Genes, environments and their interaction: song and mate choice in canaries

Miléna Trösch[*], Wendt Müller, Marcel Eens, Arne Iserbyt

Department of Biology, Behavioural Ecology and Ecophysiology Group, University of Antwerp, Wilrijk, Belgium

* **Correspondence**: M. Trösch, Department of Biology, Behavioural Ecology and Ecophysiology Group, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium.

E-mail address: milena.trosch@gmail.com.

Abstract

Male secondary sexual traits and female mate choice traits must contain heritable variation for sexual selection to operate. However, for female mate choice, especially, this is poorly known. To complicate matters, both male sexual traits and female mate choice typically show condition dependence, implying that environmental effects probably play an important synergistic role. Using a cross-fostering design, we therefore aimed to disentangle genetic, environmental and their potential interacting effects to investigate how they affect the expression of a sexually selected trait (here birdsong) and female mate choice. To assess environmental effects, we focused on the role of the social environment and thus on learned components. Among the different male song traits investigated, we found a high heritability for song bout length and song bout repertoire, as well as an intriguing gene-by-environment interaction for song bout repertoire. Specifically, the tutor appeared to negatively affect the song bout repertoire of the tutee when his genetic father had a large song bout repertoire but had a positive effect on descendants from fathers with small song bout repertoires. In contrast, we did not detect significant heritability in female mate choice. Female mate choice contained a learned component as females significantly disfavoured their foster father in the mate choice tests, indicating a learned inbreeding avoidance. Thus, our study provides important insights into the role of the social environment for both birdsong and female mate choice. However, the absence of a heritable component in female mate choice suggests a need for studies investigating the heritability of female preferences for (heritable) male song traits in order to gain a better understanding of a potential coevolution between male sexual traits and female mate choice. Overall, our results suggest that indirect genetic effects acting during the pre- and postfledging social contexts may play a prominent role in sexual selection**.**

Keywords: cross-fostering, gene-environment interaction, heritability, inbreeding avoidance, repeatability, *Serinus canaria*, sexual selection, social learning.

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Introduction

Sexual selection is characterized by intraspecific reproductive competition between individuals of one sex to get access to gametes of the other sex. It acts on those traits that enhance reproductive success, either through increased chances of success in competition for mates (intrasexual selection) or via traits that make them more attractive for the opposite sex (intersexual selection; reviewed by Kokko et al., 2006; Hosken & House, 2011). The latter is typically achieved via secondary sexual characteristics that might be reliable signals of quality and hence affect mate choice (Zahavi, 1975; Forstmeier et al., 2009; Holman, 2012). Intersexual selection may ultimately give rise to coevolution via reciprocal selective effects between signalling traits, typically in males, and the degree of preference for it by the other sex, typically the females (Ryan & Kirkpatrick, 1991; Kokko et al., 2006; Qvarnström et al., 2006).

Selection requires that traits, here male sexual traits and female mate choice, are heritable (Ryan & Kirkpatrick, 1991). Heritability of sexually selected male traits has indeed been shown in a number of previous studies (e.g. Karino & Haijima, 2001; Hadfield & Owens, 2006; Forstmeier et al., 2009; Müller et al., 2010; Evans & Sheldon, 2012; Hubbard et al., 2015). However, as reflected in the often low heritability estimates, especially for behavioural traits, sexually selected traits contain not only a genetic but also a significant environmental component.

Birdsong for example, a well-studied sexually selected trait that honestly signals male quality for female mate choice (Eens et al., 1991) and plays a role in male–male competition (Williams, 2004), is in fact a largely learned behaviour (Nottebohm et al., 1981). Male song characteristics therefore depend on the male's genetic predisposition to learn, and thus on specific brain regions that are involved in song control, which have a strong genetic component (Airey et al., 2000; Garamszegi & Eens, 2004; but see also Leitner & Catchpole,

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2004; Woodgate et al., 2014). However, what can be learned will depend on the exposure to other individuals' songs during a critical period in early life (Nowicki et al., 1998; Williams, 2004; Bolhuis & Gahr, 2006), that is their social environment. In fact, it is likely that male song will depend on an individual's capacity to learn as well as the quality of the song templates to which it is exposed, reflecting gene-by-environment interactions (GxE). Empirical studies have shown that GxE effects are widespread for male sexual traits (e.g. David et al., 2000; Lewandowski & Boughman, 2008; Rodriguez & Al-Wathiqui, 2011), including song expression, here investigated in the context of nutritional stress (Woodgate et al., 2014). However, such synergistic effects of genes and environments have received little attention in the context of song learning.

In turn, female mate choice is also an important fitness-related trait because females gain direct and indirect benefits by mating with high-quality males (Kodric-Brown & Brown, 1987; Ryan & Kirkpatrick, 1991), but our knowledge of its heritability is even more limited. Several studies, in a large range of taxa, have found significant heritability of choice-related parameters, such as the time the females invest in assessing the males or how selective the females are (Brooks & Endler, 2001; Brooks, 2002; Rodríguez & Greenfield, 2003). However, a general pattern on the heritability of preference functions (i.e. the actual outcome of female mate choice) is currently lacking despite its impact on phenotypic trait evolution via intersexual selection (Schielzeth et al., 2009; Zietsch et al., 2011; Prokuda & Roff, 2014). Moreover, repeatability estimates of these preference functions are generally low in birds (Forstmeier & Birkhead, 2004; Bell et al., 2009), while repeatabilities are often considered as upper bounds for heritability. As mentioned above, low heritability estimates indicate a relatively large influence of the environment, both abiotic (e.g. Woodgate et al., 2010, Holveck & Riebel, 2010) and biotic, given the evidence for social learning (Bolhuis & Honey, 1998; Riebel, 2000; ten Cate, 2006; Ludwig & Becker, 2008; but see Hegyi et al., 2010). As

in males, G×E effects may be very common, but studies that have investigated G×E effects for female mate choice are remarkably scarce (Ingleby et al., 2010; 2013).

In this study, we aimed to partition phenotypic variance in both male sexual traits and female mate choice traits into their genetic and their environmental variance components, as well as to investigate potential G×E effects. To this end, we manipulated the social environment of male domesticated canaries, *Serinus canaria*, to investigate three different sources of variation in male song: (1) genetic effects (via son–genetic father comparisons); (2) early environmental effects (via son–foster father comparisons); and (3) later environmental effects during the song-learning period (via son–tutor comparisons). As song represents a multifaceted signalling behaviour (Gil & Gahr, 2002), we quantified four different song parameters in male canaries (i.e. song activity, average bout duration, song bout repertoire and song consistency) that have previously been shown to be important in the context of sexual selection (see for instance: Kroodsma, 1976; Searcy, 1992; Eens et al., 1991; Gil & Gahr, 2002; Botero et al., 2009; Müller et al., 2010). In a similar and simultaneous approach, we assessed the contribution of genetic (via daughter–genetic mother comparisons) and learned early environmental effects (via daughter–foster mother comparisons and by testing the effect of male familiarity on female mate choice) to the outcome of both female mate choice and choice-related parameters. All birds experienced similar and constant husbandry conditions throughout their lives, which enabled us to specifically focus on the contribution of genetic background and social environment to the expression of our assessed traits.

<H1>Methods

<H2>Study species and animal husbandry

We used a total of 157 Fife Fancy canaries, originating from our own laboratory stock population with a known pedigree since 2006. More precisely, the songs of 44 males (hatched in 2011 or 2012) were recorded in 2014, including 38 genetic fathers (F0 generation), 36 foster fathers and 17 tutors. The songs of 51 young males, which hatched in 2014 and which descended from the 38 genetic fathers (F1 generation, subsequently referred to as 'sons'), were recorded in 2015. The mate choice experiment was performed in 2015, with 31 mothers (F0, hatched in 2011 or 2012) and their 31 genetic daughters (F1).

The F1 generation was cross-fostered as nestlings at a very early stage (day 0=hatching), with all foster nests containing four unrelated nestlings to standardize parental workload across nests (for more details, see Iserbyt et al., 2015). At cross-fostering, all nestlings of a foster nest were of similar age (maximum 12 h difference between nestlings) to equalize competitive abilities among nestlings. Furthermore, foster nestlings hatched from eggs with a different egg order to minimize inflation of maternal effects within nests (Schwabl, 1993). Each foster family was housed separately (GEHU cages measuring 50×64 cm and 40 cm high). All birds experienced a long light regime (14:10 h light:dark) throughout this period and had access to canary seeds (van Camp, Boechout, Belgium) and water ad libitum. They received enriched egg food (van Camp, Belgium) daily, as well as germinated seeds.

The second phase of the experiment started when the F1 generation reached 25 days. This period corresponds with nestling independence, when young canaries no longer rely on provisioning by the parents. After 25 days, all birds from the F1 generation were subdivided into groups of eight individuals together with an adult tutor male. Tutor males were chosen randomly from our outbred laboratory population. Tutor groups were housed in larger separate cages (50 x 128 cm and 40 cm high) in visual and semiacoustic isolation. The light

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regime was switched to a short light regime (10:14 h light:dark). Young females were removed from tutor groups after molecular sex determination (Griffiths et al., 1998) from a 100 μl blood sample taken on day 25. All F1 females were housed along with their mothers in large aviaries separated from the males. All birds stayed in these conditions for approximately 1 year until 5 weeks prior to the experiments in February 2015. At this time, the light regime was switched to long light (14:10 h light:dark) to stimulate their reproductive activities.

Ethical Note

Our research was approved by the Ethical Committee of the University of Antwerp (ID: 2014–72). Our study involved video and song recordings to monitor natural animal behaviour (see below) and was for this reason free from pain or distress. Blood sampling did not compromise the wellbeing of the birds, given that natural behaviour (e.g. brooding or singing) was resumed usually within 15 min following manipulation. Long-lasting effects of these manipulations were never observed in our study population.

Birdsong

For song recordings, all males (F0 [tutors, genetic and foster fathers] in 2014 and F1 sons in 2015) were housed individually in separate cages (GEHU cages 50 x 64 cm and 40 cm high), starting 3 days prior to the recordings so that they could get used to their new environment. During the recordings, a clear Perspex plate was placed in front of the cage to isolate the song of the focal bird from the background noise. This allowed visual contact with conspecifics at all times. Birdsong was sequentially recorded, for approximately eight birds at a time, with an omnidirectional AV-Jefe TCM141 tie clip-microphone connected to an M-audio microtrack II recorder (recording settings: WAV, 44 100 Hz, 16 bits, mono), which was placed near the middle of the trellised front of the cage, pointing to the inside of the cage. The song was

recorded for 2 h for each individual and the sonogram of the recording was then visualized in Avisoft-SASLab Pro 5.2.07 (Avisoft, Berlin, Germany; spectrogram parameters: FFT length 512, frame size 100% and overlap of 50%) to check whether it fulfilled the following conditions. First, the recording had to be of sufficient quality (i.e. the sonogram had to be clearly visible with limited background noise). Second, the bird had to be singing for at least 100 s in total, to ensure correct estimation of our four song parameters (see below). If one of these two conditions was not fulfilled, the recording was performed again.

The songs were analysed with Avisoft-SASLab Pro 5.2.07. First, the first 10 and last 5 min of the recording were discarded to avoid a possible human disturbance effect. We scored four song traits: song activity (the amount of time during which the male was effectively singing/h), average bout duration (song bouts were considered as such if they had a minimum length of 0.75 s and were delineated when a pause of at least 1.4 s was observed), song bout repertoire (here assessed by the average number of distinct syllable types within a song bout, which correlates strongly with total repertoire size in our study population: Pearson correlation: $r_{35} = 0.573$, $P = 0.0002$; based on outbred birds within our stock population; De Boer et al., 2016) and song consistency (which is defined as the similarity of specific song elements; Vergauwen et al., 2014a). To assess consistency, one specific type of syllable that most individuals in the population use was chosen and defined as a 'common syllable' (Vergauwen et al., 2014a). Ten exemplars of this specific common syllable were selected for each bird, all belonging to different bouts. The syllables were uploaded in Avisoft-CORRELATOR version 3.1 and the spectrograms were subsequently compared using an analysis of spectrographic cross-correlation. The resulting matrix of pairwise comparisons was averaged to obtain a single value per individual (Rivera-Gutierrez et al., 2012).

In a parallel study, 42 males were recorded twice with an interval of 10 days between two recordings to assess repeatability of these song traits (Iserbyt, Eens, Baetens, Vermeulen, Müller, 2017). Significant and high repeatability estimates were found for all parameters (average bout duration: $R= 0.60$; song bout repertoire: $R= 0.44$; song activity: $R= 0.62$; song consistency: $R= 0.35$). The arithmetic means of the two recordings were hence used for the F0 males for the purposes of this study.

<H2>Mate choice

Once the light regime was switched to long daylight, all experimental females were housed in groups of six to eight individuals per cage (50 x 128 cm and 40 cm high). Test males ($N = 17$) were kept individually in separate cages (50 x 64 cm and 40 cm high) in a different room. To start a mate choice test, the female was put in a double cage in front of which two small cages (33 x 33 cm and 51 cm high) were placed, one at each extremity of the cage (Lahaye et al., 2015; Fig. 1). Each small cage contained one male, which typically was settled on the central perch in the cage. The double female cage had three perches: one in the centre of the cage, which was defined as the no-choice area, as the female could not see either of the two males, and one perch per choice area, i.e. in front of each of the two small cages. Visual contact between males was prevented and the tested female could only see one male at a time. The female had access to food and water, in the no-choice area, ad libitum during the experiment. Finally, a video camera was placed in front of each set-up and the full test was video recorded. A maximum of eight females could be tested at the same time using different set-ups in the same room.

All mate choice trials were carried out in the same standardized way: Once the females were placed in their test cages, the males were placed in their respective small cages. At this stage,

visual contact between males and females was prevented by covering the trellised part of the small cages with paper sheets. These were removed after a habituation period of 30 min, and all cameras were switched on. After 1 h, the positions of the two male cages were swapped to control for a potential side preference of the female and the experiment continued for another 1 h.

Two sets of mate choice experiments were done. In the first series, a daughter, her biological mother and her foster mother were confronted with the same two unfamiliar, unrelated and otherwise randomly chosen males. The mate choice trial was performed a second time for 15 of the mothers and 15 daughters, randomly chosen, with the same males but approximately 1 week later, to estimate the repeatabilities. Finally, we performed another mate choice trial for 15 daughters to investigate the effect of male familiarity on mate choice, by confronting them with an unfamiliar male and their foster father. Male behavioural traits (e.g. song traits) or other phenotypic traits were not quantified during the mate choice tests as the major focus of these tests was to study the genetic and (foster) environmental effects on female choice-related behaviours rather than testing for the heritability of female preference for specific male traits.

The video recordings were analysed using the video tracking software The Observer XT10.5 (Noldus Information Technology, Wageningen, The Netherlands). The female's preference was estimated by scoring her position every 10 s, i.e. on the perch in front of the male on her left, on the perch in front of the male on her right or somewhere else. Four female mate choice parameters were then derived (modified from McGlothlin et al., 2004; Vergauwen et al., 2014b). Choice active time corresponded to the proportion of time that the female spent with either of the two males, relative to the total duration of the choice test. This parameter reflects

the female's sampling effort. Choosiness was estimated as the absolute deviation from 0.5 of the proportion of the choice active time that the female spent with a 'focal' male (= either of the two males, without considering whether it was the preferred male or not) reflecting her choosiness. Choosiness indicates a more skewed preference for one of the two males. Within-trial consistency was assessed as the absolute difference between the preference scores calculated for the first and the second hour of the experiment, indicating how consistent a female's preference was. A high value for this parameter indicates in this case a low repeatability of the preference score between the first and the second hour of experimentation, and hence a strong female side preference. Finally, a preference index was calculated for both males, which combined the choice active time and the time spent with a specific male. This index was calculated to obtain a more reliable measure of the actual female's interest for a specific male. The preference index, PI, was calculated for both males separately as follows:

$$PI_{male1,2} = \frac{\text{Time spent with male 1,2}}{\text{Total duration of mate choice trial}}$$

Statistics

All statistics were performed with R 3.0.2 (R Core Team, 2013), unless stated otherwise. Mixed linear models were run using the 'lmer' function in the lmerTest package (Kuznetsova et al., 2015). The normality assumption of these models was confirmed by Shapiro–Wilk tests in all cases. Heritability estimates (± SE) for both song and mate choice traits were calculated as twice the slope of the respective parent–offspring regression (Breed & Moore, 2016). An empirical example of our applied method can be found in Åkesson et al. (2007). To avoid pseudoreplication, the average value was used, in case brothers occurred in the F1 generation (birdsong) or repeated measures were taken in females (mate choice).

Birdsong

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The heritability of birdsong was assessed by investigating the relationship between the song characteristics of the sons and their biological father, their foster father and their tutor. To do so, a linear mixed-effects model was run for each of the four song parameters with the song characteristics of the sons as the dependent variable. The same characteristics of their biological father, foster father and tutor and the interaction effects between biological and foster fathers as well as between biological father and tutor were included as fixed effects. Genetic origin, foster nest identity and tutoring group were included as random effects, to account for nonindependence. Correlations between different song traits were investigated with Spearman rank tests using the function 'corr.test' in the Psych package (Revelle, 2015).

<H3>Mate Choice

The repeatability of the mate choice parameters was calculated as the ratio of the between-individual variation to the total variation (i.e. the between- and within-individual variation). A mixed modelling approach was used in SAS 9.3 (SAS Institute Inc., Cary, NC, U.S.A.) as described by Dingemanse & Dochtermann (2013). Significance was tested via the likelihood ratio test.

The heritability of female mate choice was tested in a similar way as for birdsong. Linear mixed-effects models were used with the different mate choice parameters of the daughters as the response variable and with the same characteristics of genetic mother and foster mother as explanatory variables. Random effects were added for the daughter identity, since 15 daughters were tested twice (to assess repeatability), and for foster nest, since one to three daughters were raised by the same foster parents. Fifteen of the mothers were also tested twice, for which the arithmetic mean between the values of both choice tests was used. For the preference indexes, every experiment was divided into two separate data points: the

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proportion of time the female spent with the first male and the proportion of time she spent with the second male. As these two data points were not mutually exclusive, choice test number was added as a random effect.

To determine the effect of male familiarity on female mate choice, an additional linear mixed model was performed with the preference index as response variable and the familiarity of this male (either her foster father or a random male) as categorical explanatory variable. Female identity was used as a random factor given that some females were tested twice to assess repeatability.

<H1>Results

<H2>Birdsong

<H3>Song bout duration

The average song bout duration of the sons depended on the average song bout duration of their genetic father, but not on the average bout duration of their foster father or of their tutor (Table 1). This effect was positive, and a heritability estimate ($H^2 \pm$ SE) of 0.81 ± 0.31 was obtained ($R^2 = 0.20$; Fig. 2). There were no significant interaction effects (Table 1).

<H3>Song bout repertoire

The song bout repertoire of sons strongly increased with the song bout repertoire of their genetic father (Table 1; $H^2 = 1.31 \pm 0.44$ [$R^2 = 0.26$]), although the association was modulated by the song bout repertoire of the tutor. This significant interaction effect of song bout repertoire of the genetic father by song bout repertoire of the tutor on the son's repertoire was negative (Fig. 3, Table 1). Specifically, the song bout repertoire of the genetic father had a positive effect on the song bout repertoire of the son only when the tutor possessed a small

song bout repertoire. Conversely, if the genetic father had a limited song bout repertoire, the song bout repertoire of the tutor had a positive effect on the song bout repertoire of the tutee. Males that had both a genetic father and a tutor with a large song bout repertoire had a relatively small song bout repertoire, similarly to the males that had both a genetic father and a tutor with a small song bout repertoire. Males that had a genetic father with a large song bout repertoire but a tutor with a small song bout repertoire acquired the largest song bout repertoire of all. The effect of the song bout repertoire of the foster father and the interaction between this parameter in the genetic father and the foster father was not significant (Table 1).

Song activity

The song activity of the tutor had a negative, yet nonsignificant, effect on the song activity of the sons (Table 1). Sons that stayed with a tutor that had a high song activity tended to sing less ($R^2 = 0.09$; Fig. 3). The effect of the song activity of the genetic ($H^2 = 0.17 \pm 0.55$; $R^2 = 0.004$) and foster father as well as all potential interaction effects were not significant (Table 1).

Song consistency

None of the fixed factors could explain variation in song consistency (Table 1). The heritability of song consistency was estimated at $H^2 = 0.14 \pm 0.10$ ($R^2 = 0.08$).

Mate choice

Repeatability

All but one of the mate choice parameters were repeatable for the 30 females that were tested twice. Repeatability was high and significant for choice active time ($R \pm SE = 0.55 \pm 0.25$, $\chi^2_1 = 7.9$, $P = 0.005$), within-trial consistency ($R = 0.63 \pm 0.26$, $\chi^2_1 = 10.8$, $P = 0.001$) and the

preference index ($R= 0.44 \pm 0.17$, $\chi^2_1= 9.5$, $P= 0.002$). However, the repeatability for choosiness was low ($R= 0.06 \pm 0.22$, $\chi^2_1= 0.1$, $P= 0.752$). The average within-trial consistency was 0.18 ± 0.03, indicating 82% similarity of female mate preference between the first and the second hour of the experiment.

<H3>Heritability of female mate choice

All mate choice behaviours of the daughters were unaffected by the respective mate choice parameters of their genetic and foster mother, and neither were there significant interaction effects (Table 2). All heritability estimates were comparatively low (choice active time: $H^2 = 0.29 \pm 0.42$ [$R^2 = 0.02$]; choosiness, $H^2 = 0.29 \pm 0.24$ [$R^2 = 0.05$]; within-trial consistency: $H^2 = 0.05 \pm 0.33$ [$R^2 = 0.001$]; preference index: $H^2 = 0.41 \pm 0.26$ [$R^2 = 0.09$]).

However, there was a significant effect of male familiarity on female preference ($F_{1,42}= 7.94$, $P= 0.007$). Daughters significantly preferred an unfamiliar male (mean $\pm$ SE $= 0.35 \pm 0.03$) over their foster father (0.22 ± 0.03; Fig. 4).

<H1>Discussion

<H2>Birdsong

We found that some but not all song parameters were significantly affected by the genetic father and the tutor, but none were affected by the foster father. Thus, canary song possesses both an innate and a learned component, but the critical period for song learning only occurs after fledging, which is in line with earlier reports for our study species (Weichel et al., 1986; Waser & Marler, 1977; but see Nowicki et al., 1998). Heritability estimates clearly varied between the different song traits ($H^2 = 0.14–1.31$), being particularly high for average bout duration and song bout repertoire, but relatively low for song activity and song consistency.

Below we discuss our findings for each song trait separately.

Average song bout duration was significantly and highly heritable ($H^2 = 0.81$), which is in line with a full-brother comparison in canaries (Müller et al., 2010), as well as with the outcome of a study in zebra finches, *Taeniopygia guttata* (Forstmeier et al., 2009). Interestingly, song bout duration is considered a performance-related trait (Gil & Gahr, 2002), which is thought to depend strongly on the bird's current condition. However, there is some evidence that song bout length correlates with the volume of brain song control nuclei (Bernard et al., 1996), which is known to be, at least partly, genetically determined (Gil & Gahr, 2002). Thus, bout duration is probably determined by heritable morphological, physiological and neurological aspects involved in song production. This may become more evident in captivity, when environmental factors are largely controlled for. Indeed, a recent study in zebra finches found remarkably low heritability for this trait when environmental variance had been experimentally increased by inducing nutritional stress (Woodgate et al., 2014).

However, we measured a second performance-related trait, song activity, which was not significantly heritable ($H^2 = 0.17$), but tended to be negatively influenced by the song activity of the tutor. The latter could potentially be explained by a dominance effect. Tutors with high song activity are likely to be more dominant and may suppress the singing behaviour of their tutees. Indeed, the mere presence of a dominant male was sufficient to suppress reproductive behaviours, including birdsong, in chaffinches, *Fringilla coelebs* (Hinde, 1959). Therefore, tutees with a more dominant tutor were maybe less well trained in terms of both muscular and neuronal activities involved in singing. In addition, more dominant males will have priority access to food (Ficken et al., 1990; Polo & Bautista, 2002). Therefore, tutees of dominant tutors might have been in poorer condition by the time of the recording, which reduced song

activity in zebra finches (Birkhead et al., 1998), probably because singing is thought to be energetically costly (Thomas, 1999; Gil & Gahr, 2002).

Average repertoire size per song bout (which has been shown to be strongly correlated with the total repertoire size: $r = 0.57$; De Boer et al., 2016) contained both a genetic and a learned component, which interacted in a nonadditive way (G×Es). The genetic component for song bout repertoire was particularly prominent ($H^2 = 1.31 \pm 0.44$ SE; effect size: 0.26), probably because acquiring a given repertoire size will depend on the males' cognitive capacities (e.g. on their learning performance; Boogert et al., 2008). These capacities will determine how well and how much of the input from social learning can be included in the repertoire, and these capacities are at least partly genetically determined (Airey et al., 2000; Garamszegi & Eens, 2004; but see also Leitner & Catchpole, 2004). But what is incorporated in the repertoire also depends on the postfledging acoustic environment (here, the tutor; Nottebohm et al., 1981; Kiefer et al., 2006; Nicholson et al., 2007; Vargas-castro et al., 2012). However, the interacting effects of intrinsic capacities and acoustic environment on the song bout repertoire of the sons were rather complex. Sons that had both a genetic father and a tutor with a similar (either large or small) song bout repertoire had a relatively small song bout repertoire, while sons having either a genetic father or a tutor with a large song bout repertoire developed comparatively large song bout repertoires.

To some extent, this result could be explained by large song bout repertoires being achieved only if the bird is able to practise enough. Song bout repertoire was significantly positively correlated with song activity ($r = 0.31$, $P < 0.01$), and we speculated above that tutors with high song activity potentially supressed the song behaviour of their tutees. Hence, the repertoire of these tutees could perhaps not develop optimally. However, sons that descended from genetic fathers with a small song bout repertoire still benefited from the exposure to a high-quality

template, maybe because they copied this high-quality song less accurately, which could have inflated the estimation of their song bout repertoire. Interestingly, the largest song bout repertoire was acquired by the sons of males with large song bout repertoires, suggesting that females choosing males based on their repertoire size indeed choose for genetic quality. This, however, remains to be tested.

The pattern of this gene-by-environment interaction on song bout repertoire suggests the existence of an additional cost to song learning. The juvenile males have to find not only a tutor but also one that is 'compatible' with their own genotype, or learn from a larger number of tutors. However, social learning enabled males to obtain larger repertoires. This may eventually lead to a higher reproductive success (Eens et al., 1991; Williams, 2004), consistent with the hypothesis that song learning may have evolved through sexual selection (Lachlan & Slater, 1999; Beecher & Brenowitz, 2005).

Finally, song consistency was neither significantly heritable ($H^2 = 0.14$) nor acquired posthatching via learning. Song consistency is considered as a dynamic trait that reflects the accuracy of the song control, which is probably linked to the current quality of the male (Botero et al., 2009; Vehrencamp et al., 2013). Unfortunately, we did not take any physiological measures that could explain variation in song consistency. However, as mentioned above, all birds were kept in standardized husbandry regimes, minimizing variability in male condition.

<H2>Mate choice

The repeatability estimates for the mate choice parameters we studied were relatively high ($R = 0.44–0.63$), apart from choosiness ($R = 0.06$; see also for comparable findings Vergauwen et al., 2014b: $R = 0.14$). Repeatability estimates are often considered as an upper limit to the

heritability of a trait (Bakker, 1999; but see also Dohm, 2002), but neither of the preference indexes nor the different choice-related parameters were significantly heritable in this study (H^2 = 0.05-0.41). The low heritability of these traits is generally in line with most previous studies in birds, e.g. in zebra finches (H^2= 0.10; Schielzeth et al., 2009) and in collared flycatchers, *Ficedula albicollis* (H^2= 0.04; Hegyi et al., 2010).

However, it must be considered that the heritability estimate for mate choice traits may be sensitive to the number of test males (Roff & Fairbairn, 2015). This is because the amount of phenotypic variance of a preferred male trait increases with the number of males a female is allowed to sample. Our choice trials included only two males, which may result in a 40–80% underestimation (Roff & Fairbairn, 2015). Applying such corrections to our data would result in H^2 estimates 0.07–0.74. This may partly explain the low heritability value we found.

We also did not find a significant effect of the foster mothers' mate choice behaviour on the daughters' choice, suggesting that daughters do not learn their mate choice from their mother at an early age. Similarly, Schielzeth et al. (2009) found no correlation between the mate preferences of foster sisters. However, in our study mothers were not allowed to choose their partner, so a father might not have been the preferred male of a given mother. Thus, daughters could not learn their mother's preference (i.e. mate choice copying; Swaddle et al., 2005; Kniel et al., 2015), but may have only obtained information on whether and to what extent their mother agreed on the quality of her partner. Moreover, if daughters imprinted on their foster father and showed a preference for similar males, it would have resulted in a mismatch with the foster mother's mate choice, seeing that the foster father did not necessarily correspond with the foster mother's preferences. Nevertheless, we found evidence for a learned component as daughters showed significant avoidance of their foster father in the mate choice tests, most probably to avoid inbreeding. This indicates that females use familiarity information acquired early in life as a cue for mate choice. Several other studies

have also found such learned kin recognition in birds, especially through imprinting on song or calls, but only a few of them have directly tested whether it was used as a means for inbreeding avoidance (Beecher, 1988; McGowan et al., 2005; Riehl & Stern, 2015). Unfortunately, we did not test for the existence of an innate kin recognition mechanism and further studies are thus necessary to answer this interesting question.

Thus, most variation in mate choice remains unfortunately unexplained. One potentially confounding factor could be the previous mating and breeding experience. If female mate choice involves a learning process to discriminate between traits that signal either indirect (genetic) and/or direct (e.g. parental care) benefits, it could be affected by breeding experience (but see Hegyi et al., 2010).

Possible implications for sexual selection theory

We found that several song traits in male canaries are strongly determined by genetic effects, which was not the case for all female mate choice parameters. The latter probably weakens the possibilities for coevolution, which is central particularly for intersexual selection models (e.g. Ryan & Kirkpatrick, 1991; Holland & Rice, 1998; Kokko et al., 2006). An interesting future research avenue would be to investigate the heritability of female preference for the specific male traits that we found to be heritable. This would ultimately enable us to estimate genetic correlations between female preference and male sexual traits, and hence test theoretical predictions in the context of e.g. Fisher's run-away, good-genes and direct-benefits models; Ryan & Kirkpatrick, 1991; Kokko et al., 2006; Qvarnström et al., 2006).

Our results further revealed that females discriminated between males in a repeatable and thus coherent way, but did not agree on whom they preferred, suggesting that compatibility within

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pairs may be most important. Indeed, it was shown that female mate choice will vary with female condition, implying that not all females necessarily prefer the same male (Holveck & Riebel, 2010). More information about female condition is thus needed to understand mate choice. Finally, we found that not only birdsong but also female mate choice involves an early learning process, stressing the importance of the social environment at an early age.

Taken together, our results suggest that the evolutionary trajectories of male secondary sexual traits and female mate choice may be more complex than previously assumed, given both the low and insignificant heritability of female mate choice traits and the importance of environmental effects. For the latter, it was especially the social environment that affected male sexual signalling as well as female choice, via social learning. Indirect genetic effects acting during the pre- and postfledging social contexts may therefore play a prominent role in coevolutionary processes.

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Tables

Table 1: Outcome of the linear mixed model explaining variation in four song parameters of the sons

Effect	*df*	*F*	*P*
Average bout duration			
Genetic father	43.0	4.51	**0.039**
Foster father	41.0	0.81	0.37
Tutor	40.0	0.68	0.42
Interaction Genetic*Foster	38.0	0.19	0.67
Interaction Genetic*Tutor	39.0	0.82	0.37
Song activity			
Genetic father	42.0	0.38	0.54
Foster father	38.7	0.1	0.75
Tutor	13.6	4.44	*0.054*
Interaction Genetic*Foster	38.0	0.01	0.92
Interaction Genetic*Tutor	39.0	0.95	0.33
Song bout repertoire			
Genetic father	36.2	12.13	**0.001**
Foster father	39.0	2.76	0.11
Tutor	36.0	6.34	**0.016**
Interaction Genetic*Foster	38.0	0.84	0.36
Interaction Genetic*Tutor	35.9	8.38	**0.006**

Song consistency

	df	F	p
Genetic father	34.0	0.31	0.58
Foster father	29.7	1.14	0.29
Tutor	32.0	0.38	0.55
Interaction Genetic*Foster	28.0	1.21	0.28
Interaction Genetic*Tutor	29.0	2.24	0.14

Numerator degree of freedom was 1 in all cases; *df* refers to the denominator degrees of freedom. Significant results are highlighted in bold, statistical trends in italic.

Table 2: Outcome of the linear mixed model approach explaining variation in four mate choice parameters of the F1 females

Effect	*df*	*F*	*P*
Choice active time			
Genetic mother	14.9	0.29	0.60
Foster mother	17.1	2.41	0.14
Interaction Genetic*Foster	14.1	0.18	0.68
Choosiness			
Genetic mother	33.0	0.62	0.44
Foster mother	17.0	0.2	0.66
Interaction Genetic*Foster	16.0	0.24	0.63
Within-trial consistency			
Genetic mother	6.7	0.07	0.80
Foster mother	12.4	0.06	0.82
Interaction Genetic*Foster	13.1	0.19	0.67
Preference index			
Genetic mother	34.7	0.5	0.49
Foster mother	20.0	0.01	0.94
Interaction Genetic*Foster	26.0	0.0	0.96

Numerator degree of freedom was 1 in all cases; *df* refers to the denominator degrees of freedom.

Figure legends

Figure 1: Set-up of the mate choice test. Black lines represent the perches, filled blue square and circle represent, respectively, the seeds and the water containers. Dashed lines represent trellised cage walls through which the birds could see each other.

Figure 2: Relationships between (a) the average song bout duration of sons and of their genetic father and (b) the song activity of sons and their tutor. Arithmetic means ($\pm$ SE) are given for two or more (a) genetically related sons (brothers) and (b) juveniles with the same tutor father. Grey lines represent the 95% confidence interval.

Figure 3: 3D surface plot representing the effects of the song bout repertoires (SBR) of the genetic father and tutor and the interaction effect between both on the SBR of the son/tutee.

Figure 4: Mean ($\pm$ SE) preference index (i.e. average percentage of time spent with a male) of the F1 females for their foster father and an unfamiliar male.