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Viability selection affects black but not yellow plumage colour in greenfinches

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Abstract Much of the debate surrounding the selective forces responsible for the expression of conspicuous plumage colouration is centred on the question of precisely which individual qualities are signalled by carotenoid- and melanin-based pigments. To examine this and other related issues, we performed viability selection analyses in wild-caught captive male greenfinches (*Carduelis chloris*) in Estonia during winters between 2003 and 2014. Based on our measurements, birds with a darker black eumelanin-based colouration of tail feathers survived better than those whose tail feathers had a paler black colouration. The carotenoid-based yellow colouration of the same feathers was not associated with mortality in captivity and showed much less between-year variation in the field than the black colouration. Between year-variation in the black (but not yellow) colouration of feathers was parallel in wild-grown feathers (on birds in the wild) and laboratory-grown ones (on birds held temporarily in captivity). Taken together, these findings imply that eumelanotic colouration in greenfinches is currently under selection and suggest the presence of sufficient genetic variation for a rapid response to selection. In particular, tail feathers have become darker black since the emergence of avian trichomonosis, which is known to selectively kill paler individuals.

Keywords *Carduelis chloris* · Carotenoids · Eumelanin · Plumage colouration · Survival selection

Introduction

Melanins and carotenoids are the two most common pigment types in animals. Carotenoids account for most of the red, orange and yellow colours while melanins are responsible for black (eumelanins), reddish and brown (pheomelanins) colouration. Animals synthesize melanins from the amino acids phenylalanine and tyrosine, but carotenoids must be acquired from the diet (reviewed by Hill and McGraw 2006; Jawor and Breitwisch 2003). Both types of pigments have been the focus of intensive study and controversy in terms of the factors which maintain their reliability as signals of individual quality (Badyaev and Hill 2000; Badyaev and Young 2004; Dunn et al. 2010; Griffith et al. 2006; Guindre-Parker and Love 2014; Hill 2006; McGraw 2008; Owens and Hartley 1998; Roulin 2015; Senar and Quesada 2006). The debate was initially dominated by the view that carotenoid-based pigments are sensitive indicators of individual condition, with a potential to signal foraging and/or digestive ability (Hill 1992; Tyczkowski et al. 1991) and a capacity for anti-parasite defences, detoxification and free radical scavenging (Lozano 1994; von Schantz et al. 1999). Melanin-based pigments in turn were considered to be under more strict genetic control and hence poor candidates for reliable signalling of phenotypic quality (Badyaev and Hill 2000). Recent insights into the biochemical and cellular processes responsible for pigment production have further complicated the issue. For

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example, it has recently been suggested that because genes controlling melanic traits can affect numerous other phenotypic traits, pleiotropy could also explain a linkage between individual condition and colouration (Roulin 2015).

It is well known that genes in the melanogenesis pathway, particularly those of the melanocortin system, have pleiotropic effects, and it has been suggested that these pleiotropic effects are responsible for the observed associations between melanin-based colouration, physiology, behaviour and life-history traits (reviewed by Ducrest et al. 2008, 2014; Moreno and Møller 2006; Roulin 2015). The expression levels of genes in this system are correlated with individual variation in melanism and stress hormones (Emaresi et al. 2013). For example, one of the main stress hormones, adrenocorticotropic hormone, is a cleavage product of the pro-opiomelanocortin (POMC) prohormone which also triggers the production of melanin pigments (Ducrest et al. 2008). Multiple products of POMC gene affect immune and inflammatory responses, providing a genetic basis for the phenotypic correlations between health state and melanin-based colouration. This correlation is exemplified by a recent study in female greenfinches (*Carduelis chloris*) which showed that individuals with paler eumelanin-based feather tips were more likely to die from avian trichomonosis than their conspecifics with darker black feathers (Männiste and Hõrak 2014). These findings suggest that infections can affect the evolution of eumelanin-based plumage colouration.

Avian trichomonosis is caused by the protozoan *Trichomonas gallinae*, which inhabits the upper digestive tract of birds, and it spreads through saliva. The pathogen causes necrotic inflammation of the oropharynx that eventually inhibits swallowing and leads to starvation and dehydration (Atkinson et al. 2008). Finch trichomonosis emerged first in the UK where it was responsible for a 35 % decline in breeding greenfinch numbers in 2006 (Robinson et al. 2010). The spread of epidemics to Fennoscandia coincided with a 47 % reduction of breeding greenfinch population in Southern Finland between 2009 and 2010 and a 20 % of reduction of greenfinch numbers in Sweden (Lehikoinen et al. 2013). Given the extreme pathogenicity and recent emergence of trichomonosis in greenfinches, it has been suggested that infection may have recently spilled over from another host species, leaving little time for host adaptations to evolve (Neimanis et al. 2010). However, there does seem to be variation in host resistance as virulent strains of *T. gallinae* have been often isolated from apparently healthy birds (McBurney et al. 2015).

Beginning in the winter of 2009, Estonian newspapers have been publishing occasional reports of dead and debilitated greenfinches with symptoms of trichomonosis at bird tables/feeding stations. The emergence of trichomonosis among wintering greenfinches in Estonia provided us with a unique opportunity to test whether greenfinches have evolved a darker black feather colouration since the infection has emerged as a more common avian disease that can take on epidemic proportions. Further, because the tail feathers of greenfinches contain both carotenoid-based yellow and eumelanin-based black pigments, this model allows us to compare the selection and between-year variation of both pigment types. The yellow colouration is interesting in this context because carotenoids modulate immune function (Chew and Park 2004; Møller et al. 2000; Simons et al. 2012). However, the answer to the question of whether carotenoid-based colouration has evolved to signal infection resistance is still disputed (Hill 2011, 2014; Svensson and Wong 2011; Vinkler and Albrecht 2009).

In the study reported here we relied on data collected on wintering male greenfinches captured in Estonia between 2003 and 2014 and on data collected from skins of male greenfinches kept in Zoological Museum of Tartu University which had been collected in the twentieth century. Greenfinches are sexually dichromatic seed-eating passerines that display a variety of carotenoid-based plumage patches, ranging from bright yellow to olive. The colour (chroma) of the contrasting yellow tail patch is sexually dimorphic and strongly correlates with the level of plasma lutein (Hõrak et al. 2010; Peters et al. 2008) and the concentration of canary xanthophylls in feathers (Saks et al. 2003). Yellow tail and wing feathers have eumelanin-based black tips (Hõrak et al. 2010). At capture, we collected one of the outermost tail feathers from each bird and subsequently collected replacement feathers from the same birds that were grown during an approximately 2-month period spent in an aviary (e.g. Hõrak et al. 2010). This approach enabled us to measure the colouration of both wild-grown feathers and those grown under standardized conditions of captivity. Altogether, 30 captive birds died during the 5 years of the study period, which enabled us to compare their plumage colouration with that of survivors.

The aims of this study included (1) to test whether the selection against birds with lighter eumelanin-based colouration, recorded in females in 2013 (Männiste and Hõrak 2014), could also be detected in a larger sample of male greenfinches measured over a decade; (2) to determine whether this selection, if present, would lead to a detectable response of selection; specifically, we predicted that greenfinches wintering in Estonia have become darker since the emergence of the avian trichomonosis epidemic in 2009; (3) to determine, assuming selection against birds with lighter eumelanin-based colouration, whether yearling birds appeared to be lighter in colour than older ones due to selective disappearance. Because older birds have been exposed to selection for a longer time, our expectation was that light-coloured individuals would be underrepresented among older cohorts. We predicted that in such a

case, the both wild-grown feathers (grown on birds in the wild) and laboratory-grown feathers (grown on birds held temporarily in captivity) of yearling birds would be lighter in terms of black colouration than those of older birds. (4) In addition, because a previous prediction assumed a high phenotypic canalization of eumelanin-based colouration (Roulin and Ducrest 2013), we checked the validity of this assumption by testing for the presence of a positive correlation between the black colouration of the wild-grown and laboratory-grown feathers of the same individual. Due to a lack of information on the link(s) between trichomonosis-induced mortality and carotenoid-based yellow colouration we could not make specific predictions on the between-year changes of the yellow tail patches of greenfinches. However, data available during the study period enabled us to test the general hypothesis that carotenoid-based colouration is a condition-dependent indicator of viability (Hill 2014; Hill and Johnson 2012). (5) We thus predicted that those birds with a higher value of saturation (chroma) of yellow (an indicator of blood and feather carotenoid content) would generally be better survivors both before and after the emergence of the avian trichomonosis epidemics. Lastly, we predicted that older birds would have more saturated yellow feathers, either because of selective mortality or due to an age-related increase in the ability to deposit carotenoids into feathers (e.g. Evans and Sheldon 2012).

Methods

Geographic field sampling

Greenfinches which overwinter in Estonia are a mixture of local birds and those of Fennoscandian origin. In the period ranging from 2001 to 2009, one ring recovery from Norway, three from Sweden and 17 from Finland were recorded in Estonia (Olavi Vainu, Estonian Ringing Centre, personal communication). The birds used in our study for various aviary experiments were captured in January or February of each year between 2003 and 2014 on the Island of Saaremaa and/or in South-eastern Estonia. The birds (different individuals) were captured as soon they started to accumulate at feeders after first cold spells, and in each year and at each study location the birds were captured in the same sites and using the same methods. Detailed information on the sites of capture and references describing aviary conditions and procedures are provided in Electronic Supplementary Material (ESM) Table S1.

Museum samples

Feather samples were obtained from skins of 21 greenfinches kept in Zoological Museum of Tartu University which had been collected at ten different locations in Estonia during the last century [1924 (2), 1926 (1), 1928 (2), 1929 (2), 1935 (1), 1938 (1), 1948 (2), 1949 (2), 1950 (2), 1951 (2), 1952 (4)]. Neither black ($r = 0.18$, $P = 0.440$, $N = 21$) nor yellow colouration ($r = 0.16$, $P = 0.475$, $N = 21$) significantly correlated with year of collection. Therefore, we pooled all data from these samples into a single sample. These skin samples from museum collections had been stored in drawers where they were protected from the light.

Aviary protocol

Captured birds were brought to an indoor aviary in Tartu where they were housed in individual cages ($27 \times 51 \times 55$ cm) with sand bedding and aged (yearlings vs. older birds) according to Svensson (1992). All birds were supplied ad libitum with sunflower seeds and filtered tap water and held on a natural daylength cycle using artificial lightening. They were released back into their natural habitat after about 2 months in captivity. A few days after each bird was placed in the aviary, we plucked the left outermost tail feather; subsequently, after 52 days, we plucked/collected the replacement feather that had grown when the bird was in captivity. The collected feathers were stored in air-tight plastic bags in the dark.

Colour measurement

Plumage colouration was measured using the spectrophotometry method (model AvaSpec-2048-2 with DH2000 UV–VIS-NIR light source; Avantes BV, Apeldoorn, the Netherlands) described in detail by Hõrak et al. (2010). Yellow colouration was measured from the inner and outer vanes of the feathers (Fig. 1) and characterized on the basis of chroma, which describes the shape of the reflectance curve. Black colouration was measured from the inner and outer vanes of the feather tips (Fig. 1) and characterized on the basis of summed reflectance (brightness) over the bird visible spectrum of 326–700 nm (see Männiste and Hõrak 2014). Brightness reflects the eumelanin content of feathers: the darker the feathers, the higher the eumelanin content and the lower the brightness (Hõrak et al. 2010). All measurements were performed in the autumn of 2013 and (for a dataset from the last year) in the spring of 2014 using the same spectrophotometer and light source. We consider it particularly important that the same spectrophotometer and light source were used as we have noticed that changing the colour measurement equipment can have a systematic effect on measures of colouration. For that reason we lack the measurements from 2011 to 2012 (feathers from those years had been measured in earlier studies using different equipment and, thereafter, the feathers were grinded to determine corticosterone content).

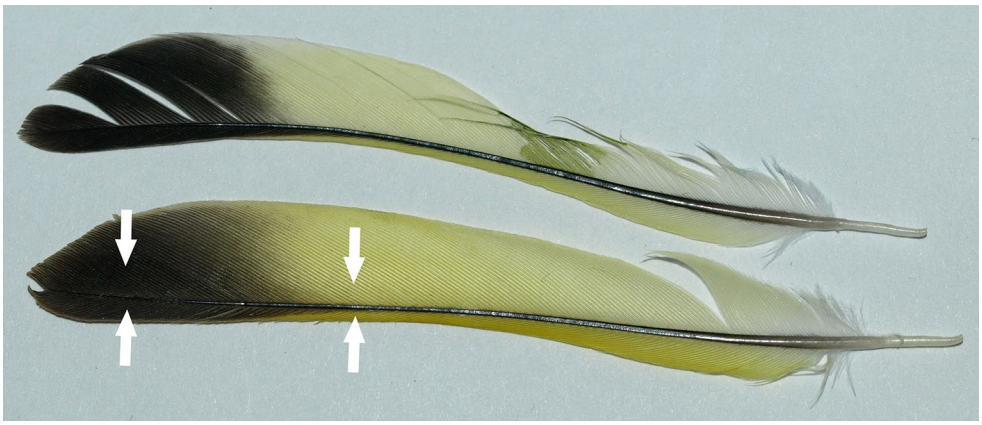

Fig. 1 Outermost tail feathers of male greenfinches. *Arrows* indicate the approximate positions of color measurements. *Lower feather* Wild-grown feather, *upper feather* lab-grown replacement feather of the same individual

Table 1 Effects of eumelanin-based black and carotenoid-based yellow plumage colouration on the survival of captive wild-caught greenfinches (*Carduelis chloris*) in a model controlling for the effects of study year and age

Effect	*df*	*F*	β (SE)	*P*
Brightness of black	1365	4.93	−0.0012 (0.0005)	0.027
Yellow chroma	1365	0.03	−1.4791 (8.6986)	0.865
Year	9365	0.44		0.914
Age[a]	1365	0.06	0.1038 (0.4383)	0.813

SE, Standard error

Logistic regression was performed using the SAS GLIMMIX procedure, type III tests

Effect of study period (before vs. after emergence of avian trichomonosis epidemic) and its interactions with colour variables appeared to be non-significant ($P = 0.8$–1; see ESM Table S4)

[a] Age effect: comparison of yearlings against older birds

Within 2 years we had captured birds from different sites which enabled us to test whether the location of the capture sites affected plumage colouration. This was not the case. Birds captured in 2008 in Saaremaa and Tartu had similar reflectance of black [summed relative reflectance units 2688 ± 389 ($N = 28$) vs. 2604 ± 290 ($N = 13$); $t = 0.69$, $P = 0.496$] and chroma of yellow [0.22 ± 0.03 ($N = 28$) vs. 0.21 ± 0.03 ($N = 13$); $t = 0.46$, $P = 0.647$]. Birds captured in 2009 in Elva and Tartu had a similar reflectance of black [2257 ± 389 ($N = 16$) vs. 2365 ± 378 ($N = 14$); $t = 0.89$, $P = 0.383$] and chroma of yellow [0.21 ± 0.03 ($N = 16$) vs. 0.22 ± 0.01 ($N = 14$); $t = 1.80$, $P = 0.082$].

Statistical analyses

Values of colour variables were normally distributed, thereby enabling the use of parametric tests. Effects of study year and age (yearling vs. older) on colour variables were tested in general linear models which treated years and age as factors, using Statistica software (v. 10, StatSoft Inc. Tulsa, OK). The dataset on the feather samples from the museum skin specimens was pooled into a single data point (year). Exclusion of this data point from the analyses of variation in plumage colouration did not affect the significance of the results. The occurrence of pairwise differences between years was confirmed with Tukey's post hoc tests for unequal sample sizes. In the case of black colouration, there were too many significant pairwise differences to enable their graphical presentation. Between-year variation in the yellow colouration of lab-grown feathers was not analysed because in most of the study years birds were subjected to manipulations which would have affected plasma carotenoid levels and, consequently, yellow chroma. In 2009, the black colouration of lab-grown feathers was physiologically manipulated in a subset of individuals (Hõrak et al. 2010); feathers from these birds were discarded. The effects of colouration on survival were analysed by logistic regression (PROC GLIMMIX, SAS/STAT version 9.2; SAS Institute Inc., Cary, NC). All models were tested for the main effects of age (yearling vs. older) and year. Because avian trichomonosis became prevalent in Estonia in 2009, we also tested whether the black tips of tail feathers of greenfinches had become darker after 2008. For this analysis we used mixed models in the SAS GLIMMIX procedure with year as a random effect. Denominator degrees of freedom for type 3 tests of fixed effects were estimated using the Kenward–Roger method. Means are reported with ± standard deviation (SD). All tests are two-tailed, and a P level of <0.05 was the criterion for significance.

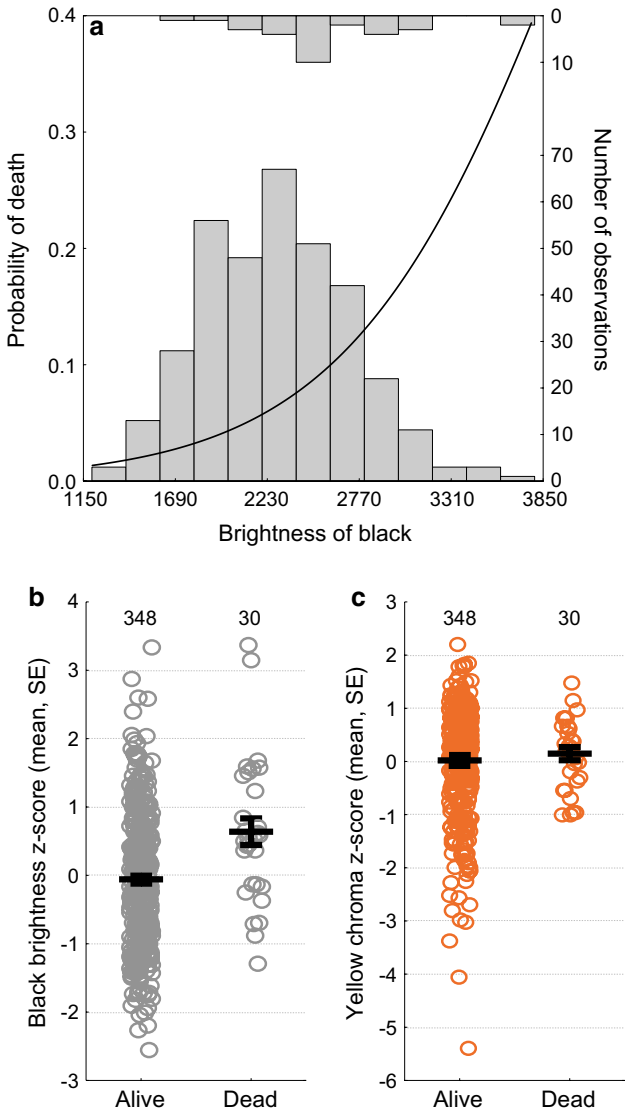

Fig. 2 **a** Probability of death in relation to brightness of black colouration. *Solid curved line* Logistic regression line. **b**, **c** Comparison of black plumage colouration (**b**) and yellow plumage colouration (**c**) of greenfinches (*Carduelis chloris*) which survived or died in captivity. *Numbers at top of graphs* Sample sizes

Results

The probability of dying increased with paler melanin-based colouration (Table 1; Fig. 2a). Feathers of the birds that had died in captivity were on average 11.7 % (0.69 SD units) paler than those of survivors (Fig. 2b), while there was no directional selection on carotenoid-based colouration of the same feathers (Fig. 2c). Mortality was independent of age and year (Table 1) and study period (i.e., before vs. after emergence of avian trichomonosis in 2009; ESM Table S4).

Table 2 Effects of study year and age (yearling vs. older) on black (brightness) and yellow (chroma) colouration of tail feathers of greenfinches

Tail feather[a]/colouration	Source	*df*	*F*	η^{2} [b]	*P*
Wild-grown/black	Year	10,387	22.9	0.37	<0.00001
	Age	1387	16.9	0.04	0.00005
Laboratory-grown/black	Year	8284	12.5	0.26	<0.00001
	Age	1284	1.2		0.231
Wild-grown/yellow	Year	10,387	2.5	0.06	0.007
	Age	1387	0.5		0.113

[a] Wild-grown feathers are those grown on birds in the wild; laboratory-grown feathers are those (re-)grown on birds held temporarily in captivity

[b] η^2 is the coefficient of partial determination, describing the proportion of total variation attributable to the predictor variable, partialling out other factors from the total non-error variation

Both black and yellow colouration of wild-grown feathers showed significant between-year variation (Table 2; Fig. 3). However, the proportion of variance explained by year was about sixfold higher for the black colouration than for the yellow colouration ($\eta^2 = 0.37$ vs. 0.06). Further, none of pairwise comparisons between years was significant in the case of yellow chroma in the post hoc tests, while for black colouration, 24 of 55 pairwise contrasts were significant at *P* values ranging from 0.011 to 0.00012 (ESM Tables S2, S3). In a model accounting for the effect of age ($F_{1,392.6} = 19.1$, $P < 0.0001$) and year as a random effect, the black tips of tail feathers had became darker since 2008, i.e. after avian trichomonosis became prevalent in Estonia ($F_{1,11.92} = 13.6$, $P = 0.0032$). In a similar model, yellow colouration did not change after 2008 ($F_{1,7.81} = 1.9$, $P = 0.208$).

Black tips of wild-grown feathers of yearlings were generally paler than those of older birds (Table 2; ESM Fig. 1a). Between-year variation in the black colouration of laboratory-grown feathers was parallel to that of wild-grown feathers (Fig. 4). Age did not affect the black colouration of laboratory-grown feathers nor the yellow colouration of wild-grown feathers; Table 2; ESM Fig. 1b). Black colouration was consistent between moults in individual birds, while the yellow colouration was not (Fig. 5). The colouration of yellow and black parts of the same feathers did not correlate ($r = -0.03$, $P = 0.546$, $N = 399$).

Discussion

Viability selection on plumage colouration has been seldom been measured (Keyser and Siefferman 2005; but see Roulin et al. 2010). The results of our study are specific

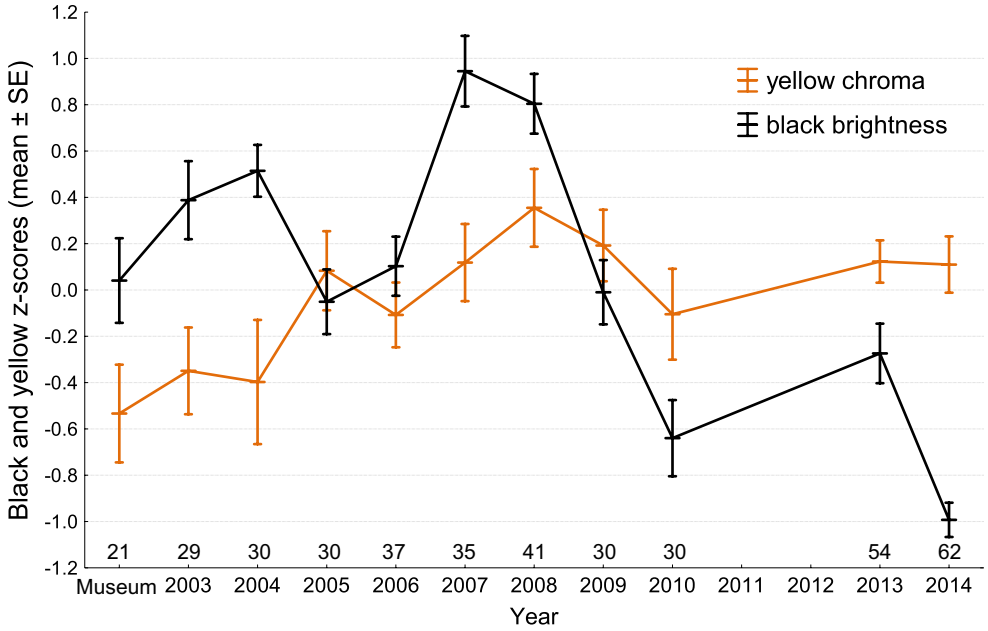

Fig. 3 Between-year variation in yellow and black plumage colouration of wild-grown feathers of greenfinches. Z-scores are calculated over the whole study period. *Numbers* Sample sizes. *Museum* Samples from skins of greenfinches in museum collections, *SE* standard error. See Table 2 for the *P* values

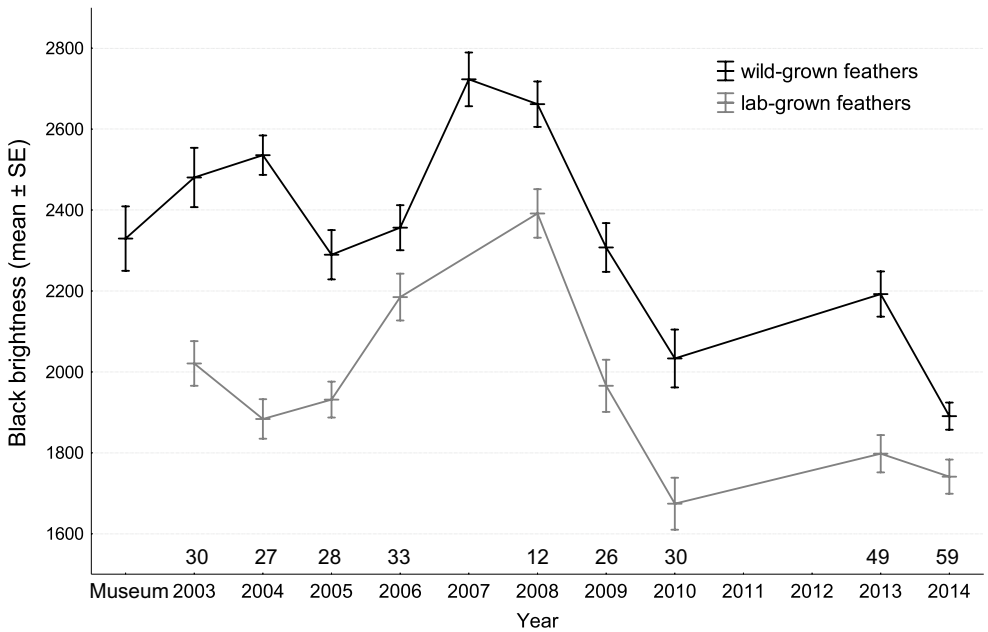

Fig. 4 Between-year variation in black plumage colouration of wild-grown (*upper line*) and laboratory-grown (*lower line*) feathers of greenfinches. *Numbers* Sample sizes for laboratory-grown feathers. Sample sizes for wild-grown feathers are given in Fig. 3. See Table 2 for the *P* values

because we measured survival selection of wild-caught birds under standardized aviary conditions. This experimental set-up clearly allowed us to exclude selection due to predation, traffic and windows, enabling us to distinguish internal causes of mortality from external ones. This is an important criterion because one might expect that diseased individuals are especially vulnerable to predation in the wild (Møller and Erritzøe 2000). Further, our data are free of the problem of distinguishing mortality from dispersal that usually complicates any measurements of

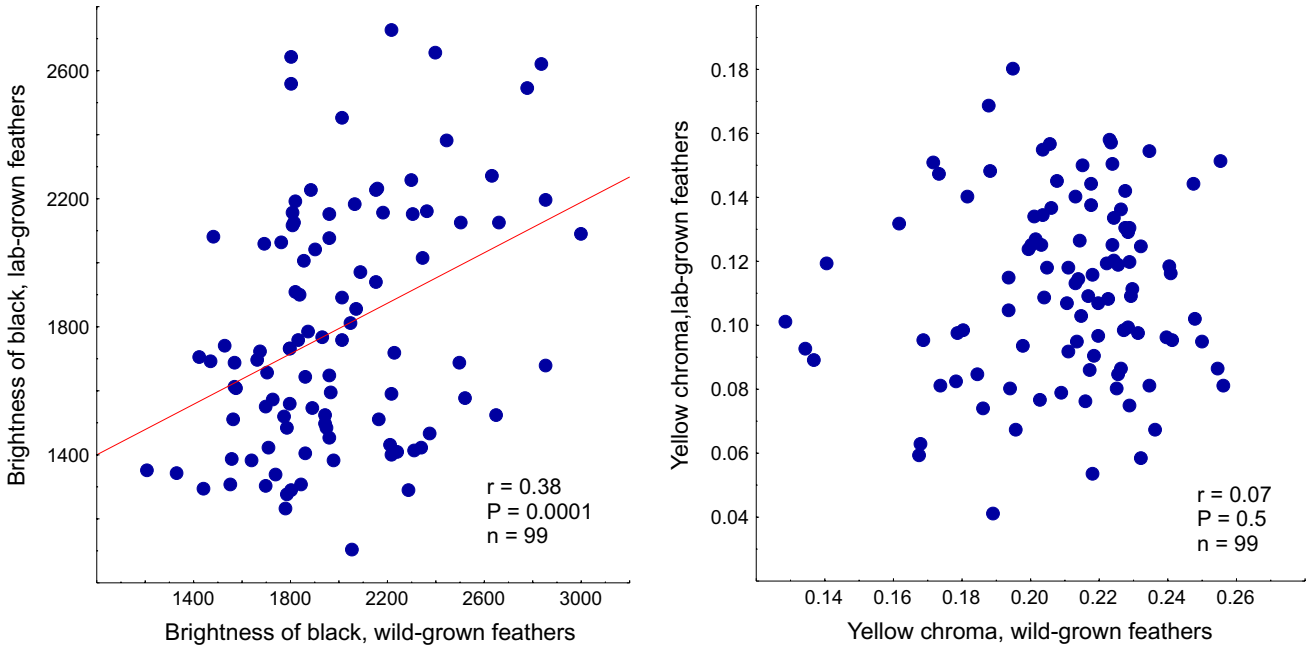

Fig. 5 Correlations between colouration of the wild-grown and lab-grown feathers of greenfinches. Data are from 2008, 2010 and 2014. The *line* is a linear regression line

survival selection in the wild. The design of our study is thus particularly suitable for the detection of selection on those plumage characteristics which reflect differences in the physiological make-up of individuals.

Black colouration

Our findings demonstrated a strong viability selection against wild-caught male greenfinches with paler eumelanin-based plumage. The black tips on the tail feathers of survivors were on average 12 % (0.7 SD) darker than those of birds which died. This finding is in the same direction as the colour difference of female greenfinches which died in captivity with symptoms of avian trichomonosis in the winter of 2013, although the magnitude of difference (22 %, 1.5 SD) was higher in the case of females (Männiste and Hõrak 2014). In both studies, mortality was independent of age. We were unable to determine the exact cause(s) of death for all of the birds in our study because those dying between 2006 and 2008 were not examined for the specific symptoms of avian trichomonosis. We also do not know exactly when the trichomonosis epidemics began to reach Estonia, but we consider it likely that it coincided with a documented spread in Finland and Sweden because greenfinches of Fennoscandian origin winter in and migrate through Estonia. The disease was first documented in Finland in 2008 (Lehikoinen et al. 2013). It thereby remains possible that avian trichomonosis is responsible for the deaths of the greenfinches in our study as early as 2008 or 2006 when the epidemics started to spread out of UK (Lawson et al. 2011).

In this context, a most interesting finding of our study is the between-year dynamics of black colouration (Figs. 3, 4). Birds captured in 2007 and 2008 were generally paler than those caught before or after that period, with the birds caught in 2014 having the darkest appearance ever recorded. One possible explanation for this colour change is that the spread of the avian trichomonosis epidemics elicited a microevolutionary change towards a darker eumelanotic colouration of feather tips since 2009. This explanation is consistent with a trichomonosis-induced selection against lighter coloured female greenfinches detected in winter 2013. However, such reasoning would not explain why greenfinches captured before 2007 and 2008 appeared to be darker than those caught during these two years, respectively. In this context it is also notable that we did not detect a change in the direction of survival selection against light-coloured birds in captivity since 2009 as the interaction term between black colouration and the study period (before 2009 vs. since 2009) was not significant ($P = 0.95$; ESM Table S4). The data from our study thus point to a temporally fluctuating—rather than persistent or recently emerged—selection against less eumelanotic individuals. An alternative explanation would be environmentally induced fluctuation in the eumelanotic colouration. Under such a scenario, yearly variations in the availability of precursors of eumelanin, tyrosine, an

essential amino acid phenylalanine or some cofactor of melanin biosynthesis (copper, calcium) would be responsible for the between-year variation in the darkness of the black colouration. However, the evidence in favour of this latter explanation is scarce and supported only by the results of a laboratory study which demonstrated that a severe (50 %) reduction in the dietary availability of phenylalanine and tyrosine increased the reflectance of black plumage in house sparrows *Passer domesticus* (Poston et al. 2005). It is currently unknown whether the micronutrients required for melanin synthesis would ever be limiting in the wild (McGraw 2008).

High between-year variation in black colouration is generally consistent with a notion of spatially and/or temporally fluctuating selection pressures maintaining the heterogeneity of melanin-based colouration between and within populations (Emaresi et al. 2014; Piault et al. 2009; Récapet et al. 2013; Roulin 2004, 2015). The degree of melanin-based colouration frequently covaries with life-history, morphological, behavioural and physiological traits, with the sign and magnitude of these correlations fluctuating in space and over time (Roulin 2004). In barn owls (*Tyto alba*), heritable eumelanotic colouration has been shown to reflect ectoparasite resistance (Roulin et al. 2001). In this framework, it is possible that darker greenfinches also are not just more resistant to avian trichomonosis but also to other temporally occurring infections. For example, diverse derivatives of the melanogenesis pathway reduce acute, allergic and systemic inflammation and septic shock (reviewed by Catania et al. 2010; Ducrest et al. 2008; Gangoso et al. 2011). Inflammation, leading to terminal septicaemia is symptomatic to *T. gallinae* infection (e.g. Neimanis et al. 2010), possibly suggesting that paler greenfinches (i.e. the ones with suppressed expression of genes involved in eumelanin production) are more likely to die due to their over-responsive inflammatory defences accompanying low eumelanin production. Yet a similar reasoning would apply to any pathogens which kill their hosts through the induction of overly strong inflammatory responses, such as salmonellosis caused by the *Salmonella* bacterium (Giovannini et al. 2013) which has been described as a major cause of death of greenfinches in UK before the emergence of the avian trichomonosis epidemics (Lawson et al. 2010).

It is also possible that greenfinches with paler eumelanotic tail colouration appeared to be more susceptible to stress (Kittilsen et al. 2012) associated with being brought into captivity than their darker conspecifics. In this scenario, stress-induced immune suppression would result in pale individuals being more susceptible to infections. Alternatively, birds with light eumelanotic colouration would die in captivity because they appear to be intolerant to captive conditions due to excessive stress responsiveness to a novel situation. One might further speculate that if the darker individuals are more dominant (see Kittilsen et al. 2012; Roulin 2015), they might require less time for satisfying their food requirements at feeders than less eumelanotic and less dominant birds. In this scenario, paler black birds would be at higher risk of infection because they have to spend more time in contact with contaminated food and water sources. Such a mechanism would also work for other infections which are spread at bird feeders, such as salmonellosis.

Our prediction that selection against birds with lighter eumelanin-based colouration results in age differences of feather colouration among both wild-grown and laboratory-grown feathers was not supported. Although the wild-grown feathers of yearlings were generally paler than those of the older birds, this age difference disappeared in feathers grown by captive birds in the laboratory under standardized conditions. This result is notable in the context that eumelanin-based colouration revealed significant phenotypic canalization, so that the colour of wild-grown feathers predicted that of laboratory-grown feathers (Figs. 4, 5). As such, this finding suggests that age-related differences in black colouration are caused by individual-level changes in pigmentation with age rather than selection. One possible explanation would be different timing of the autumnal moult of yearling and older birds which would result in differential exposure to external stressors and/or an oxidative milieu. An extensive study of moulting greenfinches in England found that yearling males started their moult on average 6 days earlier than older birds (Newton and Rothery 2005). Given that mean duration of the primary moult was 100 days, however, it would be difficult to imagine any seasonal factors related to moult that would differentially affect the plumage colouration of yearling and older birds. In this context it is notable that laboratory-grown feathers of all birds appeared to be darker than wild-grown feathers (Fig. 4). This points to the possibility that either the birds in captivity were relieved of some external constraint(s) on developing dark-black colouration that normally occurs in the wild or that captive conditions led to generally reduced organismal glutathione levels (Hõrak et al. 2010), promoting enhanced eumelanin production.

Yellow colouration

Carotenoid-based yellow chroma did not predict the survival of greenfinches in captivity. Yellow colouration showed also much less between-year variation than did the black colouration. Further, older birds did not appear consistently more yellow (in terms of carotenoid-based chroma) than yearlings. These findings suggest that carotenoid-based colouration was a poor predictor of viability or individual performance and showed little variation among the cohorts of birds under the conditions of our study. The relatively low between-year

variation in the yellow colouration resembles that expected under stabilizing selection. For example, because the yellow tail patch of male greenfinches is used in sexual display (Cramp and Perrins 1994), it remains possible that expression of this trait is maintained by the balance of sexual selection and predation on the most conspicuous individuals.

Conclusions

Our measurements of viability selection on the plumage colouration of captive wild-caught greenfinches revealed that black colouration predicted survival while yellow colouration did not. We also detected a strong between year-variation in black colouration which was strikingly parallel in wild-grown and laboratory-grown feathers. Altogether, these findings suggest that eumelanotic colouration in greenfinches is currently under selection and that this colouration has some genetic basis. In particular, tips of the tail feathers have become darker since the emergence of avian trichomonosis epidemics that is known to selectively kill paler individuals. It is thus possible that we have documented a rapid evolutionary change in a visual character of a common vertebrate species. Our findings suggest that at least under some circumstances, survival selection may drive the evolution of eumelanin-based colouration via pleiotropic effects on physiological traits.

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Author contribution statement PH and MM conceived the study; MM performed the measurements of plumage colouration; PH & and MM analysed the data; PH wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

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