PROCEEDINGS B

Research

CrossMark
click for updates

Author for correspondence:
Raïssa A. de Boer
e-mail: raissa.deboer@uantwerpen.be

THE ROYAL SOCIETY
PUBLISHING

'Out of tune': consequences of inbreeding on bird song

Raïssa A. de Boer, Marcel Eens and Wendt Müller

Behavioural Ecology and Ecophysiology Group, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

(iD) RAdB, 0000-0002-6064-6562

The expression of bird song is expected to signal male quality to females. 'Quality' is determined by genetic and environmental factors, but, surprisingly, there is very limited evidence if and how genetic aspects of male quality are reflected in song. Here, we manipulated the genetic make-up of canaries (*Serinus canaria*) via inbreeding, and studied its effects upon song output, complexity, phonetics and, for the first time, song learning. To this end, we created weight-matched inbred and outbred pairs of male fledglings, which were subsequently exposed to the same tutor male during song learning. Inbreeding strongly affected syllable phonetics, but there were little or no effects on other song features. Nonetheless, females discriminated among inbred and outbred males, as they produced heavier clutches when mated with an outbred male. Our study highlights the importance of song phonetics, which has hitherto often been overlooked.

1. Introduction

Male fitness typically increases if females consider them attractive, because females mate selectively between competing males [1]. Female choosiness evolved, among other things, because reproduction often requires larger investment of females than males (reviewed in [2]). Therefore, females should mate selectively based on male quality. Consequently, traits that signal the quality of the male will be under sexual selection, which can eventually lead to the exaggeration of such traits into ornaments [1,3].

In order to function as a signal of male quality, ornaments should be relatively more costly to express for males of low quality in comparison with high-quality males [4–7]. 'Quality' can be the result of genetic factors, but also environmental factors, which can even interact (gene–environment interactions) [4]. Thus, ornament expression can reflect environmental and/or genetic aspects of male quality.

However, most previous studies have solely focused on the effects of early environmental conditions on ornament expression and/or mate choice, without taking genetic factors into account [4]. This is quite surprising, given that females are expected to benefit from choosing males that carry 'good' genes, for example because their offspring inherits the genes for viability or attractiveness (reviewed in [1]). Alternatively, some males can be of superior quality than others because they are more heterozygous [8]. High heterozygosity—or, conversely, low heterozygosity caused by inbreeding—is expected to be heritable [9–12]. Although this can (but does not necessarily) lead to increased fitness of the offspring (reviewed in [13]), females probably gain direct benefits by choosing an outbred (i.e. more heterozygous) male over an inbred (i.e. less heterozygous) male (e.g. because the former has smaller territory sizes [14–16] or contributes less to parental care [17] than the latter).

Consequently, ornaments are hypothesized to be affected by inbreeding [8], enabling females to gain information on the genetic constitution of the male. It has, indeed, been shown that sexual signals were inbreeding-dependent in studies on fish and invertebrates [18–21]. However, studies on birds that experimentally test if the expression of song is inbreeding-dependent are extremely rare (but see [22]), despite the pre-eminent role of bird song as a model to study sexually selected traits.

Bird song is composed of many features that are constrained by different costs [23,24], and genetic and/or environmental aspects of male quality can be portrayed in different features of bird song [24,25]. Moreover, bird song is a learned behaviour, and learning capacity in itself is hypothesized to depend on male quality [26,27]. Nonetheless, there are few studies that take this into account, or look beyond features of song output (e.g. song bout length) [23,26].

Here, we aim to investigate whether song differs between inbred and outbred individuals. We expect that inbreeding may cause variation in song through differences in song learning. The development of the brain structures necessary for song learning is hypothesized to be a costly process [27], which may be hindered by inbreeding. Specifically, inbred and outbred birds may differ in what types of syllables (units of song arranged in sequences) they learn, because of a genetic predisposition to learn specific syllable types [28,29]. Syllable phonetics (e.g. mean frequency) are probably affected by inbreeding too, given that each aspect of syllable expression may represent certain neurological, morphological and physiological limits [24,30] that may be more constrained in inbred birds than in outbred birds. Furthermore, song complexity relates to cognitive capacity [31], which has been shown to be affected by inbreeding in humans [32] and rats [33], and may consequently be affected by inbreeding. Song output, finally, is perhaps the most energy-demanding trait, and thus particularly sensitive to environmental factors (e.g. food availability) [24,27]. We therefore do not expect that inbreeding affects this feature of song, at least not under benign conditions.

We used a group of inbred (parents are full siblings) and outbred (parents are unrelated) canaries (*Serinus canaria*) in order to study the effects of inbreeding on song expression. The environmental conditions were standardized from fledging until adulthood, which included the song-learning period. To this end, we applied a pairwise tutoring scheme, implying that inbred and outbred males were weight-matched, and then tutored in duos by an unrelated, older male. At adulthood, we then studied the effects of inbreeding on different dimensions of song that were divided into four categories: *song output* (song bout length and proportion spent pausing), *song complexity* (repertoire size and number of different syllable types expressed per song), *learning* (repertoire similarity and compositional similarity, and the proportion of the repertoire that was learned from the tutor) and *syllable phonetics* (mean frequency, frequency modulation, amplitude and entropy). We thereafter paired inbred and outbred males with outbred females, and quantified the reproductive investment of the females (here: clutch weight). The aim of this experiment was to test whether females could distinguish between inbred and outbred males.

2. Material and methods

(a) Study species and housing

Canaries (1 year old) originating from an outbred population kept at the University of Antwerp were used for breeding. The birds were exposed to a long light schedule (14 L : 10 D) for five weeks before breeding was commenced. Breeding occurred in two cohorts, the first one in February 2013 and the second one in April 2013 (for more details, see [34]). Breeding cages (50 × 64 × 40 cm^3, GEHU cages, The Netherlands) were equipped with two perches, shell sand, a nest cup, nesting material and constant access to seeds (Van Camp, Belgium) and water. Birds were given unlimited access to egg food (Van Camp, Belgium), supplemented with 1 tablespoon kg^{-1} Orlux hand mix (Versele-Laga), and freshly germinated seeds after the first chick hatched. In total, 39 nests with full-sibling parents were formed (breeding phase 1: $n = 29$; phase 2: $n = 10$), and 91 nests with unrelated parents (breeding phase 1: $n = 64$; phase 2: $n = 27$). There were more nests with unrelated parents, because the outbred birds were simultaneously part of a long-term study. In total, 239 outbred chicks and 73 inbred chicks survived until fledging. At fledging (± 25 days old), birds were weighed, and tarsus length was measured. Additionally, we collected a blood sample to determine sex. We only used a subset of these chicks for the here described experiment, according to their sex (male), hatching order and weight (for more details, see below). We also, with one exception, excluded siblings from the study in order to prevent biased data due to relatedness.

(b) Experimental design

We selected 19 outbred males from 18 different nests (nine nests each in the first and the second breeding phase) and 19 inbred males from 19 different nests (nine nests in the first and 10 nests in the second breeding phase). For the song tutoring, we worked with a matched-pairs design, and paired up each inbred male with an outbred male, yielding $n = 19$ pairs of 'tutees'. To control for effects of size and mass on song parameters, we ensured that two birds within a tutee pair had comparable weight/size at fledging (inbred males: 18.88 ± 0.4 g; outbred males: 18.92 ± 0.4 g; Wilcoxon signed-rank test: $V = 100$, $p > 0.8$; tarsus length: inbred males: 17.95 ± 0.2 mm; outbred males: 18.20 ± 0.1 mm; Wilcoxon signed-rank test: $V = 66.5$, $p = 0.26$). To control for effects of hatching order, only males that hatched first (day i) or second (day $i + 1$) in a brood were used in this study, because hatching order affects growth rate [34]. Each inbred–outbred tutee pair was allocated to a cage, after which a tutor was added. The tutor was an older outbred male (2 years old, $n = 5$; 3 years old, $n = 12$; 4 years old, $n = 2$) that was unrelated and unfamiliar to either tutee. Tutors were present during nearly the entire song development phase of the tutees, which in canaries lasts from approximately 40 until 240 days after hatching [35,36]: for first breeding phase, tutor was present on average 47 (range: 44–52) until 313 (range: 311–318) days after hatching; for second breeding phase, tutor was present on average 45 (range: 43–52) until 259 (range: 247–267) days after hatching. In order to mimic a seasonal cycle during this time course, the light schedule was gradually changed from a 14 L : 10 D regime towards a 10 L : 14 D regime, and then back to a long light schedule approximately one month before song recordings commenced. All tutor–tutee groups were kept in the same room, having therefore acoustic contact, but no visual contact with each other. Female canaries were kept in the same room in large flight cages, within audible/visual access to the males. The song of each bird was recorded (see below) and at the start of the female investment experiment the tutee pairs were weighed again.

(c) Song recordings

For all tutor–tutee groups originating from the first breeding phase, tutors were separated and put into a different cage (50 × 64 × 40 cm^3, GEHU cages) in order to record their song after spending on average 266 (range: 265–267) days with the tutees in the first breeding phase, and 213 (range: 202–222) days in the second breeding phase. While tutors were recorded, the inbred and outbred males remained together in a cage. Males from the first breeding phase were recorded on average 320 (range: 315–324) days after hatching, while males from the second breeding phase were recorded on average 265 (range: 255–269) days after hatching.

3

rspb.royalsocietypublishing.org   Proc. R. Soc. B 283: 20161142

Table 1. The definitions of the parameters that composed into four main song features: song output, complexity, learning and syllable phonetics.

song feature	definition of the parameters
(i) song output	— the average song bout length
	— the average proportion spent pausing per song bout: for each song bout, the summed pausing between syllables was divided by the song bout duration
(ii) complexity	— the total number of unique syllable types expressed in 240 s of song
	— the average number of different unique syllable types expressed per song bout
(iii) learning	— repertoire similarity with the use of the DICE measure [42]; this measure is defined as $DICE = 2(n_{x \cap y})/(n_x + n_y)$, where $n_{x \cap y}$ is the number of shared syllable types between individual x and individual y, and n_x and n_y the total number of syllable types sung by x and y, respectively
	— compositional similarity (CS), defined as $CS = 1 - 0.5 \times \sum (o_{ik} - o_{jk})$, where o_{xk} represents how often syllable type k occurs in the song record relative to the total number of syllables analysed, and i and j two individuals to be compared [43]
	— proportion of syllable repertoire learned from the tutor: the number of syllable types shared with the tutor, divided by the repertoire size of the tutee
(iv) syllable phonetics	— (Wiener) entropy: a measure indicating the uniformity and width of the power spectrum, with a pure tone having large negative values, whereas noisy sounds approach zero
	— frequency modulation (FM): this measure can be visualized as the difference in slope of frequency traces compared with a horizontal line; low FM accords with a more horizontal tonal structure of syllables
	— mean frequency: a measure to reflect pitch, which estimates the centre of the power distribution across frequencies
	— amplitude: the loudness of the tone

Birds were recorded for approximately 3 h consecutively, in the morning or in the afternoon. If it was not possible to extract 240 s of song from 3 h of continuous recording, the recording was repeated on another day. It was made sure that the song analysed from inbred and outbred males of each tutor–tutee group was recorded within 24 h of each other.

When making the recordings, males were separated within their cage using a separation wall, and then recorded with an omnidirectional microphone (TCM141, AV-JEFE) clipped inside the cage. To avoid effects of the presence of the experimenter, the first 300 s after the installation of the microphone were discarded in all birds. A Plexiglas plate was taped against the front of the cage at the beginning of the recording to dilute background noise. We used M-AUDIO MicroTrack II portable digital recorder (44 kHz, 16 bits) for the recordings.

(d) Song analyses

In order to estimate how many seconds of song was needed to accurately estimate syllable repertoires, we first plotted the number of new syllables appearing within 300 s of song of two tutor birds, and plotted this as a cumulative curve. The increase of new syllables over time quickly reached an asymptote in both birds (electronic supplementary material, figure S1). We decided to use 240 s of song for further analyses, because in both birds after this time span hardly any new syllables appeared (in both birds, after 240 s more than 97% of the syllable repertoire in 300 s was sung; see also [37,38]).

From the continuous recording, we sampled song bouts until 240 s of song was collected (243.12 ± 1.5 s of song per bird), using Avisoft-SASLab PRO (Specht, Germany). Song bouts were collected in order of appearance, but omitting those that had high levels of background noise (e.g. by overlapping birds), as this could affect subsequent song analyses. One 'song bout' was defined as having a minimum of 1.5 s in length, and a maximum pause duration of 0.4 s between syllables [38] (tutor $n = 696$, inbred $n = 724$, outbred $n = 677$ song bouts). The song bout length of each song for each bird was noted.

All syllables sung in the 240 s of song of each bird were segmented in SOUND ANALYSIS PRO v. 2011.104 [39], after which acoustic features for each syllable could be automatically quantified (tutor $n = 36\,731$, inbred $n = 32\,765$, outbred $n = 32\,505$ syllables). Syllables were not included in the sound feature analysis if it was noted that there was background noise (e.g. from movements of the bird (tutors 5%, inbred birds 2.5%, outbred birds 3%), but were included in repertoire analyses.

Additionally, we visually inspected spectrograms created in Avisoft (sampling frequency: 22 kHz, FFT length = 256, frame size = 75%) in order to categorize syllables into syllable types. We followed previous descriptions of canary syllable types [28,38,40,41], and different syllable types were distinguished based on the spectrogram with a special focus on the duration, FM and mean frequency of the syllables. We catalogued all different syllable types that were found in all song combined, and comparable syllable types were named the same across birds (for more details, see the electronic supplementary material, figure S2). This enabled us to compare the occurrence and use of syllable types between tutors and tutees, which were used as measures of learning (see below). All above described analyses were performed blindly with respect to the inbreeding status of the bird.

We analysed the following dimensions of song for each male (table 1): (i) song output (average song bout length and the average proportion of each song bout that was spent pausing), and (ii) complexity (size of the complete syllable repertoire and the number of different syllable types expressed per song bout). Further, we analysed (iii) learning. We measured the repertoire similarity with the 'DICE' measure (table 1) [42]. The DICE of each inbred and outbred tutee with respect to their tutor and among inbred and outbred tutees was calculated. Furthermore, we compared the DICE of tutees with their own tutor, with the average DICE of that tutee with all other tutors. This analysis was performed in order to determine if tutees had copied their tutor. Because the DICE measure does not provide information on how the repertoire is delivered, we included an additional measure that describes the CS (table 1). This measure not only takes into account how many syllable types are shared between

two birds, but also how often these syllables occur in the song recording [43]. CS of inbred and outbred tutees with respect to their tutors was calculated, and between tutees. Additionally, we compared for each tutee what proportion of their repertoire was learned. We also searched for the occurrence of two syllable types with broad frequency bandwidth, also termed 'sexy' syllables, which have been argued to play a central role in female mate choice [30,44]. However, sexy syllables were too infrequently found to allow a separate analysis, which seems typical for our population [45].

We extracted with the use of SOUND ANALYSIS PRO four key aspects regarding (iv) syllable phonetics: mean frequency, FM, entropy and amplitude. For this analysis, a subset of the data was used that, for each tutor–tutee group, only contained syllable types that were part of the repertoire of the inbred tutee of that group.

(e) Female investment
After song of all tutees was recorded, outbred females were paired with either an inbred or outbred male in a new cage ($50 \times 64 \times 40$ cm^3, GEHU cages), while ensuring that all pairs were unrelated to each other. All pairs were formed on the same day, ± 25 days after song recordings ended. All males and females were weighed and provided with a nest cup and nesting material. All cages were in the same room, and birds had auditory, but not visual access to each other. Nests were checked daily after birds were put together, and we recorded the start of laying. All eggs were weighed and marked on the day of laying. We removed the eggs after the clutch was completed (no new eggs for three consecutive days). The total weight of the clutch was taken as a measure of reproductive investment of the females according to the male (inbred or outbred) they had been paired with.

(f) Statistical analyses
We analysed all parameters with linear mixed models. For the analysis of song output, complexity and learning, the fixed effects were weight at fledging, breeding phase (first or second) and inbreeding status (inbred or outbred). We included weight at fledging to investigate whether early condition affected any of the song features. We also included the interaction of weight with inbreeding status, and breeding phase with inbreeding status in order to check if potential effects were dependent on inbreeding. For the analysis of syllable phonetics, we additionally included the syllable duration as a covariate, because especially short syllables function in female mate choice [44], and length may therefore be an important aspect for this analysis. Lastly, fixed effects in the analysis of female investment were weight (before pairing) of the male and female, inbreeding status of the male and breeding phase.

Tutor–tutee group identity was included as a random effect for the analysis of repertoire similarity, CS, repertoire size and female investment. For the analysis of the number of syllable types per song, song bout length, time spent pausing and syllable phonetics, we nested bird identity in tutor–tutee group identity, because these analyses included repeated measurements for each bird.

The statistical software package R [46] was used for all statistical analyses. The add-on package lme4 [47] was used to fit the linear mixed models. Statistical significance of terms in the linear mixed models was obtained via stepwise regression with Satterthwaite's approximation of degrees of freedom, using the add-on package lmerTest [48]. We calculated effect sizes with the use of the add-on package 'compute.es' package in R [49]. We reported the correlation coefficient r, and the associated 95% confidence interval (CI).

In order to test if relatedness among two birds affected the results, we repeated all analyses with random exclusion of one of the two tutor–tutee groups that included these birds. As this did not affect the results, we conclude these brothers did

not affect our outcome and retained them in the analyses. Results are presented as mean $\pm$ s.e.

3. Results
A summary of the effects of inbreeding on the different song features, and on female investment, can be found in table 2.

(a) Song expression
The comparison between song parameters of the inbred versus the outbred males showed no significant difference for (i) song output: average song bout length did not differ between inbred and outbred males, neither did the proportion spent pausing. Furthermore, we found little effect on (ii) complexity: syllable repertoire size did not significantly differ between inbred and outbred males. There was also no evidence that there were differences between inbred and outbred birds in the amount of unique syllable types that were uttered per song. Inbreeding status did not affect (iii) learning: the proportion of syllables that was shared with the tutor did not differ (inbred male–tutor: 0.73 ± 0.03; outbred male–tutor: 0.75 ± 0.03). Further, inbred and outbred males shared an equal amount of syllable types among each other as with their tutor (inbred male–outbred male: 0.73 ± 0.02; $F_{2,36} = 0.23$, $p = 0.80$). Males had, independent of inbreeding status ($F_{1,690} = 0.62$, $p = 0.68$), much lower repertoire similarity with tutors other than their own tutor (inbred male–other tutors: 0.47 ± 0.005; outbred male–other tutors: 0.48 ± 0.006) in comparison with the repertoire similarity with their own tutor ($F_{1,683} = 325.71$, $p < 2.2 \times 10^{-16}$). CS with the tutor did not differ between inbred and outbred males (inbred male–tutor: 0.93 ± 0.02; outbred male–tutor: 0.94 ± 0.02). There was also no difference in CS between inbred and outbred males among each other compared with their respective tutor (inbred male–outbred male: 0.89 ± 0.02; $F_{2,36} = 1.95$, $p = 0.16$). Last, a similar proportion of the repertoire of inbred and outbred birds was learned from the tutor (inbred male–tutor: 0.83 ± 0.03, outbred male–tutor: 0.81 ± 0.03). (iv) Syllable phonetics: the expression of amplitude, mean frequency, entropy and FM was strongly dependent on the duration of the syllables in interaction with inbreeding status (all F-values > 17, all p-values $< 1 \times 10^{-7}$). To explore further how the duration of syllables related to differences in phonetics according to inbreeding status, we categorized in a post hoc analysis the duration of syllables, ranging from short to long syllables. To this end, we first ensured there was no difference in the duration of syllables between inbred and outbred birds with a linear mixed model ($F_{1,17.88} = 0.73$, $p = 0.40$). Then, we examined the density distribution of the duration of syllables, which further showed that there was little difference in syllable duration between inbred and outbred birds (electronic supplementary material, figure S3). The data were split into five categories, each containing 20% of the observations in order to correct for the skewness of the data towards shorter syllables (electronic supplementary material, figure S3). The analyses were then performed again with duration of the syllable as a categorical variable, which enabled post hoc testing.

First, we found that syllable phonetics were dependent on the length of the syllable. The expression of all acoustic features differed significantly from each duration category to the next (all p-values < 0.003). The only exception was the

Table 2. Summary of the effects of inbreeding on song expression and female investment. For the analysis of syllable phonetics, syllables were classified into five categories (category 1: 4.8–52.9 ms, mean 36.4 $\pm$ 0.1 ms; category 2: 52.9–82.9 ms, mean 66.7 $\pm$ 0.1 ms; category 3: 82.9–109.4 ms, mean 96.7 $\pm$ 0.1 ms; category 4: 109.4–163.4 ms, mean 132.1 $\pm$ 0.2 ms; category 5: 163.4–439.9 ms, mean 226.4 $\pm$ 0.5 ms). Significant effects are noted with asterisks.

| song expression		inbred male (mean $\pm$ s.e.)	outbred male (mean $\pm$ s.e.)	*F*-value	*p*-value	effect size (*r* (95% CI))
(i) song output	average song bout length (s)	6.33 $\pm$ 0.1	6.76 $\pm$ 0.2	0.61	0.44	0.13 ($-$0.21, 0.44)
	proportion spent pausing	0.22 $\pm$ 0.003	0.24 $\pm$ 0.003	0.34	0.56	0.09 ($-$0.24, 0.41)
(ii) complexity	syllable repertoire size	30.0 $\pm$ 1.6	32.6 $\pm$ 1.8	2.32	0.15	0.24 ($-$0.10, 0.53)
	syllables per song	7.77 $\pm$ 0.2	8.70 $\pm$ 0.2	3.03	0.10	0.27 ($-$0.06, 0.55)
(iii) learning	repertoire similarity with tutor	0.73 $\pm$ 0.03	0.75 $\pm$ 0.03	0.33	0.57	0.09 ($-$0.24, 0.41)
	compositional similarity with tutor	0.93 $\pm$ 0.02	0.94 $\pm$ 0.02	0.15	0.70	0.06 ($-$0.27, 0.38)
	proportion of syllable repertoire learned from tutor	0.83 $\pm$ 0.03	0.81 $\pm$ 0.03	0.82	0.38	0.15 ($-$0.19, 0.45)
(iv) syllable phonetics	entropy (W)					
	category 1	$-$4.11 $\pm$ 0.008	$-$4.41 $\pm$ 0.010	$t = -3.72$	0.002*	0.53 (0.24, 0.73)
	category 2	$-$4.58 $\pm$ 0.006	$-$4.82 $\pm$ 0.006	$t = -2.58$	0.019*	0.40 (0.07, 0.64)
	category 3	$-$4.91 $\pm$ 0.006	$-$4.99 $\pm$ 0.005	$t = -1.14$	0.27	0.19 ($-$0.15, 0.49)
	category 4	$-$4.89 $\pm$ 0.006	$-$4.98 $\pm$ 0.006	$t = -1.17$	0.26	0.19 ($-$0.15, 0.49)
	category 5	$-$5.12 $\pm$ 0.006	$-$5.09 $\pm$ 0.006	$t = -0.50$	0.63	0.08 ($-$0.25, 0.40)
	mean frequency (kHz)					
	category 1	3.46 $\pm$ 0.01	3.74 $\pm$ 0.02	$t = 2.17$	0.042*	0.34 (0.01, 0.60)
	category 2	3.92 $\pm$ 0.01	4.08 $\pm$ 0.01	$t = 1.64$	0.12	0.26 ($-$0.07, 0.55)
	category 3	4.52 $\pm$ 0.01	4.68 $\pm$ 0.01	$t = 2.05$	0.054	0.32 ($-$0.01, 0.59)
	category 4	4.50 $\pm$ 0.01	4.52 $\pm$ 0.01	$t = 1.27$	0.22	0.21 ($-$0.13, 0.50)
	category 5	4.53 $\pm$ 0.01	4.50 $\pm$ 0.01	$t = -0.64$	0.53	0.11 ($-$0.23, 0.42)
	frequency modulation (FM)					
	category 1	44.56 $\pm$ 0.12	40.55 $\pm$ 0.15	$t = -4.34$	0.0003*	0.59 (0.32, 0.77)
	category 2	37.10 $\pm$ 0.09	36.14 $\pm$ 0.11	$t = -0.90$	0.38	0.15 ($-$0.19, 0.46)
	category 3	34.01 $\pm$ 0.10	33.30 $\pm$ 0.11	$t = -1.21$	0.24	0.20 ($-$0.14, 0.50)
	category 4	30.66 $\pm$ 0.10	30.76 $\pm$ 0.11	$t = -0.60$	0.55	0.10 ($-$0.24, 0.42)
	category 5	25.88 $\pm$ 0.11	25.41 $\pm$ 0.13	$t = -1.16$	0.26	0.19 ($-$0.15, 0.49)
	amplitude (dB)					
	category 1	33.60 $\pm$ 0.07	33.24 $\pm$ 0.07	$t = 0.34$	0.73	0.06 ($-$0.28, 0.38)
	category 2	37.89 $\pm$ 0.07	36.51 $\pm$ 0.06	$t = -0.58$	0.57	0.10 ($-$0.24, 0.41)
	category 3	39.57 $\pm$ 0.06	38.82 $\pm$ 0.06	$t = -1.66$	0.11	0.27 ($-$0.07, 0.55)
	category 4	39.86 $\pm$ 0.06	38.74 $\pm$ 0.06	$t = -1.22$	0.23	0.20 ($-$0.14, 0.50)
	category 5	40.70 $\pm$ 0.06	39.43 $\pm$ 0.06	$t = -1.92$	0.06	0.30 ($-$0.03, 0.58)
female investment		inbred male (mean $\pm$ s.e.)	outbred male (mean $\pm$ s.e.)	*F*-value	*p*-value	effect size (*r* (95% CI))
clutch weight (g)		7.4 $\pm$ 0.2	8.3 $\pm$ 0.3	7.18	0.011*	0.40 (0.08, 0.64)
average weight of egg (g)		1.80 $\pm$ 0.03	1.86 $\pm$ 0.03	5.62	0.029*	0.36 (0.03, 0.62)
number of eggs		4.11 $\pm$ 0.11	4.47 $\pm$ 0.16	4.06	0.05	0.31 ($-$0.02, 0.58)
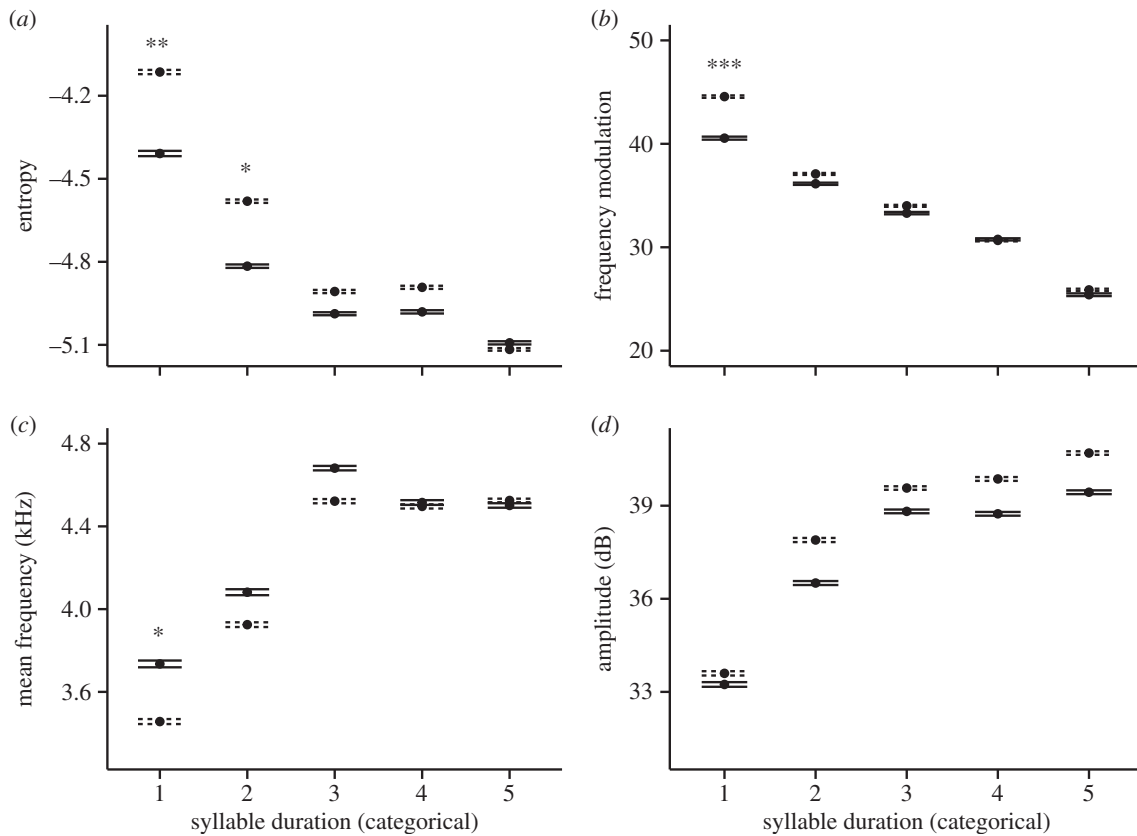
Figure 1. (a) Entropy, (b) frequency modulation, (c) mean frequency and (d) amplitude of inbred (dotted lines) and outbred (solid lines) birds. The duration of syllables is categorized, with each category containing 20% of the observations (category 1: $n = 11\,322$, 4.8–52.9 ms, mean 36.4 ± 0.1 ms; category 2: $n = 11\,322$, 52.9–82.9 ms, mean 66.7 ± 0.1 ms; category 3: $n = 11\,321$, 82.9–109.4 ms, mean 96.7 ± 0.1 ms; category 4: $n = 11\,322$, 109.4–163.4 ms, mean 132.1 ± 0.2 ms; category 5: $n = 11\,322$, 163.4–439.9 ms, mean 226.4 ± 0.5 ms). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

difference between the fourth and fifth category of mean frequency ($p = 0.1$). Entropy and FM were negatively related to syllable duration, whereas mean frequency and amplitude were positively related to syllable duration (figure 1). Further, we found again that syllable phonetics are dependent on inbreeding status in interaction with syllable duration (all F-values > 29, all p-values $< 1 \times 10^{-7}$). Post hoc analyses revealed that the entropy of the shortest syllables was significantly lower for outbred birds in comparison with inbred birds, as well as in the foremost shortest syllables. The shortest syllables were also sung with higher FM by inbred birds, but there were no differences in longer syllables. Inbred birds also sang the shortest syllables with lower frequency, and also average-length syllables, though the latter was not statistically significant. Further, there were no significant differences. On the other hand, we find that for amplitude, inbreeding only tended to affect the longest syllables, but did not have an effect in other categories (figure 1).

In none of the above-described features was there an effect of breeding phase or weight at fledging, alone or in interaction with inbreeding status (all p-values > 0.12).

(b) Female investment

Outbred females mated with an inbred male produced significantly lighter clutches compared with outbred females mated with an outbred male (figure 2 and table 2). The smaller clutch weight was the result of lighter eggs, and a tendency towards fewer eggs laid by females mated with an inbred male in comparison with those mated with an outbred male (table 2). Weight of the male was an important predictor of

clutch weight ($F_{1,34} = 8.87$, $p = 0.005$), but this was independent of inbreeding status ($F_{1,31} = 1.28$, $p = 0.27$). Further, there was no effect of female weight on clutch weight, and breeding phase of the male (p-values > 0.17). Females mated with an inbred or outbred male did not differ in weight ($F_{1,30} = 0.04$, $p = 0.85$).

4. Discussion

This study investigated whether inbreeding affects male song in canaries, a learned sexually selected trait, potentially allowing females to distinguish males according to their level of inbreeding. Inbred birds expressed syllables differently in terms of all measured phonetics: mean frequency, entropy, FM and amplitude. The effects of inbreeding were highly dependent on syllable duration: the shortest syllables were sung less pure, at lower frequency and with higher FM by inbred birds.

Phonetics are the products of complex neurological, morphological and physiological processes involved in song expression, which may have been affected by inbreeding. Such effects may arise via inbreeding–environment interactions early in life, as inbreeding can be specifically detrimental during early development [50], and changes to the early developmental trajectory often have long-lasting consequences. Although we ensured that inbred and outbred males did not differ in hatching position or size at fledging, it could be that the development of the vocal system was affected by subtle developmental differences that relate to inbreeding. These complex neural pathways of the vocal system are
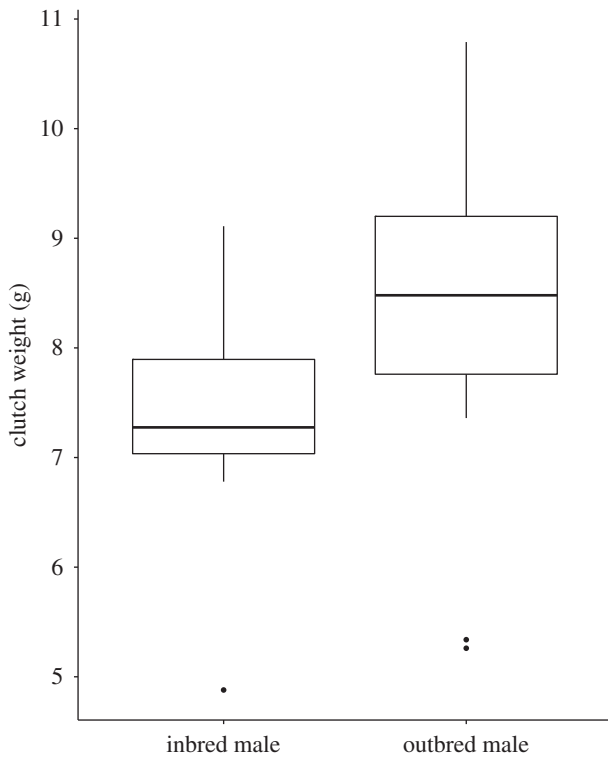
Figure 2. Reproductive investment in terms of clutch weight of females mated with an outbred male ($n = 19$) is higher compared with females that were mated with an inbred male ($n = 18$).

necessary to precisely coordinate muscles in the syrinx, vocal tract and the respiratory system [51], controlling in large part how singing behaviour is expressed with regard to acoustic features (e.g. amplitude, mean frequency) [52]. In zebra finches for example, neurons in the forebrain exhibited a unique pattern of activity for each syllable type, showing that activity in the brain structures is concentrated on the syllable [53]. If the neural pathways involved in song expression were affected by inbreeding, it could have caused distorted control of the coordination of the structures involved in syllable expression. The respiratory system has to function in coordination with the syrinx, and many muscles are involved. After expression of each individual syllable, the exhaled air is replenished with a 'mini-breath'. If syllables are repeated at a very high rate, an alternative 'pulsatile' respiratory pattern can be used, in which one breath is released with short 'puffs' [54,55]. Short syllables are often repeated at a high rate, and because these syllables were specifically affected by inbreeding, maybe this pulsatile respiratory pattern was more of a limiting factor for inbred birds than outbred birds. Other morphological or physiological differences between inbred and outbred birds could have caused differences also in song expression (e.g. beak shape) [56–58], but we did not study these mechanisms here.

Our study revealed that inbred birds were able to imitate the same syllable types of the tutor as outbred birds, and that they used these syllables equally often in the repertoire, implying no difference in learning ability. There were also few effects on song complexity. However, we expected that these features would be affected by inbreeding, because of the needed cognitive ability [31], which has been shown to be subject to inbreeding depression [32,33], and because of the costly development of complex neural structures [27,59].

In contrast with our findings, a study on zebra finches found that syllable rate was affected by inbreeding [22], which is comparable with our measure of song complexity. Likewise, a study on song sparrows found that song repertoire size was dependent on the males' inbreeding coefficient [60], showing that in different species and/or under different conditions inbreeding does have the potential to affect song complexity.

For the analysis of learning, we compared the use of syllable types based on visual inspection of spectrograms. Unfortunately, we were unable to use sound analysis software for this particular analysis, because canary song appeared to be too complex. An automated comparison of syllable types is potentially less susceptible to subjectivity, although requiring some subjective selection and setting of features. Nevertheless, all analyses were performed blind to the inbreeding status of the birds, and it is unlikely that this affected the outcome.

Furthermore, we did not follow song learning throughout the entire song-learning period in this study. We compared song of inbred and outbred birds with that of their tutors only at the end of the song-learning period, when the birds were exposed to a long light period, which should therefore reflect adult song [35]. Studies to investigate the effects of inbreeding during other stages of the song-learning process would, however, be very valuable. In this context, it should also be taken into account, especially for the syllable similarity between tutors and tutees, that tutors possibly changed their song seasonally. Consequently, there may have been changes in the syllable repertoire of the tutor throughout the song-learning period [38,41], which we controlled for via our matched-pairs design. The latter ensured that inbred and outbred birds experienced the same song-learning conditions. We also found little effect of inbreeding on song output, in contrast with a previous study that did report effects on song rate [22], a comparable measure of song output. However, this is in accordance with our expectation that song output is a feature of song that is strongly dependent on environmental conditions [24,27], and that especially under standardized conditions of the laboratory the effects of inbreeding may not become apparent.

In fact, the reason why we found effects of inbreeding on one feature of song only may be that the negative effects of inbreeding are often amplified under stressful circumstances [61]. Here, we controlled environmental conditions stringently, which may have masked the effects of inbreeding on other features. Although results remain ambiguous, previous studies have found that early adverse environmental conditions can be reflected in song output and/or complexity [62–64], and (although studied much less often) song learning [26,65]. Thus, it could be that under stressful conditions the effects of inbreeding become aggravated, and not only impinge on phonetics but also on other features of song.

Given that inbreeding affected song phonetics even under our rather benign conditions, we propose that genetic aspects of quality are specifically portrayed in this feature of song. The effects of environmental stress on phonetics are still quite unknown, but one study on zebra finches found no effects [65], suggesting that phonetics may indeed reflect other aspects of male quality, such as inbreeding status, as we found here.

This may also explain why canary females are specifically responsive to certain syllable types sung by males [44], and show preference for particular acoustic features of these syllables [66–68]. A preference for certain aspects of phonetics has also been found in other species: zebra finches [69], and

8

dusky warblers [70] prefer high-amplitude song, and swamp sparrows prefer a wide-frequency bandwidth [71].

In this study, we found that females that were paired with an outbred male produced heavier clutches in comparison with females that were paired with an inbred male, which implies that females can indeed distinguish between inbred and outbred males. The difference in clutch weight mostly related to the size of the eggs, although females mated with an inbred male also tended to lay fewer eggs than those mated with an outbred male. Taken together, females invested less in reproduction when they were mated with an inbred male. In a previous study, it was found that a preference for song was also reflected in clutch weight [72]. We therefore expect that the variance in clutch weight we found is a result of the differences in phonetics we showed. An alternative explanation is that outbred males sang certain syllable types that were not used by inbred males. Although inbred and outbred males shared a large proportion of their repertoire, outbred males perhaps expressed syllable types that were specifically important to females. Unfortunately, we have not performed song-preference experiments that would enable us to show whether females can actually distinguish between males purely based on phonetics. Nevertheless, choosing for signals that reflect genetic aspects of quality is certainly expected to benefit the female.

Choosing for outbred males over inbred males can provide indirect (genetic) benefits to the female, for example when it leads to the production of highly heterozygous offspring (reviewed in [13]). Additionally, choosing based on heterozygosity can provide direct benefits, for example

through larger territory sizes [14–16] or increased parental care [17]. However, the functional consequences of the observed differential investment with regard to inbreeding still need to be investigated, although it is known that egg size affects many traits in the offspring, including growth rate and survival [73].

In conclusion, we have shown that inbreeding is reflected in song phonetics specifically, an often overlooked feature of bird song. Females were able to distinguish between inbred and outbred males, highlighting the potential importance of song phonetics. We suggest that variation in phonetics may allow females to choose a male with 'good' genes (e.g. highly heterozygous), even when environmental factors are largely controlled for.

References

1. Andersson M. 1994 *Sexual selection*. Princeton, NJ: Princeton University Press.

2. Kokko H, Brooks R, Jennions MD, Morley J. 2003 The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B* **270**, 653–664. (doi:10.1098/rspb.2002.2235)

3. Darwin C. 1871 *The descent of man, and selection in relation to sex*. London, UK: Murray.

4. Cotton S, Fowler K, Pomiankowski A. 2004 Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B* **271**, 771–783. (doi:10.1098/rspb.2004.2688)

5. Grafen A. 1990 Biological signals as handicaps. *J. Theor. Biol.* **144**, 517–546. (doi:10.1016/S0022-5193(05)80088-8)

6. Rowe L, Houle D. 1996 The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B* **263**, 1415–1421. (doi:10.1098/rspb.1996.0207)

7. Zahavi A. 1975 Mate selection—a selection for a handicap. *J. Theor. Biol.* **53**, 205–214. (doi:10.1016/0022-5193(75)90111-3)

8. Brown JL. 1997 A theory of mate choice based on heterozygosity. *Behav. Ecol.* **8**, 60–65. (doi:10.1093/beheco/8.1.60)

9. Mitton JB, Schuster WSF, Cothran EG, de Fries JC. 1993 Correlation between the individual

heterozygosity of parents and their offspring. *Heredity* **71**, 59–63. (doi:10.1038/hdy.1993.107)

10. Nietlisbach P, Keller LF, Postma E. 2016 Genetic variance components and heritability of multiallelic heterozygosity under inbreeding. *Heredity* **116**, 1–11. (doi:10.1038/hdy.2015.59)

11. Reid JM, Keller LF. 2010 Correlated inbreeding among relatives: occurrence, magnitude, and implications. *Evolution* **64**, 973–985. (doi:10.1111/j.1558-5646.2009.00865.x)

12. Reid JM, Arcese P, Keller LF. 2006 Intrinsic parent-offspring correlation in inbreeding level in a song sparrow (*Melospiza melodia*) population open to immigration. *Am. Nat.* **168**, 1–13. (doi:10.1086/504852)

13. Kempenaers B. 2007 Mate choice and genetic quality: a review of the heterozygosity theory. *Adv. Study Behav.* **37**. 189–278. (doi:10.1016/S0065-3454(07)37005-8)

14. Seddon N, Amos W, Mulder RA, Tobias JA. 2004 Male heterozygosity predicts territory size, song structure and reproductive success in a cooperatively breeding bird. *Proc. R. Soc. Lond. B* **271**, 1823–1829. (doi:10.1098/rspb.2004.2805)

15. Höglund J, Piertney SB, Alatalo RV, Lindell J, Lundberg A, Rintamäki PT. 2002 Inbreeding depression and male fitness in black grouse.

Proc. R. Soc. Lond. B **269**, 711–715. (doi:10.1098/rspb.2001.1937)

16. Hoffman J, Boyd IL, Amos W. 2004 Exploring the relationship between parental relatedness and male reproductive success in the Antarctic fur seal *Arctocephalus gazella*. *Evolution* **58**, 2087–2099. (doi:10.1111/j.0014-3820.2004.tb00492.x)

17. García-Navas V, Ortego J, Sanz JJ. 2009 Heterozygosity-based assortative mating in blue tits (*Cyanistes caeruleus*): implications for the evolution of mate choice. *Proc. R. Soc. B* **276**, 2931–2940. (doi:10.1098/rspb.2009.0417)

18. Aspi J. 2000 Inbreeding and outbreeding depression in male courtship song characters in *Drosophila montana*. *Heredity* **84**, 273–282. (doi:10.1046/j.1365-2540.2000.00655.x)

19. Drayton JM, Hunt J, Brooks R, Jennions MD. 2007 Sounds different: inbreeding depression in sexually selected traits in the cricket *Teleogryllus commodus*. *J. Evol. Biol.* **20**, 1138–1147. (doi:10.1111/j.1420-9101.2006.01286.x)

20. Mariette M, Kelley JL, Brooks R, Evans JP. 2006 The effects of inbreeding on male courtship behaviour and coloration in guppies. *Ethology* **112**, 807–814. (doi:10.1111/j.1439-0310.2006.01236.x)

21. Van Oosterhout C, Trigg RE, Carvalho GR, Magurran AE, Hauser L, Shaw PW. 2003 Inbreeding depression and genetic load of sexually selected traits: how the

guppy lost its spots. *J. Evol. Biol.* **16**, 273–281. (doi:10.1046/j.1420-9101.2003.00511.x)

22. Bolund E, Martin K, Kempenaers B, Forstmeier W. 2010 Inbreeding depression of sexually selected traits and attractiveness in the zebra finch. *Anim. Behav.* **79**, 947–955. (doi:10.1016/j.anbehav.2010.01.014)

23. Gil D, Gahr M. 2002 The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* **17**, 133–141. (doi:10.1016/S0169-5347(02)02410-2)

24. Nowicki S, Searcy WA. 2004 Song function and the evolution of female preferences: why birds sing, why brains matter. *Annu. NY Acad. Sci.* **1016**, 704–723. (doi:10.1196/annals.1298.012)

25. Woodgate JL, Buchanan KL, Bennett ATD, Catchpole CK, Brighton R, Leitner S. 2013 Environmental and genetic control of brain and song structure in the zebra finch. *Evolution* **68**, 236–240.

26. Holveck M-J, de Castro ACV, Lachlan RF, ten Cate C, Riebel K. 2008 Accuracy of song syntax learning and singing consistency signal early condition in zebra finches. *Behav. Ecol.* **19**, 1267–1281.

27. Nowicki S, Searcy WA, Peters S. 2002 Brain development, song learning and mate choice in birds: a review and experimental test of the 'nutritional stress hypothesis'. *J. Comp. Physiol. A* **188**, 1003–1014. (doi:10.1007/s00359-002-0361-3)

28. Mundinger PC. 1995 Behaviour-genetic analysis of canary song: inter-strain differences in sensory learning, and epigenetic rules. *Anim. Behav.* **50**, 1491–1511. (doi:10.1016/0003-3472(95)80006-9)

29. Mundinger PC, Lahti DC. 2014 Quantitative integration of genetic factors in the learning and production of canary song. *Proc. R. Soc. B* **281**, 2013-2631. (doi:10.1098/rspb.2013.2631)

30. Vallet E, Kreutzer M. 1995 Female canaries are sexually responsive to special song phrases. *Anim. Behav.* **49**, 1603–1610. (doi:10.1016/0003-3472(95)90082-9)

31. Boogert NJ, Giraldeau L-A, Lefebvre L. 2008 Song complexity correlates with learning ability in zebra finch males. *Anim. Behav.* **76**, 1735–1741. (doi:10.1016/j.anbehav.2008.08.009)

32. Bashi J. 1977 Effects of inbreeding on cognitive performance. *Nature* **266**, 440–442. (doi:10.1038/266440a0)

33. Harker KT, Whishaw IQ. 2002 Place and matching-to-place spatial learning affected by rat inbreeding (Dark–Agouti, Fischer 344) and albinism (Wistar, Sprague–Dawley) but not domestication (wild rat vs. Long–Evans, Fischer–Norway). *Behav. Brain Res.* **134**, 467–477. (doi:10.1016/S0166-4328(02)00083-9)

34. de Boer RA, Eens M, Fransen E, Müller W. 2015 Hatching asynchrony aggravates inbreeding depression in a songbird (*Serinus canaria*): an inbreeding–environment interaction. *Evolution* **69**, 1063–1068. (doi:10.1111/evo.12625)

35. Nottebohm F, Nottebohm ME, Crane L. 1986 Developmental and seasonal changes in canary song and their relation to changes in the anatomy of song-control nuclei. *Behav. Neural*

Biol. **46**, 445–471. (doi:10.1016/S0163-1047(86)90485-1)

36. Weichel K, Schwager G, Heid P, Güttinger HR, Pesch A. 1986 Sex differences in plasma steroid concentrations and singing behaviour during ontogeny in canaries (*Serinus canaria*). *Ethology* **73**, 281–294. (doi:10.1111/j.1439-0310.1986.tb00810.x)

37. Halle F, Gahr M, Kreutzer M. 2003 Effects of unilateral lesions of HVC on song patterns of male domesticated canaries. *J. Neurobiol.* **56**, 303–314. (doi:10.1002/neu.10230)

38. Leitner S, Voigt C, Gahr M. 2001 Seasonal changes in the song pattern of the non-domesticated island canary (*Serinus canaria*), a field study. *Behaviour* **138**, 885–904. (doi:10.1163/156853901753172700)

39. Tchernichovski O, Nottebohm F, Ho CE, Pesaran B, Mitra PP. 2000 A procedure for an automated measurement of song similarity. *Anim. Behav.* **59**, 1167–1176. (doi:10.1006/anbe.1999.1416)

40. Mundinger PC. 2010 Behaviour genetic analysis of selective song learning in three inbred canary strains. *Behaviour* **147**, 705–723. (doi:10.1163/000579510X489903)

41. Voigt C, Leitner S. 2008 Seasonality in song behaviour revisited: seasonal and annual variants and invariants in the song of the domesticated canary (*Serinus canaria*). *Horm. Behav.* **54**, 373–378. (doi:10.1016/j.yhbeh.2008.05.001)

42. Bessert-Nettelbeck M, Kipper S, Bartsch C, Voigt-Heucke SL. 2014 Similar, yet different: male reed buntings (*Emberiza schoeniclus*) show high individual differences in song composition, rates of syllable sharing and use. *J. Ornithol.* **155**, 689–700. (doi:10.1007/s10336-014-1052-x)

43. Garamszegi LZ, Zsebok S, Török J. 2012 The relationship between syllable repertoire similarity and pairing success in a passerine bird species with complex song. *J. Theor. Biol.* **295**, 68–76. (doi:10.1016/j.jtbi.2011.11.011)

44. Vallet E, Beme I, Kreutzer M. 1998 Two-note syllables in canary songs elicit high levels of sexual display. *Anim. Behav.* **55**, 291–297. (doi:10.1006/anbe.1997.0631)

45. Müller W, Vergauwen J, Eens M. 2008 Yolk testosterone, postnatal growth and song in male canaries. *Horm. Behav.* **54**, 125–133. (doi:10.1016/j.yhbeh.2008.02.005)

46. R Development Core Team. 2014 *R: a language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing.

47. Bates D, Maechler M, Bolker B, Walker S. 2015 Fitting linear mixed-effects models Using lme4. *J. Stat. Softw.* **67**, 1–48. (doi:10.18637/jss.v067.i01)

48. Kuznetsova A, Brockhoff PB, Christensen RHB. 2015 lmerTest: tests in linear mixed effects models. R package version 20–29. See http://cran.r-project.org/web/packages/lmerTest/lmerTest.pdf.

49. Del Re A. 2013 compute.es: compute effect sizes. R package version 02–2. See http://cran.r-project.org/web/packages/compute.es/compute.es.pdf.

50. Keller LF, Waller DM. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241. (doi:10.1016/S0169-5347(02)02489-8)

51. Wild J. 2004 Functional neuroanatomy of the sensorimotor control of singing. *Annu. NY Acad. Sci.* **1016**, 438–462. (doi:10.1196/annals.1298.016)

52. Wild JM. 1997 Neural pathways for the control of birdsong production. *J. Neurobiol.* **33**, 653–670. (doi:10.1002/(SICI)1097-4695(19971105)33:5<653::AID-NEU11>3.0.CO;2-A)

53. Yu AC, Margoliash D. 1996 Temporal hierarchical control of singing in birds. *Science* **273**, 1871–1875. (doi:10.1126/science.273.5283.1871)

54. Suthers RA, Zollinger S. 2004 Producing song: the vocal apparatus. *Annu. NY Acad. Sci.* **1016**, 109–129. (doi:10.1196/annals.1298.041)

55. Hartley RS. 1990 Expiratory muscle activity during song production in the canary. *Respir. Physiol.* **81**, 177–187. (doi:10.1016/0034-5687(90)90044-Y)

56. Podos J. 1996 Motor constraints on vocal development in a songbird. *Anim. Behav.* **51**, 1061–1070. (doi:10.1006/anbe.1996.0107)

57. Podos J. 2001 Correlated evolution of morphology and vocal signal structure in Darwin's finches. *Nature* **409**, 185–188. (doi:10.1038/35051570)

58. Ryan MJ, Brenowitz EA. 1985 The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.* **126**, 87–100. (doi:10.1086/284398)

59. Scharff C, Adam I. 2013 Neurogenetics of birdsong. *Curr. Opin. Neurobiol.* **23**, 29–36. (doi:10.1016/j.conb.2012.10.001)

60. Reid JM, Arcese P, Cassidy ALEV, Marr AB, Smith JNM, Keller LF. 2005 Hamilton and Zuk meet heterozygosity? Song repertoire size indicates inbreeding and immunity in song sparrows (*Melospiza melodia*). *Proc. R. Soc. B* **272**, 481–487. (doi:10.1098/rspb.2004.2983)

61. Higginson AD, Reader T. 2009 Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. *Proc. R. Soc. B* **276**, 1153–1159. (doi:10.1098/rspb.2008.1592)

62. Spencer KA, Buchanan KL, Goldsmith AR, Catchpole CK. 2003 Song as an honest signal of developmental stress in the zebra finch (*Taeniopygia guttata*). *Horm. Behav.* **44**, 132–139. (doi:10.1016/S0018-506X(03)00124-7)

63. Buchanan KL, Spencer KA, Goldsmith AR, Catchpole CK. 2003 Song as an honest signal of past developmental stress in the European starling (*Sturnus vulgaris*). *Proc. R. Soc. Lond. B* **270**, 1149–1156. (doi:10.1098/rspb.2003.2330)

64. Spencer KA, Buchanan KL, Leitner S, Goldsmith AR, Catchpole CK. 2005 Parasites affect song complexity and neural development in a songbird. *Proc. R. Soc. B* **272**, 2037–2043. (doi:10.1098/rspb.2005.3188)

65. Brumm H, Zollinger SA, Slater PJB. 2009 Developmental stress affects song learning but not song complexity and vocal amplitude in zebra finches. *Behav. Ecol. Sociobiol.* **63**, 1387–1395. (doi:10.1007/s00265-009-0749-y)

66. Drăgănoiu TI, Nagle L, Kreutzer M. 2002 Directional female preference for an exaggerated male trait in canary (*Serinus canaria*) song. *Proc. R. Soc. Lond. B* **269**, 2525–2531. (doi:10.1098/rspb.2002.2192)

67. Pasteau M, Nagle L, Kreutzer M. 2009 Preferences and predispositions of female canaries (*Serinus canaria*) for loud intensity of male sexy phrases. *Biol. J. Linn. Soc.* **96**, 808–814. (doi:10.1111/j.1095-8312.2008.01136.x)

68. Pasteau M, Ung D, Kreutzer M, Aubin T. 2012 Amplitude modulation of sexy phrases is salient for song attractiveness in female canaries (*Serinus canaria*). *Anim. Cogn.* **15**, 639–645. (doi:10.1007/s10071-012-0492-z)

69. Ritschard M, Riebel K, Brumm H. 2010 Female zebra finches prefer high-amplitude song. *Anim. Behav.* **79**, 877–883. (doi:10.1016/j.anbehav.2009.12.038)

70. Forstmeier W, Kempenaers B, Meyer A, Leisler B. 2002 A novel song parameter correlates with extra-pair paternity and reflects male longevity. *Proc. R. Soc. Lond. B* **269**, 1479–1485. (doi:10.1098/rspb.2002.2039)

71. Ballentine B, Hyman J, Nowicki S. 2004 Vocal performance influences female response to male bird song: an experimental test. *Behav. Ecol.* **15**, 163–168. (doi:10.1093/beheco/arg090)

72. Holveck M-J, Riebel K. 2010 Low-quality females prefer low-quality males when choosing a mate. *Proc. R. Soc. B* **277**, 153–160. (doi:10.1098/rspb.2009.1222)

73. Krist M. 2011 Egg size and offspring quality: a meta-analysis in birds. *Biol. Rev.* **86**, 692–716. (doi:10.1111/j.1469-185X.2010.00166.x)