Zebra finch song parameters are affected by the breeding status of the male and the ambient temperature conditions

Maëlle Lefeuvre[1,2], Joanna Rutkowska[2]

[1] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Cracow, Poland

[2] Jagiellonian University, Faculty of Biology, Institute of Environmental Sciences, Cracow, Poland

Corresponding author:

Maëlle Lefeuvre

maelle.lefeuvre@doctoral.uj.edu.pl

Orcid: 0000-0003-4291-134X

Acknowledgment

We warmly thank Marzena Majgier for her time and her help with the birds, Mariusz Cichoń, and other group members for their support and insightful comments. We also thank Diego Gil who helped improving the manuscript.

Funding

The study was supported by the National Science Centre, Poland within the project 2018/31/B/NZ8/02388 awarded to J.R. and also supported by the Jagiellonian University statutory funds N18/DBS/000003.

Compliance with ethical standards

The study was authorized by the 2nd Local Institutional Animal Care and Use Committee (IACUC) in Cracow, Poland; permit no. 155/2019. Birds were housed and bred following the European Union Law for experimentation with animals. Birds had constant access to *ad libitum* food and water and they were monitored daily.

Conflict of interest

Authors declare no conflict of interest.

Authors' contributions

Maëlle Lefeuvre: methodology, investigation, formal analysis, writing original draft, writing-review and editing, visualization. Joanna Rutkowska: conceptualization, methodology, investigation, resources, writing-review & editing, supervision, funding acquisition.

Data availability

The data used in this study is available using this DOI: https://doi.org/10.57903/UJ/KF4AH3

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 use the singing performance to judge the quality of a potential mate (Nowicki et al. 2002). The male's quality can be indicated by its song proficiency, complexity and structure, but also by finer 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, 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). 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 guttata*) 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). Although directed and undirected songs share the same syllables and syntax, they differ in their motif duration and the number of repetitions, their tempo, amplitude and spectral variability (reviewed in Riebel 2009). 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).

Seasonal birds depend on temperature and photoperiod to start breeding, while zebra finches rely more on rainfall than seasons. In Australia, where this species originates 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).

When the temperature is manipulated in the laboratory, it is usually given a fixed value and is often accompanied by sudden disturbance (Johnson and Rashotte 2002; Coomes and Derryberry 2021). However, climate change is also characterized by increasingly variable and unpredictable environmental conditions (Coumou and Rahmstorf 2012; Cohen et al. 2021). For zebra finches in the wild, climate change can affect directly and indirectly the temperature conditions in which they breed. It directly affects the temperature variability, potentially increasing thermal stress. Moreover, by directly affecting the unpredictability of the precipitations, it indirectly affects the temporality of the breeding season and thus the ambient temperature in which males breed and sing. Temperature variability can have a long term effect on the song learning accuracy (Lefeuvre et al. 2023). Variable conditions has also been shown to increase the propensity to sing during recordings and activity during behavioral tests (Lu et al. 2022; Lefeuvre et al. 2023). Although temperature variability did not affect the complexity and consistency of zebra finch song (Lefeuvre et al. 2023), its effect on finer song features remains to be described.

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). During this period, tutors actively adapt their song to improve its memorization by their tutees (Chen et al. 2016; Carouso-Peck et al. 2020). 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. 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. 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.

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 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 used lmer models to analyze all our dependent variables except the number of trials that was analyzed with a glmer model with a poisson family (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), we used the syllable type nested within the bird ID as a random effect. The residuals of the statistical models were visualized with graphs to validate the usage of the parametric tests. When the interaction between the breeding status and the temperature condition was significant, we performed an emmeans post-hoc test using a Kenward-Roger method for degrees of freedom (package emmeans, Lenth 2021) within each temperature condition. In addition, we performed likelihood ratio tests for each of our models to determine the relevance of keeping the interaction term. For the peak frequency, average amplitude and average entropy, the p-value was highly significant, so the interaction could not be removed from the model. To be consistent in our analysis, we decided to keep the breeding status, the temperature condition and their interaction in all the models.

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, by itself and in interaction with the temperature condition, affected almost all the song parameters that we measured, except for the song bout duration (overall mean: 2.030±1.595 seconds, Table 1, Fig. 1c). Paired males generally needed more trials before singing than unpaired males. The significant effects of the temperature condition and the interaction term are driven by two paired individuals in stable condition who needed more than 40 trials to sing at least one motif (see Fig. 1a). When removing those males, only the breeding status affected the number of trials. 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).

Syllable duration (overall mean: 0.142±0.067 seconds) was only shaped by the interaction between breeding status and temperature condition (Fig. 1d). It stems from the fact that in variable conditions, average syllable duration was longer for unpaired males (results of post-hoc tests controlling for the random effects: $t_{6078} = -2.185$, $p = 0.0289$), while in the stable condition, it was longer for paired males ($t_{6078} = 2.683$, $p = 0.0073$).

Syllable consistency and peak frequency decreased after pairing to a larger extent in stable than in variable temperature conditions (results of the post-hoc tests; syllable consistency: Stable: $t_{318} = -5.839$, $p < 0.0001$; Variable: $t_{318} = -2.352$, $p = 0.0193$; peak frequency: Stable: $t_{6078} = -13.401$, $p < 0.0001$; Variable: $t_{6078} = -8.663$, $p < 0.0001$). However, average entropy decreased after pairing more clearly in variable than in stable temperature condition (Stable: $t_{6078} = -22.516$, $p < 0.0001$, Variable: $t_{6078} = -30.537$, $p < 0.0001$). Similarly, the interaction between breeding status and temperature condition had a strong effect on average amplitude, with a greater increase after pairing in variable compared to stable temperature conditions (Stable: $t_{6078} = 20.536$, $p < 0.0001$, Variable: $t_{6078} = 34.572$, $p < 0.0001$).

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 song bout duration, song rate, syllable duration and average amplitude variables were log-transformed. The models for the number of trials, song bout duration, song rate and syllable duration included random effects of the male ID. The models for the other variables included random effects of the syllable type nested in the male ID (data not shown)

	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**
Song bout duration	-0.034±0.041	$0.294_{1,1721}$	0.588	0.003±0.085	$0.076_{1,60}$	0.784	0.037±0.058	$0.401_{1,1721}$	0.527
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	-0.008±0.003	$0.211_{1,6078}$	0.646	-0.001±0.046	$0.016_{1,312}$	0.899	0.014±0.004	$11.913_{1,6078}$	**0.0006**
Syllable consistency	0.069±0.012	$34.412_{1,319}$	**1.116e-08**	0.031±0.014	$0.689_{1,315}$	0.407	-0.043±0.016	$7.001_{1,319}$	**0.009**
Peak frequency	382.14±27.22	$270.718_{1,6078}$	**< 2.2e-16**	53.66±84.30	$0.067_{1,312}$	0.796	-149.93±37.34	$16.122_{1,6078}$	**6.01e-05**
Average entropy	0.193±0.009	$1392.886_{1,6078}$	**< 2.2e-16**	-0.018±0.042	$0.045_{1,314}$	0.832	0.053±0.012	$20.425_{1,6078}$	**6.32e-06**
Average amplitude	-2.023±0.098	$1492.857_{1,6078}$	**< 2e-16**	0.0042±0.651	$0.813_{1,311}$	0.368	-1.176±0.135	$75.717_{1,6078}$	**< 2e-16**
Song parameters	**Estimate±SE**	**χ^2**	**p**	**Estimate±SE**	**χ^2**	**p**	**Estimate±SE**	**χ^2**	**p**
Number of trials	-1.090±0.129	71.279	**< 2e-16**	-0.399±0.199	4.048	0.044	0.384±0.189	4.136	0.042

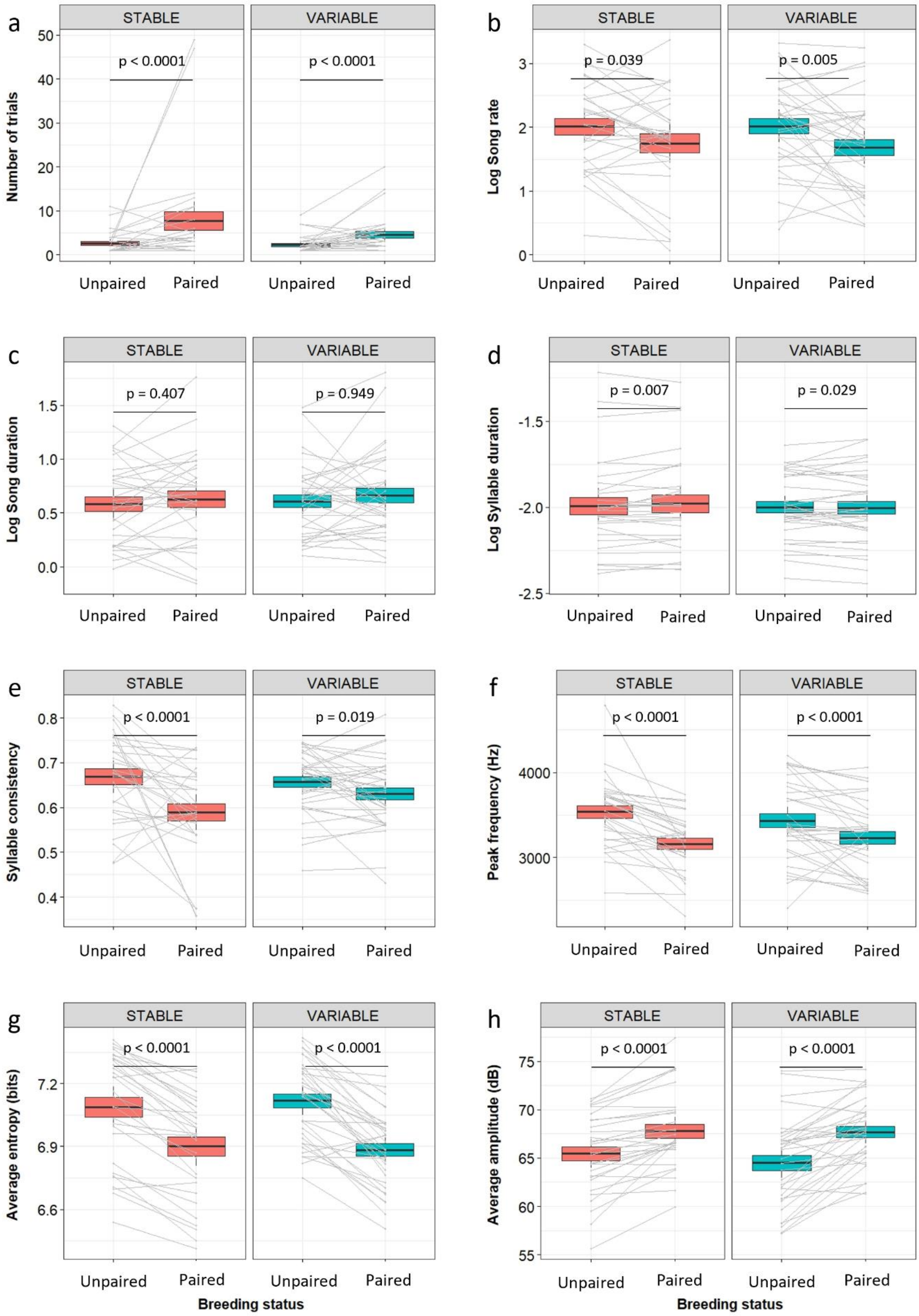

Fig.1 Plots of the effect of breeding status and temperature conditions on song parameters. The raw data are presented with the mean, standard error and 95% confidence interval. Each grey line

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. 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 the breeding status of male zebra finches affects their singing performance. 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. Their song bout duration was also affected, but this effect was modulated by environmental conditions. Throughout the study, each male was maintained in either stable or variable ambient temperature conditions. Temperature variability increased the differences in syllable amplitude and entropy between the songs produced by a given male before and after breeding, while it reduced the differences in other parameters. We first discuss our results in the light of differences in males practice and motivation, hormone levels and breeding costs. We then consider why and how variable temperature conditions could have affected song physical parameters.

Despite its syntax stereotypy, spectral and temporal aspects of the zebra finch song can vary with past (Spencer et al. 2003) and 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. 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 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. 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.

Thermoregulation in endotherms requires a lot of energy (reviewed in Rezende and Bacigalupe 2015; Yahav 2015). In cold temperatures, individuals are more likely to prioritize foraging in order to absorb the energy they need to maintain their body temperature, and in this way they allocate less time to secondary behavioral activities such as singing (Thomas 1999; Schäfer et al. 2017). Hot temperatures can also impose a physiological pressure on individuals which reduces their song production. Above their thermal threshold, zebra finches sing more stereotyped but less consistent songs with shorter syllables than under cooler temperatures (Coomes and Derryberry 2021). However, in our study, birds did not experience extreme thermal conditions. Instead, 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 the effects that we detected as interaction in our study are much more subtle than the effects of the breeding status.

In the wild, many species are susceptible to encounter variable and unpredictable conditions at different stages of their life, and those conditions can influence song production in birds. Unpredictable food resources during development generally impair song performance, leading to lower song rate, shorter and less complex songs (Buchanan et al. 2003; Spencer et al. 2004). On the other hand, food supplementation, even irregular, encourages birds to sing more (Thomas 1999). Temperature variability seems to have a different impact than food resources, since only positive effects have been reported. 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 yet still positive effect. During development, variable temperatures enhanced the syllable learning accuracy of young zebra finches, and increased their propensity to sing during recordings (Lefeuvre et al. 2023).

In our study, the temperature variability only influenced the strength of the changes induced by the breeding status. The syllable consistency and the peak frequency decreased less in variable than stable temperature, which would suggest a better conservation of syllable characteristics. In addition, the syllable entropy decreased more in variable than in stable condition, which means that the waveform became clearer. Although references about syllable entropy are very limited, one could expect that a less random sound would be of higher quality. Finally, the average amplitude increased more in variable than stable condition, improving a trait actively selected by females. Overall, one could suggest that variable temperatures slightly buffered the negative impact of breeding on song characteristics while enhancing the positive changes. However, those assumptions cannot be supported by a too scarce literature on the influence of environmental unpredictability on song performance.

Our study is the first to describe how breeding status and temperature variability 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, taking into account the natural environment of zebra finches (Griffith et al. 2017; Griffith et al. 2021). This study offers a unique experimental insight into the potential effects of temperature variability on vocal communication in songbirds (see also Lu et al. 2022; Lefeuvre et al. 2023).

References

Adam I, Riebel K, Stål P, Wood NB, Previs MJ, Elemans CPH (2023) Peak Performance Singing Requires Daily Vocal Exercise in Songbirds. bioRxiv. https://doi.org/10.1101/2023.02.23.529633

Alward BA, Balthazart J, Ball GF (2013) Differential Effects of Global versus Local Testosterone on Singing Behavior and Its Underlying Neural Substrate. Proceedings of the National Academy of Sciences 110 (48): 19573–78. https://doi.org/10.1073/pnas.1311371110

Alward BA, Mayes WD, Peng K, Stevenson TJ, Balthazart J, Ball GF (2014) Dissociable Effects of Social Context on Song and Doublecortin Immunoreactivity in Male Canaries. European Journal of Neuroscience 40 (6): 2941–47. https://doi.org/10.1111/ejn.12658

Alward BA, Rouse ML, Balthazart J, Ball GF (2017) Testosterone Regulates Birdsong in an Anatomically Specific Manner. Animal Behaviour 124 (February): 291–98. https://doi.org/10.1016/j.anbehav.2016.09.013

Aronov D, Fee MS (2012) Natural Changes in Brain Temperature Underlie Variations in Song Tempo during a Mating Behavior. PLOS ONE 7 (10): e47856. https://doi.org/10.1371/journal.pone.0047856

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting Linear Mixed-Effects Models Using Lme4. arXiv:1406.5823 [Stat], June. http://arxiv.org/abs/1406.5823

Boer RA de, Eens M, and Müller W (2016). “Out of Tune”: Consequences of Inbreeding on Bird Song. Proceedings of the Royal Society B: Biological Sciences 283 (1835): 20161142. https://doi.org/10.1098/rspb.2016.1142

Botero CA, Boogert NJ, Vehrencamp SL, Lovette IJ (2009) Climatic Patterns Predict the Elaboration of Song Displays in Mockingbirds. Current Biology 19 (13): 1151–55. https://doi.org/10.1016/j.cub.2009.04.061

Brumm H, Zollinger SA, Slater PJB (2009) Developmental Stress Affects Song Learning but Not Song Complexity and Vocal Amplitude in Zebra Finches. Behavioral Ecology and Sociobiology 63 (9): 1387–95. https://doi.org/10.1007/s00265-009-0749-y

Buchanan KL, Spencer KA, Goldsmith AR, Catchpole CK (2003) Song as an Honest Signal of Past Developmental Stress in the European Starling (Sturnus Vulgaris). Proceedings of the Royal Society of London. Series B: Biological Sciences 270 (1520): 1149–56. https://doi.org/10.1098/rspb.2003.2330

Cardoso GC, Atwell JW (2011) On the Relation between Loudness and the Increased Song Frequency of Urban Birds. Animal Behaviour 82 (4): 831–36. https://doi.org/10.1016/j.anbehav.2011.07.018

Carouso-Peck S, Menyhart O, DeVoogd TJ, Goldstein MH (2020) Contingent Parental Responses Are Naturally Associated with Zebra Finch Song Learning. Animal Behaviour 165: 123–32. https://doi.org/10.1016/j.anbehav.2020.04.019

Caryl PG (1976) Sexual Behaviour in the Zebra Finch Taeniopygia Guttata: Response to Familiar and Novel Partners. Animal Behaviour 24 (1): 93–107. https://doi.org/10.1016/S0003-3472(76)80103-0

Caryl PG (1981) The Relationship between the Motivation of Directed and Undirected Song in the Zebra Finch. Zeitschrift Für Tierpsychologie 57 (1): 37–50. https://doi.org/10.1111/j.1439-0310.1981.tb01311.x

Chen Y, Matheson LE, Sakata JT (2016) Mechanisms Underlying the Social Enhancement of Vocal Learning in Songbirds. Proceedings of the National Academy of Sciences 113 (24): 6641–46. https://doi.org/10.1073/pnas.1522306113

Clay Z, Smith CL, Blumstein DT (2012) Food-Associated Vocalizations in Mammals and Birds: What Do These Calls Really Mean? Animal Behaviour 83 (2): 323–30. https://doi.org/10.1016/j.anbehav.2011.12.008

Cohen J, Agel L, Barlow M, Garfinkel CI, White I (2021) Linking Arctic Variability and Change with Extreme Winter Weather in the United States. Science, September 373 (6559): 1116-1121. https://doi.org/10.1126/science.abi9167

Coomes CM, Derryberry EP (2021) High Temperatures Reduce Song Production and Alter Signal Salience in Songbirds. Animal Behaviour 180 (October): 13–22. https://doi.org/10.1016/j.anbehav.2021.07.020

Cordonnier M, Ridley AR, Lengagne T, Dutour M (2023) The Impact of High Temperatures on Bird Responses to Alarm Calls. Behavioral Ecology and Sociobiology 77 (7): 82. https://doi.org/10.1007/s00265-023-03354-2

Coumou D, Rahmstorf S (2012) A Decade of Weather Extremes. Nature Climate Change 2 (7): 491–96. https://doi.org/10.1038/nclimate1452

Cynx J, Bean NJ, Rossman I (2005) Testosterone Implants Alter the Frequency Range of Zebra Finch Songs. Hormones and Behavior 47 (4): 446–51. https://doi.org/10.1016/j.yhbeh.2004.11.018

Cynx J, Lewis R, Tavel B, Tse H (1998) Amplitude Regulation of Vocalizations in Noise by a Songbird,Taeniopygia Guttata. Animal Behaviour 56 (1): 107–13. https://doi.org/10.1006/anbe.1998.0746

Deerenberg C, Arpanius V, Daan S, Bos N (1997) Reproductive Effort Decreases Antibody Responsiveness. Proceedings of the Royal Society of London. Series B: Biological Sciences 264 (1384): 1021–29. https://doi.org/10.1098/rspb.1997.0141

Dooling RJ, Prior NH (2017) Do We Hear What Birds Hear in Birdsong? Animal Behaviour 124 (February): 283–89. https://doi.org/10.1016/j.anbehav.2016.10.012

Dunn AM, Zann RA (1996) Undirected Song in Wild Zebra Finch Flocks: Contexts and Effects of Mate Removal. Ethology 102 (4): 529–39. https://doi.org/10.1111/j.1439-0310.1996.tb01145.x

Fichtel C, Manser M (2010) Vocal Communication in Social Groups. In: Kappeler P (ed) Animal Behaviour: Evolution and Mechanisms, Berlin, Heidelberg: Springer, pp 29–54. https://doi.org/10.1007/978-3-642-02624-9_2

Gémard C, Planas-Bielsa V, Bonadonna F, Aubin T (2021) Contextual Variations in Calls of Two Nonoscine Birds: The Blue Petrel Halobaena Caerulea and the Antarctic Prion Pachyptila Desolata. Behavioral Ecology 32 (4): 769–79. https://doi.org/10.1093/beheco/arab020

Gill SA, Bierema AM-K (2013) On the Meaning of Alarm Calls: A Review of Functional Reference in Avian Alarm Calling. Ethology 119 (6): 449–61. https://doi.org/10.1111/eth.12097

Greene ES, Deviche P, Dridi S (2022) Chapter 40 - Reproductive Behavior. In Scanes CG and Dridi S (ed) Sturkie's Avian Physiology (Seventh Edition), San Diego: Academic Press, pp 1111–35. https://doi.org/10.1016/B978-0-12-819770-7.00052-9

Griffith SC, Crino OL, Andrew SC (2017) Commentary: A Bird in the House: The Challenge of Being Ecologically Relevant in Captivity. Frontiers in Ecology and Evolution 5:21. https://doi.org/10.3389/fevo.2017.00021

Griffith SC, Pryke SR, Mariette M (2008) Use of Nest-Boxes by the Zebra Finch (Taeniopygia Guttata): Implications for Reproductive Success and Research. Emu - Austral Ornithology 108 (4): 311–19. https://doi.org/10.1071/MU08033

Griffith SC, Ton R, Hurley LL, McDiarmid CS, Pacheco-Fuentes H (2021) The Ecology of the Zebra Finch Makes It a Great Laboratory Model but an Outlier amongst Passerine Birds. Birds 2 (1): 60–76. https://doi.org/10.3390/birds2010004

Hauber ME, Campbell DLM, Woolley SMN (2010) The Functional Role and Female Perception of Male Song in Zebra Finches. Emu - Austral Ornithology 110 (3): 209–18. https://doi.org/10.1071/MU10003

Hollén LI, Radford AN (2009) The Development of Alarm Call Behaviour in Mammals and Birds. Animal Behaviour 78 (4): 791–800. https://doi.org/10.1016/j.anbehav.2009.07.021

Hyland Bruno J, Tchernichovski O (2019) Regularities in Zebra Finch Song beyond the Repeated Motif. Behavioural Processes, Novel perspectives on avian vocal learning, 163 (June): 53–59. https://doi.org/10.1016/j.beproc.2017.11.001

Jablonszky M, Canal D, Hegyi G, Krenhardt K, Laczi M, Markó G, Nagy G, et al. (2022) Individual Differences in Song Plasticity in Response to Social Stimuli and Singing Position. Ecology and Evolution 12 (5): e8883. https://doi.org/10.1002/ece3.8883

James LS, Sakata JT (2015) Predicting Plasticity: Acute Context-Dependent Changes to Vocal Performance Predict Long-Term Age-Dependent Changes. Journal of Neurophysiology 114 (4): 2328–39. https://doi.org/10.1152/jn.00688.2015

Jesse F, Riebel K (2012) Social Facilitation of Male Song by Male and Female Conspecifics in the Zebra Finch, Taeniopygia Guttata. Behavioural Processes 91 (3): 262–66. https://doi.org/10.1016/j.beproc.2012.09.006

Johnson F, and Rashotte ME (2002) Food Availability but Not Cold Ambient Temperature Affects Undirected Singing in Adult Male Zebra Finches. Physiology & Behavior 76 (1): 9–20. https://doi.org/10.1016/S0031-9384(02)00685-6

Krause ET, Krüger O, Pogány Á (2017) Zebra Finch Nestlings, Rather than Parents, Suffer from Raising Broods under Low Nutritional Conditions. Behavioral Ecology and Sociobiology 71 (10): 152. https://doi.org/10.1007/s00265-017-2382-5

Lefeuvre M, Lu C, Botero CA, Rutkowska J (2023) Variable Ambient Temperature Promotes Song Learning and Production in Zebra Finches. Behavioral Ecology, 34 (3): 408-417. https://doi.org/10.1093/beheco/arad014

Lenth RV (2021) Emmeans: Estimated Marginal Means, Aka Least-Squares Means. https://CRAN.R-project.org/package=emmeans

Lilie ND, Riyahi S, Kalinowski A, Salazar SM, Kaiser S, Schmoll T, Korsten P (2022) Male Social Niche Conformance? Effects of Manipulated Opportunity for Extra-Pair Mating on Behavior and Hormones of Male Zebra Finches. Hormones and Behavior 146 (November): 105243. https://doi.org/10.1016/j.yhbeh.2022.105243

Logue DM (2005) Cooperative Defence in Duet Singing Birds. Cognition, Brain, Behavior 9 (3): 497–510

London SE (2019) Developmental Song Learning as a Model to Understand Neural Mechanisms That Limit and Promote the Ability to Learn. Behavioural Processes 163: 13–23. https://doi.org/10.1016/j.beproc.2017.11.008

Loning H, Verkade L, Griffith SC, Naguib M (2023) The Social Role of Song in Wild Zebra Finches. Current Biology 33 (2): 372-380. https://doi.org/10.1016/j.cub.2022.11.047

Lu C, Lefeuvre M, Rutkowska J (2022) Variability in Ambient Temperature Promotes Juvenile Participation and Shorter Latency in a Learning Test in Zebra Finches. Animal Behaviour 186: 57–66. https://doi.org/10.1016/j.anbehav.2022.01.010

Lynn SE, Stamplis TB, Barrington WT, Weida N, Hudak CA (2010) Food, Stress, and Reproduction: Short-Term Fasting Alters Endocrine Physiology and Reproductive Behavior in the Zebra Finch. Hormones and Behavior 58 (2): 214–22. https://doi.org/10.1016/j.yhbeh.2010.03.015

Mariette MM, Buchanan KL (2016) Prenatal Acoustic Communication Programs Offspring for High Posthatching Temperatures in a Songbird. Science 353 (6301): 812–14. https://doi.org/10.1126/science.aaf7049

Nowicki S, Searcy W, Peters S (2002) Brain Development, Song Learning and Mate Choice in Birds: A Review and Experimental Test of the “Nutritional Stress Hypothesis”. Journal of Comparative Physiology A 188 (11): 1003–14. https://doi.org/10.1007/s00359-002-0361-3

Ota N, Gahr M (2022) Context-Sensitive Dance–Vocal Displays Affect Song Patterns and Partner Responses in a Socially Monogamous Songbird. Ethology 128 (1): 61–69. https://doi.org/10.1111/eth.13240

Prior NH, Yap KN, Mainwaring MC, Adomat HH, Crino OL, Ma C, Guns ES, Griffith SC, Buchanan KL, Soma KK (2017) Sex Steroid Profiles in Zebra Finches: Effects of Reproductive State and Domestication. General and Comparative Endocrinology, Clinging to the edge of Gondwana: comparative endocrinology in a landscape of extremes, 244 (April): 108–17. https://doi.org/10.1016/j.ygcen.2016.02.018

Pytte CL, Gerson M, Miller J, Kirn JR (2007) Increasing Stereotypy in Adult Zebra Finch Song Correlates with a Declining Rate of Adult Neurogenesis. Developmental Neurobiology 67 (13): 1699–1720. https://doi.org/10.1002/dneu.20520

Rehling A, Spiller I, Krause ET, Nager RG, Monaghan P, Trillmich F (2012) Flexibility in the Duration of Parental Care: Zebra Finch Parents Respond to Offspring Needs. Animal Behaviour 83 (1): 35–39. https://doi.org/10.1016/j.anbehav.2011.10.003

Remage-Healey L, Adkins-Regan E, Romero LM (2003) Behavioral and Adrenocortical Responses to Mate Separation and Reunion in the Zebra Finch. Hormones and Behavior 43 (1): 108–14. https://doi.org/10.1016/S0018-506X(02)00012-0

Rezende EL, Bacigalupe LD (2015) Thermoregulation in Endotherms: Physiological Principles and Ecological Consequences. Journal of Comparative Physiology B 185 (7): 709–27. https://doi.org/10.1007/s00360-015-0909-5

Riebel K (2009) Chapter 6 Song and Female Mate Choice in Zebra Finches: A Review. In Advances in the Study of Behavior, 40:197–238. Academic Press. https://doi.org/10.1016/S0065-3454(09)40006-8

Ritschard M, Brumm H (2011) Effects of Vocal Learning, Phonetics and Inheritance on Song Amplitude in Zebra Finches. Animal Behaviour 82 (6): 1415–22. https://doi.org/10.1016/j.anbehav.2011.09.026

Ritschard M, Brumm H (2012) Zebra Finch Song Reflects Current Food Availability. Evolutionary Ecology 26 (4): 801–12. https://doi.org/10.1007/s10682-011-9541-3

Ritschard M, Laucht S, Dale J, Brumm H (2011) Enhanced Testosterone Levels Affect Singing Motivation but Not Song Structure and Amplitude in Bengalese Finches. Physiology & Behavior 102 (1): 30–35. https://doi.org/10.1016/j.physbeh.2010.10.005

Ritschard M, Riebel K, Brumm H (2010) Female Zebra Finches Prefer High-Amplitude Song. Animal Behaviour 79 (4): 877–83. https://doi.org/10.1016/j.anbehav.2009.12.038

Schäfer JE, Janocha MM, Klaus S, Tietze DT (2017) How Weather Instead of Urbanity Measures Affects Song Trait Variability in Three European Passerine Bird Species. Ecology and Evolution 7 (13): 4868–80. https://doi.org/10.1002/ece3.3032

Sierro, J, de Kort SR, Hartley IR (2023. Sexual Selection for Both Diversity and Repetition in Birdsong. Nature Communications 14 (1): 3600. https://doi.org/10.1038/s41467-023-39308-5

Slabbekoorn H, Smith TB (2002) Bird Song, Ecology and Speciation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 357 (1420): 493–503. https://doi.org/10.1098/rstb.2001.1056

Sossinka R, Böhner J (1980) Song Types in the Zebra Finch Poephila Guttata Castanotis. Zeitschrift Für Tierpsychologie 53 (2): 123–32. https://doi.org/10.1111/j.1439-0310.1980.tb01044.x

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK (2003) Song as an Honest Signal of Developmental Stress in the Zebra Finch (Taeniopygia Guttata). Hormones and Behavior 44 (2): 132–39. https://doi.org/10.1016/S0018-506X(03)00124-7

Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK (2004) Developmental Stress, Social Rank and Song Complexity in the European Starling (Sturnus Vulgaris). Proceedings of the Royal Society of London. Series B: Biological Sciences 271: S121–23. https://doi.org/10.1098/rsbl.2003.0122

Sung H, Handford P (2019) Male Song Individuality over Female Breeding Periods in Savannah Sparrows (Passerculus Sandwichensis). Vertebrate Zoology 69 (April): 161–62. https://doi.org/10.26049/VZ69-2-2019-03-1

Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP (2000) A Procedure for an Automated Measurement of Song Similarity. Animal Behaviour 59 (6): 1167–76. https://doi.org/10.1006/anbe.1999.1416

Thomas RJ (1999) Two Tests of a Stochastic Dynamic Programming Model of Daily Singing Routines in Birds. Animal Behaviour 57 (2): 277–84. https://doi.org/10.1006/anbe.1998.0956

Vleck CM (1981) Energetic Cost of Incubation in the Zebra Finch. The Condor 83 (3): 229–37. https://doi.org/10.2307/1367313

Walters MJ, Collado D, Harding CF (1991) Oestrogenic Modulation of Singing in Male Zebra Finches: Differential Effects on Directed and Undirected Songs. Animal Behaviour 42 (3): 445–52. https://doi.org/10.1016/S0003-3472(05)80043-0

Wang S, Liao C, Li F, Liu S, Meng W, Li D (2014) Castration Modulates Singing Patterns and Electrophysiological Properties of RA Projection Neurons in Adult Male Zebra Finches. PeerJ 2 (April): e352. https://doi.org/10.7717/peerj.352

Wickham H (2016) Ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://ggplot2.tidyverse.org

Woolley SC, Doupe AJ (2008) Social Context–Induced Song Variation Affects Female Behavior and Gene Expression. PLOS Biology 6 (3): e62. https://doi.org/10.1371/journal.pbio.0060062

Yahav S (2015) Chapter 37 - Regulation of Body Temperature: Strategies and Mechanisms. In Scanes CG (ed) Sturkie's Avian Physiology (Sixth Edition), San Diego: Academic Press, pp 869–905. https://doi.org/10.1016/B978-0-12-407160-5.00037-3

Zann RA (1996) The Zebra Finch: A Synthesis of Field and Laboratory Studies. Oxford University Press. Oxford

Zollinger SA, Brumm H (2011) The Lombard Effect. Current Biology 21 (16): 614-615.