Mutual mate preferences and assortative mating in relation to a carotenoid-based color trait in blue tits

Samuel Caro, Léo Pierre, Matthieu Bergès, Raldi Bakker, Claire Doutrelant, Francesco Bonadonna

To cite this version:

Samuel Caro, Léo Pierre, Matthieu Bergès, Raldi Bakker, Claire Doutrelant, et al.. Mutual mate preferences and assortative mating in relation to a carotenoid-based color trait in blue tits. Behavioral Ecology, 2021, 32 (6), pp.1171-1182. 10.1093/beheco/arab080 . hal-03434794

HAL Id: hal-03434794
https://hal.science/hal-03434794

Submitted on 10 Jan 2022

Mutual mate preferences and assortative mating in relation to a carotenoid-based color trait in blue tits

Samuel P. Caro[1,2,*], Léo Pierre[1], Matthieu Bergès[1], Raldi Bakker[2,3], Claire Doutrelant[1,#], Francesco Bonadonna[1,#]

1. CEFE, Univ Montpellier, CNRS, EPHE, IRD, Univ Paul Valéry Montpellier 3, Montpellier, France
2. Netherlands Institute of Ecology (NIOO-KNAW), Department of Animal Ecology, Wageningen, The Netherlands
3. Wageningen University (WUR), Department of Animal Science, Behavioural Ecology Group, Wageningen, The Netherlands

\# These authors contributed equally to the study

* Author for correspondence: samuel.caro@cefe.cnrs.fr

Short title: Mate-preferences in blue tits

Abstract

Choosing an appropriate sexual partner is a critical decision for many animal species. However, many mechanisms involved in mate choice are still poorly understood. Do both males and females choose their sexual partners, do both sexes use the same criteria for choosing, and do their own phenotype influence the choices they make, are questions that need further investigation. Over two successive experiments conducted in captivity with hand-reared blue tits (*Cyanistes caeruleus*), we manipulated the color of the chest plumage, a secondary sexual trait that reflects an individual's condition, to create two different color morphs (one pale and one colored). We then tested whether both sexes express a preference, whether they are attracted to the same morphs, and if the subjects' own chest color influences the preference they show. Our data reveal that both sexes are choosy, with females tending to be slightly choosier than males. We also show that both sexes preferentially select individuals with a pale chest plumage over colorful individuals, and this was again more pronounced in females. Finally, paler individuals tend to be selected by birds that are themselves pale, even if this phenotype matching was not very robust. Such a preference for paler individuals is intriguing since mates are predicted to associate with individuals displaying higher, not lower, value of quality signals. It could result from adaptive mechanisms related to avoidance of aggressiveness in confined environment, avoidance of conflicting sexual signals within individuals, or from cultural mechanisms leading to preference for individuals that match its own phenotype.

Keywords: bird, carotenoids, color, mate-choice, sexual selection

Introduction

Choosing mates is one of the most important decisions that many sexually reproducing animals have to take. Mate choice indeed impacts an individual's current reproductive success and fitness (Andersson, 1994). Both direct and indirect benefits, such as access to a high-quality territory, parental care, reduced likelihood of getting sexually transmitted disease, or genetic quality for the offspring, can be derived from selecting an appropriate partner (Fisher, 1915; Trivers, 1972; Smith, 1991). In conventional sex-role species, females are typically considered the choosing sex, while males spend their time advertising (Jones and Ratterman, 2009; Davies et al., 2012). As a consequence, mate choice has been mostly studied in terms of male traits and behaviours that affect female preference. However, conspicuous traits are present both in males and females of many species (Amundsen, 2000; Dale et al., 2015; Hare and Simmons, 2019; Doutrelant et al., 2020). While these traits in females have long been considered non-functional byproducts of sexual selection on male traits (Lande, 1980; Price, 1996; Kraaijeveld et al., 2007), the possibility that males could also choose their female partners has received comparatively less attention (Amundsen, 2000; Clutton-Brock, 2009; Courtiol et al., 2016; Doutrelant et al., 2020). Like male ornaments, female ornaments could signal reproductive or survival qualities and be involved in male mate choice (Hare and Simmons, 2019). Mutual choosiness is predicted to be particularly important when benefits of choices are evident for both sexes, in particular in monogamous species where reproductive rates are similar for males and females, and in species with biparental care (Johnstone et al., 1996; Kokko and Johnstone, 2002; Kraaijeveld et al., 2007). Male mate preferences have been documented in various taxa, including fishes (Sargent et al., 1986; Amundsen and Forsgren, 2001; Roberts and Mendelson, 2017; Schlupp, 2018), insects (Bonduriansky, 2001; Byrne and Rice, 2006), reptiles (Olsson, 1993; LeBas and Marshall, 2000), mammals (Domb and Pagel, 2001; Fitzpatrick et al., 2015), and birds (Amundsen et al., 1997; Griggio et al., 2005; Pryke and Griffith, 2007; Kimmitt et al., 2018). In birds for example, male bluethroats (*Luscinia s. svecica*) have been reported to spend more time close to colorful than drab females in a binary choice experiment (Amundsen et al., 1997). Female coloration positively correlated with tarsus length and body mass, suggesting that coloration can also signal phenotypic quality in females (Amundsen et al., 1997; Roulin et al., 2000; Griggio et al., 2005; Weiss, 2006; Doutrelant et al., 2008; 2012). In crested auklets (*Aethia cristatella*), both males and females were attracted by opposite-sex models having experimentally increased crests on the front-head (Jones and Hunter, 1993). On the other hand, evidence for male mate preference has failed to be shown in other studies (Muma and Weatherhead, 1989; Dale and Slagsvold, 1994). Since reproduction is often less costly for males than females (Williams, 2012), and additional paternities can be gained from fertilizing multiple females, males might not always need to be choosy (but see Pizzari et al., 2003). Male choosiness might even be counterselected if choosy males face a competitive disadvantage (Fitzpatrick et al 2015). These contrasted hypotheses highlight the need for additional work on male sexual preferences to understand how often males contribute to sexual selection.

For a long time, it has also been considered that individuals always try to mate with the highest quality partners. In practice, this has assumed that regardless their own condition or phenotype, females (historically considered the choosing sex, see above) should always choose the best males, and therefore that there is little inter-individual variation in preferences (Cotton et al., 2006). Several studies have however challenged this view and shown that individuals may vary substantially in their mate preferences, with preferences that can sometimes be predicted from the subject's own phenotype. Female black field crickets (*Teleogryllus commodus*) raised on a protein-rich diet were more responsive and choosy to male call quality than females reared on poorer diets (Hunt et al., 2005). Females of the smooth toadlet (*Uperoleia laevigata*), an Australian frog, selected mates that were about 70% of their own body weight (Robertson, 1990). In zebra finches (*Taeniopygia guttata*), females that were reared in enlarged broods, and that were concomitantly considered as being of poorer quality than females raised in small broods, were shown to prefer males that were themselves of lower quality (Holveck and Riebel, 2010; see Griggio and Hoi, 2010 for a similar example in sparrows; but see Wang et al., 2017). Similarly, male and female great tits (*Parus major*) have been shown to prefer opposite-sex individuals that were of similar heterozygosity levels as themselves, even if in the case of relatively homozygous individuals it meant having offspring of low heterozygosity level (Zandberg et al., 2017). In the frog and the great tit examples, the authors also showed that these preferences are adaptive because pairing with the preferred phenotypes led to higher fertilization rates and fitter offspring, respectively (Robertson, 1990; Zandberg et al., 2017). Such kind of self-referent phenotype matching, leading to assortative mating, could be quite widespread. It has however been relatively little studied experimentally so far.

Here we study experimentally the mate preferences of captive blue tits. Blue tits are socially monogamous, both sexes care for the offspring, and both sexes are ornamented (Andersson et al., 1998), characteristics that, according to what we have reported above, are ideal for testing mutual mate preference (Hunt et al., 1999) and assortative mating (Fargevieille et al., 2017). In blue tits both the UV-blue crest and the yellow chest have been suggested to function as secondary sexual signals (but see Parker, 2013 for a meta-analysis on male UV coloration). Most studies focused on the role that blue crest color variation plays on intra-sexual competition and on inter-sexual selection (Hunt et al., 1998; 1999; e.g. Delhey et al., 2003; Kurvers et al., 2010; Remy et al., 2010; Parker, 2013). The role of the yellow-colored chest has received less attention, but there is correlational and experimental evidence that the intensity of yellow chroma or brightness may reflect an individual's condition and parental ability (Senar et al., 2002; Saks et al., 2003; Peters et al., 2007; Doutrelant et al., 2008; Ferns and Hinsley, 2008; Reudink et al., 2009; 2012; Garcia-Navas et al., 2012; Midamegbe et al., 2013). The yellow color could thus potentially be used as a reliable signal of quality in a mate-choice context. Contrary to the blue color that is structurally based, the yellow color is conferred by carotenoids exclusively acquired through food (Goodwin, 1984; Hill and McGraw, 2006; Isaksson et al., 2008). In captivity, where the right balance of carotenoids is difficult to provide, blue and great tits rapidly loose their yellow-based coloration that becomes light-grey (see Fig.S1A), showing how plastic this trait is. Carotenoids are also thought to contribute to prevention of oxidative stress and regulation of immune function (Blount et al., 2003; Biard et al., 2006; Simons et al., 2012; Koch and Hill, 2018).

Here, we test whether both male and female blue tits choose their partners, and whether the expressed preferences are based on the color of their breast. For this, we manipulated the chest color of captive blue tits and tested the preferences of opposite-sex individuals for variation in this phenotypic trait over two successive experiments. In the first experiment, we manipulated the chest colors of the birds that were presented as stimuli. In the second experiment, we manipulated the chest colors of both the stimulus and the subject birds. We then tested whether both males and females express preferences (experiments 1 and 2), whether they show preferences for the same color phenotypes (experiments 1 and 2), and whether their own phenotype (chest color) influences the preference they express (experiment 2).

Methods

Ethical note

The experiments run in this study were approved by the Animal Experimentation Committee of the Royal Dutch Academy of Sciences (DEC-KNAW; permit number CTE.09-04 and NIOO11.09), and the Animal Care and Use Committee Languedoc-Roussillon (permit number CEEA-LR-1047). The work performed in the field was approved by the prefectural office of Corsica and the Regional Direction of Environment (DIREN) committee (permit numbers 2009-0379, 3467 and 2015615-147).

Subjects

In experiment 1, we used 81 blue tits (*Cyanistes caeruleus*; 44 males and 37 females) that were taken as nestlings in the long-term studied populations in Corsica (Muro and Pirio, see Reparaz et al., 2014). Whole broods of chicks (N=17 broods) were collected from their nests when they were 7 to 10-day-old, and were transferred to the laboratory for standardized hand rearing (see Drent et al., 2003; Titulaer et al., 2012). Briefly, birds were transported to the Netherlands Institute of Ecology (NIOO-KNAW) by car. During the travel, and later on at the institute, chicks were fed every half-hour, for 14 hours per day (7:00 am - 9:00 pm), with a diet consisting of a mixture of curd cheese, ground beef heart, baby cereal, multivitamin solution and calcium carbonate, supplemented with wax moth larvae and bee larvae, until independence. After reaching independence (about 35 days after hatching) and being molecularly sexed, the birds were temporarily transferred to an individual home cage of 0.9 x 0.4 m x 0.5 m, before being released in single-sex groups in outdoor aviaries, with acoustic but no visual contacts between aviaries. Adult diet consisted of a mixture of egg, cow heart, vitamins, and minerals, supplemented with dry food containing insects (Orlux Insect Patee, Versele-Laga, Deinze, Belgium) and peanuts. Birds also had access to mealworms, grit, and sunflower seeds. Food and water were provided *ad libitum*.

In experiment 2, we used 72 blue tits (40 males and 32 females) that were also taken as nestlings, aged 8 to 12-day-old, in the long-term studied population of La Rouvière, near Montpellier. Hand-rearing procedures were similar to those of experiment 1, except that it was conducted at the Center for Functional and Evolutionary Ecology (CEFE-CNRS) at Montpellier, France. Diet consisted here in a solution of hand-rearing powder (Nutribird A21 and A19, Versele-Laga, Deinze, Belgium), supplemented with wax moth larvae, bee larvae, and mealworms. After they reached independence, birds were fed with mealworms, and a cake made of eggs, sunflower margarine, sugar, flour and protein-rich pellets (Country's Best Show1-2 Crumble, Versele-Laga, Deinze, Belgium). Both mealworms and cake were supplemented with commercial powders containing mostly vitamins and minerals (Nutribird A21, Versele-Laga; and Nekton-S, Nekton GmbH, Pforzheim, Germany). Food and water were provided *ad libitum*. Birds were also housed in single-sex groups in outdoor aviaries. Here however, visual and physical contact were possible through the mesh of adjacent aviaries. Aviaries housing groups of females were away from aviaries housing groups of males, with no visual and physical contacts possible between males and females. For logistic reasons inherent to food distribution, birds from a given aviary belonged to the same color treatment (provided in the food, see below), and treatments alternated between aviaries, so that each bird could see and interact with same-sex birds from both treatments.

Color treatments

In experiment 1, we manipulated the chest color of male and female blue tits using color markers (a procedure used in other studies, Delhey et al., 2007; Remy et al., 2010; Ligon and McGraw, 2016). Only birds used as stimuli in the mate-preference apparatus (32 birds: 16 males and 16 females, see below) were artificially colored, not the ones that were used as subjects (49 birds: 28 males and 21 females). Half of the stimulus birds (8 males and 8 females) had their chest feathers colored in yellow (Yellow group, Fig. 1A), with a marker that best mimic the natural color in free-living blue tits (Prismacolor Art Marker, canary yellow PM19, Newell Rubbermaid, Atlanta, USA), while the other half of the birds (8 males and 8 females) had their chest feathers colored in grey (Prismacolor Art Marker, warm grey PM-99, Newell Rubbermaid, Atlanta, USA), the color that blue and great tits commonly acquire when held in captivity for long periods of time (Fig. 1A). Resulting colors were measured with a spectrometer (Avaspec, Avantes, The Netherlands) and with a DH-2000 Deuterium Tungsten Halogen Light Source and a 200 µm optic-fiber probe. Spectrograms obtained from the two kinds of artificially colored birds were close to spectrograms obtained in non-manipulated birds (wild yellow birds and captive grey birds, respectively; Fig. 1A and C, table S1).

In experiment 2, we manipulated the chest color of male and female blue tits through the addition of carotenoids in their diet. In order to test for self-reference phenotype matching mechanisms, the chest plumage of both stimulus and subject birds were manipulated early in life, when birds were starting to feed independently (i.e. about 35 days old, in early June). Thirty five birds had their diet (cake and mealworms, see above) supplemented with a powder containing 10% of canthaxanthin at a concentration of 5000 ppm (Carophyll-Red 10%, produced by DSM Nutritional Products Ltd, Basel, Switzerland), while 37 birds had their diet supplemented with a placebo made of the exact same powder as in the other group, but with no pigment in it (placebo specifically made for the experiment by DSM Nutritional Products Ltd, Basel, Switzerland). Treatments thus only differed in the presence/absence of canthaxanthin, which colors the chest plumage in dark yellow/orange. Spectrograms of placebo and canthaxanthin tits are shown at fig. 1B. We opted for canthaxanthin rather than other carotenoids present in the diet of wild tits like lutein, or zeaxanthin because those other carotenoids have not always been efficient in enhancing the chest color of blue tits (e.g. Biard et al., 2006), and because we had experience with canthaxanthin and knew it efficiently colors the chest plumage of blue tits (P. Perret, unpubl. data). Furthermore, using canthaxanthin allowed us to oppose a pale yellow/grey to a dark yellow/orange morph, two experimental groups that consisted in color phenotypes rarely encountered in the wild, thus avoiding to oppose a natural phenotype (natural yellow) with an artificial one (pale yellow/grey). Treatments were stopped two weeks before the start of the behavioral tests. After the first series of binary preference tests, we started the diet supplementation again, but we reversed the experimental groups, i.e. birds that were first supplemented with canthaxanthin received the placebo, and vice versa for the other group, which reversed the plumage colors of the two groups. That way each bird was tested in both groups.

Mate preference testing

About two weeks before the start of the tests, birds were settled in individual cages in rooms where opposite-sex birds could not see each other. Tested and stimulus birds were housed in separate rooms. During that time, birds were exposed to a long photoperiod (≥ 15 hours of light per day). Long photoperiods indeed trigger a cascade of neuroendocrine reactions that lead to production and secretion of sexual steroids by the gonads, particularly 17β-estradiol (E2) in females, and promote the expression of reproductive behaviors (Dawson et al., 2001). In songbirds, increasing plasma E2 in females, either directly through injection or implants of E2, or indirectly through exposure to long photoperiods, is often used to infer behavioral sexual preferences to a relevant stimulus like male song or male presence (Searcy, 1992; Byers and Kroodsma, 2009; Caro et al., 2010; Reparaz et al., 2014). By exposing blue tits to long days prior to testing, we increased the chances that the observed choices are actual sexual preferences and not social preferences, even though it is hard to firmly distinguish the two.

Experiment 1: Among the 81 birds used, 32 (16 males and 16 females) were used as stimulus birds. These 32 birds were randomly distributed across the two color treatments (yellow and grey markers, see above). The remaining 49 birds were not artificially colored and used as subjects. Each subject was tested only once and exposed to one pair of unrelated birds of the opposite sex. Sixteen pairs (8 pairs of each sex) of stimulus birds were formed and used in two to four mate-preference trials. Tests were performed in 2013 (April 23 to May 15), with birds that were born in 2010 and 2011.

Experiment 2: Among the 72 birds used, 24 (12 males and 12 females) were used as stimulus birds. Contrary to experiment 1, both stimulus and tested birds were subjected to the color treatments (canthaxanthin and placebo supplements in the diet, see above). In addition, the members of the 12 pairs of stimulus birds were not selected at random here, but pairs were made of brothers or sisters. Choosing genetically similar birds for a given pair reduces any genetically-related and early-environment-related variances between the members of a pair and hence enhances the importance of the artificially manipulated color of the chest as a choice criterion during the behavioural tests. Each pair of stimulus birds was used in three to five mate-preference trials. Tests were performed in 2015 (December 16 to December 23), with birds that were born in spring. At that age, birds were sexually mature (Silver et al., 1992; Dawson et al., 2001), and were set into a reproductive-like status by the long photoperiod (see above). After the end of the first series of tests, continuous exposure to a long photoperiod led to a state of photorefractoriness that coincides with the onset of a new molt of all feathers (Dawson et al., 2001). Once the first birds had started molting, all birds were moved to a shorter photoperiod for six weeks, which restored photosensitivity and allowed for a new cycle of stimulation of the reproductive system by the long days of April (Silverin, 1994; Dawson et al., 2001). At the end of the first series of tests the color treatments were reversed for all birds (see above), so that birds from each group could grow breast feathers of the color that previously characterized the other group. The second series of tests took place in Spring 2016 (April 28 to May 3, 2016), keeping the same pairs of stimulus birds as in the first trials.

The test apparatus used for assessing potential partner preferences was identical in both experiments and similar to the one described in Reparaz et al. (2014). Briefly, two identical test chambers were used simultaneously. Each test chamber was made of a large neutral area (approximately 4m^2) and two semi-enclosed areas (approximately

1m^2 each) in front of the cages in which the stimulus birds were placed (Fig. S1). Artificial wooden "trees" were disposed in the neutral zone and in each stimulus zone. Each stimulus cage was constructed of wood and wire mesh, and affixed to a rolling base. A ‘natural sun’ light bulb (Arcadia Compact Bird Lamp 20W, Arcadia Products, Redhill, United Kingdom) was installed in the top of each stimulus bird cage to allow for UV-coloration visibility, and each cage was equipped with four wooden perches. A curtain was installed in front of each two cages to conceal the stimulus birds from the subject bird as needed throughout the testing. Three wide-angle cameras were affixed in each test chamber, with one in the neutral area, and one in each of the two stimulus zones, to capture both subject and stimulus bird activities throughout the trials (Fig. S1).

Tested and stimulus birds were introduced in their respective compartments, with the curtains closed to prevent visual contacts between them. They were given 10 minutes to acclimate to the chamber/cages. After the acclimation period, the curtains were raised, and the first half of the mate preference trial took place for 20 minutes. After that time, the curtains were closed, and the stimulus cages were switched, in order to account for any side-bias of the subject birds. The second half of the trial took place over 20 minutes. After testing, subjects were returned to their home cages. Each bird was tested only once in experiment 1, twice in experiment 2, i.e. once as canthaxanthin bird, and once as placebo bird, with four months in between the tests to allow birds to molt and change their chest plumage color.

Video analyses and data processing

Video footages from the mate preference tests were analyzed using the computer programs Observer XT (version 10.5, Noldus, Wageningen, The Netherlands) for experiment 1, and Solomon Coder (version beta 15.11.19, https://solomoncoder.com) for experiment 2. Footages were analyzed in terms of the time that subject birds spent in each of the three possible zones (neutral, right stimulus, left stimulus). Data from the two 20-min half trials of each test were pooled together for statistical analyses. Mate preference was inferred from the amount of time (in seconds) a tested bird spent near a particular stimulus bird. We also defined two other response variables that we called "interest" and "preference strength" (see Reparaz et al., 2014). *Interest* was calculated to estimate the overall motivation of the subject birds during the experiment. This variable indicates the proportion of time that the subjects spent close to stimulus birds, and was calculated as follows:

$$\frac{Time\ spent\ with\ stimulus\ 1\ +\ Time\ spent\ with\ stimulus\ 2}{Total\ duration\ of\ the\ test}$$

The “total duration of the test” represents the cumulative time spent in the three possible zones of the test chamber. *Preference strength* indicated the strength of a subject's preference for any of the two stimulus birds it encountered in the test. Which stimulus bird is chosen is not considered here, preference strength only illustrates the magnitude of the bias for one of the two stimuli, whichever it is. In the present case, it is thus a simple way to assess how affirmative a bird is in its choice, and it has been shown to be context-dependent (Reparaz et al., 2014). Preference strength is defined as the relative amount of time spent with the chosen individual (the bird with which the subject spent >50% of the stimulus-zone time) compared to the time spent with both stimuli, and calculated as follows:

$$\frac{Time\ spent\ with\ chosen\ stimulus}{Time\ spent\ with\ stimulus\ 1\ +\ Time\ spent\ with\ stimulus\ 2}$$

Statistical analyses

We used a step approach to analyze the data from simple, starting from easily understandable tests on counts, to more elaborated tests on the exact time birds spent in the different zones of the apparatus.

Interest

For both experiment 1 and 2, we started with a simple binomial test that compared the number of subject birds that spent the majority of their time in the zone of the stimulus birds with the number of birds that spent more time in the neutral zone. We next tested the effect of several explanatory variables on the level of interest. As interest is a proportion, it was arcsine transformed to achieve normality. In experiment 1, we tested whether the level of interest differed between males and females, and varied depending on the time of the day, by including the sex of the subject birds (*female* or *male*) and time of day (continuous and centered to produce a variable with a mean of zero) as explanatory variables in a linear model. Preliminary analyses of the datasets showed that the behaviors of the birds were sometimes influenced by the time at which the birds were tested. To account for this bias, time of day will be included as a nuisance variable in all analyses that include covariates. For experiment 2, we also added an effect of the treatment of the subject birds (*canthaxanthin* or *placebo*), since in this experiment we not only compared the preferences of males and females, but also considered their color phenotypes, we used a linear mixed-model with individual ID as a random intercept to account for the fact that each bird was tested on two occasions.

Preference strength

As preference strength is a proportion, it was also arcsine transformed to achieve normality. In experiment 1, we tested whether preference strength differed between males and females, and varied depending on the time of the day, by including the sex of the subject birds (*female* or *male*) and time of day (continuous and centered to produce a variable with a mean of zero) as explanatory variables in a linear model. In the model, preference strength was weighted by interest. In other words, subjects that spent more time with the stimulus birds in general (i.e. higher interest) were given more statistical weight in the analyses than subjects that were less interested (see Reparaz et al., 2014). For experiment 2, we also added an effect of the treatment of the subject birds (*canthaxanthin* or *placebo*), since in this experiment we not only compared the preferences of males and females, but also considered their color phenotypes, and we used a linear mixed-model with individual ID as a random intercept to account for the fact that each bird was tested on two occasions.

Which birds are chosen?

For both experiments 1 and 2, we started with a simple binomial test that compared the number of subject birds that spent more time close to yellow (exp 1) or canthaxanthin (exp 2) birds than to control (exp 1) or placebo (exp 2) birds. We then tested the effect of several explanatory variables on these counts, using generalized linear (mixed) models with binomial distributions. In experiment 1, we tested whether the number of birds that spent more time close to yellow or control birds differed between males and females, and varied depending on the time of the day, by including the sex of the subject birds and time of day as explanatory variables on a binary response variable (*control* or *yellow*) in a generalized linear model. For experiment 2, we also added an effect of the treatment of the subject birds, and its interaction with sex (to test for a possible self-reference phenotype matching mechanism) on the binary response variable (*canthaxanthin* or *placebo* bird chosen) in a generalized linear mixed model with individual ID as a random intercept.

Finally, instead of only considering counts of birds (which gives the same value to all choices, independently of whether the subject spent 51 or 100% of its time with the chosen stimulus), we analyzed the exact time (in seconds) subject birds spent close to each stimulus using linear mixed models. In experiment 1, we included the sex of the subject bird (*female* or *male*), the treatment of each stimulus bird (*control* or *yellow*), and their interactions as fixed effect. Since in the dataset we considered two time values for each subject bird (one time value for each stimulus), we included subject identity as a random intercept. Like for the analysis of preference strength, time was weighted by interest. We performed the same analysis for experiment 2, adding the effect of the treatment of the subject bird (*canthaxanthin* or *placebo*), its 2- and 3-way interactions with sex of subject birds (*female* or *male*) and treatment of stimulus birds (*canthaxanthin* or *placebo*), and the effect of time of testing (continuous, centered). In experiment 2, subject identity was also introduced as a random intercept to account for the fact that each subject has been tested twice, and that there are two time values per subject for each test.

Analyses were performed in *R* (version 3.2.1)(R-Core-Team, 2015), using the functions *binom.test*, *lm*, *glm*, and the functions *lmer* and *glmer* of the package *lme4 (Bates et al., 2015).* Models were simplified using backward elimination of the non-significant terms, starting with the higher order interactions (Crawley, 2007). P-values were obtained either by model comparisons between a model that includes and another that excluded the term of interest, or using the *anova* function of the package *lmerTest (Kuznetsova et al., 2016)* in the cases of *lmer* analyses. Post-hoc tests following (nearly) significant interactions were performed using the package *lsmeans* (Lenth, 2016). Confidence intervals of model estimates plotted on the figures were calculated using the package *bootpredictlme4* (Duursma, 2017).

Results

Experiment 1

Interest for stimulus birds

On average, subject birds spent 74% of their time close to the stimulus birds, with birds spending more time in the stimulus-bird zone than in the larger neutral zone in 39 of the 49 tests performed (binomial test, $p<0.001$). The level of interest for the stimulus birds did not differ between the sexes of the subject birds, and was not influenced by the time of day the birds were tested (table 1).

Preference strength

On average female tended to express clearer preferences than males (females: 0.67±0.10, males: 0.62±0.09; mean±sd). This sex effect on preference strength was however not fully significant ($p=0.063$, see table 1). There was no effect of the time of day the birds were tested (table 1).

Which stimulus bird do they choose?

If we count the number of subjects that spent more time with yellow or control birds, significantly more subjects selected control birds than yellow birds, with controls selected in 33 out of 49 tests (binomial test, $p=0.02$). This preference for Control birds occurred both in males and females as revealed by the absence of a sex effect on which stimulus is chosen (general linear model, $z=0.30$, $p=0.76$), and was not influenced by the time birds were tested ($z=1.23$, $p=0.22$).

Analyzing the exact time the subject birds spent close to each of the two stimulus birds, instead of simply accounting for which stimulus bird was preferred overall, led to similar conclusions, i.e. subjects spent more time close to control than to yellow birds (p=0.008, table 1). In this analysis, there was also a trend for an interaction between the sex of the subject and the treatment of the stimulus birds (p=0.096, see table 1), suggesting that the preference for control birds might be more pronounced in females than in males (Fig. 2A).

Experiment 2

Interest for stimulus birds

On average, subject birds spent 61% of their time close to the stimulus birds, with birds spending more time in the stimulus-bird zone than in the, larger, neutral zone in 58 of the 90 tests performed (binomial test, p=0.008). The level of interest for the stimulus birds did not differ between the treatments or sexes of the subject birds (table 2). There was however an effect of the time of the day at which the birds were tested, with birds slightly increasing their interest level as the day progresses (table 2).

Preference strength

Males did not differ from females in their preference strength (females: 0.66±0.13; males: 0.72±0.14, mean±sd) (table 2). Time of day and the treatment in which the subjects were did not influence the strength of preference either (table 2).

Which stimulus bird do they choose?

The result of the binomial test that compared the number of occasions subject birds spent more time close to canthaxanthin than to placebo birds, reveals that in the 86 tests where the birds made a choice (i.e. did not stay all the time in the neutral zone, which only occurred four times), Canthaxanthin and Placebo stimulus birds were selected in 39 and 47 cases, respectively. Thus based on counts there is no overall preference for a stimulus category (p=0.45). This analysis does not account for the sex of the subject birds, neither for their own treatment. Incorporating those factors, their interaction and the effect of time of day in a generalized linear mixed-model with a binary response variable (Canthaxanthin or Placebo) did not lead to any significant result (table S2).

By contrast, the result of the linear mixed-model using the exact time spent close to each stimulus bird (instead of the count) reveals a significant interaction between the sex of the subjects and the treatment of stimulus birds (p=0.002, Table 2, Fig. 2B). Post-hoc analyses reveal that females spent more time close to placebo than to canthaxanthin birds (p=0.001), while males seem to spend similar amounts of times with each phenotype (p=0.33). There was also a trend for assortative choice, indicated by the (not fully significant) interaction between the treatment of the subject and the stimulus birds (p=0.058; Table 2 and fig. 3). If we run a post-hoc test on this interaction we find that Placebo subjects (males and females confounded) preferentially select Placebo stimuli (p=0.01), and canthaxanthin subjects tend to spend more time with canthaxanthin stimuli (p=0.08). This should however be interpreted cautiously since the interaction is not fully significant.

Discussion

Our experiments show that male and female blue tits do not differ in their overall interest for prospective mates. Similarly, males expressed comparable levels of preference than females, suggesting that mutual mate choice exists in this species. The only sexual difference found was in the second experiment, where the preference for paler congeners was more pronounced in females than in males. Finally, we did not find strong evidence for an assortative mate preference in blue tits, even though we found that pale birds tended to select birds that are themselves pale.

The results described here quite clearly show that male and female blue tits are mutually attracted by the opposite sex. They add to the growing body of literature that suggests that male mate choice is common in birds and other organisms (reviewed in Hare and Simmons, 2019; Doutrelant et al., 2020). We did not measure mate choice *per se* in our apparatus since birds were not allowed to pair and reproduce together, but rather a sexual, or eventually social (see below), mate preference. Actual mate choice in the wild depends on a number of other parameters than mate preferences, including environmental conditions, availability of preferred phenotypes, time and effort allocated to prospecting mates, time of season and intra-sexual competition (Jennions and Petrie, 1997; Cotton et al., 2006; Botero and Rubenstein, 2012; Kuijper et al., 2012; Auld et al., 2017; Zandberg et al., 2017). Nevertheless, mate choice is the manifestation of a mate preference (Cotton et al., 2006). In any case, similar mutual mate preferences in this species were also described in earlier works by Hunt and colleagues (1999), who specifically tested the preferences for the UV-blue coloration of the crest, and in a recent meta analysis conducted on four populations over 10 years, which suggested a low but positive assortative mating for both the blue and the yellow ornaments (Fargevieille et al., 2017).

Carotenoid-based colors have been found to signal capacity to raise an immune response (Faivre et al., 2003; Saks et al., 2003; reviewed in Simons et al., 2012) and so could potentially signal high-quality over low- quality individuals (Andersson, 1994;

Jones and Ratterman, 2009; Doutrelant et al., 2012; Wells et al., 2015). A pale plumage contains less carotenoids, it is therefore surprising that in both experiments, our blue tits did not prefer the most colored morph, corresponding to birds displaying more carotenoid in their plumage and, by extension, potentially higher quality mate. In experiment 1, pale birds were selected more often than colorful birds. This result was robust as indicated by the fact that it was already significant in the initial binomial test based on counts (number of birds that spent more time near to controls). It also suggested the existence of an assorted mating (see below). This preference for pale birds was apparent in both sexes (table 1), although it tended to be more pronounced in females (Fig. 2A). Experiment 2 confirmed these results. Even if the same binomial test did not reveal any striking preference this time, analyzing the exact time spent with the different stimulus birds, indeed showed again that female blue tits preferentially selected the least colorful males (Fig. 2B).

Preference for partners presenting lower values of signals has already been reported in the literature, both from studies conducted in the field and in captivity. Female pied flycatchers (*Ficedula hypoleuca*) and house sparrows of some wild populations were described to prefer less-ornamented partners (Saetre et al., 1997; Griffith et al., 1999). If the reason for this preference was quite clearly established in the first case, with females that would show this preference to more surely avoid hybridization with the closely related collared flycatcher (*Ficedula albicollis*) (Saetre et al., 1997), the sparrow case was less clear. In fishes, captive male sticklebacks (*Gasterosteus aculeatus*) were shown to prefer drab over red females. The author argued that avoidance of redder females might in fact be a side-effect of a mechanism of avoidance of aggressive interactions in male-male competition, and not a preference for drab females *per se* (Nordeide, 2002). As we can see, in the few cases where it has been reported, preference for less-ornamented partners generally arises from specific, context-dependent, situations (Griffith et al., 1999), and not from a general preference for dull colors. In the case of blue tits, we can only speculate on why this preference for pale-chested birds occurred, and we think that four main hypotheses can be drawn from these results, hypotheses that will need to be tested in the future.

First, we know that mate preference can be influenced by genetic and cultural factors (i. e. Dugatkin, 1996). A genetically inherited preference for paler individuals seems highly improbable, but a mating preference mechanism based on sexual imprinting early in life, or on the most common social phenotype encountered thereafter, could explain the results found, at least in experiment 1. In this experiment we only colored the stimulus birds, not the subject birds, and only soon before the start of the experiment. All subjects and their aviary mates have had a pale chest since their first pre-basic molt, and were therefore used to encounter and interact with other pale congeners. However, while pale birds was the most common phenotype they met for most of the time, birds had a natural, yellow-colored chest during their first months of life in captivity (pers. observations), thanks to the carotenoids present in the food brought by their parents at the nest (chicks were brought to the lab when they were around 10-days old, but were raised in natural conditions by their parents before that, see methods). Birds only acquired a pale chest after their first pre-basic molt, which occurred a few months after birth (July for birds born in May). In addition, the parents that feed them at the nest for the first 10 days of life were of the natural, yellow phenotype. An early sexual imprinting on pale-chest birds could therefore not explain the observed preference for pale birds. It remains however possible that social associations later in life could have played a role, and this was taken into account in the design of experiment 2. In this experiment, bird food was indeed supplemented with canthaxanthin or a placebo *before* the onset of their first pre-basic moult, meaning that contrary to what happened in experiment 1, birds in experiment 2 went directly from their initial yellow plumage to either one of the two experimental colors, only half of which were pale. Furthermore, birds were housed in aviaries in a way that they could always see both color morphs (see methods). The preference for pale-chested birds in this second experiment could thus hardly be explained by early sexual imprinting, or by social habituation later in life.

Second, more colored individuals might be more aggressive individuals (Senar, 2006; Tobias et al., 2012) (but see McGraw and Hill, 2000). If pairing with an aggressive partner might confer direct and indirect benefits in terms of territory defense and genes conferring more vigor to offspring, it may also come at the cost of being more aggressed. In captivity, where space is often limited, pairing with an aggressive partner can lead to reproductive failure (Caro et al., 2007). Therefore, in confined environment like the one in which our birds are raised, mate preference rules might differ from what has been theorized (Wang et al., 2017), and selecting a paler, potentially less aggressive, partner might be advantageous, especially for females that are slightly smaller than males (Blondel et al., 1999). We know in our populations that males and females with higher value of yellow chroma are more aggressive toward human intruders (Mercier-Gauthier, Doutrelant, Dubuc Messier, Charmantier, Réale, unpubl. data), and that duller females are less aggressive toward other blue tit females (Midamegbe et al., 2011). Alternatively, even if we believe that the results reported here depict a sexual preference because birds were primed into a reproductive state (see methods), we cannot exclude that there might have been a social dimension to the preferences expressed, with males and females choosing a partner with which to spend time rather than to breed. In this case, selecting a less aggressive social partner would seem more appropriate. A different experimental setup with more than two possible choices, and which would include one or several individuals of the same sex as the focal bird, could have shed some light on this social paring possibility (e.g. Griggio et al., 2011). In any case, like in the stickelback example (Nordeide, 2002), avoiding aggressiveness might be a possible mechanism explaining the preferences for paler individuals observed in our experiments.

Third, since potential competitors and mates are most probably assessed based on a variety of signals (Bro-Jorgensen, 2010; Ligon and McGraw, 2016), it might be the overall homogeneity of signals that matters, more than one signal taken independently from the others. In blue tits, individuals with a yellower plumage also tend to have more UV in the blue part of the plumage (Mercier Gauthier et al., unpub. data). Accordingly, Kurvers et al. (2010) found that female blue tits expressed a preference for males with UV-enhanced crowns, but only if those males also had a yellower chest plumage, otherwise females selected the UV-reduced males. This suggests that choosing birds might pay attention to an ensemble of traits and prefer those individuals that are more homogenous in their different color signals. In our experiments, like in most other experiments manipulating color phenotypes, we only manipulated one single trait (i.e. the color of the chest). Since the diet that our birds receive in captivity is obviously less diversified than what free-roaming birds eat in the wild, it is possible that long-term housing in captivity induces a general decrease in plumage quality. Although we only manipulated the chest color of our birds, we also collected some blue crown feathers from the birds used in our second experiment. Comparing the brightness, hue and UV chroma of those UV-blue feathers between the two groups shows that the diet supplemented with canthaxanthin did not affect any of those parameters (Brightness: p=0.7; hue: p=0.2; chroma: p=0.9; data not shown). This suggests that only enhancing the chest color might have increased the contrast between the different parts of the plumage, which in turn could have been counterselected. This could explain why less-colored birds were preferred in both experiments. Future studies of mate-preference, adopting a multi-modal approach manipulating several plumage traits simultaneously, will shed light on whether birds assess potential mates based on a variety of signals.

Finally, assortative mating could also explain the results described in this study. In experiment 1, all subjects had a pale chest, and they preferentially selected birds that were themselves pale, leading to a clear preference for individuals that were assorted to the subjects. This result in fact led to experiment 2, where one of the goals was to further test this hypothesis of assortative mating, presumably based on a mechanism of self-referent phenotype matching. In this second experiment, where the colors of both the stimulus and the subject birds were manipulated at an early stage, there was a trend for an interaction between the treatments of the subject and of the stimulus birds, and in the predicted direction: birds tended to spend more time with congeners of the same chest phenotype as their own. Self-reference phenotype matching was certainly not robust in our second experiment (not visible in the simple tests based on counts of birds, and interaction not fully significant when analyzing the exact time spent), but sufficiently to suggest that it might exist in blue tits, and sufficiently to call for additional studies on this specific question. A recent study testing for assortative mating for coloration in wild blue tits analyzed the matching of more than 1500 pairs across multiple populations and over 10 years. If authors found some evidence for positive assortative mating across populations, there were large spatio-temporal variations (Fargevieille et al., 2017), confirming that mate preference can be very labile in the wild (Chaine and Lyon, 2008).

In conclusion, this study suggests that mutual mate preference exists in blue tits, which makes sense in a species where both sexes invest a lot in parental care (Johnstone et al., 1996; Kokko and Johnstone, 2002). Results also show that the color of the breast does influence mate preference. We additionally asked whether the phenotype of the subjects influences the preference they express, but did not fully resolve this question yet. Using a more integrative measure of quality like the one proposed by Wang et al. (2017), which takes more than one phenotypic trait into account, seems a promising way to further our understanding of the importance of phenotype matching in mate preferences. Asking this question both in captivity and in the wild also seems important to give a broader picture of the mate preference dynamics in this, and other species.

Funding

This work was supported by the Fyssen Foundation (research grant to SPC); the Netherlands Organization for Scientific research (NWO-VENI grant to SPC), and by Wallonie-Bruxelles International (WBI-World grant to SPC).

Acknowledgments

We thank Marylou Aaldering, Franca Kropman, Bart van Lith, and many other animal caretakers for their help with hand-feeding the chicks and taking good care of the birds. Maria Del Rey, Amélie Fargevieille and Benjamin Deletrez for their help with measuring feather coloration. Marcel Visser, Kate Lessells, Ralf Kurvers and Marc Naguib for helpful discussions prior to the experiments, and two anonymous reviewers for their very constructive comments. We also thank DSM Nutritional Products Ltd for providing the carotenoid products, especially the placebo, used in experiment 2. Analyses reported in this article can be reproduced using the data provided by Caro et al. (2021).

References

Amundsen T, 2000. Why are female birds ornamented? TREE 15:149-155.

Amundsen T, Forsgren E, 2001. Male mate choice selects for female coloration in a fish. Proc Natl Acad Sci USA 98:13155-13160.

Amundsen T, Forsgren E, Hansen LTT, 1997. On the function of female ornaments: male bluethroats prefer colourful females. Proc R Soc B 264:1579-1586.

Andersson M, 1994. Sexual selection. Princeton: University Press.

Andersson S, Ornborg J, Andersson M, 1998. Ultraviolet sexual dimorphism and assortative mating in blue tits. Proc R Soc B 265:445-450.

Auld HL, Ramnarine IW, Godin JGJ, 2017. Male mate choice in the Trinidadian guppy is influenced by the phenotype of audience sexual rivals. Behav Ecol 28:362-372.

Bates D, Machler M, Bolker BM, Walker SC, 2015. Fitting Linear Mixed-Effects Models Using lme4. J Stat Soft 67:1-48.

Biard C, Surai PF, Moller AP, 2006. Carotenoid availability in diet and phenotype of blue and great tit nestlings. J Exp Biol 209:1004-1015.

Blondel J, Dias PC, Perret P, Maistre M, Lambrechts MM, 1999. Selection-based biodiversity at a small spatial scale in a low-dispersing insular bird. Science 285:1399-1402.

Blount JD, Metcalfe NB, Birkhead TR, Surai PF, 2003. Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300:125-127.

Bonduriansky R, 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biol Rev 76:305-339.

Botero CA, Rubenstein DR, 2012. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. PLoS ONE 7.

Bro-Jorgensen J, 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. TREE 25:292-300.

Byers BE, Kroodsma DE, 2009. Female mate choice and songbird song repertoires. Anim Beh 77:13-22.

Byrne PG, Rice WR, 2006. Evidence for adaptive male mate choice in the fruit fly Drosophila melanogaster. Proc R Soc B 273:917-922.

Caro SP, Lambrechts MM, Balthazart J, Perret P, 2007. Non-photoperiodic factors and timing of breeding in blue tits: Impact of environmental and social influences in semi-natural conditions. Behav Proc 75:1-7.

Caro SP, Pierre L, Berges M, Bakker R, Doutrelant C, Bonadonna F, 2021 Data from: Mutual mate preference and assortative mating in relation to a carotenoid-based color trait in blue tits (doi:10.5061/dryad.p8cz8w9qq).

Caro SP, Sewall KB, Salvante KG, Sockman KW, 2010. Female Lincoln's sparrows modulate their behavior in response to variation in male song quality. Behav Ecol 21:562-569.

Chaine AS, Lyon BE, 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. Science 319:459-462.

Clutton-Brock T, 2009. Sexual selection in females. Anim Beh 77:3-11.

Cotton S, Small J, Pomiankowski A, 2006. Sexual selection and condition-dependent mate preferences. Cur Biol 16:R755-R765.

Courtiol A, Etienne L, Feron R, Godelle B, Rousset F, 2016. The evolution of mutual mate choice under direct benefits. The American naturalist 188:521-538.

Crawley MJ, 2007. The R book: John Wiley & Sons, Ltd.

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.

Dale S, Slagsvold T, 1994. Male pied flycatchers do not choose mates. Anim Beh 47:1197-1205.

Davies NB, Krebs JR, West SA, 2012. An introduction to behavioural ecology, 4th ed: Wiley-Blackwell.

Dawson A, King VM, Bentley GE, Ball GF, 2001. Photoperiodic control of seasonality in birds. J Biol Rhythms 16:365-380.

Delhey K, Johnsen A, Peters A, Andersson S, Kempenaers B, 2003. Paternity analysis reveals opposing selection pressures on crown coloration in the blue tit (*Parus caeruleus*). Proc R Soc B 270:2057-2063.

Delhey K, Peters A, Johnsen A, Kempenaers B, 2007. Fertilization success and UV ornamentation in blue tits *Cyanistes caeruleus*: correlational and experimental evidence. Behav Ecol 18:399-409.

Domb LG, Pagel M, 2001. Sexual swellings advertise female quality in wild baboons. Nature 410:204-206.

Doutrelant C, Fargevieille A, Grégoire A, 2020. Evolution of female coloration: What have we learned from birds in general and blue tits in particular. In: Naguib M, Barrett L, Healy SD, Podos J, Simmons LW, Zuk M, editors. Advances in the Study of Behavior: Academic Press. p. 123-202.

Doutrelant C, Grégoire A, Grnac N, Gomez D, Lambrechts MM, Perret P, 2008. Female coloration indicates female reproductive capacity in blue tits. J Evol Biol 21:226-233.

Doutrelant C, Grégoire A, Midamegbe A, Lambrechts M, Perret P, 2012. Female plumage coloration is sensitive to the cost of reproduction. An experiment in blue tits. J Anim Ecol 81:87-96.

Drent PJ, van Oers K, van Noordwijk AJ, 2003. Realized heritability of personalities in the great tit (*Parus major*). Proc R Soc B 270:45-51.

Dugatkin LA, 1996. Interface between culturally based preferences and genetic preferences: Female mate choice in Poecilia reticulata. Proc Natl Acad Sci USA 93:2770-2773.

Duursma R, 2017. bootpredictlme4: predict method for lme4 with bootstrap.

Faivre B, Gregoire A, Preault M, Cezilly F, Sorci G, 2003. Immune activation rapidly mirrored in a secondary sexual trait. Science 300:103-103.

Fargevieille A, Gregoire A, Charmantier A, Granado MD, Doutrelant C, 2017. Assortative mating by colored ornaments in blue tits: space and time matter. Ecology and Evolution 7:2069-2078.

Ferns PN, Hinsley SA, 2008. Carotenoid plumage hue and chroma signal different aspects of individual and habitat quality in tits. Ibis 150:152-159.

Fisher RA, 1915. The evolution of sexual preference. Eugen Rev 7:184-192.

Fitzpatrick CL, Altmann J, Alberts SC, 2015. Exaggerated sexual swellings and male mate choice in primates: testing the reliable indicator hypothesis in the Amboseli baboons. Anim Beh 104:175-185.

Garcia-Navas V, Ferrer ES, Sanz JJ, 2012. Plumage yellowness predicts foraging ability in the blue tit *Cyanistes caeruleus*. Biological Journal of the Linnean Society 106:418-429.

Goodwin TW, 1984. The biochemistry of the carotenoids. Doordrecht: Springer.

Griffith SC, Owens IPF, Burke T, 1999. Female choice and annual reproductive success favour less-ornamented male house sparrows. Proc R Soc B 266:765-770.

Griggio M, Biard C, Penn DJ, Hoi H, 2011. Female house sparrows "count on" male genes: experimental evidence for MHC-dependent mate preference in birds. Bmc Evolutionary Biology 11:7.

Griggio M, Hoi H, 2010. Only females in poor condition display a clear preference and prefer males with an average badge. Bmc Evolutionary Biology 10.

Griggio M, Valera F, Casas A, Pilastro A, 2005. Males prefer ornamented females: a field experiment of male choice in the rock sparrow. Anim Beh 69:1243-1250.

Hare RM, Simmons LW, 2019. Sexual selection and its evolutionary consequences in female animals. Biol Rev 94:929-956.

Hill GE, McGraw KJ, 2006. Bird coloration: function and evolution. Cambridge: Harvard University Press.

Holveck M-J, Riebel K, 2010. Low-quality females prefer low-quality males when choosing a mate. Proc R Soc B 277:153-160.

Hunt J, Brooks R, Jennions MD, 2005. Female mate choice as a condition-dependent life-history trait. Am Nat 166:79-92.

Hunt S, Bennett ATD, Cuthill IC, Griffiths R, 1998. Blue tits are ultraviolet tits. Proc R Soc B 265:451-455.

Hunt S, Cuthill IC, Bennett ATD, Griffiths R, 1999. Preferences for ultraviolet partners in the blue tit. Anim Beh 58:809-815.

Isaksson C, Ornborg J, Prager M, Andersson S, 2008. Sex and age differences in reflectance and biochemistry of carotenoid-based colour variation in the great tit Parus major. Biological Journal of the Linnean Society 95:758-765.

Jennions MD, Petrie M, 1997. Variation in mate choice and mating preferences: A review of causes and consequences. Biol Rev Cambridge Philosophic Soc 72:283-327.

Johnstone RA, Reynolds JD, Deutsch JC, 1996. Mutual mate choice and sex differences in choosiness. Evolution 50:1382-1391.

Jones AG, Ratterman NL, 2009. Mate choice and sexual selection: What have we learned since Darwin? Proc Natl Acad Sci USA 106:10001-10008.

Jones IL, Hunter FM, 1993. Mutual sexual selection in a monogamous seabird. Nature 362:238-239.

Kimmitt AA, Dietz SL, Reichard DG, Ketterson ED, 2018. Male courtship preference during seasonal sympatry may maintain population divergence. Ecology and Evolution 8:11833-11841.

Koch RE, Hill GE, 2018. Do carotenoid-based ornaments entail resource trade-offs? An evaluation of theory and data. Funct Ecol 32:1908-1920.

Kokko H, Johnstone RA, 2002. Why is mutual mate choice not the norm? Operational sex ratios, sex roles and the evolution of sexually dimorphic and monomorphic signalling. Phil Trans R Soc B 357:319-330.

Kraaijeveld K, Kraaijeveld-Smit FJL, Komdeur J, 2007. The evolution of mutual ornamentation. Anim Beh 74:657-677.

Kuijper B, Pen I, Weissing FJ, 2012. A guide to sexual selection theory. In: Futuyma DJ, editor. Ann Rev Ecol Evol Syst Palo Alto: Annual Reviews. p. 287-311.

Kurvers RHJM, Delhey K, Roberts ML, Peters A, 2010. No consistent female preference for higher crown UV reflectance in Blue Tits *Cyanistes caeruleus*: a mate choice experiment. Ibis 152:393-396.

Kuznetsova A, Brockhoff PB, Christensen RHB. 2016. lmerTest: Tests in Linear Mixed Effects Models. R package version 2.0-30.

Lande R, 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. Evolution 34:292-305.

LeBas NR, Marshall NJ, 2000. The role of colour in signalling and male choice in the agamid lizard Ctenophorus ornatus. Proc R Soc B 267:445-452.

Lenth RV, 2016. Least-Squares Means: The R Package lsmeans. J Stat Soft 69:1-33.

Ligon RA, McGraw KJ, 2016. Social costs enforce honesty of a dynamic signal of motivation. Proc R Soc B 283:7.

McGraw KJ, Hill GE, 2000. Carotenoid-based ornamentation and status signaling in the house finch. Behav Ecol 11:520-527.

Midamegbe A, Gregoire A, Perret P, Doutrelant C, 2011. Female-female aggressiveness is influenced by female coloration in blue tits. Anim Beh 82:245-253.

Midamegbe A, Gregoire A, Staszewski V, Perret P, Lambrechts MM, Boulinier T, Doutrelant C, 2013. Female blue tits with brighter yellow chests transfer more carotenoids to their eggs after an immune challenge. Oecologia 173:387-397.

Muma KE, Weatherhead PJ, 1989. Male traits expressed in females: direct or indirect sexual selection? Behav Ecol Sociobiol 25:23-31.

Nordeide JT, 2002. Do male sticklebacks prefer females with red ornamentation? Can J Zool 80:1344-1349.

Olsson M, 1993. Male-preference for large females and assortative mating for body size in the sand lizard (*Lacerta agilis*). Behav Ecol Sociobiol 32:337-341.

Parker TH, 2013. What do we really know about the signalling role of plumage colour in blue tits? A case study of impediments to progress in evolutionary biology. Biol Rev 88:511-536.

Peters A, Delhey K, Johnsen A, Kempenaers B, 2007. The condition-dependent development of carotenoid-based and structural plumage in nestling blue tits: Males and females differ. Am Nat 169:S122-S136.

Pizzari T, Cornwallis CK, Lovlie H, Jakobsson S, Birkhead TR, 2003. Sophisticated sperm allocation in male fowl. Nature 426:70-74.

Price DK, 1996. Sexual selection, selection load and quantitative genetics of zebra finch bill colour. Proc R Soc B 263:217-221.

Pryke SR, Griffith SC, 2007. The relative role of male vs. female mate choice in maintaining assortative pairing among discrete colour morphs. J Evol Biol 20:1512-1521.

R-Core-Team. 2015. R: A language and environment for statistical computing.: R Foundation for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Remy A, Gregoire A, Perret P, Doutrelant C, 2010. Mediating male-male interactions: the role of the UV blue crest coloration in blue tits. Behav Ecol Sociobiol 64:1839-1847.

Reparaz LB, van Oers K, Naguib M, Doutrelant C, Visser ME, Caro SP, 2014. Mate preference of female blue tits varies with experimental photoperiod. Plos One 9:e92527.

Reudink MW, Studds CE, Marra PP, Kurt Kyser T, Ratcliffe LM, 2009. Plumage brightness predicts non-breeding season territory quality in a long-distance migratory songbird, the American redstart *Setophaga ruticilla*. J Avian Biol 40:34-41.

Roberts NS, Mendelson TC, 2017. Male mate choice contributes to behavioural isolation in sexually dimorphic fish with traditional sex roles. Anim Beh 130:1-7.

Robertson JGM, 1990. Female choice increases fertilization sucess in the Australian frog, *Uperoleia laevigata*. Anim Beh 39:639-645.

Roulin A, Jungi TW, Pfister H, Dijkstra C, 2000. Female barn owls (*Tyto alba*) advertise good genes. Proc R Soc B 267:937-941.

Saetre GP, Moum T, Bures S, Kral M, Adamjan M, Moreno J, 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. Nature 387:589-592.

Saks L, Ots I, Horak P, 2003. Carotenoid-based plumage coloration of male greenfinches reflects health and immunocompetence. Oecologia 134:301-307.

Sargent RC, Gross MR, Vandenberghe EP, 1986. Male mate choice in fishes. Anim Beh 34:545-550.

Schlupp I, 2018. Male mate choice in livebearing fishes: an overview. Curr Zool 64:393-403.

Searcy WA, 1992. Measuring responses of female birds to male song. In: McGregor PK, editor. Playback and studies of animal communication New York: Plenum Press. p. 175-189.

Senar JC, 2006. Color displays as intrasexual signals of aggression and dominance. In: Hill G, K. M, editors. Bird Coloration 2: Function and Evolution Cambridge, Massachusetts: Harvard University Press.

Senar JC, Figuerola J, Pascual J, 2002. Brighter yellow blue tits make better parents. Proc R Soc B 269:257-261.

Silver R, Ramos C, Machuca H, Silverin B, 1992. Immunocytochemical Distribution of Gnrh in the Brain of Adult and Posthatching Great Tit Parus-Major and Ring Dove Streptopelia-Roseogrisea. Orn Scan 23:222-232.

Silverin B, 1994. Photoperiodism in male great tits (Parus major). Ethology,Ecology and Evolution 6:131-157.

Simons MJP, Cohen AA, Verhulst S, 2012. What does carotenoid-dependent coloration tell? Plasma carotenoid level signals immunocompetence and oxidative stress state in birds-A meta-analysis. Plos One 7:e43088.

Smith JM, 1991. Theories of sexual selection. TREE 6:146-151.

Titulaer M, van Oers K, Naguib M, 2012. Personality affects learning performance in difficult tasks in a sex-dependent way. Anim Beh 83:723-730.

Tobias JA, Montgomerie R, Lyon BE, 2012. The evolution of female ornaments and weaponry: social selection, sexual selection and ecological competition. Phil Trans R Soc B 367:2274-2293.

Trivers RL, 1972. Parental investment and sexual selection. In: B C, editor. Sexual selection and the descent of man, 1871–1971 London: Heinemann Education. p. 136-179.

Wang D, Forstmeier W, Kempenaers B, 2017. No mutual mate choice for quality in zebra finches: Time to question a widely-held assumption. Evolution 71:2661-2676.

Weiss SL, 2006. Female-specific color is a signal of quality in the striped plateau lizard (Sceloporus virgatus). Behav Ecol 17:726-732.

Wells SJ, Ji WH, Dale J, Jones B, Gleeson D, 2015. Male size predicts extrapair paternity in a socially monogamous bird with extreme sexual size dimorphism. Behav Ecol 26:200-206.

Williams TD, 2012. Physiological adaptation for breeding in birds: Princeton University Press.

Zandberg L, Gort G, van Oers K, Hinde CA, 2017. Direct fitness benefits explain mate preference, but not choice, for similarity in heterozygosity levels. Ecol Let 20:1306-1314.

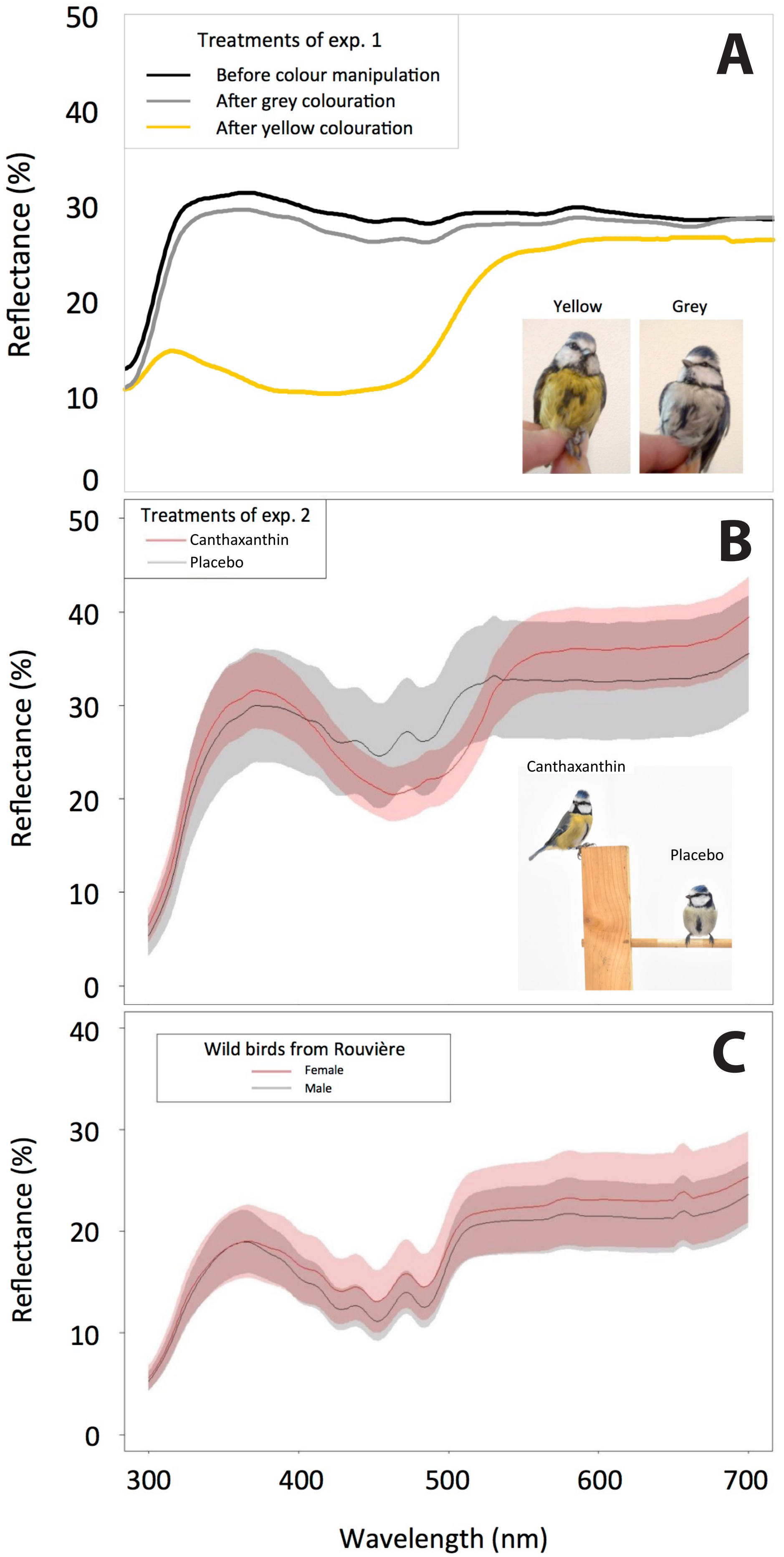
A
Treatments of exp. 1
Before colour manipulation
After grey colouration
After yellow colouration
Yellow
Grey
B
Treatments of exp. 2
Canthaxanthin
Placebo
Canthaxanthin
Placebo
C
Wild birds from Rouvière
Female
Male
Reflectance (%)
50
40
30
20
10
0
Wavelength (nm)
300
400
500
600
700

Figure 1: **Reflectance spectra of blue tit breast feathers**. (A) Spectra of birds used in experiment 1. Colours obtained by painting the feathers with colour markers. (B) Spectra of birds used in experiment 2. Colours obtained by manipulation of the diet of the birds. (C) Spectra from wild blue tits at La Rouvière, France (measurements performed on breast feathers : see Fargevieille et al., 2017).

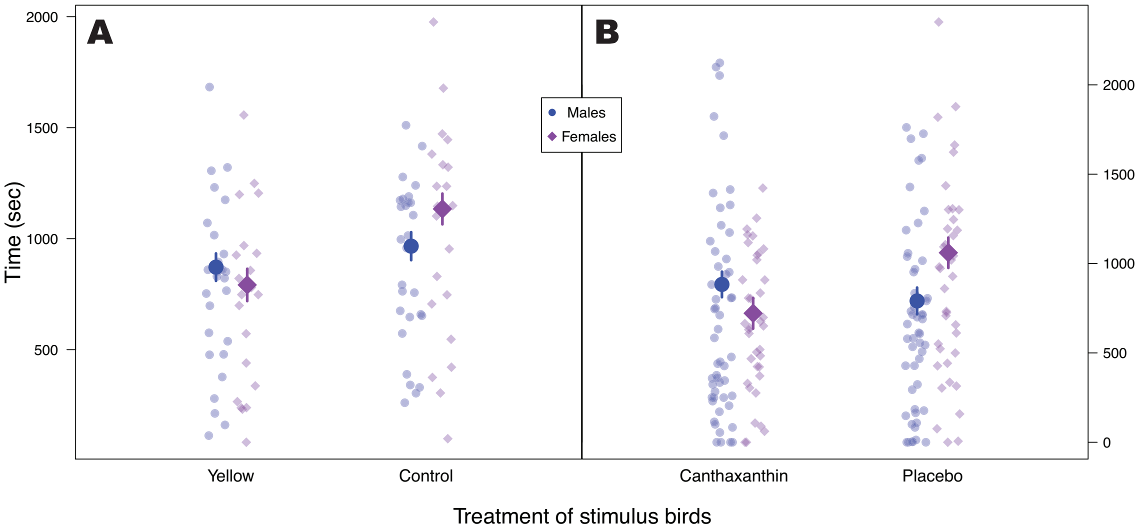

Figure 2: **Sexual differences in the color morphs selected by captive blue tits**. (A) In experiment 1, control birds were preferred over yellow birds (p=0.008), and this preference for pale individuals tended to be slightly more pronounced in females than in males (interaction between sex of the chooser and treatment of the stimulus bird: p=0.096). (B) In experiment 2, this interaction was significant (p=0.002) and reveals that female blue tits spent significantly more time close to Placebo than Canthaxanthin stimuli (post-hoc test: p=0.001), while males spent similar amounts of time with each phenotype (post-hoc test: p=0.331). Small symbols represent individual data points, bigger symbols and error bars represent the statistical model estimates and S.E.

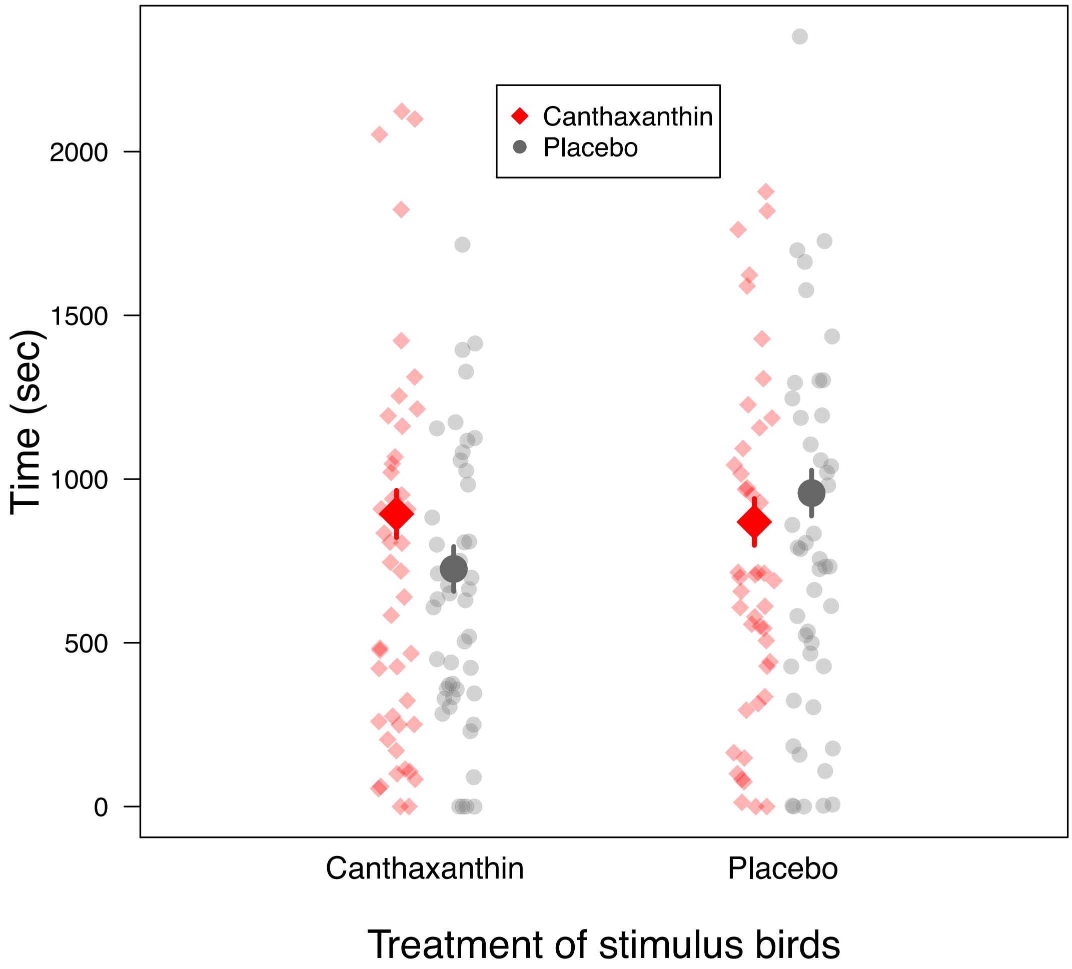

Figure 3: **Assortative mating in captive blue tits?** The nearly significant interaction between treatments of the subjects and of the stimulus birds (P=0.058) suggests that blue tits tend to prefer birds that are of the same color phenotype as theirs. This is particularly visible in placebo subjects (post-hoc test: p=0.01), less in canthaxanthin birds (p=0.08). Small symbols represent individual data points, bigger symbols and error bars represent the statistical model estimates and S.E. For the sake of simplicity, the estimates presented here come from a model including the treatments of stimulus and chooser birds, their interaction, and time of day. Sex and its interactions (table 2) were not included.

Table 1: **Analysis of the variables that potentially influence the interest of the subject birds, their preference strength, and the time spent close to the stimulus birds in experiment 1 (n=49).** Variables in bold represent the minimal adequate models, eliminated variable (in grey) are presented in the reverse order in which they were removed from the model. Intercept includes Sex of subject = female, Treatment of stimulus = control.

Trait analysed	Variable	Estimate	S.E.	F	p-value
Interest					
	(Intercept)	1.06	0.04		
	Time of day	-3.5e-04	2.5e-04	2.01	0.163
	Sex of subject	0.05	0.08	0.12	0.733
Preference strength					
	(Intercept)	0.96	0.02		
	Sex of subject	-0.06	0.03	3.62	*0.063*
	Time of day	1.2e-04	8.6e-05	1.83	0.183
Time					
	(Intercept)	**1039.49**	**52.57**		
	Treatment of stimulus	**-202.62**	**74.34**	**7.43**	**0.008****
	Sex of subject	-43.77	74.82	0.34	0.560
	Time of day	-0.12	0.21	0.33	0.568
	Treatment stimulus x Sex subject	247.76	147.28	2.83	*0.096*

Table 2: **Analysis of the variables that potentially influence the interest of the subject birds, their preference strength, and the time spent close to the stimulus birds in experiment 2 (n=47).** Variables in bold represent the minimal adequate models, eliminated variable (in grey) are presented in the reverse order in which they were removed from the model. Intercept includes Sex of subject = female, Treatment of subject and stimulus = Canthaxanthin.

Trait analysed	Variable	Estimate	S.E.	F	p-value
Interest					
	(Intercept)	**0.90**	**0.04**		
	Time of day	**3.5e-04**	**1.5e-04**	**5.71**	**0.019***
	Sex of subject	-0.09	0.08	1.26	0.268
	Treatment of subject	-0.04	0.06	0.50	0.482
Preference strength					
	(Intercept)	0.97	0.03		
	Sex of subject	0.05	0.04	1.78	0.190
	Time of day	1.1e-04	7.9e-05	2.12	0.150
	Treatment of subject	-0.04	0.04	1.29	0.262
Time					
	(Intercept)	**721.74**	**91.52**		
	Sex of subject	**161.50**	**123.59**	**0.28**	**0.609**
	Treatment of stimulus	**338.82**	**103.54**	**3.08**	**0.081**
	Time of day	**0.35**	**0.17**	**4.27**	**0.044***
	Treatment stimulus x Sex subject	**-431.27**	**140.39**	**9.44**	**0.002****
	Treatment of subject	-39.26	72.94	0.29	0.591
	Treatment subject x Treatment stimulus	264.13	138.37	3.64	*0.058*
	Treatment subject x Treatment stimulus *(placebo)* x Sex subject	76.13	203.51	0.07	0.932