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**Female great tits (*Parus major*) reproduce earlier  
when paired with a male they prefer.**

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**Abstract**

Mate choice is a key component of reproductive biology. Females often prefer certain males but do females modulate their reproductive investment depending on whether they are mated with their preferred partner? We investigated this question in great tits (*Parus major*) where we subjected 36 females to a six-choice mate preference test. Male morphological traits and the female's own characteristics did not influence the preference females expressed. We however found that females spent more time near more exploratory males. We then paired females with one of the males in indoor aviaries, and subsequently monitored their reproductive investment (through measurement of plasma  $17\beta$ -oestradiol concentrations, first egg date, clutch size, and egg size). Females that were mated with a male for which they had a strong preference laid their first clutch significantly earlier in the season than females paired with a male they less preferred. Our results show that mate preference influences reproductive investment in great tits, thereby linking mate choice to bird reproductive decisions.

**Keywords:** reproduction; mate preference; birds; laying date

## Introduction

Individuals can derive direct (for themselves and their offspring in the present generation) and indirect benefits (for their offspring in the following generation) from carefully choosing their mates (Andersson & Simmons, 2006; Jones & Ratterman, 2009; Kokko et al., 2003). Indeed, female preference for a male ornament might be beneficial if this ornament reflects the ability of the male to provide high-quality territory, nutrition, parental care or protection for example (Andersson & Simmons, 2006). Individuals consequently need reliable signals to evaluate potential partner quality. Criteria used by animals to choose their mate can be extremely different from one species to another. Birds might use morphological characteristics to select their mate, like plumage coloration (Hill, 2006; Siitari et al., 2002) or ornament size (Price, 1984; Romero-Pujante et al., 2002; Sheldon et al., 1997). They can also use behavioural traits such as song characteristics (Byers & Kroodsma, 2009; Searcy, 1992b), courtship (Ota et al., 2015; Zuk et al., 1990), personality (Schuett et al., 2011; van Oers et al., 2008) or even cognitive abilities (Boogert et al., 2011). Additionally, all these criteria may be used separately or in combination (Burley, 1981; Candolin, 2003).

For long, it has been assumed that individuals always prefer the highest quality partners and that mate preferences are absolute, meaning that individuals assess each potential partner independently and assign a fixed value to a certain trait (Jennions & Petrie, 1997; Zandberg et al., 2020). Under these circumstances little inter-individual variation is expected both in female preference and in male phenotype. However, individual females vary substantially in their mate preferences (Cotton et al., 2006; Jennions & Petrie, 1997), for example depending on their experience (Caro et al., 2010), their diet (Hunt et al., 2005), or their own quality (Holveck & Riebel, 2010). Condition-dependent preferences have for example been shown in zebra finches, where females that had been raised in enlarged brood, and therefore considered to be lower quality females, preferred lower quality males (Holveck & Riebel, 2010). Such kind of self-reference phenotype matching has been suggested in birds (Andersson et al., 1998; Caro et al., 2021; Fargevieille et al., 2017; Zandberg et al., 2017), reptiles (Sacchi et al., 2018), mammals (Farrell et al., 2011), fishes (Verzijden & Ten Cate, 2007) and insects (Malauza et al., 2005).

Besides the challenge of understanding complex mate choice mechanisms, the effect of partner choice on reproductive timing, investment and physiology remains poorly understood. Females have classically been considered the choosing sex (Jones & Ratterman, 2009; Wong & Candolin, 2005), and the ones that drive several reproductive decisions such as onset of

reproduction or clutch size (Caro et al., 2009). However male sexual traits (Limbourg et al., 2004; Walling et al., 2010) and social solicitations can also modulate female reproductive decisions and investment (Hinde & Steel, 1976). For example, female mallard (*Anas platyrhynchos*) lay larger eggs and produce better quality offspring after copulating with a preferred male (Cunningham & Russel, 2000) and female zebra finches incorporate higher level of testosterone in their eggs when mated with a preferred male (Gil et al., 1999). Gonadal development and hormone secretions in females are also under the influence of male behaviour (Hinde & Steel, 1978; Lehrman et al., 1961; Perfito et al., 2015; Stevenson et al., 2008). Having a non-preferred partner could thus lead to a reduction in the motivational state or to physiological stress altering reproductive performance (Bolund et al., 2009; Griffith et al., 2011). On the contrary, females could opt for a compensatory strategy in which females would invest more in reproduction to compensate for the lower quality of, or at least the lower preference for, its male partner (Bolund et al., 2009; Gowaty, 2008; Gowaty et al., 2007). Therefore, we predict that the deviation between the male traits a female prefers and the traits of the male with which the female is effectively mated, can influence its investment in its current reproduction.

In this study, we tested female preferences for a range of male traits, and how well its reproductive decisions and physiology are influenced by the deviation between its mate preference and its actual mate, using the great tit as our model. Previous work has suggested that females rely on characteristics of the male to decide with whom to mate, suggesting that mate choice could play a role for reproductive success in this species (Norris, 1990, 1993; Patrick et al., 2012; Zandberg et al., 2017). First, we investigated the characteristics used by females for mate choice. We then tested if this choice has an impact on reproductive parameters of the female. We hypothesised that females showing a stronger preference for a given male would show a stronger reproductive physiological development when mated to this male. We measured this through  $17\beta$ -oestradiol ( $E_2$ ) level, a key reproductive hormone that links brain neuroendocrine mechanisms to downstream mechanisms in the liver and thus stimulates the production of yolk precursors (Caro et al., 2019; Williams, 2012). This would, furthermore, imply that females mated to a more preferred mate would lay earlier, and produce more and larger eggs (Balzer & Williams, 1998; Cunningham & Russel, 2000).

## Methods

### *Ethical note*

All ethical permits requested for this experiment have been provided by the Animal Welfare Body of NIOO-KNAW (IVD - NIOO 20.09 AVD8010020209246/IVD 1556a).

### *Preference test*

#### *Experimental subjects and housing*

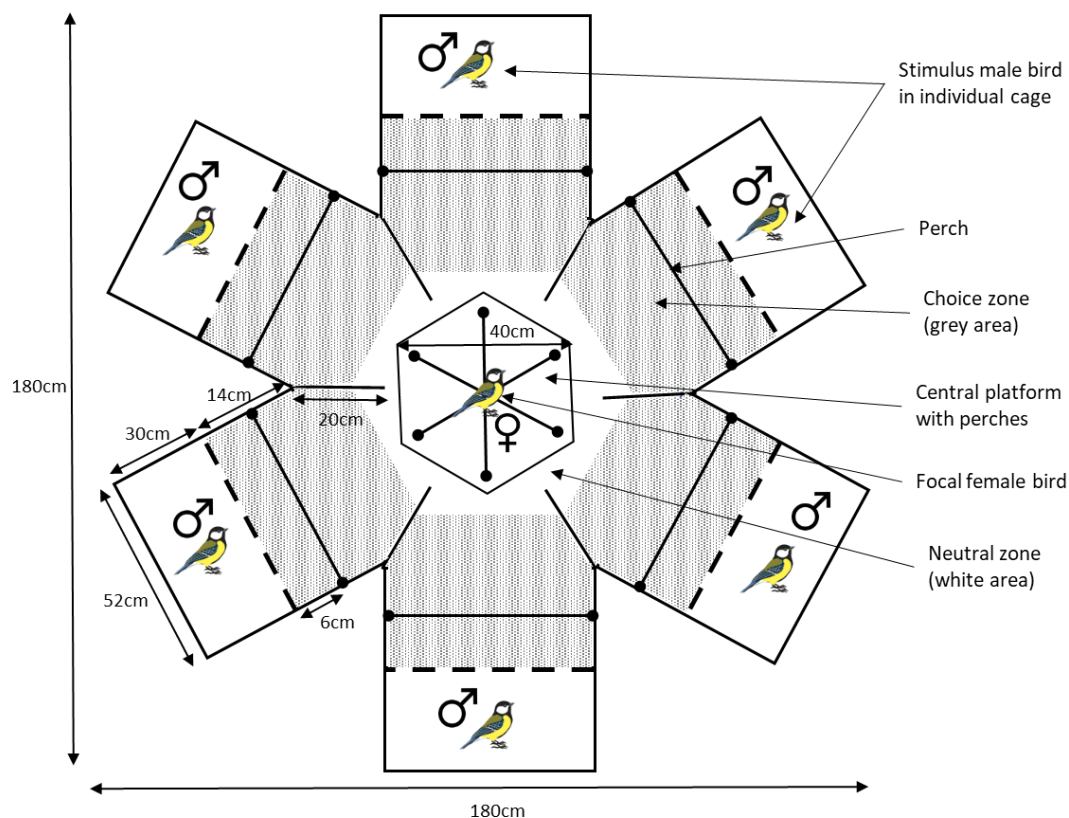
The experiment took place at the Netherlands Institute of Ecology (NIOO-KNAW) in Wageningen, the Netherlands. We used 36 female and 54 male great tits born in 2020, and hand-reared at the NIOO following the procedure described in previous studies (Drent et al., 2003). Fully-grown birds were fed daily with a mixture of minced beef heart, canary egg-food (CéDé, Evergem, Belgium), proteins, vitamins, minerals and trace elements (Carnizoo 2%, Avian, Raalte, The Netherlands, and Calci-Lux 1,5 %, Versele-Laga, Deinze, Belgium), *ad libitum* dry food (Canary egg food, CéDé, Evergem, Belgium, Uni patee, Versele-Laga, Deinze, Belgium), a piece of apple three times a week, and water for drinking and bathing.

On 20<sup>th</sup> January 2021, the 54 males were moved from outdoor aviaries (same-sex groups) to individual indoor cages (0.9 x 0.4 x 0.5 m) in two different rooms. Females were moved to individual cages in three successive groups of 12 females. They were housed in a separate room from the males, and returned to outdoor aviary once tested. Windows in rooms allowed birds to be exposed to natural light, and supplementary artificial lighting was provided from natural sunrise until sunset. Sunrise and sunset times were determined using the ptaff.ca data base (<http://ptaff.ca/soleil/>) for Nijmegen, the Netherlands.

#### *Experimental setup*

Females were tested in a carousel-shaped six-choice chamber inspired by Zandberg et al. (2017) (figure 1, figure S1) from 22<sup>nd</sup> to 31<sup>st</sup> January 2021. The carousel was placed in a room (4.0 x 2.4 x 2.5 m) with white walls, and high-frequency fluorescent lights. In the six-choice setup, each female could see all stimulus male birds from the central platform, but when in the choice zone of a stimulus male, she could not see the other males. The males were visually isolated from each other. Before being tested, each female was given 15 min to habituate to this new environment. In the meantime, the six stimulus males were transferred from their home cage to six individual mobile cages (47 x 28 x 26 cm) in an adjacent room. At the end of the 15 min habituation period, each male cage was placed into one of the six compartments of the

carrousel. Each female was tested once with one group of six stimulus males during 90 minutes. The experimenter left the room at the beginning of the test, which was video-recorded using a central camera (Panasonic WV-CP500) affixed above the test chamber (figure S1B). After each test, all birds were returned to their individual home cages. Males were returned to the pool of available males from which to select the six males to enter the next test. Each group of six males was selected randomly among the 54 males available, with the constraint that each male could not be tested more than four times, and that the males could not belong to the family of the female tested. The positions of the males in the device were randomised for each test.



**Figure 1. Experimental setup for mate preference test.**

Females were tested for their preference in a carrousel-shaped six-choice chamber for 90 min. The stimulus male birds could not see each other. From the hexagonal platform, the focal female could observe all males, whereas in the choice zones, only the stimulus male was visible.

### *Video analysis*

Videos were analysed using EthoVision XT software (Noldus Information Technology, Wageningen, The Netherlands). The time spent by the females in the choice zone of each stimulus male bird was extracted from each video, and used as a proxy for mate preference. In numerous species, time spent in a choice zone has been shown to predict courtship behaviours or pair formation (Caro et al., 2021; Clayton, 1990; Dechaume-Moncharmont et al., 2011;

Drickamer et al., 2003; Hill, 1990; Jeswiet & Godin, 2011; Lehtonen & Lindström, 2008; Mays & Hill, 2004; Senar et al., 2013; Witte, 2006). For example, in zebra finches, the time females spent in front of a male correlates positively with the number of solicitation displays directed to that male, therefore linking mate preference measured by courtship displays with preference measured through the time a female spends with a male (Witte, 2006).

### *Phenotypic trait measurements*

To identify on which criteria females choose their mate, a set of phenotypic traits was measured on each male and female that was used in the mate choice trials. We measured breast stripe width from photographs using GIMP software (version 2.10.22, <https://www.gimp.org/>). We collected five yellow breast feathers, placed them on top of each other, and measured brightness, UV chroma and hue using a spectrophotometer (AVASPEC 2048, Avantes, Apeldoorn, the Netherlands) equipped with an AVALIGHT-DHS deuterium-halogen lamp (Avantes, Apeldoorn, the Netherlands) and a 200 µm optical probe (FCR-7UV200-2-45-ME, Avantes, Apeldoorn, the Netherlands) whose tip contained a 45° quartz window, which guaranteed a constant distance between the light and the sample and highly repeatable measurements. Reflectance was calculated in relative terms, via comparison with a black and a white reference (WS1, Ocean Optics, Dunedin FL, USA). We also took morphological measures, including body mass, tarsus, beak and wing lengths and beak depth. Exploration score in a novel environment was measured following methods described in Dingemans et al. (2002). Briefly, each bird was released individually, without handling, in an observation room (4.0 × 2.4 × 2.3 m) that was equipped with five artificial wooden trees. Exploration score was calculated as the sum of movements (flights and hops) performed by each bird during the first two minutes after entering the room. Scores ranged from 0 to 43, with higher scores indicating faster exploration. Exploratory behaviour is repeatable and this exploration test is a well-validated test in passerine birds (Reparaz et al., 2014; van Oers & Naguib, 2013).

### *Reproduction*

#### *Pair formation and bird housing*

The preference test was a preliminary test to ensure that birds would breed in aviaries for the purpose of another experiment. All females were therefore preferentially paired with one of the males with which they spent the most time. If several females selected the same male (eight males were selected more than once), we paired the female that had the smallest “interest” for males (see below) with her second choice ( $n = 10$  out of 36 females), i.e. with the male for

which she spent the second most time. Preliminary analyses showed that being paired with the first or second choice male did not affect their reproductive traits (data not shown). Despite this, females differed in the total time they spent with their preferred male during the mate choice test, thus we had variability in the strength of female preference. From early February, we housed each 36 opposite-sex pairs in 36 indoor aviaries (2 x 2 x 2.25 m) under an artificial light regime mimicking a natural daylight pattern. Sunrise and sunset times were determined using the ptaff.ca data base (<http://ptaff.ca/soleil/>) for Nijmegen, the Netherlands. The main source of light consisted of three high frequency fluorescent light tubes. An additional 7W incandescent light bulb mimicked dawn and dusk five minutes before natural sunrise and five minutes after sunset to respect the natural increase and decrease of light intensity every day. Temperatures of the next eight days in aviaries were set to mimic the temperatures of the past eight days in the wild. Hourly temperatures were determined using The Royal Netherlands Meteorological Institute (KNMI) data base for the weather station at Deelen (<https://www.knmi.nl/home>). A perch and an artificial tree were installed in the aviaries so that birds could perch and hide. Three nest-boxes were available in each aviary. Pairs had no visual or acoustic contacts with the other pairs.

#### *Reproduction monitoring*

Nesting material consisting of moss and dog hair was provided from 8th March 2021. From mid-March, we monitored nest building and egg laying activities, first once a week, and then twice a week as birds were closer to lay (Dufva, 1996). The date that the first egg was found was recorded as the pair laying date. All eggs were weighted (to the nearest 0.01 mg with an electronic balance, Eidyer), and measured (to the nearest 0.05 mm with a calliper, Ecotone Measy DG). Egg volume was calculated as follows:  $\text{egg volume} = 0.4673 \cdot \text{length} \cdot \text{breadth}^2 + 0.042$  (Dufva, 1996). After the birds laid the last egg, the clutch size was determined.

#### *STRANGE statement*

The birds used in this study could be considered STRANGE as defined by (Webster & Rutz, 2020) due to their rearing history and acclimation and habituation. Indeed, birds were collected from the wild but hand-reared, which may have affected their development as well as their exploration, activity, or personality traits. However, all of the subjects used in this study were of the same age and none had ever experienced a breeding season. Therefore, none of the birds could have been influenced by their previous experience in choosing a mate or in deciding



on their investment in reproduction. We therefore considered the STRANGE character of our sample to be low.

### Physiological measures

#### *Blood samples*

Females were caught in the aviary with a net and transported in bags to a laboratory room, where blood sampling was performed from the jugular vein (max. 150 $\mu$ l). Each bird was always sampled at the same time of day to control for daily rhythms in hormone levels. Blood was transferred to a 0.5 mL heparinised tube. Plasma was separated by centrifugation (14800 rpm, 10 min), kept on ice until the last blood sample of the day was collected, and stored in freezers ( $-80^{\circ}\text{C}$ ) until assayed for hormones. After blood sampling, females were released in their aviary. We blood sampled half of the birds (i.e. 18 females) every week, so that each bird was sampled every two weeks. In total seven blood samples were performed on each bird, between 18<sup>th</sup> March (before first laying) and 17<sup>th</sup> June 2021. Mean time between catching and blood sampling was 3 min 22 s  $\pm$  46 s.

#### *Estradiol assay*

Hormonal assays were conducted at the Institut de Génomique Fonctionnelle in Montpellier, France. Plasma 17 $\beta$ -estradiol ( $\text{E}_2$ ) was measured using a commercially available double-antibody  $^{125}\text{I}$ - $\text{E}_2$  radioimmunoassay (DSL-4800, Ultra-sensitive Estradiol RIA, Beckman Coulter, Brea, CA, USA) that was modified to increase the sensitivity of the assay (Caro et al., 2019; Charlier et al., 2010). Steroids were first extracted from 30  $\mu\text{L}$  of plasma using solid phase extraction with C18 columns (100 mg C18 material, Sep-Pak Vac 1 cc, Waters, Milford, MA, USA), dried under nitrogen gas at  $40^{\circ}\text{C}$ , and reconstituted overnight with PBSg (PBS with 0.1% gelatin) containing 0.7% ethanol (Caro et al., 2019). Recovery value after extraction was estimated at 91.5%. Concentration was adjusted for samples that did not have 30  $\mu\text{L}$  of plasma available ( $n = 10$  of 250, no plasma for 2 samples). Resuspended samples were then assayed in duplicate and tubes were counted on a gamma counter (Automatic Gamma Counter, Perkin Elmer, Waltham, MA, USA). Concentrations of  $\text{E}_2$  were obtained using a linear regression with the log-transformed concentrations of the standards provided in the assay kit. Samples were run in seven assays, and the intra- and inter-assay coefficients of variation, as estimated by assaying one high and one low concentration  $\text{E}_2$  standard in duplicate, were 4.27% and 12.27%, respectively. Assay sensitivity, defined as the highest point on the standard curve whose standard deviation did not overlap that of the blank standard (Wingfield & Farner, 1975),

was 0.65 pg/mL. No sample was found to be below the detection limit. The assay procedure has previously been validated for blue and great tits (Caro et al., 2019).

### Statistical analyses

All the analyses were performed in R version 1.3.1093 (R CoreTeam). Linear mixed-effects models and generalized linear mixed-effect models (for details see below) were conducted using the lme4 package (Bates et al., 2015). P-values were obtained with the lmerTest package in the case of mixed-model analyses (Kuznetsova et al., 2017). Plots were created with the ggeffects package (Lüdtke, 2018). Principal component analyses were run with FactoMineR (Husson et al., 2016) and factoextra (Alboukadel & Mundt, 2017) packages. Models were simplified using backward elimination of the nonsignificant terms, starting with the higher-order interactions (Crawley, 2007). We set  $\alpha = 0.05$ .

### *Preference test*

We first ran three principal component analyses (PCA) on (i) body size, (ii) feather coloration and (iii) beak size for males and females separately, as these variables were represented by measures that were correlated. PCA for body size included body mass, wing and tarsus lengths (Freeman & Jackson, 1990; Wiklind, 1996) (figure S2 A-B), PCA for feather coloration of the breast included yellow brightness, as well as yellow and UV chroma and hue (figure S2 C-D) and the PCA for beak size included beak depth and length (figure S2 E-F). Score of the PC1 for each PCA were then used in the statistical models. For colouration, we also used PC2 scores as this axis was well represented by three variables of feather colouration (figure S2 C-D). Detailed results of the PCAs are available in supplementary materials (figure S2).

To identify the criteria used by female great tits during the preference test to choose their mate, we ran a linear mixed effect model using the time (in seconds) spent by the female in the choice zone of individual males as the dependent variable. The model included the following male characteristics: breast stripe size, PC1 and PC2 of colouration, PC1 of beak size, PC1 of body size and exploration score as explanatory variables, as well as one female\*male interaction for each characteristic assessed. ID of the female was added as random intercept to account for the fact that each female occurred six times in the dataset (once for each six different males). The time spent in the choice zone of the males was weighted by the “interest” of the female to give more statistical weight to the females who spent more time evaluating the males during the test rather than staying in the neutral zone (Caro et al., 2021; Reparaz et al., 2014).

“Interest” depicts the overall motivation of the female to visit the males during the mate choice test, defined as the proportion of time that the female spent close to the six stimulus males (i.e. not in the central neutral zone), and was calculated as follows (Caro et al., 2021; Reparaz et al., 2014):

$$\text{Interest} = \frac{\text{Time spent with (male 1 + male 2 + male 3 + male 4 + male 5 + male 6)}}{\text{Total duration of the test (90min)}}$$

### *Reproduction*

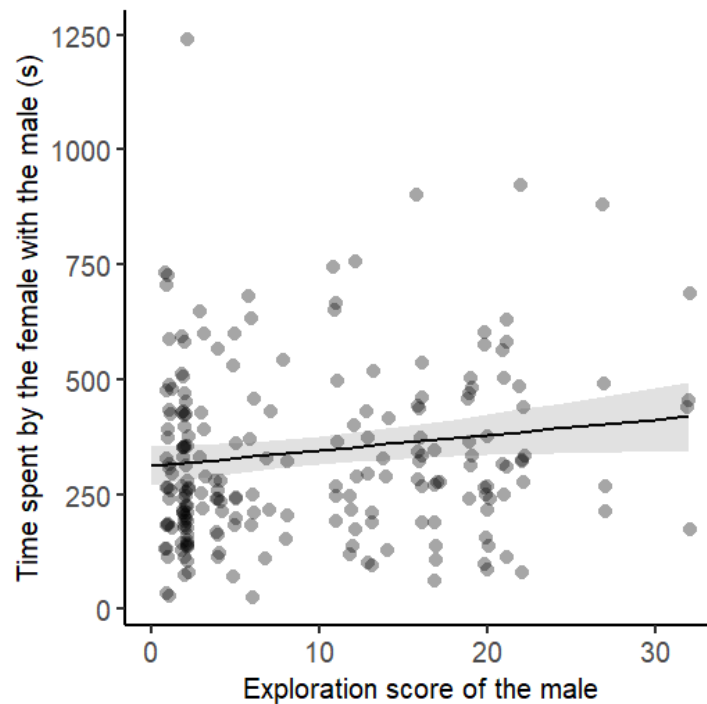
First, we ran a PCA on egg size including weight, length, breadth and volume of the eggs, as these variables were correlated (figure S3). We tested the effect of the intensity of the female preference for a male (i.e. the time the female had spent close to the male with which she was subsequently paired, as explanatory variable) on her reproductive parameters. Specifically, we performed (i) a linear model on lay date, (ii) a linear mixed-effect model on egg size (iii) a generalized linear model with Poisson distribution on clutch size. In the mixed effect model, female ID was included as a random intercept to account for the fact that each female laid several eggs. For reproductive hormone analyses, data were first log transformed to achieve normality. In order to assess if a female paired with a more preferred male would increase E<sub>2</sub> levels earlier in the breeding season, we ran a linear mixed effect models on all E<sub>2</sub> levels measured during the season, with sampling date, time spent with the male, and their interaction as explanatory variables. Female identity was added as a random intercept to account for the fact that each female occurred seven times in the dataset (seven sampling points). We also ran a linear model on the E<sub>2</sub> level measured closest to the laying date of the first egg ( $n = 17$  sampled before the first egg,  $n = 14$  sampled during the laying period,  $n = 1$  after laying. For two additional females, we used the average of two successive samples since the first-egg date was exactly in between) with time spent close to the male with which the female was paired included as explanatory variable. Linearity of the data, normality of the residuals, homoscedasticity and independence of residuals have been checked for all models.

## **Results**

### *Characteristics selected during the preference test*

All the 36 females tested visited the choice zone of all six males at least once. Time spent in the choice zone of males ranged from 24.84 to 1240.88 seconds (mean  $325.42 \pm \text{SD } 186.51$ ). We found no evidence for an assortative mating as none of the interaction terms between corresponding male and female characteristics were significant (Table S1). We found

that females showed a stronger preference for more exploratory males ( $F_{1,206.71} = 3.87$ ,  $p = 0.050$ , Table S1, figure 2). None of the quality measures (i.e. body size, beak size, tie size or coloration) were clearly preferred by the females.

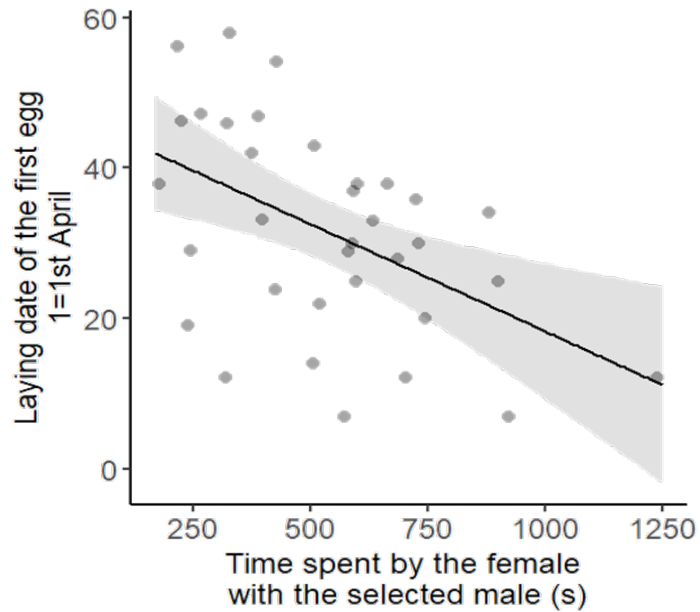


**Figure 2. Females show a stronger preference for more exploratory males.**

Females spent more time in the choice zone of more exploratory males ( $F_{1,206.71} = 3.87$ ,  $p = 0.050$ ). Points represent raw data ( $n = 216$ ) and the line represents the predicted values of the model with 95% confidence interval.

### *Reproduction*

35 out of the 36 pairs laid eggs during the breeding season. Females that had shown a stronger preference for the male with which they were paired laid earlier than females that had shown a lower preference for their male ( $F_{1,32} = 10.31$ ,  $p = 0.003$ , figure 3). Females showing stronger preference for their male also laid larger clutches ( $z_{1,32} = 2.69$ ,  $p = 0.007$ , figure S4A). However, one female was an outlier in our dataset with 28 eggs laid. When this female was removed, the effect of preference intensity on clutch size was no longer significant ( $z_{1,31} = 0.14$ ,  $p = 0.891$ , figure S4B). We did not find an effect of the female preference for its mate on egg size ( $F_{1,31.55} = 2.80$ ,  $p = 0.104$ ) and they did not show a faster increase in E<sub>2</sub> level when paired with a more preferred male (date\*time spent with the male interaction:  $F_{1,15.12} = 0.51$ ,  $p = 0.484$ , figure S5A), nor a higher concentration of E<sub>2</sub> close to laying ( $F_{1,31} = 0.29$ ,  $p = 0.589$ , figure S5B).



**Figure 3. Females showing a stronger preference for their male lay earlier.**

Females laid earlier when paired with a more preferred male ( $F_{1,32} = 10.31$ ,  $p = 0.003$ ). Each point represents a female ( $n = 34$ ) and line represents the predicted values of the model with 95% confidence interval. Laying date: 1=1<sup>st</sup> April.

## Discussion

We found that female great tits seem to express a stronger preference for more exploratory males. Male size, colours, tie size and beak size did not reliably influence female preference. Subsequently, we found that females that had shown a stronger preference for the male with which we paired them reproduced earlier. We did not find statistically significant effects of female preference strength on clutch size, egg size or  $E_2$  levels during the breeding season.

One of the main findings of our study is that females seem to spend more time close to more exploratory males ( $p = 0.050$ ). This is in accordance with a previous study in great tits (Carere et al., 2000); and Wang et al (2022) who found similar attraction for more exploratory males without assortative mating in Java sparrow (*Lonchura oryzivora*). Exploratory behaviour has been well studied in birds and is known to be representative of animal personality, referring to consistent differences between individuals in behaviour across time or contexts (Groothuis & Carere, 2005; Sih et al., 2004). This trait is also highly repeatable and heritable, both in wild populations (Dingemanse et al., 2002; Quinn et al., 2009) and under controlled laboratory conditions (Drent et al., 2003; van Oers et al., 2004). The 'fast-slow exploration' personality axis thus contrasts fast individuals that are more exploratory, or more likely to take risks (van

Oers et al., 2005), with 'slow' individuals that are less exploratory, more risk averse and more responsive to changes in their environment (Groothuis & Carere, 2005). In tits, it was shown that fast explorers were more aggressive (Verbeek et al., 1996), competitive (Cole & Quinn, 2012), and showed more foraging flexibility (Coomes et al., 2021). Consequently, being a fast explorer male could be advantageous for birds and may reflect their individual quality (Réale et al., 2007), in terms of territory defence capabilities (Amy et al., 2010) or in the number of offspring that they are able to produce (Dingemanse et al., 2004; McCowan et al., 2014), but see (Barnett et al., 2012; Wischhoff et al., 2018). With such a relationship between exploratory behaviour and fitness (Dingemanse & Réale, 2005), female great tits may have expected to obtain a high-quality male by selecting a more exploratory male.

We did not find any evidence that females preferred males of higher quality, as indicated by body size, beak size or feather coloration. Female preferences were thus not related to morphological characteristics of the males. While in the United Kingdom, longer bills have been associated with increased fitness in great tits (Bosse et al., 2017), this is not the case for Dutch great tit populations (Bosse et al., 2017), from where our birds originates. It may therefore not be surprising that females do not reliably choose their mate based on beak size. Although UV reflectance of the plumage has been involved in mate choice in different bird species (Doutrelant et al., 2012; Siitari et al., 2002), and that in the wild the intensity of the yellow coloration is often considered an honest indicator of the quality of an individual and of its territory (Casagrande et al., 2006; Isaksson & Andersson, 2007; Weaver et al., 2018), the great tits that we used were hand-reared captive birds, which diet is relatively poor in carotenoids, the pigments responsible for their yellow plumage coloration (Brush, 1990; Ferns & Hinsley, 2008; Isaksson et al., 2008). As a consequence, there was some inter-individual variation in the reflectance spectra among our males but all birds involved in the present study had a paler chest than what is observed in wild great tits (figure S6). It may therefore no longer carry any relevant signal to choose among individuals.

Another important finding of the study is that females laid significantly earlier when they had spent more time close to (i.e. expressed a stronger preference for) the male with which we subsequently paired them. This result is in accordance with our initial hypothesis of a higher reproductive investment when females are paired with their more preferred male. Indeed, egg production is both nutritionally and energetically costly for females (Nilsson & Råberg, 2001; Vézina & Williams, 2002; Visser & Lessells, 2001) and these costs might constrain the start of reproduction (Perrins, 1970). Laying earlier therefore represents an additional investment because females start laying at a time when food resources and temperatures are not favourable

yet. In the literature, there are several examples where mating with its preferred male increases reproductive success and offspring performance. Female house mice (*Mus domesticus*) mated with their preferred partners have higher reproductive success and better progeny performance than individuals mated with non-preferred partners (Drickamer et al., 2003). In zebra finches, females display a reduced readiness to copulate and males reduce parental care if they are force-paired (Ihle et al., 2015), while pairs that resulted from free mate choice achieve higher reproductive success (Balzer & Williams, 1998; Ihle et al., 2015). These results could be explained by synchronization between partners, for example. Ihle and colleagues (2015) found that individuals of freely-chosen pairs stayed closer together and behaved more synchronously than those of non-chosen pairs. Many studies show that pair coordination and bond duration is important for breeding success (Griggio & Hoi, 2011; Leniowski & Węgrzyn, 2018; Van De Pol et al., 2006) and that incompatible pairs take longer to breed than compatible ones (Griffith et al., 2011). In our case, great tit females expressing higher preference for their male may have been more stimulated by their partner and more synchronized with it, leading to earlier reproduction. One could however argue that females expressing stronger preferences for males were closer to reproduce than the others, explaining why those females laid earlier. However, contrary to males that become ready to reproduce early in spring (Caro et al., 2006; Dawson et al., 2001), females do not reach sexual readiness until a few days before they actually start laying, in April (Caro et al., 2009; Williams, 2012). This is supported by the fact that 17 $\beta$ -estradiol ( $E_2$ ) did not start to increase until April 10 (figure S5A). At the time of preference testing, in January, all females were thus still far from reproducing.

Female pairing did not seem to affect their reproductive hormonal physiology, nor their clutch size. This goes against our initial predictions. We expected females showing stronger preferences for their males to have higher levels of  $E_2$  and to lay larger clutches. Indeed in zebra finches, females lay on average 0.5 eggs more when paired with their preferred male (Balzer & Williams, 1998) and barn swallows tend to lay larger clutches when paired with an attractive male (De Lope & Moller, 1993).  $E_2$  has been shown to enhance female sexual behaviours and has even been used to promote pre-copulatory displays in avian mate-choice experiments (Searcy, 1992a).  $E_2$  has also been associated with nest-building behaviour, oviduct growth and yolk synthesis (Hunt & Wingfield, 2004; Williams, 2012). However, other studies showed that elevating  $E_2$  in female do not change their preferences (Enstrom et al., 1997) and that reproductive hormones do not always predict laying dates (Schaper et al., 2012). Schwabl and colleagues also found in female red-backed fairy-wrens (*Malurus melanocephalus*) that  $E_2$  levels varied with reproductive stage, but that these stage-dependent patterns were not affected

by the male phenotype with which they were paired (Schwabl et al., 2014). Finally, Bottoni and colleagues showed that plasma luteinizing hormone (LH) levels, the hormone regulating gonadal steroidogenesis and initiating ovulation (Williams, 2012), did not differ between pairs that had freely chosen themselves and those that had been forced-paired (Bottoni et al., 1993), which is consistent with what we find here with E<sub>2</sub>.

In conclusion, this study highlights the importance of female preference on reproductive timing. We have shown that partner preference is not only based on morphological characteristics (Andersson & Simmons, 2006), but may also depend on consistent behavioural trait. This provides some potential for selection on behavioural phenotype in the wild, as suggested in other studies (van Oers et al., 2008). Taken together, our results suggest that there could be major fitness consequences of mate preferences in socially monogamous species.

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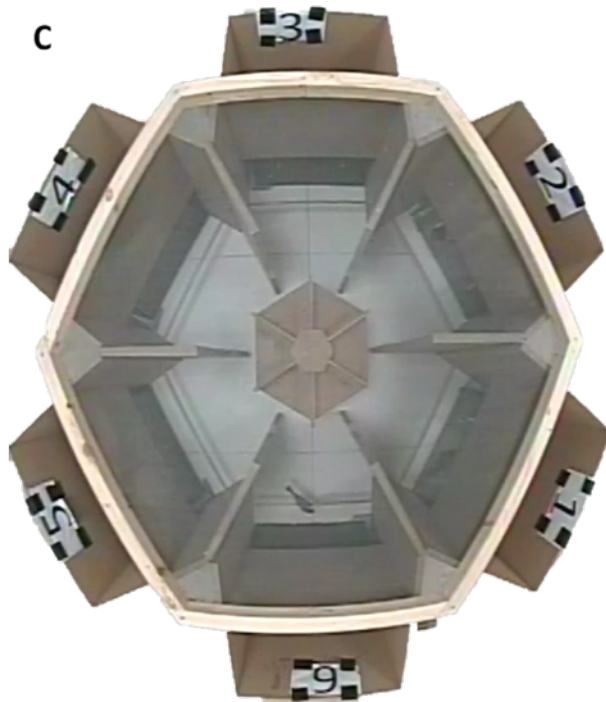
## Supplementary material

**Female great tits (*Parus major*) reproduce earlier when paired with a male they prefer.**

**Table S1: Results from linear mixed effect models, exploring the variables that influence the time spent by the female in the choice zone of the males during the 90 minutes of the test ( $n = 36$  females).**

Variables in bold had a significant effect on the response variable ( $p \leq 0.05$ ), italicized variables depict trends ( $p \leq 0.10$ ). Variables in light grey were removed during the reduction of the model, and are presented in the reverse order in which they were removed.

<b>Response variable</b>	<b>Explanatory variable</b>	<b>Estimate</b>	<b>S.E</b>	<b>df</b>	<b>F value</b>	<b>p-value</b>
<i>Time spent by the female with the males (s)</i>	Intercept	315.68	21.29			
	<b>Exploration score male</b>	<b>3.02</b>	<b>1.53</b>	<b>206.71</b>	<b>3.87</b>	<b>0.050</b>
	<i>Axis 1 PCA Body size male</i>	-15.99	9.26	204.69	2.99	0.085
	Tie size male	-20.29	19.39	205.70	1.09	0.297
	Axis 2 PCA Coloration male	-10.18	11.44	206.08	0.79	0.375
	Axis 1 PCA Coloration male	-4.72	9.35	204.06	0.25	0.614
	Axis 1 PCA Beak size male	-2.59	14.27	195.16	0.03	0.856
	Axis 1 PCA Beak size female	0.24	13.71	34.26	0.01	0.986
	<i>Axis 1 PCA Beak size male*Axis 1 PCA Beak size female</i>	21.16	11.63	176.20	3.31	0.071
	Exploration score female	-1.17	2.19	82.42	0.29	0.593
	Exploration score male*Exploration score female	0.12	0.15	167.76	0.57	0.451
	Axis 1 PCA Coloration female	-2.87	11.04	29.78	0.07	0.796
	Axis 1 PCA Coloration male*Axis 1 PCA Coloration female	2.75	6.04	163.91	0.21	0.649
	Axis 1 PCA Body size female	17.19	16.78	27.35	1.05	0.314
	Axis 1 PCA Body size male*Axis 1 PCA Body size female	2.29	8.39	155.32	0.07	0.786
	Axis 2 PCA Coloration female	-26.51	16.33	31.19	2.63	0.115
	Axis 2 PCA Coloration male*Axis 2 PCA Coloration female	2.58	7.59	150.55	0.12	0.735
	Tie size female	12.12	167.95	163.49	0.01	0.943
	Tie size male*Tie size female	-7.96	39.07	155.96	0.04	0.839



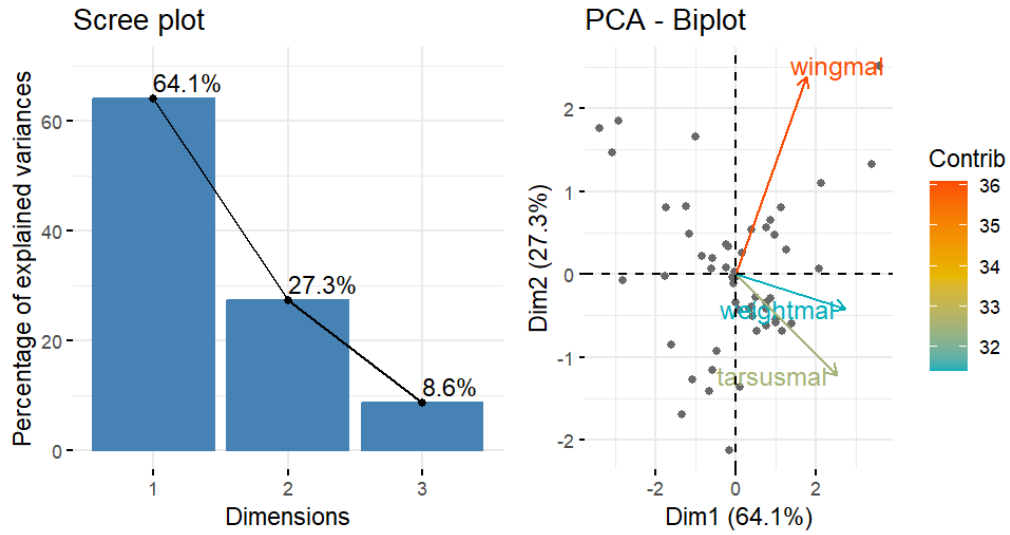
**Figure S1: Pictures of the experimental setup for the mate preference test.**

A: Picture of the carousel-shaped six-choice chamber. The roof can be moved to introduce the female into the setup.

B: Picture of the camera pointing down from the ceiling. The camera was centred to point in the middle of the setup.

C: Screenshot of the video of a test, the female is in the choice zone of the male 6. The males are not visible on the video.

**A**



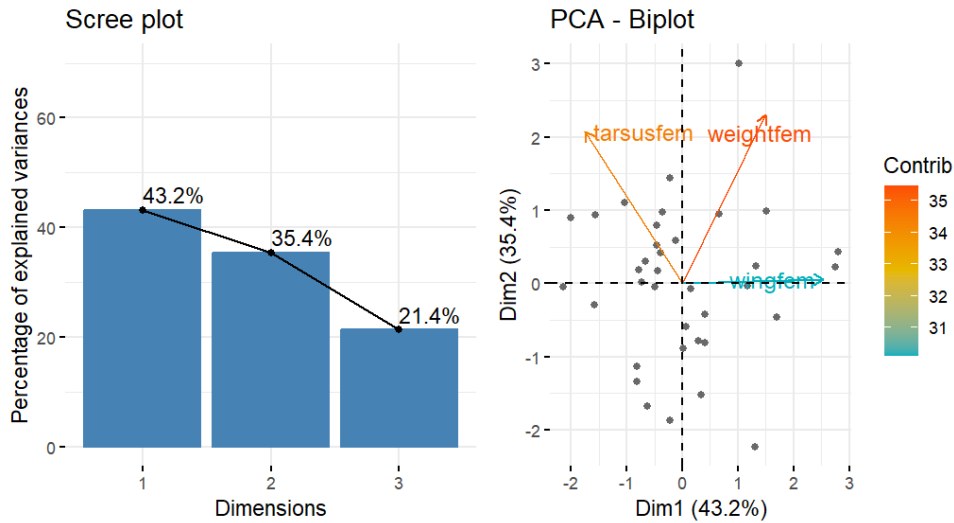
Bartlett's test:  $X_2(3) = 24.2, p = 0$

**Table S2: Correlation matrix of variables (Pearson coefficient) and loadings of the PCA of body size for males ( $n = 35$ ).**

<b>Correlation</b>	Tarsus	Wing
Tarsus	1	
Wing	0.289	1
Weight	0.606	0.505

<b>Loadings</b>	PC1	PC2	PC3
Tarsus	0.615	-0.450	0.648
Wing	0.429	0.880	0.203
Weight	0.662	-0.153	-0.734

**B**



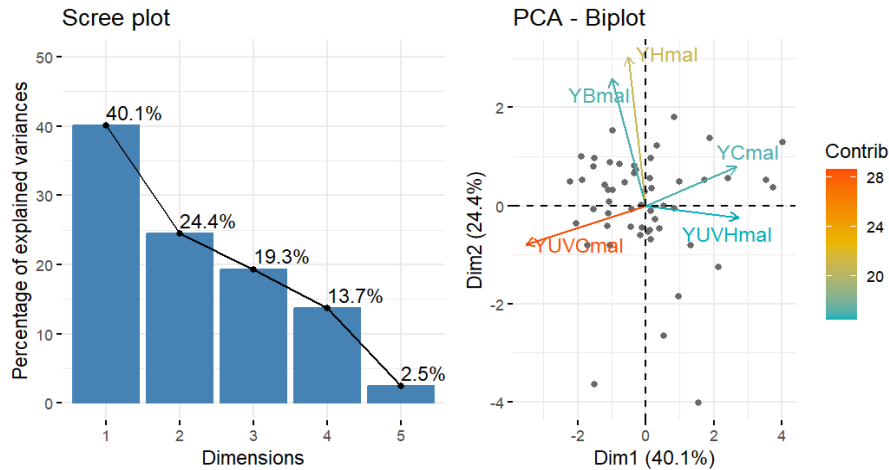
Bartlett's test:  $X_2(3) = 2.5, p = 0.476$

**Table S3: Correlation matrix of variables (Pearson coefficient) and loadings of the PCA of body size for females ( $n = 36$ ).**

<b>Correlation</b>	Tarsus	Wing
Tarsus	1	
Wing	-0.207	1
Weight	0.039	0.207

<b>Loadings</b>	PC1	PC2	PC3
Tarsus	-0.510	0.668	0.541
Wing	0.740	0.021	0.672
Weight	0.438	0.744	-0.505

**C**

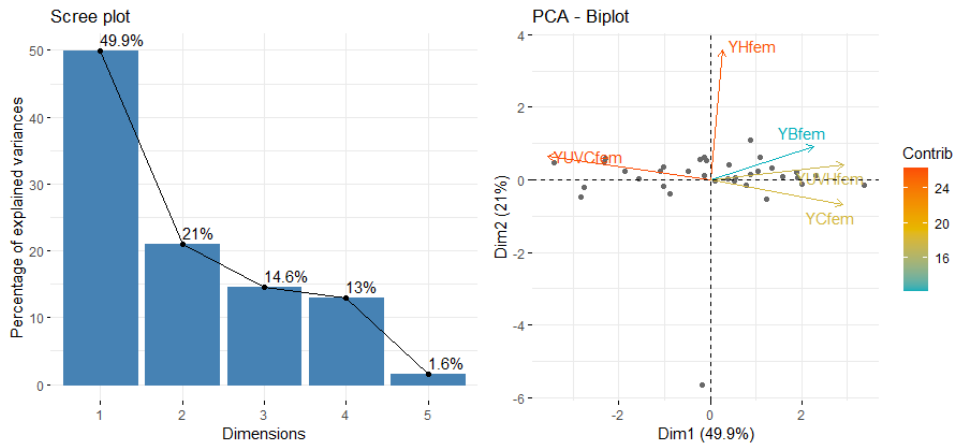


YB: achromatic yellow; YC: chromatic yellow; YUVC: chromatic UV yellow; YH: yellow hue (maximal slope in yellow part of the spectrum); YUVH: UV yellow hue (maximal reflectance in UV)  
 Bartlett's test:  $X_2(10) = 81.3, p = 0$

**Table S4: Correlation matrix of variables (Pearson coefficient) and loadings of the PCA of colouration for males ( $n = 54$ ).**

<b>Correlation</b>	YBmal	YBmal	YBmal	YBmal	<b>Loadings</b>	PC1	PC2	PC3	PC4	PC5
YBmal	1				YBmal	-0.189	0.625	0.487	-0.567	0.121
YUVCmal	0.0422	1			YUVCmal	-0.663	-0.189	-0.089	0.089	0.714
YCmal	-0.176	-0.639	1		YCmal	0.501	0.190	-0.587	-0.359	0.488
YUVHmal	-0.099	-0.653	0.103	1	YUVHmal	0.513	-0.059	0.594	0.377	0.488
YHmal	0.238	0.020	0.051	-0.1285	YHmal	-0.099	0.731	-0.239	0.632	-0.006

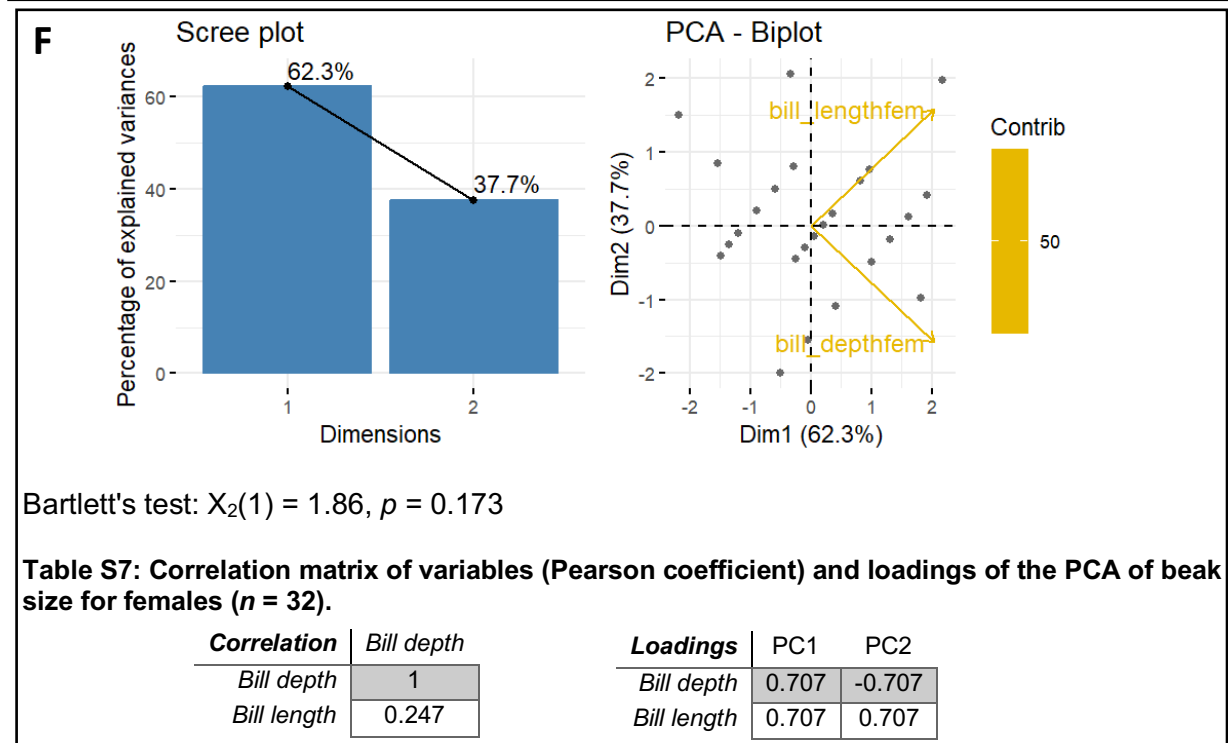
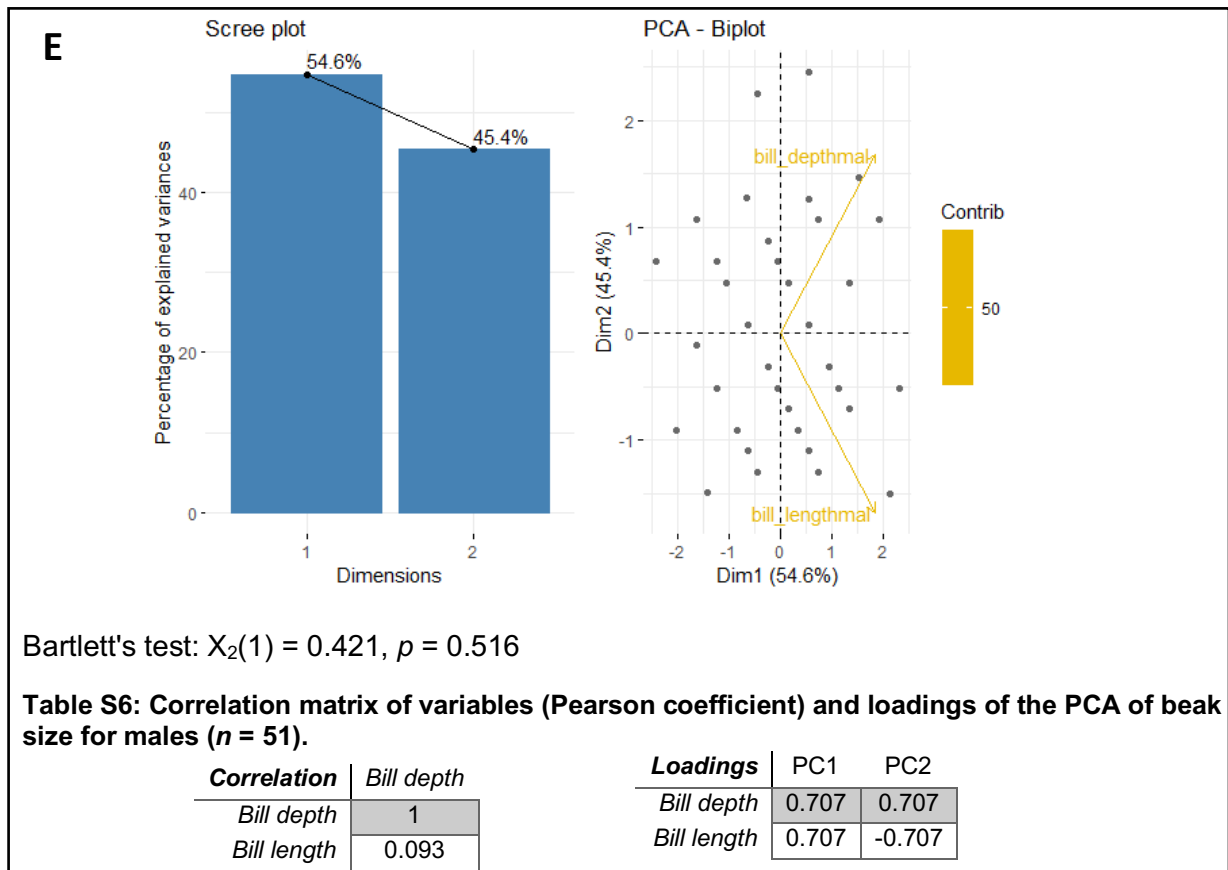
**D**



YB: achromatic yellow; YC: chromatic yellow; YUVC: chromatic UV yellow; YH: yellow hue (maximal slope in yellow part of the spectrum); YUVH: UV yellow hue (maximal reflectance in UV)  
 Bartlett's test:  $X_2(10) = 72.7, p = 0$

**Table S5: Correlation matrix of variables (Pearson coefficient) and loadings of the PCA of colouration for females ( $n = 35$ ).**

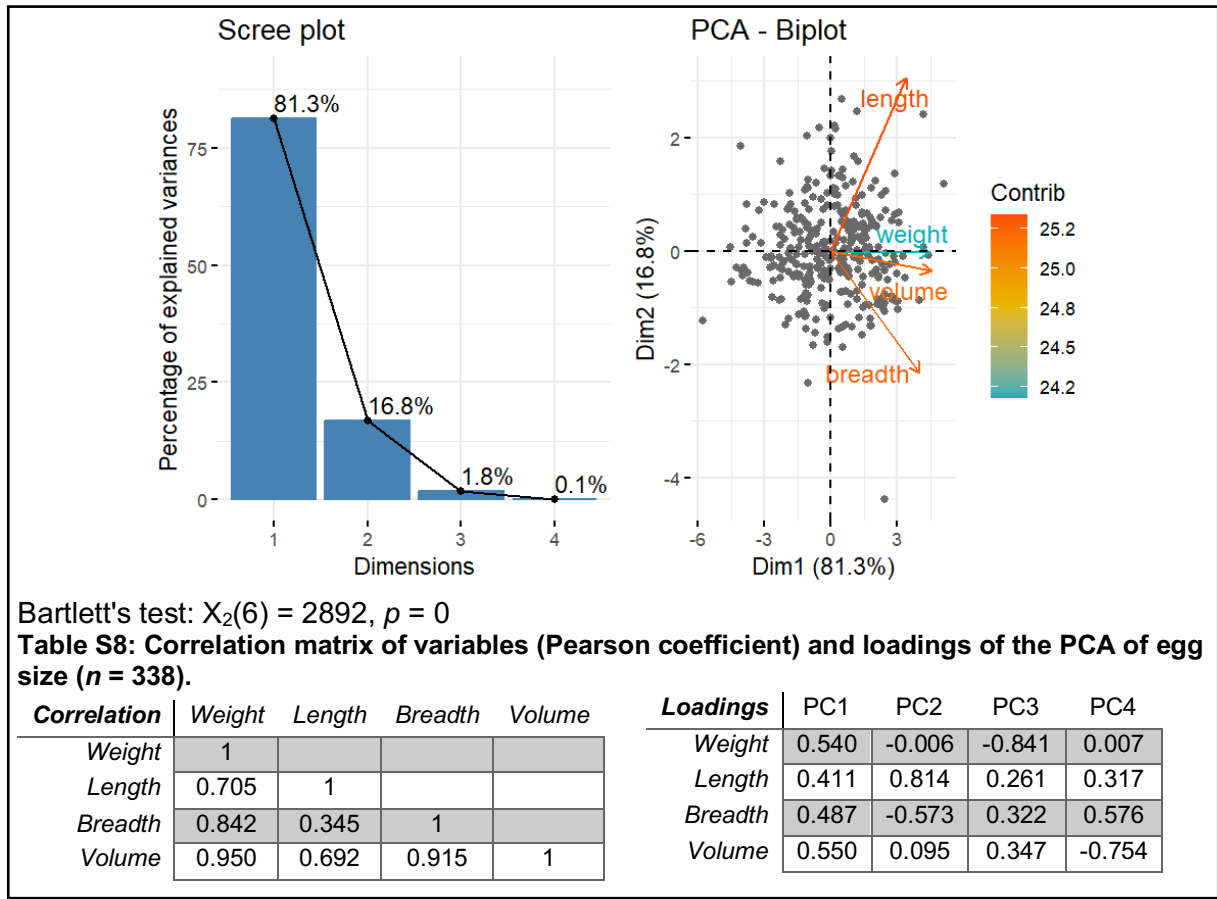
<b>Correlation</b>	YBfem	YBfem	YBfem	YBfem	<b>Loadings</b>	PC1	PC2	PC3	PC4	PC5
YBfem	1				YBmal	0.385	0.242	0.847	0.269	0.058
YUVCfem	-0.425	1			YUVCmal	-0.602	0.169	0.155	0.057	0.763
YCfem	0.291	-0.765	1		YCmal	0.493	-0.175	0.395	0.596	0.464
YUVHfem	0.344	-0.729	0.329	1	YUVHmal	0.495	0.108	-0.048	-0.745	0.432
YHfem	0.105	0.059	0.016	0.111	YHmal	0.045	0.933	-0.317	0.118	-0.116



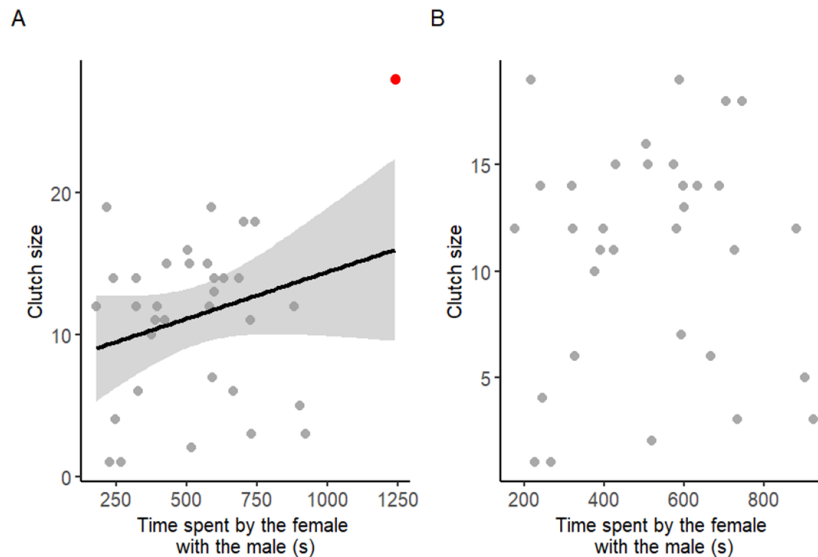
**Figure S2: Results of the PCA ran for body size, colouration, and beak size of male and female great tits.**

Correlation matrix was used for all PCAs.

A: Male body size; B: Female body size; C: Male colouration; D: Female colouration; E: Male beak size; F: Female beak size



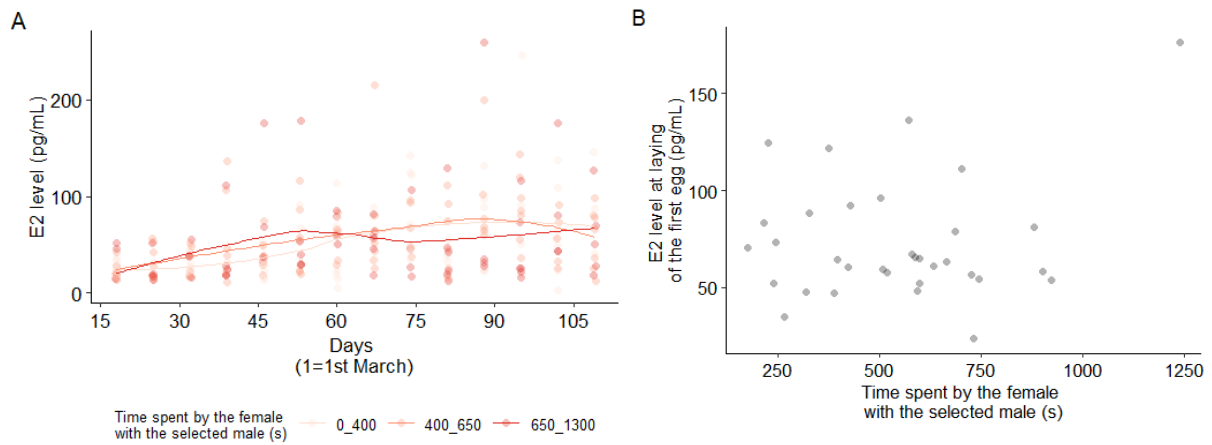
**Figure S3: Results of the PCA ran for egg size.**  
High value of the first axis represents bigger eggs.



**Figure S4: The strength of the preference of the female for the male did not affect clutch size, when excluding an outlier.**

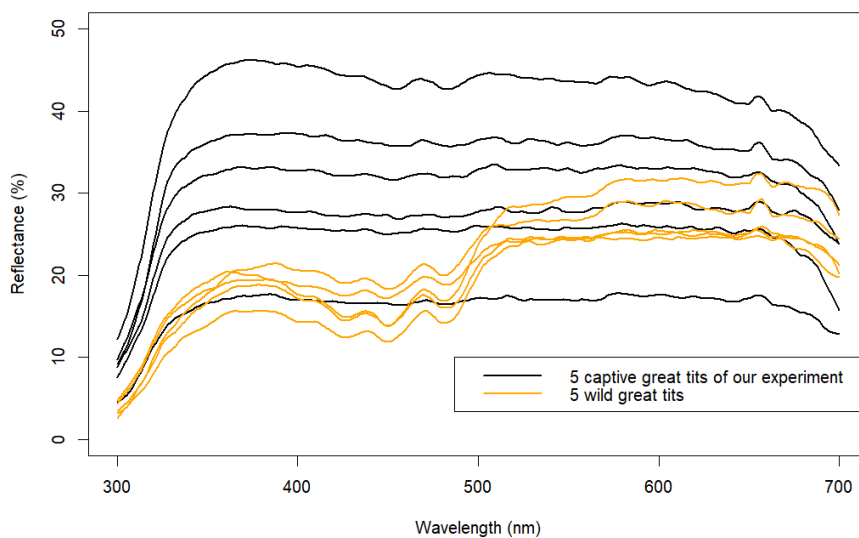
(A) Females showing stronger preference for their male laid larger clutches ( $z_{1,32} = 2.69, p = 0.007$ ). However, one female, depicted by the red point on the graph, was an outlier in our data with 28 eggs laid. (B) When this female was removed from the data, the effect of preference intensity on clutch size was no longer significant ( $z_{1,31} = 0.14, p = 0.891$ ).





**Figure S5: The strength of the preference of the female for the male did not affect E<sub>2</sub> level.**

(A) E<sub>2</sub> concentrations during the breeding season did not increase sooner in females paired with a more preferred male (date\*time spent with the male interaction:  $F_{1,15.12} = 0.51$ ,  $p = 0.484$ ). (B) Concentrations closest to the laying date of the first egg ( $F_{1,31} = 0.29$ ,  $p = 0.589$ ) were not significantly influenced by the strength of the preference of the female for her male.



**Figure S6: Reflectance spectra of male great tit breast feathers.**

Reflectance spectra of breast feathers of ten great tit males revealing variability between individuals. Each line represents the mean of three measures of one individual. Black lines represent the data of five captive males that we used in our mate preference test. Yellow lines represent the data of five wild great tits. Wild great tits have a yellow breast, whereas captive great tits have a pale grey breast.