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Original Article

Plumage color manipulation has no effect on social dominance or fitness in zebra finches

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Colorful plumage ornaments may evolve because they play a role in mate choice or in intrasexual competition, acting as signals of species identity or of individual quality. The zebra finch (*Taeniopygia guttata*) is a model organism for the study of mate choice and its colorful plumage ornaments are thought to be used in both of these contexts. Numerous genetic color variants have been described for this species, but they are rare in the wild. This raises the question whether discrimination against deviant phenotypes maintains the species' uniform plumage color (rare-mate disadvantage). Furthermore, comparison to closely related species suggests that the lack of colorful ornaments in female zebra finches is a derived condition. Male preferences for less-ornamented females may have led to sexual dichromatism in the zebra finch. Here, we test the role of plumage ornaments experimentally by altering male and female coloration to mimic 2 types of naturally occurring genetic color variants. We estimated effects on social dominance and reproductive success in large breeding aviaries in one domesticated and 2 recently wild-derived populations. Hypotheses, methods, and analyses were preregistered to ensure maximal objectivity of the results presented. Despite a fairly drastic manipulation and a powerful experimental design, we found no effect of the treatment on social dominance or on reproductive success. Our results suggest that mate choice is not the mechanism that maintains homogeneity of zebra finch plumage coloration, or that can explain the loss of ornaments in females.

Key words: **color polymorphism, color mutation, mate choice, male–male competition, rare-mate disadvantage, sexual dichromatism, species recognition.**

INTRODUCTION

One of the most striking features of birds is that they have evolved an outstanding diversity of plumage coloration and patterning both within and among species. Within species, males and females often differ in the degree of colorful ornamentation of the plumage (sexual dichromatism). Such differences have typically been interpreted in relation to sex-related differences in the strength of sexual versus natural selection. Indeed, recent comparative studies indicate that more intense sexual selection on males led to the evolution of more colorful males and more cryptic plumage in females (Kraaijeveld 2014; Price and Eaton 2014; Dale et al. 2015; Dunn et al. 2015). The evolutionary shift towards more cryptic females might have followed from a change to female-only brood care (increased benefits of crypsis), or from reduced female–female competition (reduced benefits of ornamentation) or from both (Dale et al. 2015). Sexual dichromatism would then be maintained by ongoing selection favoring crypsis in one sex and/or favoring ornamentation during mate choice or intrasexual competition in the other sex.

The zebra finch (*Taeniopygia guttata*) is an estrildid species that has been studied extensively with regard to sexual selection and mate choice (Ten Cate and Vos 1999; Riebel 2009; Hauber et al. 2010; Adkins-Regan 2011). The plumage of both sexes is characterized by 2 distinctive features: a white stripe between beak and eye, bordered by black lines (Figure 1A), and white spots on the upper tail-coverts. However, males show 4 additional plumage ornaments: orange cheek patches, a black breast band, fine black and white stripes on the chin and upper breast, and chestnut-brown flanks with white spots (Figure 1D). This type of ornamentation likely is ancestral, because most of the related Australian estrildid species show similar color patterns (Forshaw et al. 2012). However, in contrast to zebra finches, in most of the related Australian estrildid finches males and females are either equally ornamented or females show ornaments that are reduced in size compared to male ornaments (Forshaw et al. 2012). Why female zebra finches are less ornamented than females of most other estrildids remains unclear.

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Figure 1
Photographs of the control and painted females (a–c) and males (d–f). (a) Untreated female, (b) female with added breast band and untreated cheek feathers, (c) female with blackened cheek patches and untreated breast band, (d) untreated male, (e) male with enlarged breast band and untreated cheek patches, (f) male with blackened cheek patches and untreated breast band. All photos were taken on the second day of the experiment. Note that the female in (c) shows natural traces of a black breast band (incomplete suppression of a male ornament).

Assuming that plumage dichromatism in zebra finches is maintained by ongoing sex-specific selection on ornamental traits, testable, non-mutually exclusive hypotheses about the underlying selective processes can be formulated. 1) Selection favoring inconspicuousness is stronger in females than in males. This seems unlikely, given that zebra finches show biparental care (incubation and feeding offspring) and that males and females do not differ in foraging behavior (Mariette and Griffith 2012). 2) Unlike males, females expressing a more ornamented "male-like" phenotype derive no or little competitive benefit. 3) Females prefer the more ornamented male phenotypes. 4) Males prefer the less-ornamented female phenotypes.

Sexual dichromatism in zebra finches may arise via suppression of male ornaments in females, given that females sometimes show traces of a black breast band (Figure 1C), and such suppression may work via hormonal mechanisms (Owens and Short 1995; Kimball 2006; Kraaijeveld 2014; Lindsay et al. 2016). Males may then benefit from choosing less ornamented females (hypothesis d), if such females show, for example, higher fecundity via a shared hormonal mechanism.

In zebra finches, female preference for a "typical," ornamented male (hypothesis c) and male preference for a "typical," non-ornamented female (hypothesis d) may also be maintained through mechanisms that help avoid mating with the wrong species. Species recognition is an important explanation for plumage variation among species, because distinct signals of species identity prevent hybridization with other species (which usually results in reduced fitness, (e.g. Veen et al. 2001). Species-specific coloration is most effective when it is uniform intraspecifically and clearly contrasts with heterospecifics (Ryan and Rand 1993; Saetre et al. 1997). Nevertheless, about 3.5% of all bird species (Galeotti et al. 2003) show color polymorphisms, which could reduce the effectiveness of the species identity signal. The mechanisms that prevent such color mutants from becoming more frequent may lie in discrimination against deviating phenotypes during mate choice. In zebra finches, components of species recognition are learnt through sexual imprinting during early development, based on features of plumage, vocalizations, and maybe also smell (Immelmann 1959; Immelmann et al. 1978; Clayton 1990; Burley 2006; Campbell and Hauber 2009; Krause et al. 2014). Thus, constraints on changes in coloration may arise in the zebra finch, because individuals of deviating coloration are not recognized as conspecifics and rejected as mates.

Here, we experimentally altered plumage ornaments in male and female zebra finches to test the general avoidance of individuals with deviant coloration, and specifically male discrimination against ornamented females. Our manipulations focus on the 2 plumage ornaments identified by Immelmann (1959) as most important for species recognition (females recognizing males) that are lacking in females: the cheek patches and the breast band. Immelmann gave females the choice between 2 white males, one of which carried one specific painted ornament while the other was unchanged. Females strongly preferred males with an orange cheek patch (Figure 97 in Immelmann 1959), showed some preferences for males with a black breast band (Figure 98), but did not care about the added chestnut flanks (Figure 99). These results can be interpreted in light of species recognition or in light of sex recognition. However, it seems less likely that the differential response of females to the 2 types of courting males is because they consider one of them to be a male and the other a female, and more likely that the ornamented male, but not purely white one, comes close enough to the imprinted target (a conspecific male).

Furthermore, our manipulations mimic 2 types of known zebra finch color mutations. *De novo* mutations in color-determining genes are well documented in the zebra finch. Amateur zebra finch breeders recognize at least 24 different heritable color variants (recessive or dominant mutations, 3 of them sex-linked; (Landry 1997)). Many of these color mutants have also been observed as isolated cases in the wild (Landry 1997; Forshaw et al. 2012). First, we manipulated individuals of both sexes by painting their cheeks black (Figure 1C and F). This mimics individuals that are homozygous for the recessive autosomal mutation "black cheek," occurring in both sexes in some domesticated populations (Landry 1997). Although males of this mutant also typically show black flanks with white spots rather than chestnut-brown flanks with white spots, we did not attempt to mimic this trait, as it is a difficult pattern to replicate convincingly using dye. Second, we manipulated the black breast band in both sexes. Three known mutations increase the size of the breast band in males ("black-breasted," "black-fronted," and "black-faced"), one of which has also been observed in the wild ("black-fronted") (Landry 1997). Although each of them also comes with pleiotropic effects on other parts of the plumage (Landry 1997), we only experimentally increased breast band size in males (Figure 1E, resembling the "black-fronted" phenotype). As none of the experimental birds had been exposed to such deviant phenotypes prior to the experiment, this "partial replication" of the naturally occurring mutant should be sufficient to test biologically relevant responses to deviant phenotypes. In females, we dyed an artificial breast band of typical male size (Figure 1B) to mimic a "non-suppressed," possibly ancestral state.

Our study was primarily designed to test the a priori hypothesis that males and females with artificially painted ornaments show reduced reproductive success due to discrimination by the opposite sex. However, previous studies on a variety of bird species suggested that black ornaments signal dominance (Ducrest et al. 2008). It is therefore important to assess the effect of our treatment on dominance, because increased dominance might compensate the fitness costs of being discriminated against by the opposite sex. We, therefore, also tested the a priori hypothesis that manipulated (blackened) individuals are dominant over unmanipulated, same-sex individuals.

To increase the effectiveness of our experimental test, we combined the painting treatment with a sex-ratio bias towards the painted sex, such that all untreated (wild-type) individuals could pair assortatively. This would leave all painted birds unpaired and hence with zero or low reproductive success. Our approach was also designed to mimic the natural rarity of deviant phenotypes in a population. Thus, we introduced only one of each mutant type (2 painted individuals) in each experimental aviary, together with 10 wild-type individuals.

METHODS

To maximize the objectivity of the presented research, we preregistered this study with its 2 hypotheses, its planned methods, and its complete plan of data analysis before the start of data collection (https://osf.io/eb8ua/register/565fb3678c5e4a66b5582f67, accessed 29 December 2017). We closely adhered to our plan.

Study populations and experimental set up

This study was carried out at the Max Planck Institute for Ornithology in Seewiesen, Germany. Experimental birds were of wild-type plumage and originated from 3 independent populations:

1) 'Melbourne': wild-derived, brought into captivity in Australia about 3–4 generations ago; 80 individuals exported to Seewiesen in December 2015.
2) 'Bielefeld': wild-caught in Australia about 12–15 generations ago; 24 individuals exported to Bielefeld (Germany) in 1992 (population # 4 in (Forstmeier et al. 2007)), and 109 individuals transferred from Bielefeld to Seewiesen in 2009.
3) 'Cracow': F1 and F2 hybrids of 2 domesticated populations, one from Cracow, Poland (population # 11 in (Forstmeier et al. 2007); 50 individuals transferred to Seewiesen in 2011 and 2013) and one from Sheffield, U.K. (population # 18 in (Forstmeier et al. 2007); 468 individuals transferred to Seewiesen in 2004).

Before the start of the experiment, we kept individuals in same-sex groups (on average 29.8 ± 7.6 SD individuals per group, $n = 12$ groups). Because in such groups pair bonds between same-sex individuals sometimes form, we color-banded all birds and carried out about 50 h of observations (on 34 observation days within a 66 day period, between 10:00 and 20:00 h) of pair bonding (body contact and allopreening) before the experimental period. We split all observed potential same-sex couples by putting the individuals in different aviaries to break up these pair bonds.

We used a total of 18 experimental aviaries (5 for birds from the Melbourne population, 5 for the Bielefeld population, and 8 for the Cracow population). Nine aviaries (3 Melbourne, 2 Bielefeld, and 4 Cracow) were used to study effects of male ornamentation and contained 7 males (3 untreated wild-type, 2 sham-treated wild-type (one with the cheek treatment and one with the breast band treatment) and 2 painted (one with the cheek treatment and one with the breast band treatment)) and 5 untreated females. The other 9 aviaries (2 Melbourne, 3 Bielefeld, and 4 Cracow) were used to study effects of female ornamentation and contained 7 females (3 untreated wild-type, 2 sham-treated wild-type (one with the cheek treatment and one with the breast band treatment) and 2 painted (one with the cheek treatment and one with the breast band treatment)) and 5 untreated males.

Plumage ornament manipulation

We treated all experimental birds with a black dye solution made by dissolving 1 g of gum arabic (Sigma, Schnelldorf, Germany) saturated with *p*-phenylenediamine (Sigma, Schnelldorf, Germany), a white powder that turns black when mixed with hydrogen peroxide, in 8 mL of warm distilled water. We filtered the saturated solution to remove any remaining crystals of *p*-phenylenediamine and stored it at −20 °C in Eppendorf tubes for later use (200 µL per tube). Before manipulation of the birds, we defrosted each tube and added 200 µL of 6% hydrogen peroxide (diluted from 30% hydrogen peroxide stock solution, Sigma-Aldrich, Schnelldorf, Germany). The oxidized dye turns the feathers black without affecting feather structure and water resistance capacity (Götmark 1993). For the sham treatment, we used the same solution without *p*-phenylenediamine. We carried out measurements of reflectance spectra as previously described (Bolund et al. 2007) to verify that the black coloration closely matched natural patches of black plumage (male breast band), particularly in the UV part of the spectrum (Supplementary Figure S1).

We carried out all experimental manipulations on 19 May 2016 and 20 May 2016. Individuals were randomly assigned to one of four treatments. 1) Black breast band enlargement. For males, the existing black breast band size (measured using Image J software, Abràmoff et al. 2004; mean ± SD, initial size: 172 ± 65 mm^2, n = 9) was extended towards the beak and covered the entire breast (final size: 587 ± 76 mm^2, n = 9, Figure 1E, Supplementary Figure S2). For females, we painted a breast band typical for a male (using a plastic stencil, final size: 205 ± 20 mm^2, n = 9, Figure 1B). 2) Black cheek patches. In males, we applied the black dye such that it covered the orange cheek patches (final size: 116 ± 9 mm^2, n = 9, Figure 1F). In females, we applied the black dye on the gray feathers using a plastic stencil based on the natural (orange) cheek patches of a typical male (final size: 96 ± 17 mm^2, n = 9, Figure 1C). 3) Sham treatment: we painted individuals in the same area (breast band or cheek patch) with the sham solution, following the same procedure as for the black dye. Sham-treated birds were used as a control for potential undesired effects of the manipulation. 4) Untreated: individuals did not undergo any manipulation.

Each dye- or sham-solution tube was used for 2–3 birds. We applied the solution directly to the feathers with a small paintbrush (5–10 min), placed the birds inside a dark box (measuring 30 cm × 28 cm and 15 cm high) to keep the birds inactive for 20 min to prevent spread of dye to other body regions and then washed the treated feathers for about 2 min with fresh water using a cotton swab.

Measurement of reproductive success

The experiments took place in large semi-outdoor aviaries (2 m × 5 m and 2.5 m high) containing 9 nest boxes each. Mondays to Fridays, we checked every nest box, noted its content (eggs, offspring), and identified the individuals attending it. To measure reproductive success, we conducted a parentage analysis of all offspring that reached independence (minimum 35 days old, n = 350 offspring). We determined a 3-month window a priori (24 May 2016 to 24 August 2016, https://osf.io/eb8ua/), within which birds were allowed to lay eggs and raise young. Eggs laid after 24 August 2016 were replaced by dummy eggs and were not considered for measurements of reproductive success. Pairs were allowed to continue to raise the offspring from eggs laid before this date. For the parentage analysis, we collected a small (~10 µL) blood sample from the brachial vein of each 8–10 day old nestling and used 15 microsatellite markers, as previously described (Wang et al. 2017). We identified the genetic parents of all 350 offspring (mean ± SD offspring in female-biased aviaries per female = 3.0 ± 2.9, range = 0–11, and per male = 4.2 ± 4.5, range = 0–18; mean ± SD offspring in male-biased aviaries per female = 3.5 ± 2.9, range = 0–13, and per male = 2.5 ± 3.2, range = 0–15).

For this study, the primary outcome variable is the relative reproductive success of individuals. This was calculated (following classical measurements of relative fitness) as an individual's total number of genetic offspring produced (see above) divided by the average number of genetic offspring produced by the seven same-sex individuals within the respective aviary (9 aviaries for each sex).

Behavioral observations and measurement of dominance

Within each aviary, all males and females were randomly assigned 2 plastic bands of the same color (one on each leg; previous color bands removed) for individual identification during behavioral observations, using 7 colors (black, white, yellow, green, red, for both sexes in all aviaries, plus light blue and dark blue for the overabundant sex in all aviaries). Note that band colors (including red and green) affected neither relative reproductive success (males: p = 0.84, females: p = 0.51, n = 108), nor the dominance index (males: p = 0.48, females: p = 0.88, n = 108).

Between 24 May 2016 (release) and 7 June 2016, four observers carried out a total of 79 bouts of observations (minimally 3 and maximally 9 per day, between 08:00 and 20:00 h) through 1-way glass, using binoculars. This period was chosen a priori, because we expected the highest level of aggressiveness among birds that were placed in new groups. Each observation bout lasted 5 min per aviary × 18 aviaries = 90 min in total. For each bout, we monitored the 18 aviaries in a randomized order and recorded all observations of relevant behaviors by any individual. We noted the following aggressive events: 1) initiation of beak fencing ("pecking"), where the attacked bird does not leave its place, 2) attacking and supplanting another individual without following it ("attack"), and 3) attacking and following another individual as it flies away ("chase"). We recorded the sex and band color of both individuals involved in an aggressive event. Repeated occurrences of the same event were recorded maximally once per 30-s interval (maximally 10 times per 5-min observation period). Over the 2-week observation period, we recorded 4576 aggressive events (1276 chases, 2816 attacks, and 484 peckings). The number of events (active plus passive) recorded for one individual ranged from 4 to 139. Observations could not be performed blindly with respect to treatment (except for sham-treated), as dyed birds were readily visible. However, to minimize observer bias, the observers were instructed to only pay attention to band colors and to avoid focusing on treatment categories. Observers were asked about their a priori expectations regarding treatment effects, which in fact differed between observers (see https://osf.io/eb8ua/). Yet, the data suggested that observer bias was minimal (Supplementary Figure S5).

We calculated a "dominance index" that takes both performed and received aggression into account and weights each type of aggressive event (chase, attack or pecking) by a coefficient reflecting the intensity of the interaction of interest (a priori determined, see https://osf.io/eb8ua/).

$$\text{index} = \ln \frac{\begin{pmatrix}\text{number of performed chases} \times 3\\ +\text{number of performed attacks} \times 2\\ +\text{number of performed peckings}\end{pmatrix}+1}{\begin{pmatrix}\text{number of received chases} \times 3\\ +\text{number of received attacks} \times 2\\ +\text{number of received peckings}\end{pmatrix}+1}$$

Dominance indices were calculated for the 7 individuals of the overabundant sex (5 control and 2 painted birds) considering all interactions among the 12 birds per aviary, to comprehensively capture all aspects of dominance. Across all 4576 aggressive events, 67% were performed by males, and 70% were directed at a same-sex individual. Incomplete observations that lacked the identification of either the performer or the receiver of aggression were discarded ($n = 43$ events, decision taken before the first data analysis). To account for between-individual differences in the precision of the dominance index, each value was weighted by $\sqrt{n-3}$ (Nakagawa and Cuthill 2007), where n is the total number of aggressive events (active and passive) observed for a certain individual. Overall, the dominance index varied from −3.30 to 4.20 (mean ± SD = -0.05 ± 1.06).

Statistical analyses

All statistical analyses closely followed our preregistered analysis plan (https://osf.io/eb8ua/). We implemented general linear mixed-effect models in R (R Core Team 2016) using the "lmer" function from the "lme4" package version 1.1.12 (Bates et al. 2015). *P* values for mixed models (lmer) were obtained from model comparison (with and without the fixed effects) with the "anova" function. Residuals of the models were visually inspected (QQ plots, fitted versus residual plots and histograms) to verify model assumptions. The models of dominance index showed normally distributed residuals. Data on relative reproductive success showed some skew and zero-inflation (see Supplementary Figure S5), but we decided a priori to do the analyses on the original scale.

Because sex- and ornament-specific analyses suffer from a multiple-testing problem combined with low statistical power ($n = 9$ treated individuals), we focused initially on one overall test for dominance and one for fitness ($n = 36$ treated birds against $n = 90$ controls). For this purpose, sham-treated ($n = 36$) and untreated birds ($n = 54$) of the overabundant sex were grouped as control birds ($n = 90$), after confirming that there was no difference between untreated and sham-treated birds in their relative reproductive success (Supplementary Table S1, model 1) or dominance index (Supplementary Table S1, model 2). Furthermore, for statistical validity, we confirmed that variances did not differ strongly between sexes (Levene-test for relative reproductive success: $P = 0.05$; for dominance index: $P = 0.12$, $n = 216$; using the "leveneTest" from the "Rcmdr" package), although the greater variance in male reproductive success is likely to be biologically real. To maximize statistical power, the treatment effect was initially analyzed—irrespective of ornament and sex—as a fixed effect with 2 levels ("treatment2"): "painted" (36 birds) and "control" (90 birds). We added "aviary" and "population" as random-slope effects to account for the non-independence of the data within the 18 aviaries and the 3 populations.

Given that some of our hypotheses were sex-specific, we also analyzed the treatment effects on relative reproductive success and dominance separately for the 2 sexes. In these sex-specific analyses, we also distinguished between the 2 ornaments, so we contrasted all 3 treatment levels within the overabundant sex ("treatment3"): "painted breast" (9 males or females), "painted cheek" (9 males or females), and "control" (45 males or females). These models have lower power and hence can only detect stronger effects. In these models, we added "population" (but not "aviary" since there is no replication of "painted breast" or "painted cheek" within aviaries) as a random-slope effect to account for the non-independence of the data.

RESULTS

Analysis of reproductive success

Overall, painted individuals did not differ significantly in relative reproductive success from control birds of the same (overabundant) sex (mean: painted = 1.12, $n = 36$; control = 0.95, $n = 90$, $P = 0.47$, Figure 2A and Supplementary Figure S3A, Supplementary Table S2-Model 1). The estimate of the random-slope effect for "population" was zero (Supplementary Table S2-Model 1) indicating that the treatment effect did not differ between the 3 populations.

When each treatment was analyzed separately for males and females, neither individuals with painted cheeks nor those with painted breast differed significantly in relative reproductive success from same-sex control individuals (males: mean painted breast = 0.88, $n = 9$; painted cheeks = 1.10, $n = 9$; control = 1.00, $n = 45$; $P = 0.96$, Figure 2B and Supplementary Figure S3B, Supplementary Table S2-Model 2; females: painted breast = 1.27, $n = 9$; painted cheeks = 1.19, $n = 9$; control = 0.91, $n = 45$; $P = 0.42$, Figure 2C and Supplementary Figure S3C, Supplementary Table S2–3).

Analysis of dominance

Overall, painted individuals did not differ significantly in dominance index from the control individuals of the same sex (all 36 painted versus 90 control birds, $P = 0.92$, Figure 2D and Supplementary Figure S4A, Supplementary Table S3-Model 1). The estimate of the random-slope effect for "population" was zero (Supplementary Table S3-Model 1) indicating that the treatment effect did not differ between the 3 populations. Sex- and ornament-specific analyses also did not reveal any significant differences, neither in males ($P = 0.28$, $n = 9$, 9, and 45 for painted breast, painted cheeks and control individuals, respectively; Figure 2E and Supplementary Figure S4B, Supplementary Table S3-Model 2) nor in females ($P = 0.74$, $n = 9$, 9, and 45 for painted breast, painted cheeks and control individuals, respectively; Figure 2F and Supplementary Figure S4C, Supplementary Table S3-Model 3).

DISCUSSION

Species recognition and color mutants

Our experiment involved striking manipulations of those ornaments that are among the most salient for species recognition in zebra finches (Immelmann 1959). However, these manipulations resulted in no detectable consequences for individual reproductive success and social dominance. Given that birds of natural coloration and those with artificially augmented pigmentation had equal reproductive success, it appears that painted birds were not discriminated against during mate choice, despite a sex-ratio bias that was introduced to facilitate discrimination against deviant phenotypes (rare mate disadvantage). Our results also suggest that there was no rare mate advantage, which has been reported for the guppy, *Poecilia reticulata* (Zajitschek and Brooks 2008).

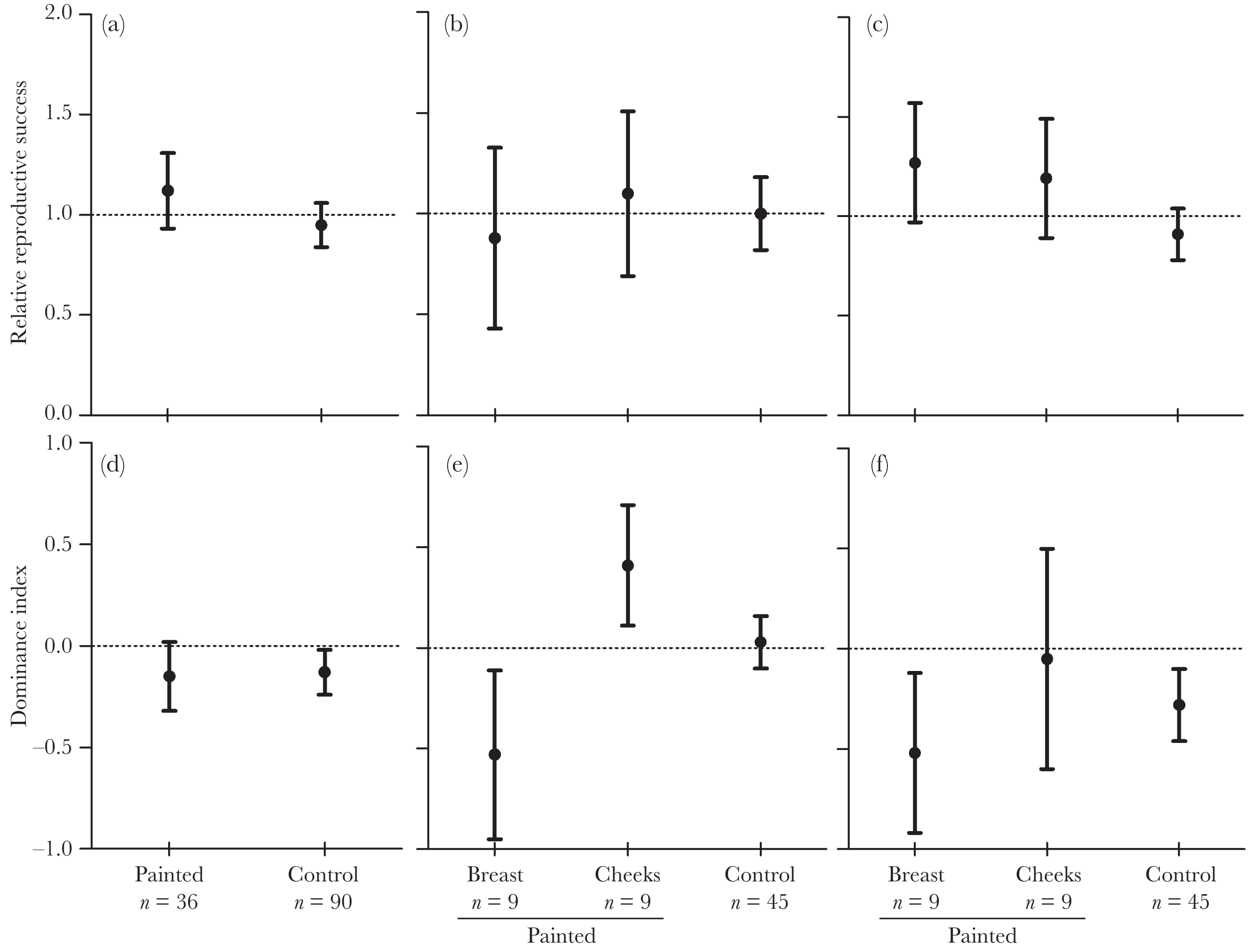

Figure 2

Parameter estimates (mean ± SE) for effects of the plumage color manipulations (see Figure 1) on relative reproductive success (a–c) and on the dominance index (d–f). Relative reproductive success for the painted and the control birds (a), for the males with painted breast, the males with painted cheeks and the control males (b), and for the females with painted breast, the females with painted cheek and the control females (c). Dominance index estimates for the painted and the control birds (d), for the males with painted breast, the males with painted cheeks and the control males (e), and for the females with painted breast, the females with painted cheek and the control females (f). All estimates and SE are obtained from the general mixed-effect models (Supplementary Table S2 and S3).

Our results stand in contrast to earlier studies (see below) that showed a clear effect of plumage characteristics on mate choice. Studies on sexual imprinting using a color mutant with completely white plumage together with wild-type birds reported strong patterns of plumage-dependent mate choice. Birds generally preferred a partner that looked like the own or foster parents (sexual imprinting), irrespective of their own plumage type (no self-inspection; Walter 1973; Immelmann et al. 1978; Vos et al. 1993). In these studies, wild-type zebra finches reared by wild-type parents and exposed only to this phenotype clearly discriminated against white zebra finches. In contrast, our modifications of the plumage (Figure 1) apparently did not trigger a strong avoidance. Conceivably, white birds are no longer recognized as conspecifics, while the phenotypes resulting from our manipulation (Figure 1) may not have fallen outside the range of acceptable mates for zebra finches raised with wild-type birds only.

A study focusing on incipient speciation (Clayton 1990) reported that zebra finches mate assortatively by subspecies (using Australian *T. g. castanotis* versus Indonesian *T. g. guttata*), despite lacking experience with the other subspecies. Interestingly, manipulations of the width of the breast band alone (which is narrower in the Indonesian subspecies) induced assortative mating by subspecies phenotype (Clayton 1990). Female preferences for the “right” width of breast band alone were apparently sufficient to induce assortment by subspecies phenotype, despite the fact that Indonesian males with enlarged breast bands paired with females of the Australian subspecies that they had not been imprinted on. In our study, painted birds had been raised by wild-type parents and hence are not expected to refrain from pairing with a wild-type partner. However, we did not find any evidence that females avoided pairing with painted phenotypes, although the manipulations were less subtle than those in Clayton (1990).

Another study based on a non-existent plumage feature (an artificial white crest with a horizontal versus vertical black stripe) also reported type-specific mate preferences arising from sexual imprinting on the parents (Burley 2006). That study suggested that zebra finches pay attention even to small plumage details, conflicting with our observation that manipulations of presumably important signals did not result in discrimination during mating. However, in Burley (2006) birds experienced a parental "population" consisting of 2 types of zebra finches that were mated assortatively. Such a situation could facilitate the required discrimination learning. In contrast, in our study, birds never encountered deviant phenotypes during the presumed critical phase, which may have led to a less discriminating species-recognition mechanism. Thus, we propose the testable hypothesis that previous exposure to 2 phenotypes with individuals being mated assortatively by phenotype (e.g. exposure to pairs of wild-type/wild-type and pairs of mutant/mutant phenotype, as in Burley 2006) is required for discrimination against the phenotype that differs from the parents of an individual to develop. In line with this idea, Kniel et al. (2017) found that female zebra finches did not prefer or reject a single male that had an artificial red feather attached to its forehead. However, after observing such a male paired to a female that also wore a red feather on the forehead, wild-type females avoided such deviant males. More generally, it is possible that birds need exposure to true heterospecifics (absent in our captive conditions) to "narrow down" their image of what represents a conspecific and to develop discrimination against deviating phenotypes (Campbell and Hauber 2010).

In conclusion, our study cannot answer which factors maintain uniformity in the plumage of wild zebra finches, but raises doubts about intersexual selection (mate choice) as the mechanism that eliminates initially rare genetic variants coding for deviating plumage coloration.

Effects of ornaments on male and female attractiveness

We found that males did not discriminate against females with painted black ornaments (breast band or cheek patch) during social mate choice (contrary to hypothesis d, see Introduction). This suggests that male preference for less ornamented females (more female-like plumage characteristics) is not the mechanism that has led to the loss of ornamentation in female zebra finches.

The size of the breast band in male and female zebra finches varies naturally, and a substantial part of this variation is heritable (Bolund et al. 2010). Price and Burley (1994) examined how this variation correlates with fitness, and concluded that the patterns were consistent with weak sexually antagonistic selection (favoring larger breast bands in males and smaller ones in females). Our manipulations went far beyond the natural range in breast band size (except for the rare color mutants) and thus allowed us to explore underlying preferences as a possible cause of sexually antagonistic selection. Fitness consequences of the manipulation showed weak and non-significant trends (see Figure 2B and C) in the direction opposite to those found in the previous study (Price and Burley 1994). This argues against the idea that sexually antagonistic selection acts on breast band size via opposing mate choice preferences, i.e. by females preferring larger and males preferring smaller breast bands. Sexually antagonistic selection could in principle still occur if breast band size is linked to a fitness-related trait, such as female fecundity. However, this should lead to rapid evolution of associated preferences (see Introduction), making the breast band phenotype an unlikely candidate trait for sexually antagonistic selection in the zebra finch.

Several studies proposed that the orange cheek patch of male zebra finches plays a role in female choice. Roberts et al. (2007) suggested that the color of the cheek patch (within the normal range) influences male attractiveness to females, whereas Naguib and Nemitz (2007) as well as Tschirren et al. (2012) reported that females prefer males with larger cheek patches. In addition, Immelmann (1959) showed that female zebra finches strongly preferred white males with a painted orange cheek patch over white males without such an orange patch (see Figure 97 in Immelmann 1959). Our study shows that a dramatic manipulation of the same signal (from orange to black) did not have any measurable effect on male reproductive success (contrary to hypothesis c, see Introduction). While our manipulation maintained the natural variation in cheek patch size and did not change the presence of the cheek patch per se, the results are not easily reconciled with the findings from earlier studies emphasizing the role of the male's cheek patches, and in particular their coloration. This calls for further investigation.

Effects of ornaments on male and female dominance

One possible explanation for the presence of some plumage ornaments only in male zebra finches is that these ornaments affect dominance and that males benefit more than females from expressing these ornaments (see hypothesis b in the Introduction). Our results do not support this explanation, because males with artificially increased breast bands tended to show reduced rather than enhanced dominance (see Figure 2E). This provides no explanation for the maintenance of the male breast band through male–male competition. Furthermore, males whose orange cheek patches were blackened showed a non-significantly higher dominance index than control males (see Figure 2E). Hence, orange cheek patches do not appear to enhance dominance either, and, if anything, the black-cheeked color mutant would be predicted to spread in the wild, if it indeed conveyed competitive benefits.

Exploratory analyses and the value of preregistration

Preregistration ensures against post hoc modification of hypotheses (making them match the data) and against selective reporting of effects that reach significance under a particular analysis strategy chosen after data exploration (Forstmeier et al. 2017). In this study, we specified—prior to data inspection—1) the primary outcome measures of the study (here: relative reproductive success and dominance index) and 2) the intended data analysis model (here: comparison of treated birds to all control birds in prespecified mixed-effect models). The conclusions of our study rest on the interpretation of the outcomes of planned tests, and the resulting effect size estimates are, hence, not inflated by confirmation bias (Greenwald 1975; Nickerson 1998; Simmons et al. 2011).

Different conclusions can be drawn from the same data if analyses are designed post hoc, namely when 1) using additional outcome measures in combination with 2) alternative ways of analysis. For example, a paired *t*-test between painted and sham-treated birds from the same aviary ($n = 9$ paired comparisons) shows that breast-painted males conducted significantly fewer attacks than their sham breast-painted counterparts (though not fewer attacks than the more representative 45 control birds together). Further, cheek-painted females produced significantly more fledglings than the sham cheek-painted females (paired *t*-test, $n = 9$ comparisons; though not more fledglings than all 45 control females). Such post hoc data exploration is possible and often helpful for generating new hypotheses with testable predictions. However, our study shows that selective reporting of significant tests can lead to different and misleading conclusions.

CONCLUSIONS

Our study suggests that deviant phenotypes in the zebra finch 1) are not avoided during mate choice and 2) do not show reduced or enhanced competitive ability. Thus, the maintenance of sexual dichromatism and species monomorphism with rare natural variants remains puzzling. Our study also questions existing hypotheses about the function of the various plumage ornaments of the zebra finch.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Jerónimo et al. (2017).

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REFERENCES

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. Biophotonics Intern. 11:36–42.

Adkins-Regan E. 2011. Neuroendocrine contributions to sexual partner preference in birds. Front Neuroendocrinol. 32:155–163.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Soft. 67:1–48.

Bolund E, Schielzeth H, Forstmeier W. 2007. Intrasexual competition in zebra finches, the role of beak colour and body size. Anim Behav. 74:715–724.

Bolund E, Schielzeth H, Forstmeier W. 2010. No heightened condition dependence of zebra finch ornaments–a quantitative genetic approach. J Evol Biol. 23:586–597.

Burley NT. 2006. An eye for detail: selective sexual imprinting in zebra finches. Evolution. 60:1076–1085.

Campbell DLM, Hauber ME. 2010. Conspecific-only experience during development reduces the strength of heterospecific song discrimination in Zebra Finches (*Taeniopygia guttata*): a test of the optimal acceptance threshold hypothesis. J Ornithol. 151:379–389.

Campbell DL, Hauber ME. 2009. The disassociation of visual and acoustic conspecific cues decreases discrimination by female zebra finches (*Taeniopygia guttata*). J Comp Psychol. 123:310–315.

Clayton NS. 1990. Assortative mating in zebra finch subspecies, *Taeniopygia guttata guttata* and *T. g. castanotis*. Philos Trans R Soc Lond B Biol Sci. 330:351–370.

Dale J, Dey CJ, Delhey K, Kempenaers B, Valcu M. 2015. The effects of life history and sexual selection on male and female plumage colouration. Nature. 527:367–370.

Ducrest AL, Keller L, Roulin A. 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol Evol. 23:502–510.

Dunn PO, Armenta JK, Whittingham LA. 2015. Natural and sexual selection act on different axes of variation in avian plumage color. Sci Adv. 1:e1400155.

Forshaw JM, Shephard O, Pridham A. 2012. Grassfinches in Australia. Collingwood, Australia: CSIRO Publishing.

Forstmeier W, Segelbacher G, Mueller JC, Kempenaers B. 2007. Genetic variation and differentiation in captive and wild zebra finches (*Taeniopygia guttata*). Mol Ecol. 16:4039–4050.

Forstmeier W, Wagenmakers EJ, Parker TH. 2017. Detecting and avoiding likely false-positive findings - a practical guide. Biol Rev Camb Philos Soc. 92:1941–1968.

Galeotti P, Rubolini D, Dunn PO, Fasola M. 2003. Colour polymorphism in birds: causes and functions. J Evol Biol. 16:635–646.

Götmark F. 1993. An experimental study of the importance of plumage coloration in breeding males of the white-crowned sparrow. Ornis Scandinavica. 24:149–154.

Greenwald AG. 1975. Consequences of Prejudice against Null Hypothesis. Psychol Bull. 82:1–19.

Hauber ME, Campbell DLM, Woolley SMN. 2010. The functional role and female perception of male song in Zebra Finches. Emu. 110:209–218.

Immelmann K. 1959. Experimentelle Untersuchungen über die biologische Bedeutung artspezifischer Merkmale beim Zebrafinken (*Taeniopygia castanotis* Gould). Zoologische Jahrbücher, Abteilung für Systematik. 86:437–592.

Immelmann K, Kalberlah HH, Rausch P, Stahnke A. 1978. The possible role of sexual imprinting in intraspecific sexual isolation in zebra finches. J Ornithologie. 119:197–212.

Jerónimo S, Khadraoui M, Wang D, Martin K, Lesku JA, Robert KA, Schlicht E, Forstmeier W, Kempenaers B. 2017. Data from: plumage colour manipulation has no effect on social dominance or fitness in zebra finches. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.sm819.

Kimball RT. 2006. Hormonal control of avian coloration. In: Hill GE, McGraw KJ, editors. Birds Coloration, Vol 1: Mechanisms and Measurements. Cambridge (MA): Harvard University Press. p. 591–644.

Kniel N, Müller K, Witte K. 2017. The role of the model in mate-choice copying in female zebra finches. Ethology 123:412–418.

Kraaijeveld K. 2014. Reversible trait loss: the genetic architecture of female ornaments. Ann Rev Ecol Evol Syst. 45:159–177.

Krause ET, Brummel C, Kohlwey S, Baier MC, Müller C, Bonadonna F, Caspers BA. 2014. Differences in olfactory species recognition in the females of two Australian songbird species. Behav Ecol Sociobiol. 68:1819–1827.

Landry GP. 1997. An almost complete color guide to varieties and genetics of the zebra finch. Franklin: Poule d’eau Publishers.

Lindsay WR, Barron DG, Webster MS, Schwabl H. 2016. Testosterone activates sexual dimorphism including male-typical carotenoid but not melanin plumage pigmentation in a female bird. J Exp Biol. 219:3091–3099.

Mariette MM, Griffith SC. 2012. Nest visit synchrony is high and correlates with reproductive success in the wild zebra finch *Taeniopygia guttata*. J Avian Biol. 43:131–140.

Naguib M, Nemitz A. 2007. Living with the past: nutritional stress in juvenile males has immediate effects on their plumage ornaments and on adult attractiveness in zebra finches. PLoS One. 2:e901.

Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. Biol Rev Camb Philos Soc. 82:591–605.

Nickerson RS. 1998. Confirmation bias: A ubiquitous phenomenon in many guises. Rev Gen Psychol. 2:175–220.

Owens IP, Short RV. 1995. Hormonal basis of sexual dimorphism in birds: implications for new theories of sexual selection. Trends Ecol Evol. 10:44–47.

Price DK, Burley NT. 1994. Constraints on the evolution of attractive traits - selection in male and female zebra finches. Am Nat. 144:908–934.

Price JJ, Eaton MD. 2014. Reconstructing the evolution of sexual dichromatism: current color diversity does not reflect past rates of male and female change. Evolution. 68:2026–2037.

R Core Team. 2016. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Riebel K. 2009. Song and female mate choice in zebra finches: a review. In: Naguib M, Zuberbuhler K, Clayton NS, Janik VM, editors. Advances in the study of behavior. Vol. 40. San Diego: Academic Press. p. 197–238.

Roberts ML, Buchanan KL, Bennett ATD, Evans MR. 2007. Mate choice in zebra finches: does corticosterone play a role? Animal Behav. 74:921–929.

Ryan MJ, Rand AS. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution. 47:647–657.

Saetre G-P, Moum T, Bures S, Král M. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. Nature. 387:589.

Simmons JP, Nelson LD, Simonsohn U. 2011. False-positive psychology: undisclosed flexibility in data collection and analysis allows presenting anything as significant. Psychol Sci. 22:1359–1366.

Ten Cate C, Vos DR. 1999. Sexual imprinting and evolutionary processes in birds: A reassessment. In: Slater PJB, Rosenblat JS, Snowden CT, Roper TJ, editors. Advances in the study of behavior. Vol. 28. San Diego: Academic Press. p. 1–31.

Tschirren B, Postma E, Rutstein AN, Griffith SC. 2012. When mothers make sons sexy: maternal effects contribute to the increased sexual attractiveness of extra-pair offspring. Proc Royal Soc B. 279:1233–1240.

Veen T, Borge T, Griffith SC, Saetre GP, Bures S, Gustafsson L, Sheldon BC. 2001. Hybridization and adaptive mate choice in flycatchers. Nature. 411:45–50.

Vos DR, Prijs J, Tencate C. 1993. Sexual imprinting in zebra finch males - a differential effect of successive and simultaneous experience with 2 color morphs. Behaviour. 126:137–154.

Walter MJ. 1973. Effects of parental colouration on the mate preference of offspring in the zebra finch, *Taeniopygia guttata castanotis* Gould. Behaviour. 46:154–173.

Wang D, Kempenaers N, Kempenaers B, Forstmeier W. 2017. Male zebra finches have limited ability to identify high-fecundity females. Behav Ecol. 28:784–792.

Zajitschek SR, Brooks RC. 2008. Distinguishing the effects of familiarity, relatedness, and color pattern rarity on attractiveness and measuring their effects on sexual selection in guppies (*Poecilia reticulata*). Am Nat. 172:843–854.

Supplementary Material

Plumage colour manipulation has no effect on social dominance or fitness in zebra finches

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Table S1. Effects of the sham treatment (comparing untreated controls versus sham-treated controls) on relative reproductive success (model 1) and on the dominance index (model 2). Parameter estimates with standard errors (SE) are given relative to the sham-treated birds. Data from both sexes combined to achieve maximal power for possible effects of sham-treatment. The percentage of variance explained by the random effects of aviary (Av) and population (Pop) are indicated. The numbers refer to variation in intercepts (Intercept) and in sham treatment effects (Untreated). The number of birds in each treatment category is indicated in the last column.

Model	Fixed effects	Estimate	SE	χ^2	*p*	Av (%)	Pop (%)	N
1	Intercept	1.03	0.19			9.4	0	36
	Untreated	-0.12	0.28	0.20	0.65	32.1	0	54
2	Intercept	0.03	0.22			0	0	36
	Untreated	-0.28	0.30	0.87	0.35	0	0	54

Table S2. Effects of the plumage colour manipulations on relative reproductive success. Model 1: data from both sexes and both manipulations combined; Model 2: data from males only; Model 3: data from females only. Parameter estimates with standard errors (SE) are given for the painted treatments relative to the control birds. *P*-values refer to the removal of treatment as a fixed effect (1 df in Model 1, 2 df in Models 2 and 3). The percentage of variance explained by the random effects of aviary (Av) and population (Pop) are indicated where applicable. The numbers refer to variation in intercepts (Intercept) and in treatment effects (Painted contrasts). Note that variation in treatment effects between populations is unlikely to be estimated reliably in Models 2 and 3 (on average based on 3 treated individuals per sex and ornament and population).

Model	Fixed effects	Estimate	SE	χ^2	*p*	Av (%)	Pop (%)	N
1	Intercept	0.95	0.11			1.4	0	90
	Painted	0.16	0.23	0.51	0.47	17.4	0	36
2	Intercept	1.00	0.18	0.08	0.96		0.1	45
	Painted breast	-0.12	0.50				9.9	9
	Painted cheeks	0.10	0.45				1.5	9
3	Intercept	0.91	0.13	1.75	0.42		0	45
	Painted breast	0.37	0.33				0	9
	Painted cheeks	0.28	0.33				0	9

Table S3. Effects of the plumage colour manipulations on dominance. Model 1: data from both sexes and both manipulations combined; Model 2: data from males only; Model 3: data from females only. Parameter estimates with standard errors (SE) are given for the painted treatments relative to the control birds. *P*-values refer to the removal of treatment as a fixed effect (1 df in Model 1, 2 df in Models 2 and 3). The percentage of variance explained by the random effects of aviary (Av) and population (Pop) are indicated where applicable. The numbers refer to variation in intercepts (Intercept) and in treatment effects (Painted contrasts). Note that variation in treatment effects between populations is unlikely to be estimated reliably in Models 2 and 3 (on average based on 3 treated individuals per sex and ornament and population).

Model	**Fixed effects**	**Estimate**	**SE**	χ^2	***p***	**Av (%)**	**Pop (%)**	**N**
1	Intercept	-0.13	0.11			0	0	90
	Painted	0.02	0.20	0.01	0.93	0	0	36
2	Intercept	0.03	0.13	2.51	0.28		0	45
	Painted breast	-0.56	0.44				0.3	9
	Painted cheeks	0.37	0.33				0	9
3	Intercept	-0.28	0.18	0.60	0.74		0.8	45
	Painted breast	-0.24	0.42				1.0	9
	Painted cheeks	0.23	0.63				34.7	9

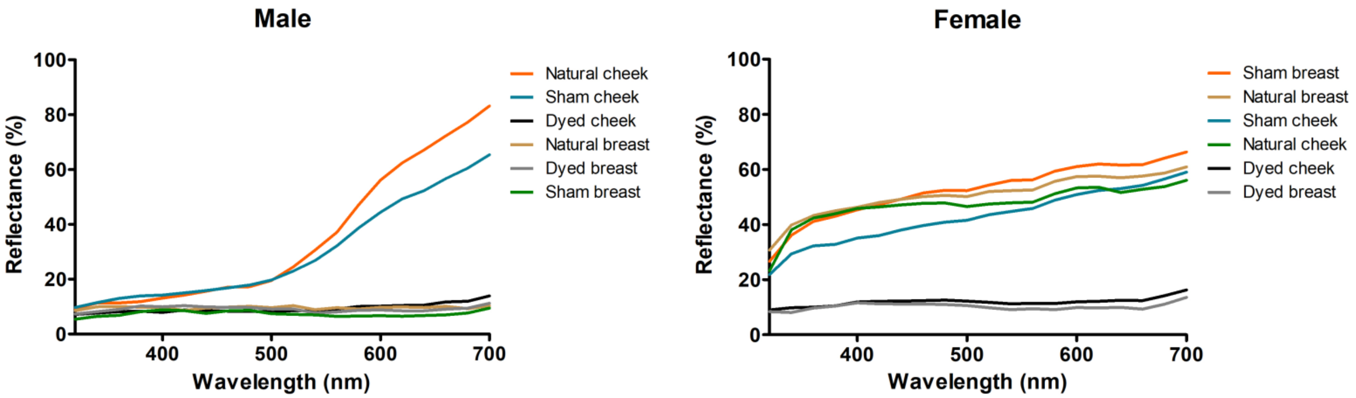

Figure S1. Examples of reflectance spectra of natural, sham-treated and dyed plumage areas in males and females. In both sexes, the black colouration closely matched natural patches of black plumage, particularly in the UV part of the spectrum.

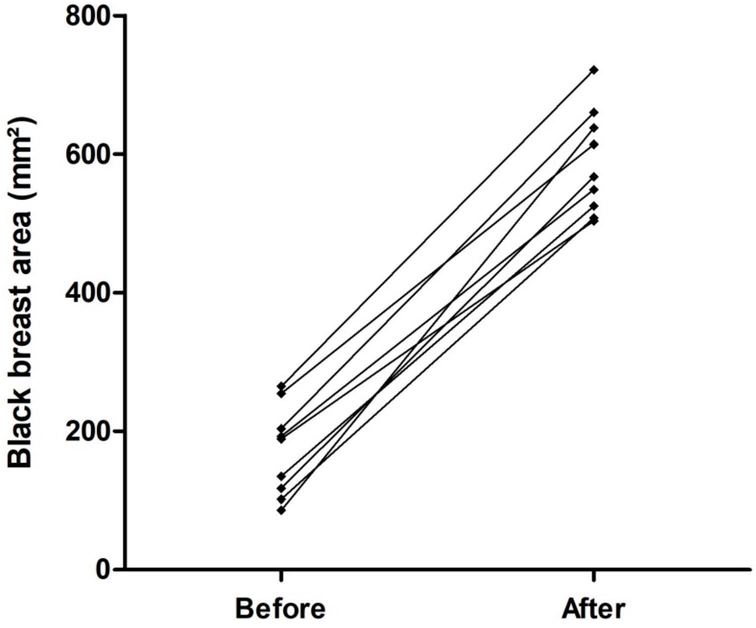

Figure S2. The size of breast bands before and after experimental enlargement (n = 9 males, repeated measures connected by lines). Ornament sizes were measured from standardized photographs using the software Image J (Abràmoff et al., 2004).

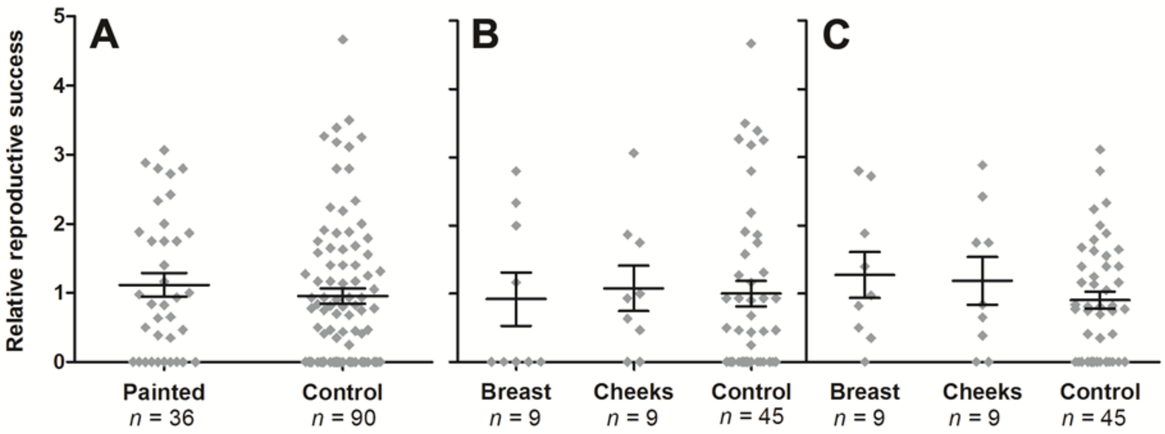

Figure S3. Raw data of relative reproductive success of the painted and the control birds. (A) Both sexes and plumage colour treatments (breast and cheeks) combined. (B) Data for males only. (C) Data for females only. Mean values ± SE are indicated by horizontal lines with error bars.

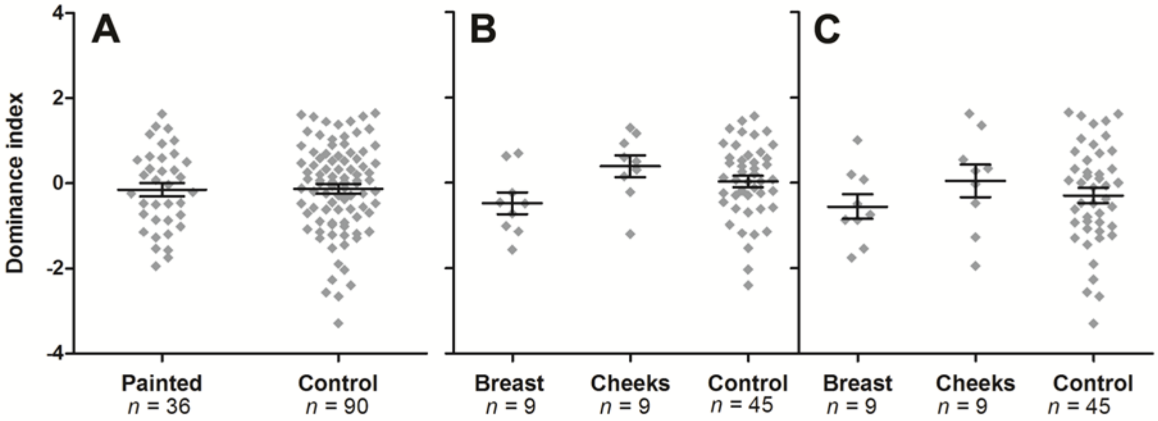

Figure S4. Raw data of dominance index of the painted and control birds. (A) Both sexes and plumage colour treatments (breast and cheeks) combined. (B) Data for males only. (C) Data for females only. Mean values ± SE are indicated by horizontal lines with error bars.

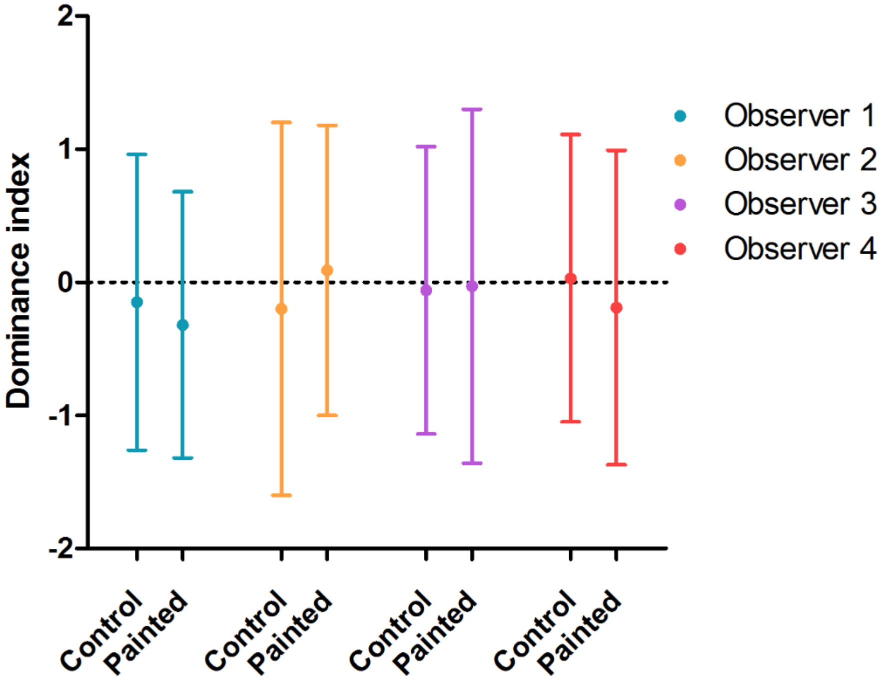

Figure S5. Dominance index (mean ± SD) for control and painted birds based on data from each of the 4 observers. Observer 1 and 2 predicted in advance that the painted birds would be dominant over the control birds, whereas observer 3 and 4 predicted that the dye manipulation would not affect dominance. Shown are mean ± SD for 90 control and 36 painted birds per observer. The effect size per observer was small: $d_{observer1} = -0.16$, $d_{observer2} = 0.23$, $d_{observer3} = 0.03$ and $d_{observer4} = -0.20$. Observer 2 showed a weak trend in line with the preconception, but observer 1 showed an almost equally strong trend against the expectation. Overall, the treatment by observer interaction was not significant (mixed-effect models based on n = 504 indices, controlling for n = 126 individuals as a random effect, comparing models with and without the interaction term, df = 3, $\chi^2 = 5.89$, p = 0.12, while keeping the non-significant main effects of observer and treatment).