Zebra finch song parameters are affected by the breeding status of the male, but not temperature variability

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Abstract

Bird song is a crucial feature for mate choice and reproduction. Song can potentially communicate information related to the quality of the mate, through song complexity, structure or finer changes in syllable characteristics. It has been shown in zebra finches that those characteristics can be affected by various factors including motivation, hormone levels or extreme temperature. However, although the literature on zebra finch song is substantial, some factors have been neglected. In this paper, we recorded male zebra finches in two breeding contexts (before and after pairing) and in two ambient temperature conditions (stable and variable) to see how those factors could influence song production. We found strong differences between the two breeding contexts: compared to their song before pairing, males that were paired had lower song rate, syllable consistency, frequency and entropy, while surprisingly the amplitude of their syllables increased. Temperature variability did not affect the song parameters that we measured. Our results describe for the first time how the breeding status can affect zebra finch song, and give some new insights into the subtleties of the acoustic communication of this model species.

Keywords

Taeniopygia guttata, social context, temperature variability, syllable characteristics, acoustic communication

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Vocal communication has a major role in social interactions, be they inter- or intraspecific, agonistic or mutualistic, directed toward a short or long distance audience (Fichtel and Manser 2010). Birds rely on vocal communication in a variety of contexts including reproduction (Slabbekoorn and Smith 2002), territory defense (Logue 2005), resources acquisition (Clay et al. 2012) and predator avoidance (Gill and Bierema 2013; Hollén and Radford 2009; Cordonnier et al. 2023). One very well studied example is the courtship song that males produce to attract a female (Greene et al. 2022). Females can potentially use the singing performance to judge the quality of a potential mate (Nowicki et al. 2002). In order to be chosen, males exhibit high song proficiency and complexity, but they can also modulate finer song features at the syllable level (de Boer et al. 2016; London 2019).

Song parameters are also thought to indicate the male’s interest in the female (Riebel 2009). Male mating interest has usually been studied by comparing courtship and spontaneous songs. Indeed, courtship song is driven by reproductive motivation, while spontaneous song can occur even in social isolation (Sossinka and Böhner 1980). These two types of song rarely differ in their structure and syntax, but rather in their spectral characteristics such as song stereotypy (Ota and Gahr 2022), length and frequency (Gémard et al. 2021; Jablonszky et al. 2022), tempo and amplitude (Riebel 2009). The reproductive status of a male can also be communicated by its song parameters. For example, male canaries (*Serinus canaria*) have been shown to sing less when they are paired with a female than when they are single (Alward et al. 2014). In petrel species, breeding males produce calls with shorter syllables, higher frequency and narrower bandwidth than non-breeding males (Gémard et al. 2021). In blue tits (*Cyanistes caeruleus)*, males also adapt their song performance to the breeding stage of the females to increase their breeding chances (Sierro, de Kort, and Hartley 2023).

Zebra finches (*Taeniopygia castanotis*) are widely used in avian bioacoustics research because of their relatively simple and stereotyped song. Males develop a single song motif based on their tutor’s song, most often their social father, and repeat this motif profusely on a daily basis, and as part of their courtship display (reviewed in Riebel 2009; London 2019). For this reason, courtship (female-directed) and spontaneous (undirected) songs have been compared extensively in zebra finches (Sossinka and Böhner 1980; Caryl 1981; Walters et al. 1991; Woolley and Doupe 2008; Jesse and Riebel 2012; Hyland Bruno and Tchernichovski 2019). However, despite the light shed on the difference between directed and undirected song, differences between other social contexts have been overshadowed. For example, the zebra finch literature seems to lack comparisons between the directed song of single and paired males (but see Dunn and Zann 1996 for undirected song). To the best of our knowledge, there are no such studies for captive zebra finches. In the wild, recorded males are usually paired (Loning et al. 2023).

In Australia, where zebra finches originate from, there is a strong seasonality of temperature with hot summers and cold winters, but a high variability of precipitation throughout the year (see Griffith et al. 2021). It implies that, although zebra finches preferably breed in spring, they can also reproduce at any time of the year in very different temperature conditions (Griffith et al. 2008). It is reasonable to expect that those differences in temperature conditions can affect courtship song production and characteristics. It has been shown that in high temperatures, zebra finches spend less time singing and produce fewer song motifs (Coomes and Derryberry 2021). In addition to affecting the singing behavior, heat also impacts song characteristics such as syllable length and consistency (Coomes and Derryberry 2021), song tempo (Aronov and Fee 2012) or bandwidth (Schäfer et al. 2017).

Although climate change is associated with higher temperatures, it is also characterized by increasingly variable and unpredictable environmental conditions (Coumou and Rahmstorf 2012; Cohen et al. 2021). Temperature variability have been shown to have a long term effect on the song learning accuracy (Lefeuvre et al. 2023). Variable conditions also increase the propensity to sing during recordings and activity during behavioral tests (Lu et al. 2022; Lefeuvre et al. 2023). Although temperature variability does not seem to affect the complexity and consistency of zebra finch song (Lefeuvre et al. 2023), its effect on finer song features remains to be described. In addition, paired males mainly sing to their mate and communicate their breeding condition (Loning et al. 2023), but if temperature variability influences parental behavior and breeding decisions, song characteristics could be affected accordingly.

The aims of this paper are 1) to describe, for the first time in zebra finches, the difference between directed songs produced by males at two different breeding contexts (unpaired versus paired context). It has been shown that the song rate of wild zebra finches decreases after egg laying (Loning et al. 2023). We expect that zebra finches can also adapt their song characteristics to inform other birds about their breeding situation and mating availability. Higher competition for mates when males are unpaired should boost the song traits selected by females such as the song rate, amplitude or stereotypy (Riebel 2009). Once males are paired, we expect a decrease of their motivation to attract a female and to exacerbate their song characteristics accordingly. In addition, we aim at 2) investigating the potential effect of temperature variability on the vocal communication in different social contexts. Zebra finches can detect and interpret small changes in song characteristics (Dooling and Prior 2017), and environmental conditions such as temperature variability could affect singing performance and alter the clarity of the message. Temperature has been shown to affect animal social interactions, namely responses to alarm calls (Cordonnier et al. 2023). Yet, it is not known whether variable temperature treatment would also affect sexual vocal interactions. This study would give us a better understanding of the effects of ambient temperature on male-female communication in songbirds.

Material and methods

Birds and housing

This study was carried out at the Jagiellonian University in Cracow, Poland. Adult zebra finches (88 males and 88 females) were transferred from outdoor aviaries to two indoor climatic chambers for acclimation and breeding. Each chamber had 44 breeding cages (75 × 70 × 40 cm), all of them equipped with perches, *ad libitum* water and food (commercial mix by Megan, Poland), and cuttlebone. During the breeding period, the birds received a mixture of hard boiled egg and carrots with a vitamin supplement (Dolfos Pets, Poland) three times a week.

Experiment

The two chambers had the same day length (day/night: 13h/11h), humidity (60%) and night temperature (set at 17°C but 16°C measured), but different day temperature. The first chamber (stable) reached 20°C everyday, while the temperature in the second chamber (variable) was set randomly each day between 11°C and 30°C, the day temperature across the experiment period following a normal distribution with a mean of 20°C and a standard deviation of 5°C. The temperature regime chosen for this study intends to mimic biologically relevant climatic conditions for zebra finches. In the arid desert of Australia, the average year temperature is ca. 19°C (Griffith et al. 2021), but during the breeding season, temperature ranges from mean daily minimum 12.4 °C to mean daily maximum 26.4 °C (Griffith et al. 2017) and maximum temperatures can vary day-to-day in a range of approximately 20°C (see the supplementary materials for Mariette and Buchanan 2016). The measures of the actual temperature in our chambers can be found in Supplementary materials.

At the beginning of the experiment, all birds were between 12 and 15 months old and never mated. They were randomly assigned to each chamber, we only controlled their identity to avoid inbreeding in the cohort. They were kept in same-sex pairs in the breeding cages from 2 February 2020 to 23 April 2020. They were able to hear and see other birds in the chamber but could not physically interact with them. On 23 and 24 April, males and females were paired for the purpose of other studies (Lu et al. 2022; Lefeuvre et al. 2023). They were provided with a nest box and nest material. Nests, eggs and chicks were monitored daily until the offspring became independent.

Song recordings

Adult males were recorded twice: within the first month of acclimation, before breeding (thereafter: unpaired status) and during the critical period for song learning of their offspring (thereafter: paired status), which occurs between 25 and 65 days after hatching in zebra finches (Riebel 2009; London

2019). At this stage, offspring fledged and are nutritionally independent or soon-to-be (more self-feeding than parental feeding at ca. 27 days old, only self feeding at ca. 31 days old, see Rehling et al. 2012). We started recording trials at the beginning of their offspring critical learning period for each tutor and repeated it as many times as needed to record its song. A majority of the males were recorded during the first half of the critical song learning period of their chicks (see Supplementary materials for details about the range of recording days). Recordings were performed throughout the day, our cohort did not show variation in the propensity to sing according to the time of day (data not presented). The same equipment and setup was used for all the recordings of all the individuals to ensure comparable data.

We recorded males in a soundproof room, using a Telinga Pro 5W shotgun microphone and Raven Pro (The Cornell Lab, USA, version 1.6). The recording room was maintained at approximately 21-22°C, and males from the variable chamber were recorded when the day temperature in their chamber was between 20°C and 24°C. Males were recorded in an individual cage (70 x 30 x 40 cm) equipped with a single perch in the center, which ensured that all males were in the same position and orientation towards the microphone. The microphone was positioned in front of the male's cage, at 55 centimeters from the side of the perch that was closest to the female, where males stood to sing. An unrelated and unfamiliar female was placed beside in a similar cage, for recordings in both breeding status (see below). The two cages were separated by an opaque partition which was removed only during recording, enabling the birds to see and hear each other but not physically interact to avoid copulation. Recordings lasted 5 minutes, then the males were released in their home cage. If they did not sing, they were presented to another female on another day. Birds were recorded until they had produced 10 song motifs in each social context. Recordings of the paired males were used in another study investigating the song learning abilities of their offspring (Lefeuvre et al. 2023). It was not possible to record data blind because birds were kept in different chambers according to their temperature treatment. However, the song measurements and analysis were performed blindly.

The stimulus females were initially randomly caught in the same chamber as the focal male. They showed a wide variety of behavioral responses to the recording chambers, some females sat still in a corner of the cage, while other females actively interacted with the male. The first never elicited a song while the latter significantly increased the propensity to sing of the males during the recordings. For this reason, we identified 8 very active females and used them repeatedly but in a random order during both recording sessions (of unpaired and paired males) as stimuli. Paired males regularly sing to their female (Loning et al. 2023), but the extent to which this song differs from courtship song is not common knowledge. To obtain comparable songs for unpaired and paired males in our study, we decided to record them in similar conditions, i.e., with an unfamiliar female that would elicit courtship song.

Song analysis

The recordings were analyzed with Raven Pro 1.6. We reported the number of trials needed to record at least one motif, and we measured song rate (time spent singing over the total recording time) and song bout duration, i.e. the duration of each song bout. We defined a song bout as a sequence of one or more song motifs, separated from the next song bout by either introductory notes or by at least 0.5 seconds of silence.

We selected the first 10 clear renditions of all the different syllable types in the males' repertoire. For these selections, we measured their duration, peak frequency (frequency at which power reaches its maximum), average amplitude (calculated as the root-mean-square amplitude) and average entropy (measure of randomness; the messier the waveform, the higher the entropy, see Wang et al. 2014; Tchernichovski et al. 2000). Due to a high number of duplicated values of peak frequency, we reduced the frequency grid spacing from 86.1 Hz to 10.8 Hz in Raven Pro. This setting had no influence on the other measurements except the average entropy which shifted up but without impacting the distribution of the values. We calibrated the amplitude measurement in Raven Pro using a pure tone of 1kHz recorded in the identical setup as used to record the birds, at the same position and distance from the microphone. We converted the uncalibrated root-mean-square amplitude (measured in dimensionless unit in Raven Pro) to calibrated SPL (sound pressure level, in dB) at 55 cm, and then corrected the values to get the average amplitude at 1 meter. For the details of the calibration process and formulas, refer to the Supplementary materials.

We also measured the consistency of each syllable type using spectral cross-correlation matrices, the method used to construct these matrices can be found in Lefeuvre et al. 2023. Those matrices compare all the renditions of a given syllable type and compute a similarity score between 0 and 1, 0 being a complete absence of similarity and 1 being a perfect match. We used the mean of those similarity scores (45 scores without duplicates) for each syllable type as the syllable consistency measure.

Statistical analysis

All statistical analysis were performed in R (version 4.2.0, R Core Team, 2022). We performed random slope mixed-effect models (i.e., models including random intercepts and slopes, Schielzeth and Forstmeier 2009) to avoid pseudoreplication due to repeated measured on the same syllables and individuals. We used lmer models with a Gaussian error distribution to analyze all our dependent variables (lme4 package, Bates et al. 2014). All models included the breeding status and the temperature condition as well as their interaction. We added random effects according to the level at which the variable was measured. For measures at the individual or song bout level (i.e., number of trials, song rate and song bout duration), the bird ID was considered as a random effect. For measures at the level of the syllable type or the syllable rendition (i.e., syllable duration and consistency, peak frequency, average amplitude, and average entropy), there were two random effects: the male ID (64 levels) and the syllable ID coded within each male (320 levels). Both random effects were fitted as random slopes over the breeding status. Hence, for each male and for each syllable there was an intercept being estimated and a slope of parameter change from being unpaired to being paired. The residuals of the statistical models were visualized with graphs to validate the usage of the parametric tests. We performed an emmeans post-hoc test using a Kenward-Roger method for degrees of freedom (package emmeans, Lenth 2021) within each temperature condition, the values are presented in Fig. 1.

Results

The males which did not have offspring at the time of the second recording were excluded from this study (n = 24). Out of the 64 remaining males, 30 were in stable ambient condition and 34 were in variable ambient temperature condition.

The breeding status affected almost all the song parameters that we measured, except for the song bout duration (overall mean±SD: 2.030±1.595 seconds, n = 1750 song bouts, Table 1, Fig. 1c) and syllable duration (overall mean±SD: 0.142±0.067 second, n = 6400 syllables renditions, Table 1, Fig. 1d). Paired males generally needed more trials before singing than unpaired males. Song rate was significantly higher for unpaired than paired males regardless of the temperature condition (Fig. 1b). Syllable consistency (Fig. 1e), peak frequency (Fig. 1f) and average entropy (Fig. 1g) were clearly higher for unpaired than paired males (see Table 1). On the contrary, average amplitude was significantly higher for paired than unpaired males (Fig. 1h). The temperature condition had no significant effect on song production and characteristics at any stage (Table 1).

Table 1 Summary of the results. The effect of the breeding status, the temperature conditions and the interaction between these two factors are presented for each song parameter. The estimates (reference for breeding status: paired; reference for temperature condition: stable) and their standard error, F-statistics with degrees of freedom, and p-values are reported. The variables song bout duration, song rate, syllable duration, average amplitude and number of trials were log-transformed. The dataset for the number of trials and the song rate had 64 rows; the dataset for the syllable consistency had 640 rows; the dataset for the song bout duration had 1750 rows; the dataset for the other variables had 6400 rows. The models for the number of trials, song bout duration, song rate and syllable consistency included random effects of the male ID (64 levels). The models for the other variables included random effects of the male ID (64 levels) and the syllable type (320 levels) (data not shown). Both random effects were fitted as random slopes over the breeding status.

	Breeding status			**Temperature conditions**			**Breeding status × Temp. conditions**		
Song parameters	**Estimate±SE**	**F_{df}**	**p**	**Estimate±SE**	**F_{df}**	**p**	**Estimate±SE**	**F_{df}**	**p**
Number of trials	-0.867±0.173	$40.800_{1,62}$	**2.439e-08**	-0.291±0.196	$1.398_{1,62}$	0.242	0.215±0.238	$0.816_{1,62}$	0.370
Song bout duration	-0.057±0.050	$1.138_{1,45}$	0.292	0.009±0.094	$0.126_{1,60}$	0.724	0.040±0.070	$0.316_{1,45}$	0.577
Song rate	0.259±0.123	$12.366_{1,62}$	**0.0008**	-0.066±0.185	$0.029_{1,62}$	0.866	0.075±0.169	$0.200_{1,62}$	0.657
Syllable duration	-0.013 ±0.012	$0.472_{1,61}$	0.495	0.002±0.056	$0.041_{1,92}$	0.841	0.016±0.015	$1.220_{1,61}$	0.274
Syllable consistency	0.072±0.020	$13.724_{1,64}$	**0.0004**	0.035±0.023	$0.574_{1,60}$	0.452	-0.045±0.027	$2.818_{1,64}$	0.098
Peak frequency	385.57±65.14	$46.223_{1,53}$	**9.13e-09**	86.33±105.70	$0.001_{1,62}$	0.971	-165.43±89.09	$3.448_{1,53}$	0.069
Average entropy	0.192±0.025	$164.848_{1,58}$	**< 2e-16**	- 0.024±0.054	$0.0001_{1,63}$	0.993	0.049±0.034	$2.097_{1,58}$	0.153
Average amplitude	-2.298±0.489	$67.050_{1,61}$	**2.13e-11**	0.153±0.888	$0.102_{1,61}$	0.751	-0.882±0.669	$1.738_{1,61}$	0.192

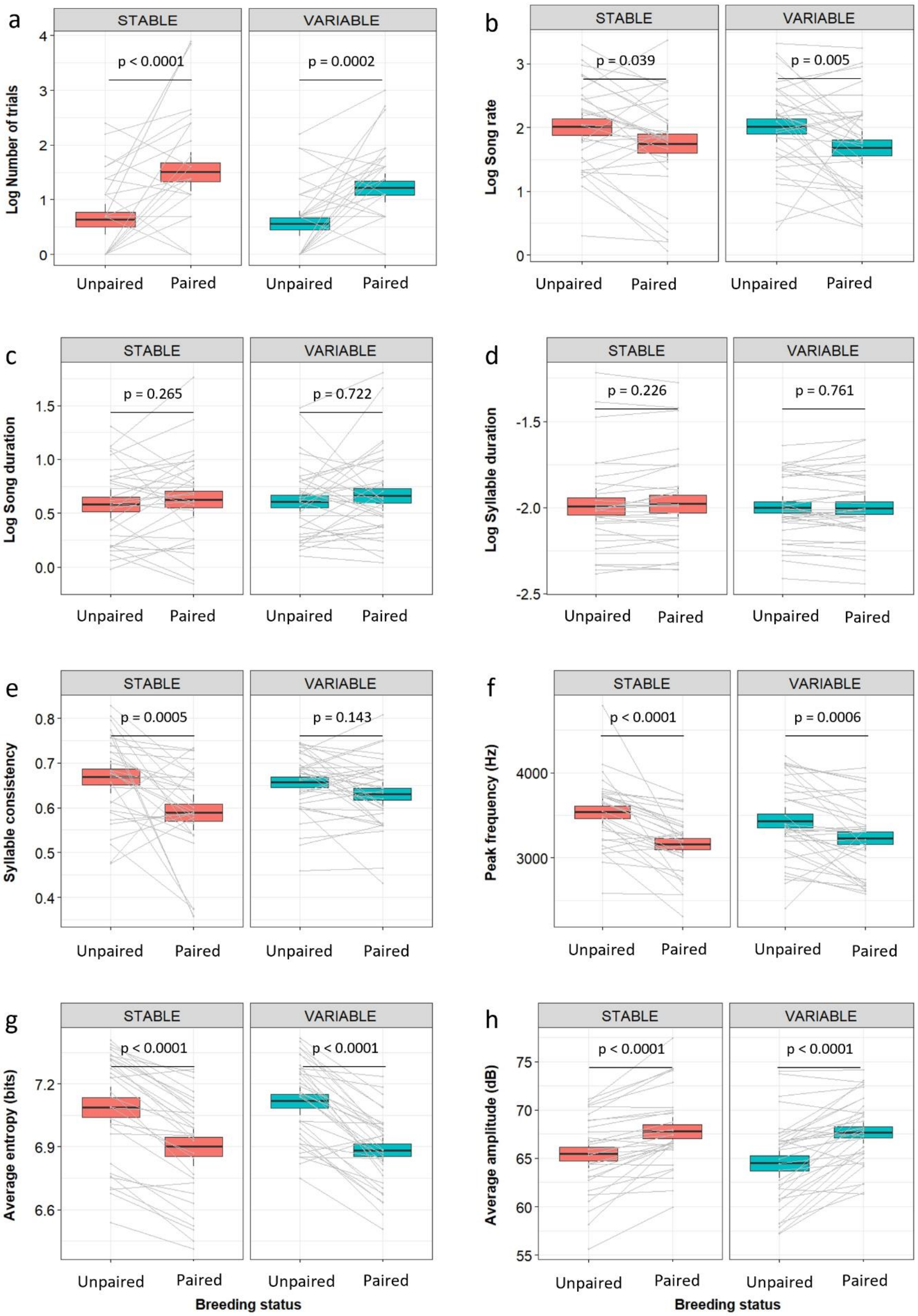

Fig.1 Plots of the effect of breeding status and temperature conditions on song parameters. The raw data are presented with the mean, standard error (box) and 95% confidence interval (whiskers).

Each grey line represents a male, the mean value for each individual is presented here for the song traits with more than one measure per individual. The p-values of the post-hoc tests are reported for each temperature condition. a: Number of trials before singing at least one motif (log-transformed variable). b: Song rate (log-transformed variable). c: Song bout duration (log-transformed variable). d: Syllable duration (log-transformed variable). e: Syllable consistency. f: Peak frequency. g: Average entropy. h: Average amplitude. Graphs were plotted using the ggplot2 package (Wickham 2016).

Discussion

Our study shows that zebra finch singing performance reliably changed from the initial unpaired to the later paired breeding status. Specifically, after a given male was paired and breeding, the following parameters of his song decreased: song rate, syllable consistency, peak frequency, and average entropy. Breeding males needed more trials before singing during recordings and sang with higher average amplitude than before breeding. On the other hand, their song bout and syllable duration were not affected. Throughout the study, each male was maintained in either stable or variable ambient temperature conditions. These conditions, per se or in interaction with the breeding status, did not affect song production and syllable characteristics. We first discuss our results in the light of differences in males practice and motivation, hormone levels and breeding costs. We then consider the lack of temperature effect in the light of the zebra finches biology and their natural conditions.

Despite its syntax stereotypy, spectral and temporal aspects of the zebra finch song can vary with present conditions (Ritschard and Brumm 2012), as well as practice. In our study, one could wonder whether differences in singing performance observed in the two social contexts were due to the fact that recording in the paired context were done after additional 4 month of singing practice. To disentangle the effect of social context and longer practice time, one would have to record some males in the reversed order, first in the paired and then in the unpaired context. Despite their breeding failure, males without chicks could also have been recorded at the same time as successful males. Unfortunately, that was not possible for technical reasons. Nevertheless, older finches generally sing more stable song sequences at a higher speed than younger birds, with no consistent change in frequency, entropy or pitch (Bengalese finches, *Lonchura striata domestica*, James and Sakata 2015; zebra finches, Pytte et al. 2007). Our results show very different trends, namely a decrease of the syllable consistency, variation of the syllable duration according to the temperature treatment, no change in song bout or syllable duration, and strong shifts of spectral parameters in the second recording compared to the first one. Thus, the effects that we present are unlikely to be caused by practice during the four months separating the two recordings.

Zebra finches are known to form strong and lifelong pair bonds with their mate, unless the mate disappears (Zann 1996). Disappearance in the wild usually means the death of the partner, and in captivity it has been shown that visual and auditory isolation from their mate causes males to immediately re-bond with an available female. In our study, paired males produced courtship song to the unfamiliar female encountered during the recordings, however, the higher number of trials that they needed to sing shows a reduced eagerness to mate compared to unpaired males. Because we used a small number of stimulus females and some males encountered the same ones a few times, it also corroborates the results of another study showing that males sing less to familiar than new females, including females that are introduced to the male several times (Caryl 1976). In addition, song rate is commonly used as a proxy for motivation in zebra finches (Riebel 2009; Hauber et al. 2010), and was higher for unpaired males in our study. As expected, zebra finches that already have a partner (with which they conducted a successful breeding) are less motivated to mate than single males. Paired males could also decrease their song rate due to stress related to separation from the mate (Remage-Healey et al. 2003), although no link between stress hormones and song rate has been drawn yet in zebra finches.

Some studies have also proposed amplitude as a measure of motivation (Brumm et al. 2009; Cardoso and Atwell 2011), especially due to females preference for louder songs (Riebel 2009; Ritschard et al. 2010; Ritschard and Brumm 2011). Surprisingly, however, the amplitude increased significantly after pairing in our study. The separation from the family could be perceived as a breeding failure and/or a divorce and could encourage males to rebond as quickly as possible because resources for breeding are time-limited, but our motivation variables say otherwise. Additional source of stress could have been the novelty of the recording setup for unpaired males, and this stress could have decreased during the second recording session as males already knew the sound-proof room. This familiarity could have encouraged the males to sing louder, but it did not help them to sing more and after less trials. Another possibility, although completely hypothetical, could be related to the Lombard effect which stipulates that individuals in a loud environment involuntarily adapt their vocal performance, by increasing the amplitude or frequency of their voice (Zollinger and Brumm 2011). When nestlings grew, our experimental chambers were more crowded and loud, which could have forced males to sing at a higher amplitude to be heard (Cynx et al. 1998). However, the Lombard effect is not a lasting adaptation of the vocal production, and it is unlikely that the loudness of the songs that males produced in their home chamber persisted in the quiet recording room.

The singing performance could also be affected by different factors related to breeding, such as hormone levels or metabolic cost. Testosterone levels in male zebra finches increase during mating, decrease during egg laying and remain relatively low during chicks rearing (Prior et al. 2017; Lilie et al. 2022). Many studies, on zebra finches, as well as on other oscine bird species, show that testosterone levels positively influence the song rate (Riebel 2009; Alward et al. 2013). It is more debated for other song parameters. The song stereotypy is thought to increase with higher testosterone levels, but conflicting results have been published (see Wang et al. 2014). The amplitude is not affected by testosterone levels in Bengalese finches (Ritschard et al. 2011), but seems to decrease when testosterone is injected in some particular areas in the brain of canaries (Alward et al. 2017). Testosterone levels also decreased the syllable entropy (i.e., increased syllable stability, Wang et al. 2014) and fundamental frequency (Cynx et al. 2005; Riebel 2009), at least in zebra finches, since the frequency of Bengalese finches song was not affected by testosterone levels (Ritschard et al. 2011). Since we did not measure testosterone levels in our birds, we can only assume that male zebra finches in our study had higher testosterone levels before pairing than during breeding. This could explain that before breeding, the song rate (Fig. 1b) and syllable consistency (Fig. 1e) were higher while the amplitude was lower (Fig. 1h) than during nestlings phase. However, in our study the syllable frequency (Fig. 1f) and entropy (Fig. 1g) decreased during breeding, contrary to what we could have expected based on previous work.

In zebra finches, both males and females are involved in parental care at considerable cost (Vleck 1981; Zann 1996). Some studies showed that body mass (Krause et al. 2017) and immunocompetence (Deerenberg et al. 1997) decrease during parental care, while metabolic rate increases (Vleck 1981). In some other bird species, breeding costs have been suggested to affect vocal production, with breeding males producing shorter calls and songs than non-breeding males during territorial defense (Sung and Handford 2019; Gémard et al. 2021). Other factors that impose metabolic stress, such as fasting, have been shown to reduce courtship behavior in zebra finches, including song production (Lynn et al. 2010). High investment in breeding could also reduce the time males can allocate to singing, and may decrease the practice they need to maintain a high performance song. One week without song practice resulted in shorter motifs, lower syllable amplitude and variation in the fundamental frequency and Weiner entropy of the syllables (Adam et al. 2023). Even if offspring were soon-to-be nutritionally independent when we recorded the males, parents conducted a complete breeding attempt and carried the cost of it. Taken together, the breeding costs experienced by the birds in our study may be another explanation for the lower song rate and peak frequency of paired males, although additional measures would be required to verify this suggestion.

In the wild, many species are susceptible to encounter variable and unpredictable conditions at different stages of their life. At an evolutionary timescale, mockingbird species living in areas with a more unpredictable weather display a larger repertoire size, sing at a higher rate and with more consistent syllables than species living in more stable environments (Botero et al. 2009). At a smaller timescale, temperature variability has a less pronounced effect. During development, variable temperatures slightly enhanced the syllable learning accuracy of young zebra finches, and increased their propensity to sing during recordings (Lefeuvre et al. 2023). When the temperature is manipulated in lab studies, it is usually given a fixed value and is often accompanied by sudden disturbance (Johnson and Rashotte 2002; Coomes and Derryberry 2021). However, zebra finches in captivity are kept either in constant indoor or in variable outdoor conditions, which can imply important thermal fluctuations. Differences in song development and production between stable or variable thermal environments could potentially compromise the comparisons between indoor and outdoor studies. Nonetheless, the present study showed no significant difference between songs produced by males housed in either stable or variable temperatures, suggesting that these comparisons should not be an issue.

Zebra finches in the wild experience highly variable thermal conditions with extreme maximums during summer heatwaves (Griffith et al. 2021) and can physiologically handle temperatures reaching 40°C without perceptible changes in their weight or blood parameters (Cooper et al. 2020). Above this threshold, zebra finches sing more stereotyped, but less consistent songs with syllables shorter than under cooler temperatures (Coomes and Derryberry 2021). The thermal regime used in our study did not reach the above-mentioned extremes, but resembled the conditions experienced by wild zebra finches during their breeding season (Griffith et al. 2017; Griffith et al. 2021). In our study, the temperature was limited to 30°C and probably did not induce a significant physiological stress that could have impacted song production. In addition, both treatment groups experienced the same temperature during the recordings, and the same mean ambient temperature in the chambers throughout our experiment. That could be the reason why we detected no effect of the temperature variability on song production and syllable characteristics.

Our study is the first to describe how breeding status can affect directed song in male zebra finches. Although we could not identify the main cause of the differences in several song parameters, we explored some possible explanations for our results. Our study sheds some light on how cognitive performance and communication can be shaped by current conditions of the male. This study, although results were not significant, offers an experimental insight into the potential effects of temperature variability on vocal communication in songbirds (see also Lu et al. 2022; Lefeuvre et al. 2023).

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