unknown whether within-individual variation in these plumage traits is also associated with variation in fitness components. Preliminary studies with a limited number of individuals showed that individuals significantly and consistently change their plumage traits between yearling and adult stages (i.e. between 1 and 2 years old), whereas the plumage does not vary significantly during the two subsequent years, at least not in a consistent way. Males and females become less pheomelanic when they reach 2 years old (Taylor, 1993; Roulin, 1999a), and females become more eumelanic, whereas males become less eumelanic (Roulin, 1999a). This study did not examine which eumelanic traits (size or number of spots) changed with age because only the proportion of the plumage surface covered by black spots was considered. Because age-related patterns have been shaped by natural selection, we expect that as *T. alba* change in plumage traits in a consistent way in the population, the extent of the within-individual change in melanin synthesis would be positively correlated with an improvement in reproductive success.

Using a data set collected over a period of 12 years, our aims are: (1) to describe the lifetime fluctuations in the three melanin-based traits; (2) to examine whether these fluctuations are explained by within-individual changes, or by survival or migration, by comparing within- and among-individual changes; (3) as melanin-based traits are highly heritable in *T. alba*, we also aimed to gain insight into the genetic basis of within-individual variation in melanin-based traits, by estimating individual repeatability of plumage changes in different years, and testing whether a change in plumage traits could be heritable; and (4) to determine whether within-individual variation in melanin-based traits is related to concurrent changes in reproductive parameters. Although our study is mainly descriptive, support for a covariation between the changes in melanin-based and life-history traits can provide important information on the ultimate function of melanin.

MATERIAL AND METHODS

Model species

Tyto alba are monogamous within each reproductive season, with very little extra-pair paternity (Roulin *et al*., 2004). Individuals can breed from the age of 1 year, and in our study area breeding females can produce up to two annual broods from late February to mid-October. The hatching date is an important parameter in *T. alba* reproduction, because an early start of reproductive activities at the first annual breeding attempt increases the chance to produce a second annual brood (A. Dreiss & A. Roulin, unpubl. data). Clutch size ranges from two to 11 eggs (mean ± SE of 923 clutches: 6.0 ± 1.6), and brood size at fledging ranges from one to nine nestlings (mean ± SE of 764 broods that produced at least one fledgling: 4.3 ± 1.4).

General procedures

The study was performed in western Switzerland (46°4′N, 6°5′E, 430–520 m a.s.l.) between 1996 and 2007 in an area covering 190 km^2 where we mounted 110 nest boxes on the external wall of barns. Each year, breeding pairs and their offspring were captured and ringed. Breeding females were recognized by the presence of a brood patch. When breeders had not been ringed as nestlings, we considered them as 'yearlings' (1 year old) if all primary and secondary wing feathers belonged to the same generation, as 2 years old if only the sixth primary had already been renewed, and as 3 years old if more primary or secondary wing feathers were already renewed (Taylor, 1993). We termed individuals of 2 years or older as 'adults'. Nests were visited regularly to record the reproductive parameters of hatching date (i.e. date when the first egg of a clutch hatched), clutch size, egg volume (mm^3), and number of hatchlings. As in a wide range of bird species egg size is positively correlated with hatchling size, nestling growth, and survival (Williams, 1994), we measured eggs to the nearest 0.1 mm for maximum length (L) and breadth (B) to estimate egg volume (V) with the formula $V = B^2L\pi/6$ (Stonehouse, 1966). The within-clutch repeatability of egg volume was higher ($r = 0.90 \pm 0.01$, $F_{745,3077} = 59.22$, $P < 0.0001$) than repeatability in egg length and breadth (respectively: $r = 0.70 \pm 0.03$, $F_{606,3216} = 15.54$, $P < 0.0001$; $r = 0.70 \pm 0.03$, $F_{606,3216} = 15.95$, $P < 0.0001$). In the present study, we consider the first annual reproductive attempt exclusively, as the second clutch presents different characteristics in terms of hatching date, clutch size, and clutch success than the first, and as only a small proportion of individuals (approx. 10%) produced a second clutch. Reproductive parameters were not correlated ($N = 395$ females, reproductive parameters of the females breeding more than once were averaged; Pearson correlation between hatching date and clutch size, $r = -0.09$, $P > 0.12$; hatching date and number of hatchlings, $r = -0.03$, $P > 0.5$; hatching date and egg volume, $r = -0.05$, $P > 0.2$; clutch size and egg volume, $r = -0.01$, $P > 0.9$; number of hatchlings and egg volume, $r = -0.05$, $P > 0.3$), except clutch size and number of hatchlings ($r = 0.80$, $P < 0.0001$).

Plumage traits

Alexandre Roulin assessed individual pheomelanin-based colour on four body parts (breast, belly, one flank, and one underside of the wings), by visual comparison with eight colour chips, ranging from 1 for dark reddish-brown to 8 for white; hereafter, this pheomelanin-based trait is referred to as 'plumage colour'. On the same four body parts the number and diameter (in mm) of eumelanin-based spots were recorded using the methods described in previous studies (Roulin, 2004). Because plumage traits measured in the different parts of the body are correlated (Roulin, 2004), the four measurements of plumage features were averaged. Methods of assessing plumage traits are accurate: by measuring plumage traits in 149 individuals twice in the same season, the repeatability of assessing plumage traits is on average 0.90 (Roulin, 1999b, 2004). In a subset of individuals for which we disposed of feather samples, we have also estimated an objective parameter of pheomelanin-based colour, brown-chroma (spectral purity, R600–700/R300–700). This parameter represents the reflectance in the reddish-brown part of the spectrum. For each individual, we computed the mean of four reflectance spectra, measured with an Ocean Optics S2000 spectrophotometer (Dunedin, FL, USA) on two different feathers sampled with scissors on individual flank. Flank brown-chroma and visual estimation of flank colour are highly correlated (Pearson correlation: $r = -0.78$, $P < 0.0001$, $N = 1107$). Because of this strong correlation between visual and spectral parameters of flank colour, and because of the high repeatability of the visual parameter, we are confident that the visual parameter of pheomelanin-based colour is reliable. We have thus chosen to use this visual parameter rather than the spectral parameter brown-chroma as: (1) some individuals in the population have not been

Table 1. Plumage traits were not related to wing length and body condition (estimated as the residual of the regression between body mass measured during young feeding and wing length). Results of a mixed model with plumage traits as the dependent variables, individual as a repeated factor, year as a random factor, and age and sex as covariates

		Wing length		Body condition	
	d.f.	*F*	*P*	*F*	*P*
Plumage colour	1,1414	0.53	*0.47*	2.55	*0.11*
Spot diameter	1,1326	0.02	*0.88*	0.54	*0.46*
Number of spots	1,1326	1.62	*0.20*	0.30	*0.50*

sampled for feathers; (2) measures and analyses of spectra are highly time-consuming; (3) visual and spectral measurements are highly correlated, and thus give a similar estimation of plumage colour.

Roulin & Dijkstra (2003) found a significant heritability for plumage colour [$h^2 = 0.81 \pm 0.09$ (SE)], and for the proportion of the plumage surface covered by black spots ($h^2 = 0.65 \pm 0.05$), which correlates with both spot number and diameter. Maternal and paternal contributions on the plumage traits of daughters and sons suggest that plumage colour and number of spots are autosomally inherited, whereas spot diameter is partly sex-linked (Roulin & Dijkstra, 2003; Roulin, 2004; Roulin *et al.*, 2010). Change in plumage trait was defined as the trait a given year minus the trait of the previous year. Plumage traits were not related to wing size or body condition estimated during reproduction as the residual of the regression between body mass measured during young feeding and wing length (Table 1).

We analysed change in plumage from the first molt (change in plumage from yearling to the adult stage) and during subsequent adult molts. By marking feathers, Roulin (1999a) has already shown that no molt occurs between the fledging and yearling stages in *T. alba*. The only significant plumage change that occurs between fledging and yearling stages was a decline in the number of spots (see results). This decrease in spot number is probably the result of feather loss during the first year of life: because females present a higher decrease in spot number than males, this decrease is consistent with a loss of some feathers as females lose more feathers in the incubation patch, and usually molt during this period (A. Roulin, pers. observ.).

STATISTICAL ANALYSES

Age patterns of melanin-based traits: at the population level and within-individual level

We used mixed-model procedures (Littell *et al.*, 1996), with the individual as a repeated variable, year as a random effect, and plumage traits or the change in plumage traits between two successive years as the dependent variables. Sex was included in the initial models as a fixed factor, and age was included as a covariate. Because the relationship between melanic traits and age may be nonlinear, we also included in the models square age (age^2) and age cubed (age^3). When age was involved in a significant interaction (age^2, age^3, or age × sex), we additionally separated individuals according to their age (e.g. age $> x$ or age $< x$) or sex. We provide these additional tests to illustrate the complex age effect.

We performed an analysis across individuals of the plumage traits in relation to age (Figs 1A, 2A, and 3A) with 395 different females and 329 different males including all observations for a given individual. The individuals included in this analysis were ringed as nestling or adult, and thus there may be some doubt about exact individual age (Table 2). Results were nevertheless qualitatively similar if we only kept the individuals ringed as nestling (i.e. 165 different females and 187 different males, Table S1). To analyze the within-individual changes in plumage traits, breeders captured in two consecutive years were used to analyse the within-individual changes in plumage traits (139 different females and 136 different males; Figs 1B, 2B and 3B).

Repeatability of age-related changes in melanin-based traits

Repeatability analyses were calculated by one-way ANOVA (Lessells & Boag, 1987). We examined the resemblance between biological parents and their offspring at the same ages to examine whether individual plumage change could potentially be heritable. In 10 of the 12 years, cross-fostering experiments were performed (see Roulin *et al.*, 2007), but in the present paper we considered offspring raised either by their biological or foster parents to maximize sample size. Therefore, our heritability estimations may be inflated by environmental effects. We performed mixed-model procedures, with changes in offspring plumage traits as dependent variables, offspring status (raised by biological or foster parents) as a

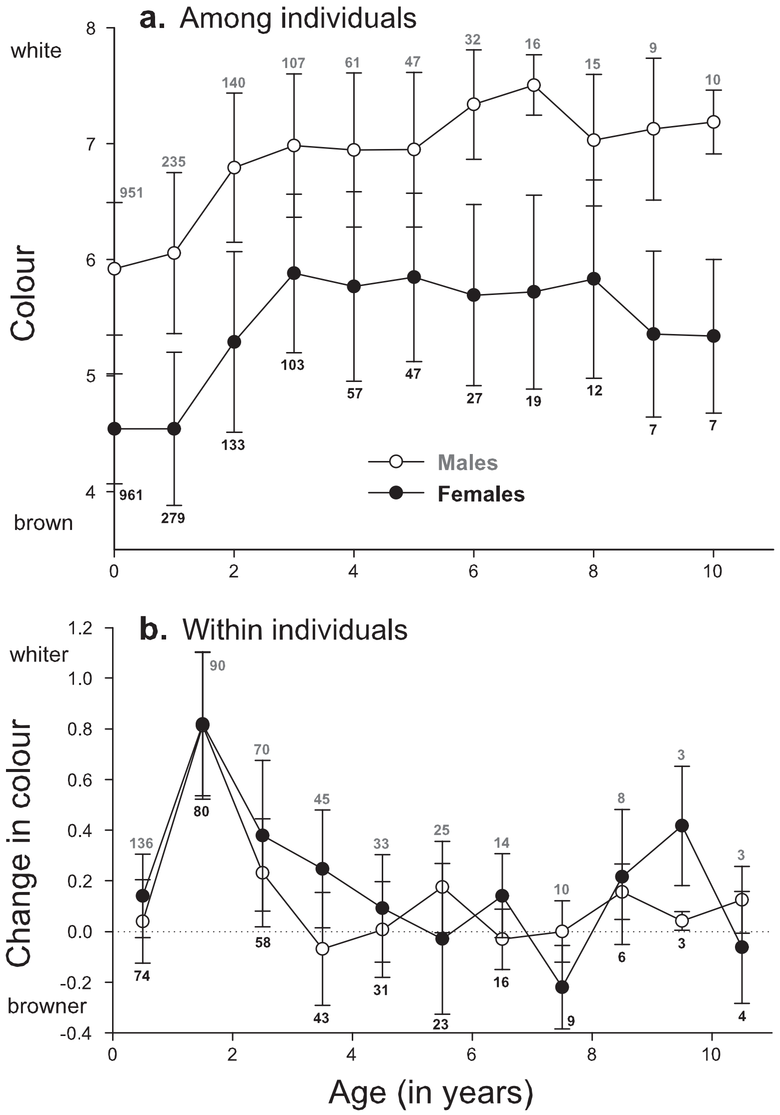

Figure 1. Age-related variation in pheomelanin-based colour in female (●) and male (○) *Tyto alba*. A, mean ± SD colour in the population calculated across similarly aged individuals. B, mean change in colour between two age classes (sample sizes are quoted above and below error bars for males and females, respectively).

factor, changes in biological parental plumage traits as a covariate and the interaction between nestling status and change in parental traits. Offspring in this analysis were all originated from different fostered or non-fostered parents.

Covariation between changes in melanin-based traits and reproductive parameters

We used mixed-model procedures with the individual as a repeated variable, year as a random effect, changes in reproductive parameters as dependent variables (hatching date, clutch size, egg volume, and number of hatchlings), sex as cofactor, and age and changes in the three plumage traits as covariates. Additionally, to take into account the effect of the breeding partner, changes in the three plumage traits in the partner of the focal individual were included as covariates. Some interactions between age and change in plumage were significant for males, so for these analyses we only considered males ringed as nestlings for which the exact age is known, but results are qualitatively similar if all males are taken into account (not shown). In order to describe the interactions involving age, we performed additional tests in which males were separated according to their age, because the significant age interaction implied that age affected the studied variable from a certain age onwards, or only until a certain age.

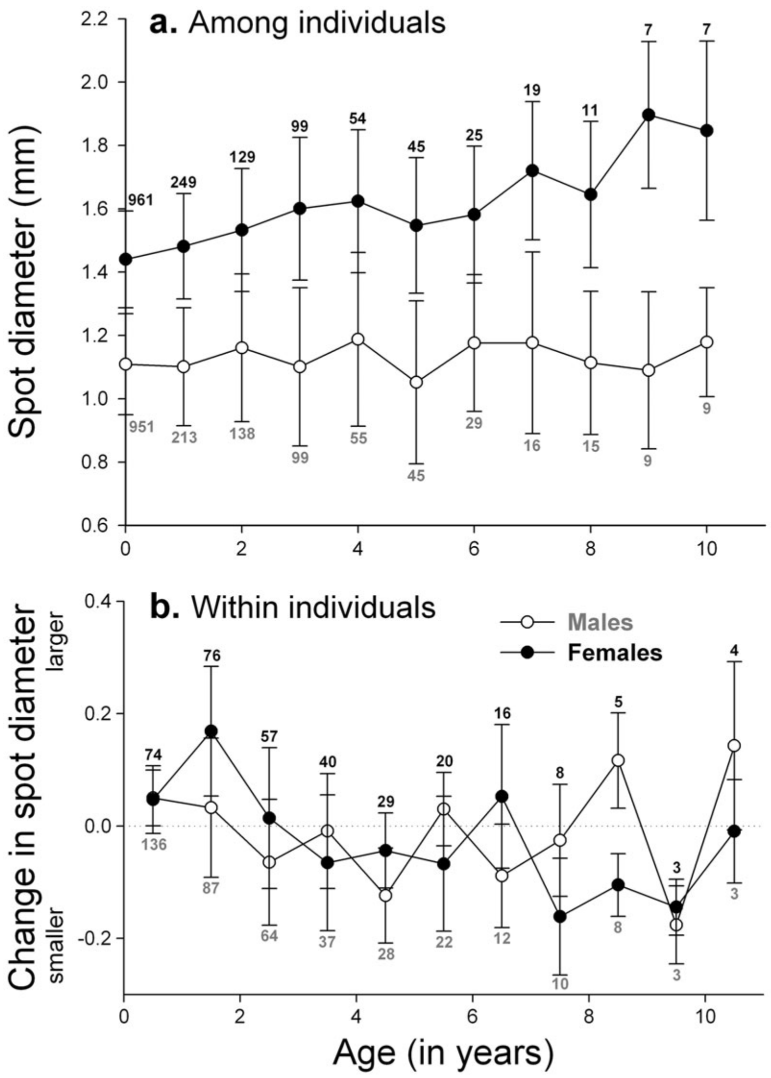

Figure 2. Age-related variation in spot diameter (mm) in female (●) and male (○) *Tyto alba*. A, mean ± SD spot diameter in the population calculated across similarly aged individuals. B, mean change in spot diameter between two age classes (sample sizes are quoted below and above error bars for males and females, respectively).

Parameter estimates and standard errors are provided in the text for significant results.

We have also performed the same analyses with changes in reproductive parameters as dependent variables, and the original plumage trait value was included as a covariate instead of the plumage change, in order to verify that the relationships found between changes in reproductive parameters and plumage traits were not caused by the original trait value. The change in plumage traits could indeed simply reflect the trait itself if the magnitude of the change in plumage trait is correlated with the absolute initial trait value (Hegyi *et al.*, 2008). However, original trait value did not explain changes in reproductive parameters (see Results).

All statistical analyses have been computed with the computer program SAS v9.1. Assumptions for the parametric tests used (homoscedastic and normal distributions of variables or residuals) were verified in each test. We performed backward model selection using $P = 0.05$ as the threshold value for elimination. Final models only contained significant effects, and main effects involved in significant interactions. We verified that the final models selected presented the smallest Akaike information criterion (AIC), compared with initial and intermediate models, which was always the case. Means are quoted ± SD unless otherwise stated. A slightly smaller sample size for eumelanic than pheomelanic traits arose from an absence of measurement of mean spot number and

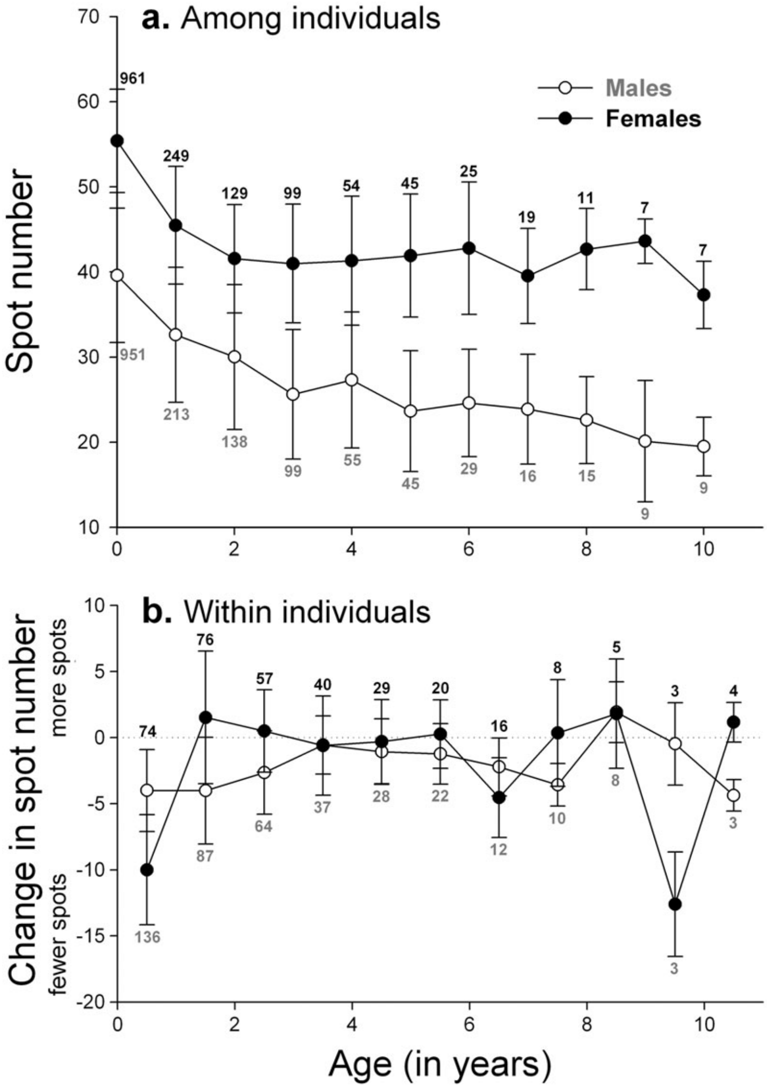

Figure 3. Age-related variation in the number of spots in female (●) and male (○) *Tyto alba*. A, mean ± SD number of spots in the population calculated across similarly aged individuals. B, mean change in number of spots between two age classes (sample sizes are quoted below and above error bars for males and females, respectively).

diameter in 2005 (spots were only recorded on the breast).

RESULTS

AGE PATTERNS OF MELANIN-BASED TRAITS AT THE POPULATION LEVEL

Pheomelanin-based colour

Plumage colour varied nonlinearly with age in the population (Fig. 1A; Table 2, the term age^3 was significant). Two-year-old adults were on average whiter than yearlings ($F_{1,771} = 47.69$, $P < 0.0001$), and 3-year-old individuals were whiter than 2-year-old birds ($F_{1,468} = 3.93$, $P = 0.048$); subsequently, plumage colour was not correlated with age (for age > 2 years, $F_{1,560} = 0.53$, $P > 0.4$; Fig. 1A).

Spot diameter

Among individuals, the diameter of plumage spots was related to age in a sex-specific way (Fig. 2A; Table 2, the interaction between age and sex was significant). The spot diameter was not only larger in adults compared with yearlings, but increased continuously with age in females ($F_{1,631} = 37.18$, $P < 0.0001$; Fig. 2A). Spot diameter was not related to age in males ($F_{1,614} < 0.01$, $P > 0.9$; Fig. 2A).

Table 2. Melanin-based traits in relation to age and sex. Results of mixed models with plumage traits or plumage changes as the dependent variables, with individual as a repeated variable and year as a random effect. Results of final models are written in bold and non-significant (NS) results of the initial full models in plain

			Age		Age^2		Age^3		Sex		Sex × age		N	
		d.f.	F	P	F	P	F	P	F	P	F	P	Male	Female
Across individuals	Colour	1,1345	**75.82**	***< 0.0001***	**38.89**	***< 0.0001***	**25.01**	***< 0.0001***	**347.91**	***< 0.0001***	NS	*> 0.5*	329	395
	Spot diameter	1,1257	**15.82**	***< 0.0001***	NS	*> 0.5*	NS	*> 0.5*	**57.54**	***< 0.0001***	**15.8**	***< 0.0001***		
	Spots number	1,1257	**24.84**	***< 0.0001***	NS	*> 0.5*	NS	*> 0.5*	**52.76**	***< 0.0001***	**14.18**	***< 0.001***		
Within individual	Change in colour	1,518	**98.34**	***< 0.0001***	**50.99**	***< 0.0001***	**30.13**	***< 0.0001***	NS	*0.07*	NS	*> 0.03*	136	139
	Change in spot diameter	1,518	**12.04**	***< 0.0001***	NS	*> 0.5*	NS	*> 0.5*	**17.51**	***< 0.0001***	**11.78**	***< 0.0001***		
	Change in number of spots	1,518	**NS**	***0.16***	NS	*> 0.5*	NS	*> 0.5*	**25.08**	***< 0.0001***	**10.58**	***0.001***		

Number of spots

Similarly to spot diameter, we found a significant interaction between age and sex in the number of plumage spots in the population (Fig. 3A; Table 2). The pattern was opposite to spot diameter, as the number of spots was negatively correlated with age in males ($F_{1,614} = 32.93$, $P < 0.0001$; Fig. 3A), whereas it stayed constant with age in females ($F_{1,631} = 0.62$, $P > 0.4$; Fig. 3A).

WITHIN-INDIVIDUAL CHANGES OF MELANIN-BASED TRAITS IN RELATION TO AGE AND SEX

Change in pheomelanin-based colour

Consistent with the pattern at the population level, within-individual change in colour from one year to the next varied nonlinearly with age, independently of sex (Fig. 1B; Table 2). Colour change was maximum at the first molt, where 92% of non-white individuals (i.e. individuals with a mean colour score of less than 8) became whiter (mean colour change in yearlings: 0.82 ± 0.57; significantly different from zero, $t = 18.59$, $P < 0.0001$, $N = 170$). On average, individuals also became slightly whiter between 2 and 3 years of age (0.29 ± 0.51; significantly different from zero, $t = 6.57$, $P < 0.0001$, $N = 128$). At subsequent ages, although the colour change showed a tendency towards birds becoming lighter coloured between two successive years, this relationship was on the borderline of significance (0.06 ± 0.40; $t = 1.96$, $P = 0.052$, $N = 188$). The extent of the change in plumage colour was not related to age in adults (age ≥ 2 years, $F_{1,261} = 0.04$, $P > 0.8$; Fig. 1B).

Change in spot diameter

The within-individual change in spot diameter between two consecutive years was differently related to age in the two sexes (Fig. 2B; Table 2), with a decline in spot size change with age in females ($F_{1,246} = 22.49$, $P < 0.0001$), but not in males ($F_{1,262} < 0.01$, $P > 0.9$; Fig. 2B). Females increased in spot diameter between 1 and 2 years old (mean spot diameter change, 0.17 ± 0.23 mm; significantly different from zero, $t = 6.38$, $P < 0.0001$, $N = 76$). At subsequent ages, the change in spot diameter was no longer significant at any age (globally, –0.03 ± 0.23 mm; $t = -1.92$, $P = 0.057$, $N = 182$). This was dissimilar to the pattern at population level where the spot diameter increased continuously with age. For males, the within-individual change in spot diameter was not significantly different from zero at any age (globally, –0.02 ± 0.22 mm; $t = -1.34$, $P = 0.18$, $N = 274$).

Change in number of spots

The within-individual change in spot number between two consecutive years also varied with age in a sex-specific way (Fig. 3B; Table 2). Males lost fewer spots with age ($F_{1,261} = 5.58$, $P = 0.019$; Fig. 3B). In males, spot number decreased between 1 and 2 years old, and between 2 and 3 years old (mean change in spot number, respectively, –4.0 ± 8.1 spots and –2.6 ± 6.3 spots; both significantly different from zero, respectively $t = -4.65$, $P < 0.0001$, $N = 87$ and $t = -3.35$, $P = 0.001$, $N = 64$). For older males, however, the change in spot number between two age classes was not significantly different from zero (all $P > 0.1$), but changes were overall negative for these males, as shown by an analysis considering all between-year changes after 3 years of age (–1.4 ± 4.7 spots; $t = -2.72$, $P = 0.008$, $N = 86$). By contrast, the change in spot number did not change with age in females ($F_{1,245} = 1.60$, $P > 0.2$; Fig. 3B), and did not overall differ from zero (0.1 ± 7.9 spots; $t = 0.17$, $P > 0.8$, $N = 258$; Fig. 3B).

REPEATABILITY OF AGE-RELATED CHANGES IN MELANIN-BASED TRAITS

Melanin-based traits were highly repeatable within reproductive individuals across their lifetime, showing that the change in coloration at the first molt was moderate (between-years repeatability within 156 males: 0.83 ± 0.02 for colour; 0.87 ± 0.02 for spot diameter; 0.86 ± 0.02 for spot number; between-years repeatability within 147 females: 0.85 ± 0.02 for colour; 0.85 ± 0.03 for spot diameter; 0.77 ± 0.03 for spot number). The repeatability was slightly higher when we consider only adult individuals (males: 0.89 ± 0.02 for colour; 0.88 ± 0.02 for spot diameter; 0.91 ± 0.01 for spot number; females: 0.91 ± 0.01 for colour; 0.85 ± 0.03 for spot diameter; 0.82 ± 0.03 for spot number). The lower repeatability of number of spots is consistent with the fact that females lose feathers during reproductive activities. Conversely, the between-year changes in melanin-based traits were not repeatable (all repeatability values < 0), even if we only consider adult individuals that already have matured plumage. We therefore do not predict strong genetic effects for the change in plumage traits between successive years. Accordingly, considering the mean plumage changes in individuals measured at adulthood (i.e. 2 years of age or older), parents and offspring did not significantly resemble each other (Table 3b). Conversely, the change in pheomelanin-based colour at the first molt correlated between mother and offspring (Table 3a). Other changes in plumage between the first and second years of age were not related between parents and offspring (Table 3a). Comparing mothers with daughters, mothers with sons, fathers with daughters, and fathers with sons provided nonsignificant results (not shown), except for mother–son spot diameter change between the first and second years of age ($F_{1,9} = 10.68$, $P = 0.010$, $N = 11$).

COVARIATION BETWEEN CHANGES IN MELANIN-BASED TRAITS AND REPRODUCTIVE PARAMETERS

As changes in melanin-based traits varied nonlinearly with age and were maximal in yearlings, we performed separate analyses for yearlings and adults. Within adult females the change in colour was positively related to the change in spot diameter, as

Table 3. Mother–offspring and father–offspring comparisons for the changes in plumage traits between two consecutive years (mixed models with change in offspring plumage as dependent variables). Initial models contained changes in parent plumage as covariates, chick status (fostered or non-fostered) as a factor, and the interaction between changes in parent plumage and chick status

		Change in mother plumage		Change in father plumage	
a. First molt	d.f.	*F*	*P*	*F*	*P*
Change in colour	1,17	**7.09**	***0.016***	0.04	*0.85*
Change in spot diameter	1,16	3.58	*0.08*	0.01	*0.97*
Change in number of spots	1,16	0.55	*0.47*	1.61	*0.22*
		Change in mother plumage		Change in father plumage	
b. Subsequent molts	d.f.	*F*	*P*	*F*	*P*
Change in colour	1,25	0.03	*0.86*	2.18	*0.15*
Change in spot diameter	1,22	1.55	*0.23*	2.94	*0.11*
Change in number of spots	1,22	1.02	*0.32*	0.01	*0.93*

Table 4. Change in reproductive parameters in relation to change in melanin-based traits in adult *Tyto alba*. Results of mixed models with change in reproductive parameters as dependent variables, individual as a repeated variable, and year as a random effect. Results of final models are written in bold and non-significant results of the initial models in plain

	Change in hatching date			Change in clutch size			Change in brood size			Change in egg volume		
	d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*	d.f.	F	*P*
Change in colour (C)	1,328	0.41	*0.52*	**1,282**	**9.38**	***0.002***	**1,260**	**11.24**	**< *0.001***	1,189	0.01	*0.97*
Change in spot diameter (D)	**1,368**	**1.94**	***0.16***	1,243	0.55	*0.46*	1,230	0.02	*0.9*	**1,205**	**0.29**	***0.59***
Change in number of spots (N)	1,328	2.39	*0.12*	**1,282**	**0.38**	***0.54***	**1,260**	**0.16**	***0.69***	1,189	0.51	*0.48*
Change in colour in partner	1,328	0.36	*0.55*	1,243	0.28	*0.6*	1,230	1.71	*0.19*	1,189	1.29	*0.26*
Change in spot diameter in partner	1,328	2.78	*0.1*	1,243	1.10	*0.29*	1,230	1.07	*0.3*	1,189	1.48	*0.23*
Change in number of spots in partner	1,328	0.22	*0.64*	1,243	1.63	*0.2*	1,230	0.27	*0.6*	1,189	0.13	*0.72*
Age	**1,368**	**8.22**	***0.004***	**1,282**	**0.67**	***0.41***	**1,260**	**0.03**	***0.85***	1,189	0.66	*0.42*
Sex	**1,368**	**1.96**	***0.16***	**1,282**	**0.03**	***0.87***	**1,260**	**0.71**	***0.4***	**1,205**	**0.81**	***0.37***
Age × C	1,323	0.90	*0.34*	1,239	0.20	*0.65*	1,226	0.07	*0.79*	1,184	0.12	*0.73*
Age × D	1,323	2.00	*0.16*	1,239	1.11	*0.29*	1,226	2.28	*0.13*	1,184	0.34	*0.56*
Age × N	1,323	0.11	*0.74*	**1,282**	**0.56**	***0.45***	**1,260**	**1.18**	***0.28***	1,184	0.75	*0.39*
Sex × C	1,323	0.88	*0.35*	1,239	0.89	*0.35*	1,226	2.36	*0.13*	1,184	2.33	*0.13*
Sex × D	**1,368**	**8.50**	***0.004***	1,239	1.62	*0.2*	1,226	3.00	*0.86*	**1,205**	**4.11**	***0.044***
Sex × N	1,323	0.14	*0.71*	**1,282**	**4.11**	***0.044***	**1,260**	**3.89**	**0.049**	1,184	0.01	*0.94*
Age × Sex × C	1,322	2.69	*0.11*	1,237	0.05	*0.82*	1,224	0.05	*0.83*	1,181	2.51	*0.12*
Age × Sex × D	1,320	0.95	*0.33*	1,237	0.01	*0.95*	1,224	0.04	*0.84*	1,181	0.42	*0.51*
Age × Sex × N	1,320	2.26	*0.13*	**1,282**	**5.82**	**0.016**	**1,260**	**6.17**	**0.014**	1,181	0.68	*0.41*

females becoming whiter also increased their spot diameter ($r = 0.21$, $P = 0.004$, $N = 179$; all other comparisons are not significant, $P > 0.2$). In yearling and adult males, changes in different plumage traits were not correlated (Pearson correlation between changes in the different melanin-based traits in yearlings or adults: all $r < 0.13$, all $P > 0.05$). Changes in plumage traits in one individual were not correlated with the changes in plumage traits in its breeding partner (Pearson correlation between changes in the different melanin-based traits in yearlings or adults: all $r < 0.15$, all $P > 0.05$; Table S2).

Yearlings

Changes in plumage traits between the first and second years of age were not significantly related to any changes in reproductive parameters (all P values > 0.05, Table S3).

Adults

At adulthood, changes in hatching date and egg volume of the first annual clutch were related to the interaction between sex and change in spot diameter (Table 4). When spot diameter increased between two years, adult females laid earlier and bigger eggs in the second than in the first year, and inversely females laid later and smaller eggs when spot diameter decreased (hatching date parameter estimate: –26.2 ± 8.7, $F_{1,174} = 9.01$, $P = 0.003$, Fig. 4A; egg size (mm^3): 48.0 ± 22.9, $F_{1,97} = 4.40$, $P = 0.038$, Fig. 4B). These two relationships were not confounded by a change in plumage of the breeding partner (Table 4). In adult males, no such relationship was found between changes in spot diameter and hatching date or egg volume (hatching date, $F_{1,181} = 0.31$, $P = 0.58$; egg size (mm^3), $F_{1,104} = 0.42$, $P = 0.51$). When considering only females of known age (i.e. ringed as nestlings) we found similar results (relationship between changes in spot diameter and egg volume, $F_{1,43} = 4.39$, $P = 0.042$; changes in spot diameter and hatching date, $F_{1,76} = 3.40$, $P = 0.069$). These analyses are consistent with an analysis among individuals showing that larger-spotted females produced first annual clutches earlier in the season (mixed models with hatching date as the dependent variable, age and spot diameter as covariates, in yearlings, $F_{1,209} = 4.02$,

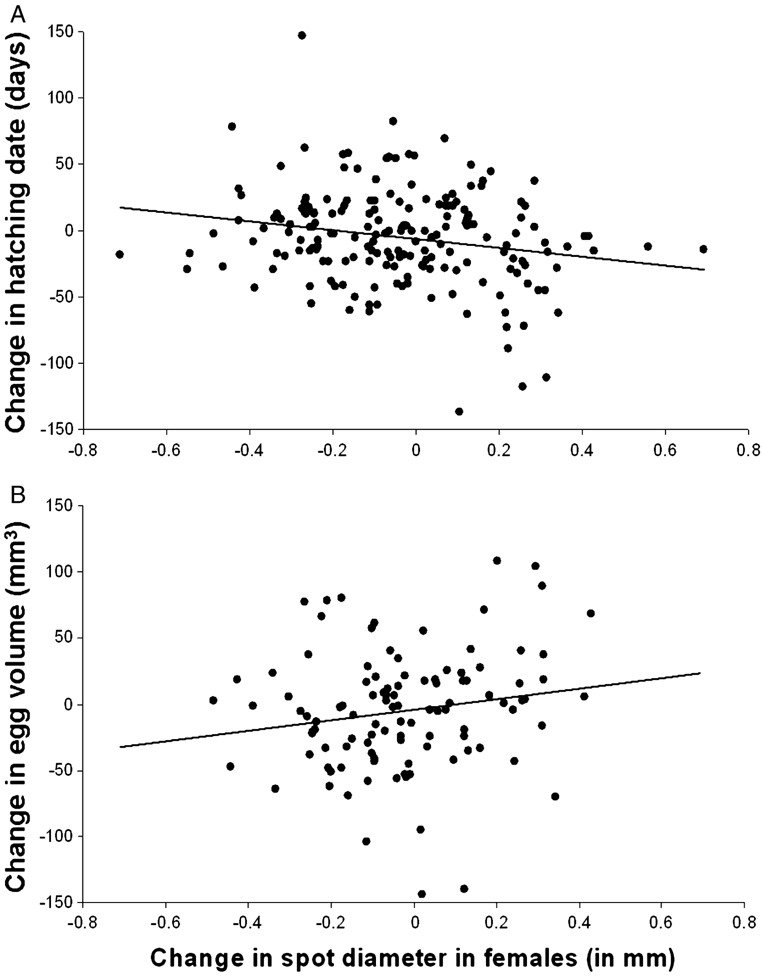

Figure 4. Change in hatching date (in days) (A) and change in egg volume (mm^3) (B) of the first annual clutch in relation to change in spot diameter (mm) in female *Tyto alba*. Changes were given by subtracting values found in one year with values found in the same individuals in the previous year.

$P = 0.046$, $N = 233$; in adults, $F_{1,369} = 7.69$, $P = 0.006$, $N = 197$) and produced bigger eggs as adults (adults, $F_{1,279} = 7.49$, $P = 0.007$, in this sample of birds the relationship is independent of exact age; yearlings, $F_{1,177} = 0.05$, $P > 0.8$). Note that changes in adult hatching date and egg volume were not associated with spot diameter measured in the first or second years ($P > 0.05$ in similar mixed models with change of reproductive parameter as the dependent variable).

The changes in clutch and brood sizes between two successive years were positively related to the change in adult colour in both females and males (Table 4; clutch size, 0.7 ± 0.2, $F_{1,282} = 9.38$, $P = 0.002$; number of hatchings, 0.9 ± 0.3, $F_{1,260} = 11.24$, $P < 0.001$, Fig. 5A), and were significantly related to the interaction between sex, age, and change in spot number (clutch size, $F_{1,282} = 5.82$, $P = 0.016$; number of hatchings, $F_{1,260} = 6.17$, $P = 0.014$). These results were not caused by a change in plumage of the breeding partner (Table 4). When adults became whiter between two years, their clutch and brood size congruently increased, and inversely adults had smaller clutches and brood sizes when plumage darkened (Fig. 5). These changes in clutch and brood sizes could not be imputed to absolute plumage colour or spot number measured in the first or second year (all $P > 0.1$ in mixed models with change of reproductive parameter as the dependent variable and plumage traits in the first or the second year as covariates). Moreover, in an analysis across individuals, there was no significant correlation between reproductive variables and adult plumage traits (all $P > 0.4$).

The significant interaction between sex, age, and change in spot number is explained by the fact that in adult males the changes in clutch and brood sizes between two successive years were positively related to the interaction between male age and change in male spot number (clutch size, $F_{1,87} =$

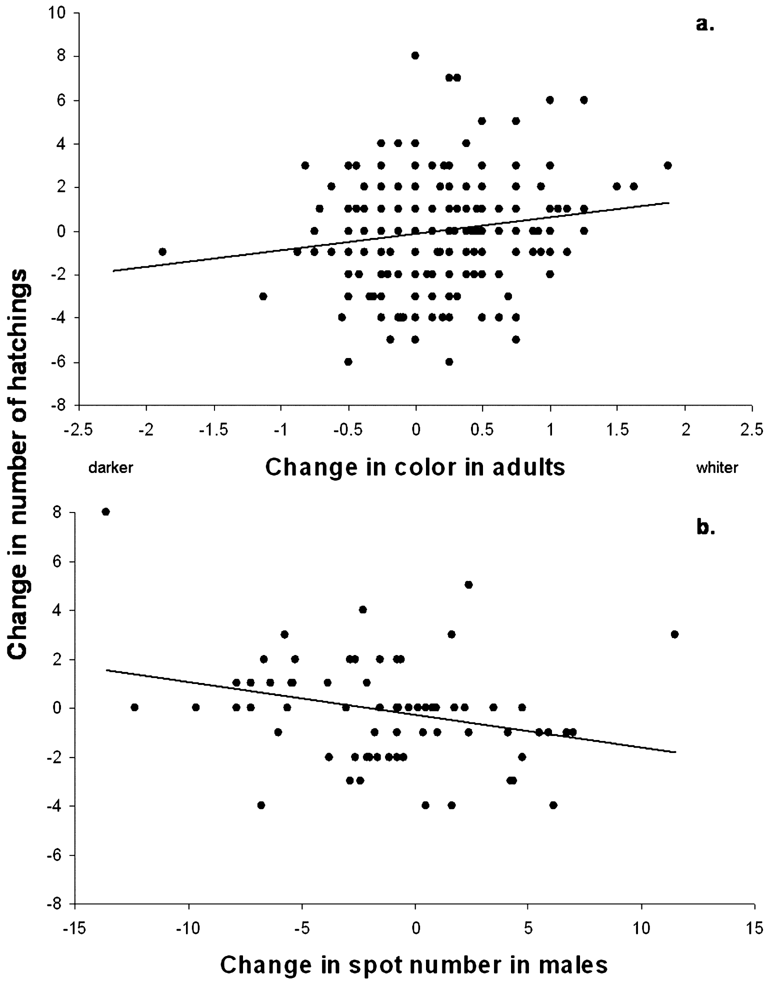

Figure 5. Change in number of hatchings of the first annual clutch in relation to (A) change in pheomelanin-based colour in adult *Tyto alba* and (B) change in spot number in males 3 years of age or older. Changes were given by subtracting values found in one year with values found in the same individuals in the previous year.

4.76, $P = 0.032$; number of hatchings, $F_{1,82} = 5.85$, $P = 0.018$). For males of 3 years in age or older, brood size increased with decreasing spot number (-0.13 ± 0.06, $F_{1,52} = 4.85$, $P = 0.032$; Fig. 5B). This relationship was not significant in 2-year-old males ($F_{1,23} = 0.24$, $P > 0.6$). Similarly, males 4 years of age or older had a larger clutch the year following a larger loss of spots (-0.19 ± 0.07, $F_{1,17} = 8.74$, $P = 0.009$), a relationship that was not verified in 2–3-year-old males ($F_{1,59} = 0.12$, $P > 0.7$). In adult females no covariation between change in plumage spot number and change in clutch and brood sizes were significant (clutch size, $F_{1,172} = 0.20$, $P > 0.6$; number of hatchings, $F_{1,158} = 0.05$, $P > 0.8$).

DISCUSSION

The present study shows that melanin-based traits present a significant change at the first molt between the first and second years of age: with females and males becoming whiter (i.e. less reddish pheomelanic), females displaying larger black spots, and males displaying fewer spots in the second year comapred with the first year. However, the extent of these phenotypic changes was not correlated with reproduction. Only at subsequent molts, when the sign and magnitude of the changes in plumage traits was not consistent among individuals, did these changes in plumage covary with concomitant changes in reproductive parameters.

COVARIATION BETWEEN CHANGES IN MELANIN-BASED TRAITS AND REPRODUCTIVE PARAMETERS

Although individuals remain similarly colored throughout their lifetime, as shown by a high repeatability of plumage traits measured in different years, we detected small between-year changes that present interesting patterns. The largest changes in plumage features were found during the first molt, between the yearling and the adult stages. Individuals of the two sexes became lighter coloured, and females became more female-like by displaying larger spots, whereas males became more male-like by displaying less spots. This kind of pattern is common in bird species where plumage usually changes after a maturation period, i.e. the bird reaches sexual maturity before its fledgling plumage has been totally replaced by the adult-specific plumage (Rohwer *et al*., 1980). This delayed maturation is usually studied in birds where yearling plumage is qualitatively different from adult plumage (see Vergara *et al*., 2009). In this case, delayed plumage maturation may present a selective advantage for juvenile survival, because juvenile plumage reduces the aggressiveness of adult individuals towards younger conspecifics (Conover, Reese & Brown, 2000; VanderWerf & Freed, 2003; Berggren, Armstrong & Lewis, 2004). Alternatively, the change in plumage may be explained by constraints arising from energy and nutritional demands that prevent yearlings from producing adult plumage before their first breeding attempt (Rohwer & Butcher, 1988). In *T. alba*, where we found a substantial quantitative change between yearling and adult plumage, we did not find evidence for this constraint hypothesis, as individuals reaching a more 'adult-like' plumage at 2 years of age (whiter, less spotted for males, and with larger spots for females) or yearlings being more 'fledgling-like', did not increase reproductive success. This plumage shift may still be favoured if fledgling-like or adult-like plumages present some other dissimilar fitness benefits at the different stages. The shift may be beneficial to reproductive status, as adult *T. alba* increased their reproductive success when they changed their plumage in the same direction as yearlings did, although we cannot exclude that this yearling shift in plumage coloration is not selected per se, but instead reflects hormonal and social changes taking place after the first reproduction (Leader & Nottebohm, 2006).

Between-year variations in plumage were less pronounced in adulthood, but the individuals that changed in the same direction as yearlings gained in reproductive success. In particular, adults that became whiter (less pheomelanic) gained in breeding success by producing larger broods. Moreover, females that became more female-like (with larger eumelanic spots) bred earlier and laid larger eggs, and males that became more male-like (with less eumelanic spots) produced larger broods (the latter relationship was only found in older males). According to these covariations, the increase in one eumelanin-based ornament is positively favoured in adult females, as well as the decrease in another eumelanin-based trait in adult males, whereas the decrease in pheomelanin-based colour is positively favoured in adults of both sexes. We found that intra-individual changes in plumage traits were not repeatable in adulthood, and were apparently not heritable, although we acknowledge that more data are needed on this issue.

Changes in plumage features and reproduction could be concomitantly regulated, or could be strategically adjusted, by individuals in order to optimize fitness (Gross, 1996). For instance, a change in condition may simultaneously modify bird plumage and breeding success (Møller, 1989; Gustafsson *et al*., 1995; Griffith, 2000). Oxidative stress, which is related to ageing, could influence both breeding potential and melanin-based traits (Galván & Alonso-Alvarez, 2008). Melanin-based plumage ornaments and reproduction may also be controlled by the same biochemical factors, like some regulators of melanogenesis (including melanocortins, glucocorticoids, steroids, glutathione, etc.) that are known to have pleiotropic effects that can affect sexual activity (Slominski *et al*., 2004; Ducrest *et al*., 2008). Alternatively, the covariation found may be causal, for instance if ornament expression influences reproductive success. This mechanism may explain why female *T. alba* that gained larger spots bred earlier the next year. Spot diameter in females has indeed been shown experimentally to affect the probability of breeding (Roulin & Altwegg, 2007) and the reproductive investment by the mate (Roulin, 1999b).

ADAPTIVE VALUE OF MELANIN-BASED TRAITS AND MAINTENANCE OF GENETIC VARIATION

In vertebrates, melanin-based traits are usually supposed to play an important role in predator–prey interactions, for instance by enhancing the degree of camouflage (Majerus, 1998). Such traits are also known to be important in social interactions as dark individuals are usually more aggressive than pale conspecifics (Ducrest *et al*., 2008). Despite that melanin-based traits play a role in mate choice (e.g. Burley, 1977; Houtman & Falls, 1994; Fox *et al*., 2002; Dunn *et al*., 2008), why and how such colour traits could signal aspects of individual quality is poorly known. Results reported in the present study provide some new information on the adaptive function of melanin-based coloration.

Although adult *T. alba* that became whiter performed better than the previous year, pheomelanin-based colour did not vary significantly with age in the population. Hence, this plumage change, although associated with an improvement in reproductive success, does not appear to be directionally selected in the population. This suggests that white and reddish-brown plumage may provide different advantages in various situations, and the absolute selective pressure on colour is still unclear. Indeed, previous studies have shown that reddish-brown males produce more offspring (Roulin *et al*., 2001), whereas white male yearlings had a higher probability of breeding than their darker nestmates, and white individuals were heavier than reddish-brown ones (Roulin, 2006; Roulin & Altwegg, 2007). These results may partly explain the maintenance of genetic variation in pheomelanin-based colour in our population through balancing selection (Losey *et al*., 1997).

Among individuals, we found that spot diameter increased with female age, whereas spot numbers decreased with male age. In females, this pattern could either result from selection of individuals that increased their spot diameter or of individuals that presented large spots. The first hypothesis could be rejected because the change in female spot diameter from one year to the next was no longer significant after 1 year in age. The second hypothesis is more likely, as spot diameter has been shown to be positively related to survival in females, but not in males (Roulin & Altwegg, 2007; Roulin *et al*., 2010). In contrast to females, the decrease in spot number with age among males may result from within-individual changes in spot number, as males lost spots throughout their lifetime. The loss of spots seemed to be favoured within an individual, as this plumage change (and not the absolute number of spots) was associated with an increase in reproductive success. Further studies are needed to determine whether the decrease in spot number with age among males may result not only from within-individual changes in spot number, but also from a higher survival of less-spotted males. Finally, these results suggest that spot diameter is positively selected in females, whereas spot number is negatively selected in males. This is an important finding that provides a potential explanation as to how genetic variation in plumage traits can be evolutionarily stable despite directional selection. These two traits are positively genetically correlated within (i.e. individuals with more spots display larger spots) and between (e.g. fathers displaying many spots produce sons and daughters with larger spots, Roulin *et al*., 2001; Roulin, 2004) sexes, thus if directional selection acts on eumelanic traits in an opposite way in the two sexes, this may create a counterbalancing selection, and explain the maintenance of a genetic variation in spottiness in this population.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article:

Table S1. Melanin-based traits in relation to age and sex for *Tyto alba* ringed as nestlings.
Table S2. Change in melanin-based plumage traits in *Tyto alba* were not significantly related to change in plumage traits in the breeding partner (Pearson correlations).
Table S3. Change in reproductive parameters in relation to change in melanin-based traits in yearling *Tyto alba*.