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Low evolutionary potential for blue-green egg colouration in a wild bird population

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Abstract

Exploring the evolutionary architecture of female sexual traits and their potential evolvability is important to understand their possible role as postmating sexual signals. Egg colouration has been proposed to be one of these postmating sexual signals, honestly advertising female quality in birds, especially in blue-green laying species. In this study, we used an animal model in a Bayesian framework to estimate the evolvability of multiple descriptors of blue-green egg colouration and egg size in a wild long-term monitored population of spotless starlings (*Sturnus unicolor*). Our results show low to moderate heritability ($h^2 = 0.31–0.44$) for 3 egg colour descriptors (blue-green chroma, chroma, and lightness) and egg size. Using the coefficient of additive genetic variance (CV_A) and the evolvability (I_A) as proxies of evolutionary potential of all components of this trait, we found low values of CV_A for all these variables, suggesting a small evolutionary potential of these phenotypic traits, contrasting to previous results reported in another blue-green egg laying species. Our results indicate a modest raw genetic material of this trait on which sexual selection can act upon and, therefore, a small probability for these traits to respond easily to selection.

Keywords: quantitative genetics, animal model, sexual selection, eggshell coloration, spotless starling

Introduction

The origin and maintenance of numerous conspicuous traits are usually interpreted by evolutionary ecologists as a result of sexual selection (Andersson & Iwasa, 1996). Many of these characters involve the incorporation of pigments in the skin, feathers, iris, and beak, and they are predominantly bright colours shown by males, often concerning multiple traits appearing in combination (Møller & Pomiankowski, 1993) and used both in mate choice by females and male–male competition. Although comparatively less studied, females are subjected to mate choice by males too (Amundsen, 2000; Hill, 1993; Lyu et al., 2017), providing indirect and direct benefits to males (Martinez-Padilla et al., 2012) and favouring the evolution of conspicuous characters in females of some species. Recent research shows that female ornaments can be as informative as males’ (Hernández et al., 2021) and are not simple evolutionary by-products of sexual selection acting on male sexual traits (Amundsen & Pärn, 2022). Selection on female signalling traits can occur before mating (pre-mating) during mate choice. But sexual selection may also operate after mating. Typical examples of this so-called postmating sexual selection happen primarily at the zygotic level, in the form of sperm competition or sperm selection (Birkhead, 1987). However, they may also take the form of differential investment of males in reproduction with the current female partner.

Among female birds, examples of postmating sexual traits are nest size (Jelínek et al., 2016) or nest decorations (García-Navas et al., 2015; Polo & Veiga, 2006). In addition, eggshell colouration has been proposed to be a postmating sexual signal (Moreno & Osorno, 2003). According to the sexually selected egg colouration hypothesis (Moreno & Osorno, 2003), eggshell colour intensity would act as a condition-dependent trait, thus indicating the quality of the laying female to the male and the expected gain in fitness that would be obtained through an increased level of paternal care. In species where parental care is performed mostly by females or in polygynous species, expressing an enhanced version of this phenotype would imply a cost to females, pushing their partners to invest more in the current breeding attempt. Nevertheless, differences in egg colour in a cavity nest might be difficult to detect. However, this has been shown to be possible under certain light conditions (Avilés et al., 2006, 2011). To be an evolutionary selected trait, these traits should convey information about the quality of the female, which should give the male a fitness benefit when choosing more attractive females. In addition, theoretical models have shown that postmating sexual signals can evolve in females (Lyu et

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al., 2017), provided that there is variation in male quality and that male preference provides direct benefits to the chosen female (Fitzpatrick & Servedio, 2016), such as those obtained by parental care. However, the evolutionary potential of such traits can only be quantified by exploring their genetic basis.

Overall, the evidence for a role of egg colouration as a sexual signal is inconclusive to this day (Hodges et al., 2020; Randall & Dawson, 2018), and empirical and experimental support for its hypothetical role as a maternally derived resource is still pending (Morales, 2020). A significant additive genetic component of eggshell colouration should be substantial to evolve (Moreno & Osorno, 2003). Previous studies investigating the genetic basis and the inheritance of egg colouration are scarce and focus on diverse aspects of egg pigmentation and appearance (Francesch et al., 1997; Gosler et al., 2000; Höltje et al., 2016; Morales et al., 2013). To the best of our knowledge, there is only a single study investigating the additive genetic variance of blue-green colouration of eggs in a wild population of birds, namely pied flycatchers (*Ficedula hypoleuca*) (Morales et al., 2010). Through REML models, Morales et al. calculated the heritability, the additive genetic variance (V_A), the coefficient of additive genetic variance (CV_A), and other sources of variance of three colour measurements of blue-green (blue-green chroma [BGC], chroma, and lightness) in eggs of pied flycatchers. They found significant heritability for some components of egg colour (especially for intraclutch variation in lightness and BGC, and mean BGC), along with some relatively high levels of evolvability in other egg colour components. Though relevant, a single study in a single population of a given species seems limited evidence to assess the genetic basis of a trait (biliverdin-based eggshell colouration) that is widely distributed along the Class Aves. Addressing the genetic architecture of this trait in other species (specially in those for which empirical evidence supporting a signalling role has been provided) is therefore necessary to test the generality of this pattern.

Here we analyse the genetic architecture of chromatic and achromatic colour components (blue-green chroma, chroma, and lightness) and size (volume) of the blue-green eggs of a wild population of spotless starlings. Previous field studies have shown that males of this species respond to increased egg colouration by increasing parental care, consistent with the predictions of the sexually selected eggshell colouration hypothesis (Soler et al., 2008), thus making it an ideal study species for addressing this topic. Apart from egg coloration, we also considered egg size in our analyses because it is one of the most directly interpretable traits linked to the reproductive capacity of the female, and, given its effect on fitness, it can be considered a proxy for prenatal maternal investment (Smith et al., 1993). After building a social pedigree on eight years of field data, we use the animal model in a Bayesian framework to calculate the heritability, evolvability, coefficient of additive genetic variance, repeatability, and the components of total phenotypic variance for different egg colour components and size to disentangle their evolvability. Given previous findings in other species (Morales et al., 2010), we expect to find a low to moderate evolutionary potential for blue-green egg colouration.

Methods

Data collection

We monitored the breeding of a population of spotless starlings (*S. unicolor*) from 2012 to 2019 nesting in 250 nest boxes in an open woodland in Soto del Real, central Spain, mainly composed of oaks (*Quercus pyrenaica*) and ashes (*Fraxinus angustifolius*), and subject to cattle grazing. The spotless starling is a nonmigratory, facultative polygynous species that produces up to two broods per season, the first one in March/April and the second one in May/June. The nest is constructed primarily by the male, and both pair members defend it from intruders. Each female lays an average of 4.5 eggs of a blue-green colour that incubates for about 11 days.

At the beginning of every season, from early March until about a week before the first eggs were found, we captured the pairs roosting in the nests before sunrise, or when they came in the morning to prepare the nest. Bird identities of previously captured individuals were confirmed by both the ring number and the unique code of a passive integrative transponder tag (PIT-tags: Trovan Ltd., Douglas, UK). Previously unmarked individuals were ringed and PIT-tagged by inserting the transponder under the skin of the upper third of the back. Later in the season, fledglings were similarly marked to ease their future identification as recruits.

Once pairs were established and the eggs laid, mothers were identified at night during incubation through a medium-distance PIT-tag reader (GR-250, Trovan Ltd., Douglas, UK) allowing us to unambiguously assign each clutch to a female.

Most spotless starlings are monogamous, but minor levels of polygyny also occur, and extra-pair paternity (EPP) and intraspecific clutch parasitism are not uncommon (Celis et al., 2021). The identity of the social fathers was determined by a combination of PIT-tag monitoring sessions before breeding and during nestling rearing through an antenna installed at the entrance of the nest and connected to a PIT-tag reader (Trovan Ltd, Douglas, UK). The male providing the highest number of visits to the nest during brooding was assigned as the father. If male feedings were lower than 20% of those provided by the female, no male was assigned as a father since we assumed that an un-tagged bird was attending the brood. A small subset of genetic data for 2013–2016 confirmed that our assignments were highly reliable (Gil et al. in prep). These data offer a similar picture of EPP, also consistent with previous studies for this population (Celis et al., 2021): EPP was found in 34.3% of broods, but only 11.7% of nestlings were extra-pair young. Although precise extra-pair parentage should be considered when building a social pedigree, quantitative genetics methods are robust to rates of EPP lower than 20% (Charmantier & Réale, 2005). Given that we found much lower levels of EPP than those considered problematic, we can trust that our social pedigree closely reflects real parentage and can be used in the animal model. Brood parasitism was less common, being found in only 12.5% of broods and in 3.5% of nestlings (Gil et al. in prep). We decreased the introduction of potential errors due to brood-specific clutch parasitism by eliminating from the database those eggs identified as parasites when found in the nests. Eggs were identified as parasites when different in colour and/or size from the other eggs in the clutch or when two new eggs were found on the same day of the laying sequence. All eggs that could not be unequivocally assigned to identified starlings were not considered in any of the analyses reported here. On the basis of the inferred identities of mothers (dams) and fathers (sires), we built a social pedigree used for the quantitative genetic analyses (see below).

Egg colour and size measurements

When the first eggs were found in a given nest, we checked the nest every day (seasons 2012–2014 and 2019) or every two days (seasons 2015–2018) in order to determine laying date and follow the laying order. Five days after the first egg was laid, each egg was measured in a shady area around the nest with a portable spectrophotometer (Minolta CM-2600d) that recorded the reflectance in the range 360–700 nm at 10 nm steps. Their length and width were measured at the widest point with a digital calliper (Mitutoyo Absolute, Kawasaki, Japan, precision = 0.01 mm). Our analysis of a subset of eggs measured twice for a different study reported high repeatability for egg size measurements ($r = 0.96$, $t = 47.8$, $p < 0.001$, D'Arpa et al., 2022).

From the reflectance spectra, we obtained the values of BGC, chroma and lightness. BGC is the proportion of reflectance in the blue-green part of the spectrum over the total reflectance ($R_{400\text{–}570}/R_{360\text{–}700}$). Biliverdin peak of reflectance falls in the same region of the spectrum (Falchuk et al., 2002), and biliverdin content has been found to correlate positively with BGC in eggs of this species (López-Rull et al., 2008). Chroma (also known as saturation) is the degree of spectral purity of a colour and is a function of how sharply reflectance changes with wavelength (Endler, 1990). We calculated chroma according to the segment classification method as $\sqrt{(R-G)^2+(Y-B)^2}$, where R, G, Y, and B are the relative lightness of each colour segment of the spectrum (respectively, $R = R_{620\text{–}700}/R_{360\text{–}700}$, $G = R_{470\text{–}550}/R_{360\text{–}700}$, $Y = R_{550\text{–}630}/R_{360\text{–}700}$, and $B = R_{400\text{–}480}/R_{360\text{–}700}$) (Endler, 1990). Lightness is the achromatic component of the colour, and it is equal to the integral of the radiance of the considered spectra. It is calculated as the sum of the values of the radiance between 360 and 700 nm: $\sum_{\lambda 360}^{\lambda 700} R$. Finally, from the measurements of length and width of each egg, we calculated egg volume (mm^3) using the formula: 0.51 × length × width^2 (Hoyt, 1979).

The final database consisted of 11,002 eggs, laid by 962 females through eight breeding seasons (2012–2019) in 2,401 different clutches.

Data analysis

We used animal models to quantify the additive genetic variance of egg colour and size measured as described above. The animal model is a particular generalized linear mixed model in which a matrix of relatedness is included in the model as a random term (de Villemereuil et al., 2018). The advantage of the animal model is twofold. First, the use of a complex pedigree allows a comparison between distant relatives, which results in a better estimation of the similarity effects due to shared environment, that may otherwise be mistreated as shared genes (Postma & Charmantier, 2007). Second, it allows a robust differentiation between additive genetic (V_A) and nonadditive sources of variance (Wilson, 2008). This allows a more precise calculation of heritability (h^2) (Houle, 1992), evolvability (I_A), and the coefficient of additive genetic variation (CV$_{A)}$ (Garcia-Gonzalez et al., 2012), which are different measures of evolutionary potential. CV$_A$ and I_A are the mean-scaled measure of evolvability and are more suitable than h^2 for comparisons between populations and/or traits (Garcia-Gonzalez et al., 2012; Hansen et al., 2011).

We run all the analysis on R version 3.4.5 (R Core Team, 2021). Estimation of variance components was based on a six-generation deep social pedigree of 12,244 individuals ringed between 2011 and 2019, including 753 maternal identities and 944 paternal identities. A pruned pedigree included 645 individuals, including 157 maternal identities and 134 paternal identities, and was four generation deep. We built an animal model in a Bayesian framework for each of the chromatic (BGC and chroma) and achromatic (lightness) colour components and for egg volume running models using MCMCglmm, version 2.33 (Hadfield, 2010). Each model considered laying order, clutch (first or second in the same year—coded as *clutch wave* in the models), and female age category (see below) as fixed factors. We controlled for laying order in the models since laying order is known to affect the degradation of the biliverdin when the eggs are exposed to air, meaning that the colour of the first-laid eggs within the clutch decreases in relation to those laid at the end (D'Arpa et al., 2021; Moreno et al., 2011). Laying order was registered daily most years, but from 2015 to 2018 data were collected every two days. As a result, the exact sequence of the laying order was not available for all eggs. To be able to pool the data together, we transformed laying order data in an ordinal variable thus: 1, first or second egg; 2, third or fourth egg; and 3, fifth or sixth egg, as last-laid eggs. Lastly, we considered the age of the females. Since 56.8% of eggs were laid by immigrant females, which could only be aged as yearlings or older than that, we treated age as a factor with these two levels to maximize sample size. As random effects, we considered the matrix of relatedness, year, and female ID. We thus quantified additive genetic variance V_A, year accounted for the environmental variance between years and ID for repeated measures of the same individuals and to evaluate the permanent environment variance (V_{PE}). In preliminary models, we also tried to evaluate parental effects and a common environment variance considering as random factors the identity of the mother (maternal effect), the identity of the father (paternal effect), the nest, and the unique identity of each clutch. However, none of these variables returned acceptable diagnostic parameters and were therefore discarded from definitive models. For the election of the priors, we started with a simple uninformative prior for all the random terms, and then proceeded to change it for the terms that did not perform well. We tried different expanded and nonexpanded priors and found no significative changes (results not shown) and used the prior that produced the best posterior distribution to avoid autocorrelation. Priors used in the definitive models were uninformative priors ($V = 1$ and $\nu = 0.002$) for the animal term in all the models, for the year in the lightness and the volume models, and for the ID in the BGC and chrome models. As for the other variables, a simple uninformative prior did not return acceptable posterior distributions. For the year in the BGC and in the chrome models, a prior with $V = 1^{-10}$ and $\nu = -1$ was necessary, and for the ID in the lightness and the volume models, an expanded prior was used ($V = 1$, nu = 1, alpha.mu = 0, alpha.V = 1,000; see (Morosinotto et al., 2020) for a previous use of different priors for different random terms).

We set MCMC chains to run for 2,500,000 iterations to achieve satisfactory effective sample sizes (i.e., ≈10,000), a thinning interval of 200, and a burn-in of 30,000 iterations. We then checked that autocorrelation was <0.1 in the first thinning lag, and visually inspected posterior distribution plots (de Villemereuil, 2012).

For each model, we obtained the variance component for each egg trait (see Table 2): additive genetic variance (V_A), permanent environment (V_{PE}), year effects (V_Y), residual variance (V_R), and narrow sense heritability (h^2). Then, we calculated the coefficient of additive variance ($\mathrm{CV}_A = \frac{\sqrt{V_A}}{\bar{x}}$), evolvability ($I_A = \frac{V_A}{\bar{x}^2}$), and repeatability ($\mathrm{Rep} = V_A + V_{PE}/V_P$). CV$_A$ and I_A

values were not given for the BGC and the chroma because they are proportions, so their mean scale does not have biological meaning (Pélabon et al., 2020). The significance of the fixed factors was obtained through an approximation of the probability calculated by the MCMC with a threshold of 5%. The significance of random terms was obtained by running every model each time without a given random term and evaluating the difference in DIC values (a Bayesian analogous to the AIC) compared to the full model (Wilson et al., 2010).

Results

Our results showed a relatively high proportion of additive genetic components for all the traits considered in relation to total phenotypic variance (Table 1, Figure 1), and the resulting heritability values were also high. The highest value was for chroma, with a heritability of 0.438 (CI = 0.349–0.523), followed by lightness ($h^2 = 0.415$ [CI = 0.282–0.587]), BGC ($h^2 = 0.34$ [CI = 0.324–0.471]) and volume ($h^2 = 0.312$ [CI = 0.174–0.5]). Nevertheless, the calculation of the evolvabilities, that is, the scaling of the V_A against the mean value of the trait, returned low values (Table 2). Repeatability was high for all traits (Table 2). The comparison of the DIC values revealed that the values of V_A for colour descriptors were significant (i.e., DICs > 5), but not so in the case of egg volume. V_Y was statistically significant for all the models, while V_{PE} was only significant for lightness.

We found that clutch wave and laying order influenced colour components, but female age only showed a significant effect in lightness (Table 1). In particular, BGC increased from the first to the second clutch wave ($p < 0.001$) and along the laying order ($p < 0.001$). The same pattern was found for chroma (clutch wave: $p < 0.001$; laying order: $p < 0.001$). Lightness decreased across clutch waves ($p < 0.001$) and laying order ($p < 0.001$). Volume, on the other hand, increased from first year to older females ($p < 0.001$) and decreased with increasing laying order ($p < 0.001$). Estimates and CI of all colour components are shown in Table 1.

Discussion

In this study, we used data from a four-generation deep social pedigree, built over eight years of monitoring a spotless starling population to evaluate the evolutionary potential of biliverdin-based eggshell colouration and egg size. Our results show that the characteristic blue-green colour of starling eggs has a low to moderate heritability, a variable additive genetic variance (depending on the colour parameter considered), but consistently low values of evolvability for all colour parameters. Our results suggest that egg colour has a small evolutionary potential in this population and that the total phenotypic variation of all these traits can be due to other sources of nongenetic variance (e.g., maternal effects or environmental factors).

Table 1. Random term and fixed effects for the colour descriptors and egg size models and their credible intervals.

Dependent variable	Random terms (CI)				Fixed effects (CI)			
	V_A	V_Y	V_{Pe}	V_r	Intercept	Age	Clutch wave	Laying order
BGC	**0.833^{-04} (0.656^{-04}; 0.985^{-04})**	**0.042^{-04} (0.012^{-04}; 0.211^{-04})**	0.524^{-04} (0.394^{-04}; 0.678^{-04})	0.608^{-04} (0.592^{-04}; 0.625^{-04})	**0.579 (0.577; 0.581)**	-2.556^{-04} (-11.774^{-04}; 5.824^{-04})	**8.815^{-04} (5.153^{-04}; 12.438^{-04})**	**18.770^{-04} (16.754^{-04}; 20.824^{-04})**
Chroma	**1.785^{-04} (1.427^{-04}; 2.223^{-04})**	**0.114^{-04} (0.028^{-04}; 0.49^{-04})**	0.924^{-04} (0.608^{-04}; 1.271^{-04})	1.243^{-04} (1.25^{-04}; 1.282^{-04})	**0.08 (0.077; 0.084)**	-4.391^{-04} (-17.655^{-04}; 8.387^{-04})	**13.843^{-04} (8.624^{-04}; 19.165^{-04})**	**25.34^{-04} (22.53^{-04}; 28.323^{-04})**
Lightness	**7818.71 (5154.5; 11399.6)**	**314.63 (101.7; 1359.18)**	**3453.77 (184.55; 5700.4)**	7353.16 (7129.91; 7535.94)	**1408.5 (1387.92; 1430.01)**	**10.27 (0.58; 19.98)**	**−16.24 (−20.13; −12.12)**	**−20.95 (−23.12; −18.57)**
Volume	119837.6 (60708.5; 185072.8)	11132.9 (3886.9; 47121.1)	**99722.9 (41223.2; 153932.9)**	128630.3 (125366.7; 132537.4)	**7169.38 (7057.02; 7279.96)**	**123.2 (82.27; 163.97)**	−15.32 (−32.7; 1.189)	**−87.16 (−96.25; −77.54)**

Note. For random terms, variance components in bold denote Δ DIC >|5| between the DIC value of the full model and that of the model without the considered variable. Models with a Δ DIC <|5| are considered equivalent. Fixed effects in bold denote pMCMC values < 0.001. V_A = additive genetic variation; V_{Pe} = permanent environment effect (i.e., individual identity); V_r = residual variance; V_Y = yearly variation.

Table 2. Estimations of evolutionary potential for colour descriptors and egg size and their credible intervals.

Trait	Estimations of evolutionary potential (CI)				Phenotypic mean (SD)
	CV_A	I_A	h^2	Rep	
BGC	–	–	0.34 (0.324; 0.471)	0.670 (0.613; 0.698)	0.582 (0.014)
Chroma	–	–	0.438 (0.349; 0.523)	0.673 (0.606; 0.698)	0.085 (0.02)
Lightness	0.066 (0.053; 0.078)	0.004 (0.003; 0.006)	0.415 (0.282; 0.587)	0.597 (0.557; 0.626)	1380.7 (134.4)
Volume	0.049 (0.036; 0.062)	0.002 (0.001; 0.004)	0.312 (0.174; 0.5)	0.605 (0.547; 0.639)	7088.5 (586.2)

Note. CV_A = coefficient of additive genetic variation; h^2 = heritability; I_A = evolvability; rep = repeatability. The CV_A and I_A of BGC are not shown (see text).

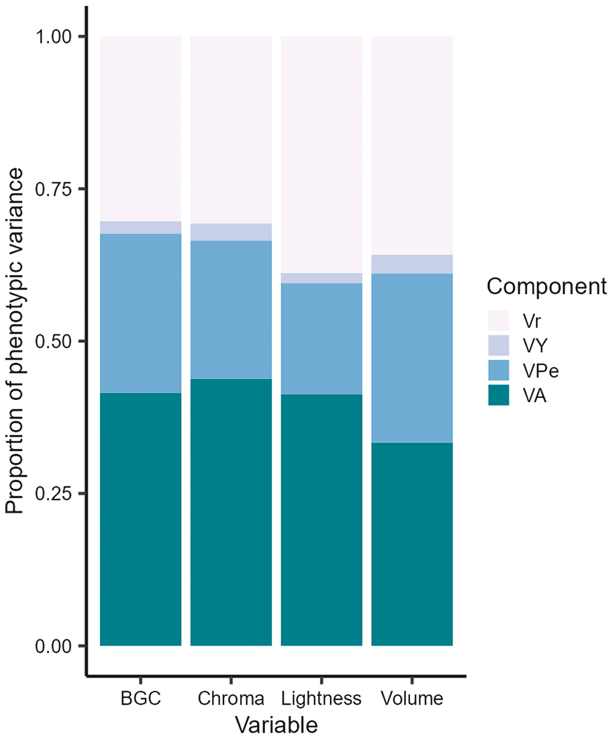

Figure 1. Relative contribution of the variance components to the total phenotypic variance for colour descriptors and egg size. V_A = additive genetic variation; V_{PE} = permanent environment effect (i.e., individual identity); V_r = residual variance; V_Y = yearly variation.

Our results seem to suggest a relation between egg colouration and the genetic quality of the female, given the amount of additive genetic variation we found in the considered egg traits. This seems to contradict previous findings in the same population, where the characteristics of the eggs were not predicted by female body condition (D'Arpa et al., 2021, 2022).

Sexual selection is expected to deplete genetic variation, and thus reduce the value of selection, leading to the so-called "lek paradox" (Kirkpatrik & Ryan, 1991). Under this context, we found high levels of genetic variation, suggestive that sexual selection is unlikely to erode both genetic and phenotypic variation. In this context, the result of high variance of the phenotypic trait and genetic variance results in a low evolvability of the studied traits. Altogether, we can speculate that although selection on this trait occurs, its impact in evolutionary terms is weak at most. Consequently, the additive genetic variation we find might be unlikely linked to the genetic quality of females and perhaps is maintained by pleiotropic effects associated with genetic variation of other phenotypes or environmental variation. Since the response to selection is the product between heritability and selection pressure, a low evolvability implies a low response to selection, whatever the selection pressures, and then a low probability that egg colour components are responsive to sexual selection.

We considered variance-scaled and mean-scaled parameters to obtain robust estimations of total additive genetic variance controlling for the magnitude of the focal trait. Additive genetic variation and therefore CV_A (and I_A) is a more robust and precise proxy of evolutionary potential than heritability estimates, demonstrated by weak associations between heritability and CV_A (Postma, 2014). For example, Houle (1992) notes that morphological traits tend to have high heritability, but low evolvability, and the opposite is true for life-history traits. Thus, using heritability as a measure of evolutionary potential is inaccurate in wild population data (Hansen et al., 2011). Meanwhile, evolvability, being independent of unpredictable sources of environmental variance, is considered a more suitable measure of evolutionary potential for comparison between traits and populations (Garcia-Gonzalez et al., 2012, Hansen et al., 2011).

In the only previous study investigating the quantitative genetics of blue-green eggshell colouration, Morales et al. (2010) found moderate heritability for lightness ($h^2 = 0.42$) and chroma ($h^2 = 0.36$) and high heritability for BGC ($h^2 = 0.54$). Interestingly, we found a different pattern, in which lightness and chroma have a moderate heritability similar to Morales et al.'s (lightness $h^2 = 0.42$; chroma $h^2 = 0.44$, Table 2), but BGC heritability was noticeably lower ($h^2 = 0.34$, Table 2). However, despite the similarity of the figures, heritability values are not appropriate to compare the evolutionary potential of traits, as heritability is a specific attribute that changes between traits and populations and over time.

The values of CV_A that we found for the different colour descriptors spanned between two and three orders of magnitude between them. This may indicate that colour descriptors possibly respond differently to selective pressures, which in turn is consistent with the idea that their biological roles are different and independent from one another, as it has been found in other kinds of pigment-dependent colour traits. In kestrels, the chroma of a carotenoid-based signal has been shown to be more heritable than lightness and hue, indicating different levels of environmental dependency (Vergara et al., 2015). In carotenoid-based signals, chroma has been linked with carotenoid content (Peters et al., 2012). Our results suggest that chroma would have the highest evolutionary potential among all colour components.

Consistently with the finding of low values of CV_A, we found very low values of I_A (Table 2). In a recent publication, Hansen & Pélabon (2021) reviewed the evolvability values for different traits. They found that the mean evolvability for life history traits was much larger than for morphological traits. In particular, the median value for volume/mass traits was 0.5%, 1.28% for fitness traits, 1.45% for physiology, and 2.28% for sexually selected behavioural traits (percentages as in Hansen & Pélabon, 2021). Postma (2014) found an I_A for morphological traits in wild vertebrates of 4.48 ± 1.46 (mean ± SE). The high difference between the magnitude of these values and those we found for all egg colour descriptors in our study gives little room for evolutionary change of this trait. Specifically, its low evolvability and V_A suggest that these traits would hardly respond to indirect models of sexual selection, that would have very little raw genetic material to act upon. This is in contrast to sexually selected traits that, despite active selection, have substantial heritability and evolvability, suggesting that some of the proposed mechanisms that allow the resolution of the lek paradox could be at work.

Egg size, considered one of the most directly interpretable female traits associated with reproductive capacity, is generally acknowledged as a feature with low plasticity, primarily governed by genetic factors (Christians, 2002; Kontiainen et al., 2008). However, studies suggest a nuanced circumstance, indicating that egg size is, to some extent, dependent on the condition of the laying female (Christians, 2002; D'Arpa et al., 2021; Nilsson & Svensson, 1993). Egg size significantly influences the allocation of resources available to the developing embryo (Krist, 2011). Larger eggs, beneficial for offspring, produce chicks with a competitive advantage over smaller siblings, thereby contributing to enhanced fitness (Krist, 2011). Considering its impact on reproductive success, egg size serves as a proxy for reproductive investment (Smith et al., 1993) and is expected to be under strong selection, although with low evolvability (Hansen & Pélabon, 2021). However, in species with a considerable level of parental care, egg size effect is quickly exceeded by the postnatal care effect (Hadfield et al., 2013). Considering that prenatal investment is subjected to indirect selection, and its effects are transient, it is possible that the overall selection pressure on egg size in such a system is limited.

In our analysis, fixed effects reveal a darkening and increased saturation of egg colour across laying order and between clutch waves. The observed effect of laying order on colour is attributed to biliverdin degradation postdeposition due to light exposure (D’Arpa et al., 2021; Moreno et al., 2011), an effect we expected due to an artefact derived from the data collection protocol. Additionally, a small yet significant clutch wave effect indicates increased pigmentation in the second wave, contrary to the expected honest signal of condition hypothesis. Environmental factors during the second wave, characterized by worsened conditions and reduced female investment, challenge the notion of egg coloration as a reliable indicator of reproductive capacity. Egg size increased with female age (yearlings vs. older) and decreased with laying order, partly aligning with prior findings (D’Arpa et al., 2021; Monclús et al., 2017). Surprisingly, contrary to previous results, our study indicates that first-year females lay smaller eggs than older counterparts. This discrepancy may stem from the smaller sample size and inability to separate the effects of year and age in the previous study (D’Arpa et al., 2021).

In conclusion, we found a very low evolutionary potential for any egg colour trait considered. Thus, the phenotypic variance of this trait seems to be associated with other nonadditive, environmental sources of variance. However, further research would be needed to quantify eventual variation in fitness among females based on difference in eggshell colouration and the relative response to selection for these traits.

Data availability

The dataset and pedigree used in this study are available at DOI: 10.5061/dryad.d7wm37q8s.

Author contributions

Stefania D’Arpa (Conceptualization [Lead], Data curation [Lead], Formal analysis [Lead], Investigation [Equal], Methodology [Equal], Visualization [Lead], Writing—original draft [Lead]), Diego Gil (Conceptualization [Equal], Data curation [Equal], Investigation [Equal], Project administration [Lead], Resources [Lead], Validation [Equal], Writing—review & editing [Equal]), Jaime Muriel (Data curation [Equal], Investigation [Equal], Writing—review & editing [Equal]), Raquel Monclús (Investigation [Supporting], Writing—review & editing [Supporting]), Lorenzo Pérez-Rodríguez (Conceptualization [Supporting], Data curation [Supporting], Funding acquisition [Equal], Investigation [Equal], Project administration [Equal], Resources [Equal], Supervision [Equal], Writing—review & editing [Equal]), and Jesus Martinez-Padilla (Conceptualization [Supporting], Formal analysis [Supporting], Methodology [Lead], Software [Lead], Supervision [Equal], Validation [Lead], Writing—review & editing [Lead])

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Conflicts of interest

None declared.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (RD 53/2013). Capture and manipulation of birds were authorized by the Consejería de Medio Ambiente (Comunidad de Madrid, Spain) under licence from the Spanish institutional authorities (Consejería de Medio Ambiente and Centro de Migración de Aves de SEO/BirdLife). Permission to work in the area was granted by both the Parque Regional de la Cuenca Alta del Manzanares (Consejería de Medio Ambiente de la Comunidad Autónoma de Madrid: Ref. 10/129049.9/11) and the Ayuntamiento de Soto del Real, Spain.

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