The effects of rearing conditions on sexual traits and preferences in zebra finches

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Chapter 4

Song syntax learning and singing consistency as long-term signals of past developmental condition in zebra finches

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Chapter 4

ABSTRACT
Birdsong is a sexually selected and culturally transmitted multidimensional signal. As for other sexually selected traits, it is generally assumed that variation between male songs is partly condition dependent. In oscine songbirds, song is learned early in life, thus variation arising from the song learning process provides yet another source of variation between males, raising the question of how conspecifics can judge male quality by song. The developmental stress hypothesis proposed that poor early developmental condition can adversely affect song learning. As a consequence some learned features of song might indicate male quality to females. Surprisingly, most studies testing this hypothesis compared adult males' song repertoires without looking at song learning. The only two studies that did to date (Gil et al., 2006 *J Neurobiol 66*; Nowicki et al., 2002 *J Comp Physiol a 188*) reported inconsistent effects and analyzed a limited number of song features. Here, we examined the effects of early condition (by manipulation of brood size) on song learning and song features in the zebra finch, *Taeniopygia guttata*. Our song analysis compared repertoire and the number of specific elements copied from the tutor, and also included a great number of syntax and performance related song parameters. Birds from large broods (i.e. of poor early condition) sang with less consistent sound duration between song motif renditions, but did not have a smaller repertoire size, than the birds from small broods. In addition, the birds from large broods were not as good as those from small broods to copy accurately syntactical dependencies of song elements from the song motif of their tutor. These findings support the developmental stress hypothesis. We discuss the potential constraints underlying condition-dependent expression of song features signaling male past developmental history. We also discuss the potential role of such long-term signals of condition in female mate choice.

KEYWORDS: Developmental stress hypothesis, song learning, syntax learning, singing consistency, condition-dependent signal, male quality, brood size manipulation, zebra finch, *Taeniopygia guttata*.

Darwin (1871) first proposed that songs of songbirds evolved in response to sexual selection by female mate choice. Since then, many studies have found that individual variation in a variety of song features affects reproductive success through mate choice and male-male competition (Andersson, 1994; Searcy and Yasukawa, 1996). In the case of an exaggerated mating signal such as birdsong, the handicap principle of signaling predicts that song features should be costly to develop or maintain in order to constitute reliable indicators of male quality (Grafen, 1990; Zahavi, 1975). Bird song is thus traditionally seen as a condition-dependent sexual signal. Condition (or quality) in this case is defined as the pool of resources an individual can accumulate and then allocate to the production or maintenance of traits that enhance fitness (Hunt et al., 2005; Rowe and Houle, 1996). Differences in song production can therefore signal differences in genetic or phenotypic quality between males and might facilitate female choice of high quality mates (Andersson, 1994; Searcy and Yasukawa, 1996).

While condition dependence of song output has been demonstrated experimentally in a number of species (Gil and Gahr, 2002), evidence for condition dependence of other song features is, however, scant and sometimes controversial (Gil and Gahr, 2002; ten Cate et al., 2002). Birdsong varies along many dimensions but studies often focus only on a few aspects of song performance although different song features could be constrained by different costs. For some song features known to be preferred by females these costs are easier to estimate than for others. An increase in singing performance, which is usually measured by the quantity of song output in terms of length or rate of songs, is likely to require added time or energy expenditure (Nowicki et al., 2002a; but see Oberweger and Goller, 2001). The production costs are much less obvious for other song features also important in female choice such as song complexity, which is often set equal to a measure of repertoire size e.g. the number of different song types or song elements an individual learns and sings (Gil and Gahr, 2002; Nowicki et al., 2002a; but see Vallet et al., 1998).

Nowicki and coauthors (Nowicki et al., 1998; Nowicki et al., 2002a) proposed that learned features of song could indicate male condition or quality because the development of costly-to-build brain structures mediating song learning and production occurs during the period of fastest development, i.e. when young birds are most vulnerable (see also Buchanan et al., 2003; Catchpole, 1996; Doutrelant et al., 2000). Different song features might thus signal different aspects of male condition simultaneously or at different moments in time (multiple message hypothesis, Møller and Pomiankowski, 1993). Some dimensions of the signal could respond rapidly to momentary variations in condition such as song rate (Birkhead et al., 1998; but see de Kogel and Prijs, 1996) while others might reflect past condition. The latter ones could be those song features learned early in life (e.g. repertoire size). Based on such long-term signals of condition, females may therefore gain reliable information about how well males fared in the face of an early developmental stress.

Several studies have now demonstrated effects of early environmental stressors (i.e. direct manipulation of food availability, corticosterone administration or parasite infection) on nestling and adult condition, song control brain nuclei, song complexity and singing performance (Buchanan et al., 2004; Buchanan et al., 2003; Spencer et al., 2003; Spencer et al., 2004; Spencer et al., 2005a). However, there is limited evidence that developmental stress causes differences in male song learning although it is a fundamental prerequisite of the developmental stress hypothesis. Indeed, most studies testing this hypothesis compared adult males' song repertoires only without looking at song learning. To date, the only two studies that did (Gil et al., 2006; Nowicki et al., 2002a) reported inconsistent effects. Nowicki et al. (2002a) showed that hand-reared male swamp sparrows, *Melospiza georgiana*, with limited food availability and controlled quality of song exposure by tape-tutoring produced less accurate copies of the model songs from which they learned, but did not have smaller repertoire size than the control males. In zebra finches, *Taeniopygia guttata*, Gil et al. (2006) experimentally altered early condition by brood size manipulations (Naguib et al., 2004) but found no effect of the treatment on the amount of song elements learned from an adult model or total number of elements (Spencer et al., 2003). This latter effect had been reported in both experimental groups in a study using two different early stressors: reduced food availability and corticosterone administration (Spencer et al., 2003). It is currently unclear which of a number of possible factors can explain these different outcomes. Next to the different treatments employed, both zebra finch studies (Gil et al., 2006; Spencer et al., 2003) did not fully control the quantity and quality of song exposure. Spencer et al. (2003) poorly controlled for potential effects of the treatment on the behavior of the song tutor and Gil et al. (2006) found song learning to be affected by the number of males learning from the same song tutor, which has also been observed in other song learning studies in this species (Tchernichovski et al., 1999; Tchernichovski and Nottebohm, 1998). As shown by Nowicki et al. (2002a) in their study in swamp sparrows these problems can be addressed methodologically.

Building on these earlier studies, we here reinvestigate effects of developmental condition on male song quality in zebra finches with a carefully controlled song tutoring procedure and a much more detailed song analysis. We used brood size manipulations as a means to manipulate male condition because earlier studies in this species have shown that this does indeed induce phenotypic variation affecting fitness and survival (de Kogel, 1997; de Kogel and Prijs, 1996; Naguib et al., 2006; Naguib et al., 2004). Our manipulated brood sizes were within the natural range (i.e. 1 to 6 chicks per brood for zebra finches, Zann, 1996), which allowed addressing the effects of developmental conditions within an ecologically relevant range. Our design overcomes the earlier problems of potentially confounding effects of 1) the number of siblings on song learning accuracy (Gil et al., 2006) and 2) possible effects of the treatment on the tutor's song quality (Spencer et al., 2003) as follows: high and low condition males were pair-wise exposed to the same adult song tutor not previously exposed to the treatment. Moreover, other than earlier studies in this species, which either used the number and similarity of copied elements as sole measures of song learning (Gil et al., 2006) or only compared adult song structure (Spencer et al., 2003), we combined a song structure based comparison with a tutor-tutee song sharing assay. This allowed us to test for effects of early condition not only on overall adult song structure but also on the learning of element phonology and the rarely studied accuracy of syntax learning (Funabiki and Konishi, 2003).

METHODS

Brood size manipulation and song tutoring procedure

Subjects were offspring of 30 breeding pairs of wild-type out bred zebra finches housed in 80 x 40 x 40 cm cages in a large bird room at Leiden University (The Netherlands). The study was first run in 2004 (14 breeding pairs) and replicated in 2005 (16 breeding pairs). All chicks (*n* = 113) were cross-fostered at 3 ± 1.7 days post hatching (2004: 3.9 ± 1.6, *n* = 56; 2005: 2.1 ± 1.4, *n* = 57) in two different brood sizes: either small consisting of 2-3 chicks (19 broods) or large consisting of 5-6 chicks (11 broods). To control for parental differences in rearing we made sure that there was no correlation between initial and experimental brood sizes (Pearson 2004: $r_{14} = 0.18$, $P = 0.5$; 2005: $r_{16} = 0.42$, $P = 0.1$).

Chicks stayed with their foster parents until 33.5 ± 3.3 days post-hatching i.e. until nutritional independence (2004: 34 ± 3; $n = 53$; 2005: 33 ± 3, $n = 52$). We then separated the experimental birds from their foster parents for song tutoring. We assigned 26 males and 26 females from the foster broods to 13 tutoring groups (6 in 2004, 7 in 2005) of two males and two females each (1 chick per brood size and sex). Each group consisted of genetically and fosterly unrelated individuals and was housed with a song tutor, which was an unrelated mated adult male. The song tutoring procedure lasted until 68.9 ± 2.7 days post-hatching (2004: 70.2 ± 2.8, $n = 12$ males; 2005: 67.8 ± 2.2, $n = 14$ males) and was therefore well within the sensitive period for song acquisition between days 35 to 65 (for reviews of male song learning see Jones et al., 1996; Slater et al., 1988). Afterwards tutees were housed in single-sex groups of four to five birds. Throughout, birds were on a 13.30:10.30 L:D schedule (lights on at 7:00 C.E.T.) at 20-22°C and 35–50% humidity. They had *ad libitum* access to a commercial tropical seed mixture (Tijssen goed voor dieren, Hazerswoude, Holland), drinking water and cuttlebone. They were supplemented three times weekly with 3-4 g of egg food (Witte Molen, B.V., Meeuwen, Holland) per bird, twice weekly with branches of millet and once weekly with germinated tropical seeds.

This study was conducted in line with the ASAB guidelines on animal experimentation and the Dutch laws on animal experimentation and approved by Leiden University committee for animal experimentation (Dierexperimentencommissie Universiteit Leiden, DEC 04090).

Song recording

Recordings were made after birds had crystallized their stable adult song, which takes place around 90 days post-hatching (Jones et al., 1996; Slater et al., 1988). We recorded non-directed songs of the 26 male tutees at 141 ± 13 days post-hatching (2004: 141 ± 7, $n = 12$; 2005: 141 ± 16, $n = 14$) and of their 13 song tutors and 17 foster fathers (3 foster fathers were also used as tutors but not for chicks they had raised) when they were more than 180 days old. We recorded foster fathers' songs to also include similarity between foster father and tutor song in our song sharing analysis because the song heard early in life can guide the choice of a particular male as a song tutor in zebra finches (Clayton, 1987; Mann and Slater, 1994; Slater and Mann, 1990).

For recordings, birds were placed singly in a cage (70 x 30 x 45 cm) on a wooden shelf (100 x 55 cm) at 120-cm height in one of two identical sound attenuation chambers (100 x 200 x 220 cm). Songs were recorded at 75-cm distance from the cage with a Sennheiser MKH40 microphone (Wedemark, Germany) and MZN16 P48 power supply using Ishmael software (version 1.0.2, http://cetus.pmel.noaa.gov/cgi-bin/MobySoft.pl; automatic energy detection settings for 2000-10000 Hz, detection threshold 1, detection limits 0.2-100 s, buffer 3 s). The conditions in the chambers were similar to those of the bird colony room. We moved males in and out of the chamber in the afternoon (means ± 1 SD C.E.T.; in: 16:36 ± 01:52; out: 15:27 ± 01:47). Most of them (41 of 53) started singing on the next day or on the day after, usually after light went on at 7:00. The remaining males that did not do so were returned to their home cage for a resting period of at least one day before another trial (mean number of trials ± 1 SD: 3.8 ± 2.9, n = 12). Recording sessions lasted 1.6 ± 0.8 days (n = 87). We obtained 101 ± 123 recording files per birds (range = 3-533; n = 53).

Song analysis

Song motif selection

The song of zebra finches consists of a series of introductory elements followed by several repetitions (range 1-23) of one individually distinctive motif consisting of a sequence of individually distinctive elements (Sossinka and Böhner, 1980) that are delivered in a relatively fixed but not wholly stereotyped sequential order (Sturdy et al., 1999b). For our analysis, we randomly chose five renditions of each male's motif.

Within the directory with all recorded sound files of each bird, a custom-written software randomly selected five files (©copyright 2006, Niklas J. Tralles; the software can be obtained on request from the authors). Within each selected file (often containing more than one song), we selected the song with the highest number of motifs or the first song appearing in the file when several songs had the same number of motifs. Then within the selected song, we randomly selected one motif with a dice or with the custom-written software when the song had more than 6 motifs (the software randomly selected five files among dummy numbered files corresponding to the number of motifs within the selected song). Truncated songs or motifs were not included for selection. For four birds (one tutee from a large brood and three foster fathers), we had only three or four recording files. For these we randomly selected two motifs from the same sound file but from two different songs when the file had more than one song. If motifs were sampled from the same song (one instance for two foster fathers) we made sure not to select the same motif twice. As all songs begin with introductory elements that are highly similar between individuals and because the number of introductory elements is highly variable between renditions of motifs, these elements can easily exaggerate or water down the number of shared elements between two motifs. The variable number of introductory elements mostly occurs before the first motif within a song, thus comparing this motif with the subsequent rendition in a song allows one to identify the core motif. This is what we used for analysis for which we digitally deleted those excess introductory elements only appearing in some, especially the first motifs of songs, but keeping those introductory elements that occurred at the beginning of each rendition of the motif within songs (Praat software v. 4.2.07 for Windows, freely available from http://www.praat.org).

Element labeling

We carried out all subsequent bioacoustic analyses with Luscinia sound analysis software (version 1.0 for Windows, freely available from http://luscinia.sourceforge.net). Next to standard functions, this sound analysis software also offers an automated feature to compare the order of element sequences between song motifs.

For each selected motif, we let Luscinia apply a high-pass cut-off filter at 50 Hz to remove low-frequency background noise and then calculate a fast Fourier transform of the product of the waveform and the moderate-resolution Gaussian windowing function. This rendered spectrographs with a resolution of 10 KHz with a temporal resolution of 1 ms. The computational determination of fundamental frequency of song elements is sometimes difficult in zebra finches because the harmonics (multiple frequencies of the fundamental frequency that are used in its automatic calculation) can be suppressed in the elements (Williams et al., 1989). We therefore used both the automatic pitch detection and manual fine tuning feature in Luscinia (Appendix 1a). Zebra finch songs contain a number of noisy structures that are difficult to visually inspect in detail even on spectrograms made from high quality recordings with high signal to noise ratios. Luscinia offers an ‘echo reduction’ feature which allows reducing the appearance of reverberations on the spectrograph. We generally measured elements with the same settings of echo reduction (bypassed) and dynamic range (70 dB), but especially with noisy elements we could greatly improve the spectrographic quality (i.e. the signal to noise ratio) in slightly varying these settings (Appendix 1b).

The motifs were segmented into elements according to decisions made by four people experienced with the analysis of zebra finch song: M.J. Holveck, A.C. Castro, K. Riebel and C. ten Cate. Elements are not always separated by silent intervals (e.g. Sossinka and Böhner, 1980; Williams and Staples, 1992) and birds can learn small units within complex elements (Williams, 1990) and break song production between units within a complex element (Cynx, 1990). Therefore, we also based our segmentation decisions on other cues than silent intervals like rapid changes in fundamental frequency, frequency modulation, harmonics’ structure, amplitude or noisiness (Williams and Staples, 1992). We analyzed all selected motifs of the two separate years in one go. Observers were blind with regard to brood size treatment and male status (i.e. tutee, tutor or foster father), except in some difficult cases where we had a second round of comparisons and observers specifically compared tutees’ to their tutor’s motifs to help in the decision.

Measures of song structure parameters and singing consistency

From the standard measurements of Luscinia sound analysis software we retrieved several parameters per motif pertaining to song complexity and singing performance (parameters listed in Table 1). For each of the frequency parameters (Fig 1) and for the parameter ‘harmonicity’, we obtained a value per element then used the mean of all elements within the motif, so that we had one value per parameter and per motif.

We also measured repertoire size which we labeled ‘element repertoire’ (Table 1). Earlier studies have used different classifications of element categories based on mostly visual categorizations (Price, 1979; Scharff and Nottebohm, 1991; Sturdy et al., 1999b; Williams and Staples, 1992; Zann, 1993), so we decided to use Luscinia’s features to arrive at a parameter based classification. To this end, we first calculated a distance measure between each pair of elements within the complete data set (i.e. including all tutees’, tutors’ and foster fathers’

Table 1. Labels and definitions of the 12 song structure parameters measuring song complexity or singing performance

	Parameter	Definition
Song complexity	Element number	Total number of elements per motif
	Element repertoire	Total number of unique element categories in a motif (repertoire size)
Singing performance	Motif duration	Total duration of motif (ms), including silent gaps between elements
	Sound duration	Duration of motif over which sound is present (ms), excluding silent gaps between elements
	Element rate	Number of elements delivered per second, calculated in dividing 'element number' by 'motif duration'
	Peak frequency average	Mean of frequency values (Hz) with the highest amplitudes per element
	Peak frequency maximum	Maximal value of frequency (Hz) with the highest amplitude per element
	Peak frequency minimum	Minimal value of frequency (Hz) with the highest amplitude per element
	Fundamental frequency average	Mean of fundamental frequency values (Hz) per element (approximate perceptual pitch)
	Fundamental frequency maximum	Maximal value of fundamental frequency (Hz) per element
	Fundamental frequency minimum	Minimal value of fundamental frequency (Hz) per element
	Harmonicity	Mean of the noisiness over the element duration; it measures the proportion of energy in the spectrum that falls within 50% of the phase cycle as would be expected if the signal was perfectly harmonic (a high value corresponds to a low noisiness)

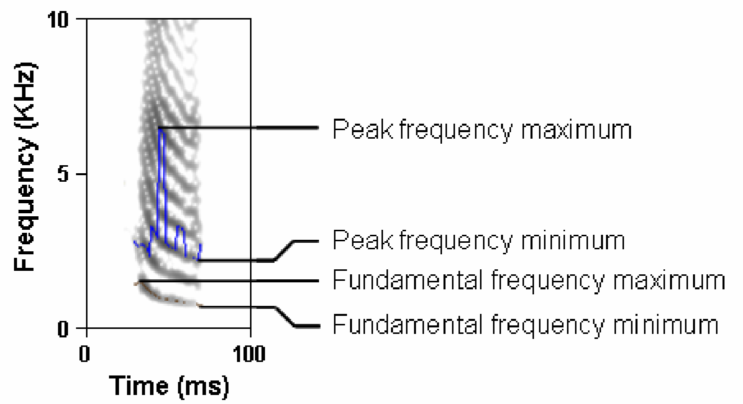

Figure 1. Spectrograms of a song element showing the automated measures of frequency parameters by the Luscinia software (see Table 1 for definitions).

motifs; 265 motifs for 3736 elements) using a refined dynamic time warping algorithm (see algorithm details at http://luscinia.sourceforge.net). We could adjust the influence of a parameter on the final distance measure. We based our choice of parameter weightings on the experience gained from a previous study (Lachlan et al., unpublished) and from several pilot trials to match our cluster analysis based element categorization (see below) and previously described categories (Price, 1979; Scharff and Nottebohm, 1991; Sturdy et al., 1999b; Williams and Staples, 1992; Zann, 1993). The resulting settings we applied as standard throughout this study are described in Appendix 1c.

Elements were clustered on basis of the distance measures of an UPGMA-algorithm (Unweighted Pair Group Method with Arithmetic mean). We used the smallest set of clusters showing element categories that had previously been described as being zebra finch element categories. The resulting grouping yielded 12 element categories including six well described ones (Table 2; Fig. 2) and two new element categories labeled based on their spectral and temporal features (Table 2; Fig. 2). The four remaining categories represented each only 34 elements or less (i.e. less than 1 % of the total number of elements) and received the label of ‘other’. Although our clustering analysis gave consistent results with previously described element categories, we want to emphasize that the UPGMA-tree presented here makes no inferences about the level of clustering used by the birds. Such assertion requires a direct test of zebra finch abilities to perceive, discriminate and categorize the elements in the set of clusters we obtained. However, the element categorization by Sturdy et al. (1999a) has been tested this way and our categorization method also identified these categories.

We estimated singing consistency in calculating the repeatability of each of the song structure parameter across the five motifs per bird following Lessells & Boag (1987) using a one-way ANOVA with parameter as the dependent variable and bird identity as between subjects factor. The standard error (1 SE) of the repeatability estimate R was calculated as the square root of the sampling variance of the intra-class correlation (Becker, 1984). We compared the repeatability estimates calculated separately for birds from small and large broods with a test of homogeneity (Sokal and Rohlf, 1995).

Measures of song sharing

If there are only a limited number of different elements and element combinations, some males will share some elements by chance. To be

Table 2. Phonology of each element category and equivalence with previously described categories

Element category	Phonology	Equivalent
Down-sweep	Frequency down-sweep	Slide note (Sturdy et al., 1999b)
Tone	Extended harmonic structure and unmodulated frequency over element duration	Tone element (Zann, 1993); Flat note (Sturdy et al., 1999b)
Noisy	Extremity tight or not clearly visible harmonic structures	Noise element (Zann, 1993)
Stack	Tight harmonic structure	Stack element (Zann, 1993)
Trill	Vertical rapid frequency modulation	Click note (Williams and Staples, 1992)
High	High fundamental frequency	High note (Sturdy et al., 1999b)
Short noisy	Extremely tight or not clearly visible harmonic structures of less than 33 ms	n.p.d.: not previously described.
High up-sweep	High fundamental frequency up-sweep	n.p.d.

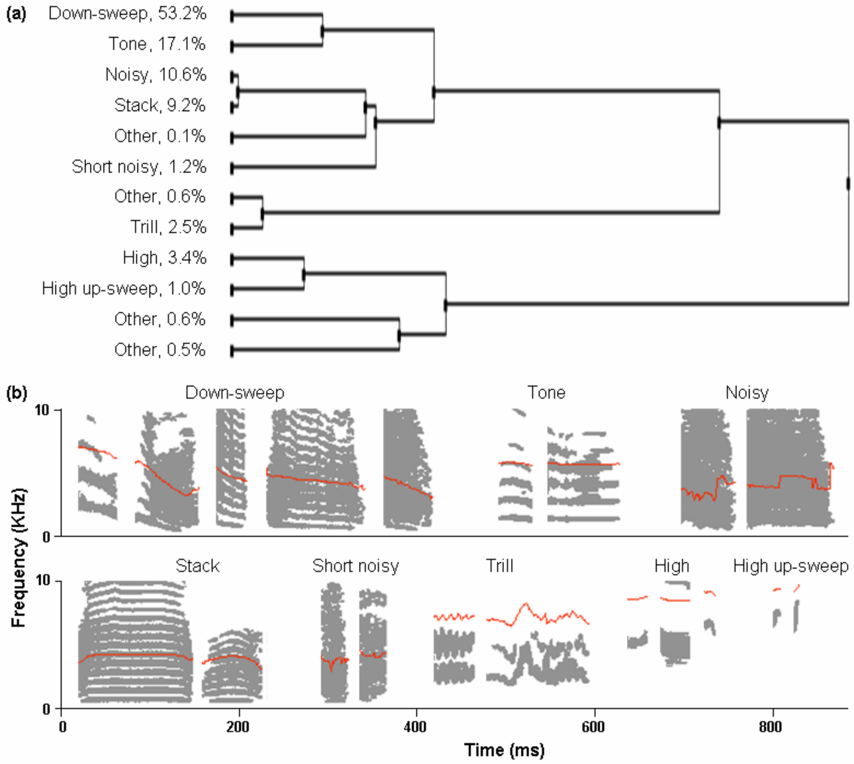

Figure 2. The UPGMA-tree clustering the 3736 elements from the 265 motifs of the 53 tutees, tutors and foster fathers and examples of elements for each of the 8 most represented categories (see Table 2 for phonology of each element category). Percentages of each element category are based on the 1730 elements of tutees' song motifs.

confident that a particular male has learned from another individual, the amount of sharing should be higher than expected by chance. For our analysis of what was learned when and from whom, we therefore did not only compare the amount of sharing between tutors and tutees and foster fathers and tutees, but also between random males from the colony (the foster father and song tutor pairs).

To measure the amount of song sharing between tutors and tutees, we compared each of the five motifs of each tutee with each of the five motifs of its tutor, resulting in 25 motif pairs per tutor-tutee pair. For song motif comparisons, we used the same distance measures and parameter weightings as above (details in Appendix 1c). A distance measure was calculated between all possible element pairs of the two compared motifs. Based on previous work (Lachlan et al., unpublished) and on visual inspection of element resemblance, we digitally set a threshold of distance measure at 0.12 below which elements were remarkably visually identical to each other (Fig. 3). Element pairs with a distance measure below this set threshold were therefore classified as shared elements. In case one element was involved in two different pairs which both got a distance measure below the set threshold, only the element pair with the lowest distance measure was classified as shared elements. Each element of the tutor's motif could be classified as shared element only once within a given motif-motif comparison.

To learn a song correctly a tutee has to copy the different elements of the model but also arrange them in the right order. To assess these two dimensions of learning, we calculated two song sharing scores for each of the 25 motif pairs per tutor-tutee pair. The first score measured the accuracy in element learning as the proportion of tutor's elements in the song motif of the tutee, which we labeled 'shared elements Tutee/Tutor'. The second score measured as an aspect of accuracy in syntax learning the proportion of tutor's element transitions in the song motif of the tutee, which we labeled 'shared transitions Tutee/Tutor'. To correct for the fact that element transition learning depends on learning the phonology of the involved elements in the first place, 'shared transitions Tutee/Tutor' must correct for the total number of shared elements. Therefore, we calculated it in dividing the number of identical transitions between the shared elements of the two motifs (see algorithm in Appendix 2) by the total number of shared elements minus 1, which is equivalent to the total number of possible shared element transitions between the two motifs. We also included in statistical analyses the proportion of shared elements as explanatory variable of

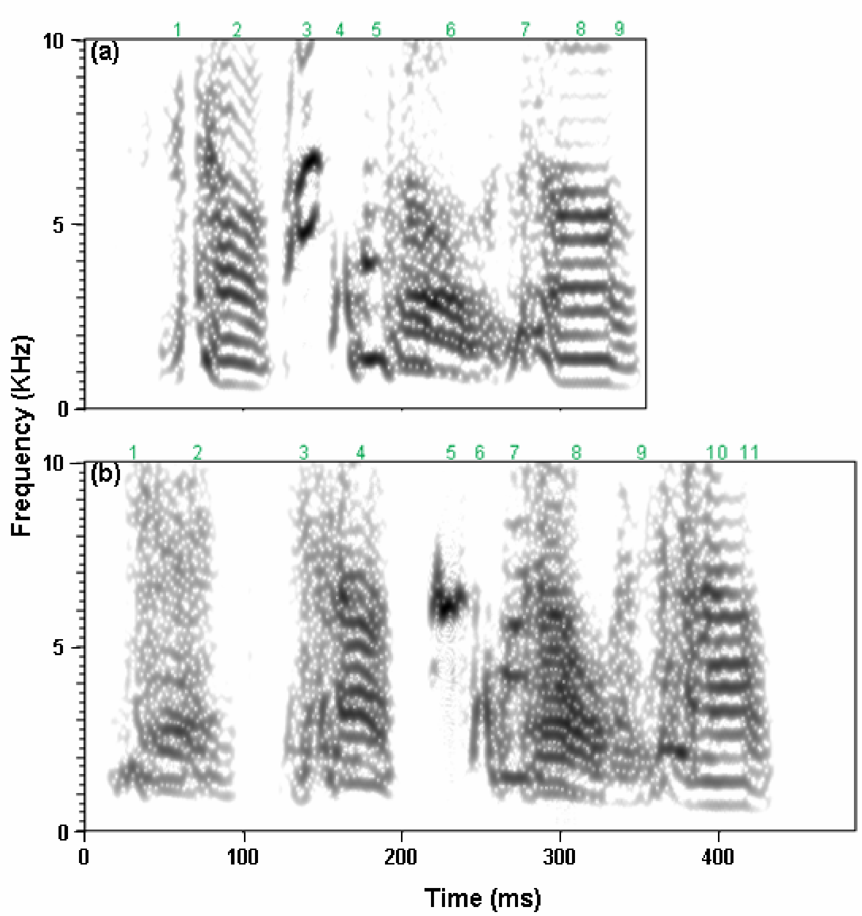

Figure 3. Spectrograms showing the song motifs of a tutor (a) and one of one of its tutees (b) to illustrate their numbers of shared elements and shared element transitions. Elements 1, 2, 5, 6, 7, 8 and 9 in the tutor motif are respectively shared with elements 3, 4, 7, 8, 9, 10 and 11 in the tutee motif[1]. Motifs thus share 7 elements and 5 element transitions.

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[1] In this example, the element 4 in the song motif of the tutor and the element 6 in the song motif of the tutee, despite their visual resemblance, were not classified as shared element by Luscinia software. Although the decisions made by Luscinia about when considering two elements as shared always agreed with visual inspection, one has to be aware that in few cases (as the one here) a human observer would probably have classified two elements as shared where Luscinia did not. Nevertheless, we used the computerized method because it has the advantage to be extremely accurate and gives an objective way to analyse the songs, not hampered by human subjective judgement.

the proportion of shared transitions.

To estimate improvised elements and element transitions in tutees' motifs, we calculated the proportions of tutee's elements and element transitions in the song motif of the tutor, which were respectively labeled 'shared elements Tutor/Tutee' and 'shared transitions Tutor/Tutee'. For these scores, each element of the tutee's motif could be classified as shared element only once.

The same scores were also calculated for the foster fathers ('shared elements Tutee/Foster father', 'shared transitions Tutee/Foster father', 'shared elements Foster father/Tutee' and 'shared transitions Foster father/Tutee') and for random overlap between song motifs in our study population. These last song sharing scores were labeled 'shared elements Tutor/Foster father' and 'shared transitions Tutor/ Foster father' and measured the proportions of foster father's elements and element transitions in the song motif of the tutor.

For each of the resulting 10 different song sharing scores, we used the means of the 25 repeated measurements per individual pair comparison in all analyses.

Statistics

We tested treatment effects on the 12 song structure parameters with repeated-measurements linear mixed models and on the amount of song sharing between tutors and tutees with generalized linear mixed models. In all models, we included the year of treatment as a fixed factor to test whether the effects of the treatment differed between the two years in which it was conducted. We first assessed the statistical significance of crossed random factors (birth nest and foster brood) in fitting a similar model without the random effect. We calculated its departure from the main model using maximum-likelihood theory (except for the proportions of shared elements where we had to use a quasi-binomial distribution and thus penalized quasi-likelihood theory). We retained the random factor 'birth nest' in only one model (footnotes Table 4). For all other models, the models without the random effects did not differ from the main model (all $P > 0.05$). We then sequentially deleted from models non-significant higher order interactions between factors and then non-significant factors until reaching the minimal adequate model (details in footnotes of Tables 4, 5 and 6).

To test the prediction that tutees learned their song only from their tutor, we tested differences in shared elements and element transitions between tutor-tutee, foster father-tutee and foster father-tutor comparisons. For the proportions of shared elements, we used one-way repeated measurements ANOVA followed by paired-sample t-tests. To correct for multiple comparisons, we applied sequential Bonferroni corrections. This procedure incurs a substantial reduction in the statistical power with a high probability of making a Type II error (false negative) for some of the tests (Nakagawa, 2004). We therefore also reported the effect sizes as Cohen's *d* computed using pooled standard deviation (Cohen, 1988; Rosnow and Rosenthal, 1996). For the proportions of shared element transitions, which could not achieve a normal distribution even after transformation (Table 7), we ranked the data and performed a Friedman test followed by post-hoc tests (Siegel and Castellan, 1988).

We ran all model analyses in R software (2.4.1 for Windows, http://www.r-project.org) and all other statistical analyses in SPSS software (12.0.1 for Windows, SPSS Inc., Chicago, IL, U.S.A.). We checked all measurements for normality before analysis with one-sample Kolmogorov-Smirnov test and transformed them when necessary (details in footnotes of Tables 3, 4, 5, 6 and 7). All statistical analyses were two-tailed with $\alpha = 0.05$ and all means (of untransformed variables) are given ± 1 SD except when stated otherwise.

RESULTS

For both treatments, the 12 song structure parameters were significantly repeatable between the five analyzed motifs per bird (all $R > 0.22$, $F_{12,52} > 2.35$, $P < 0.05$; Table 3). Next to between-individual variation in song, there were nonetheless systematic differences between the treatments. Tutees from small broods were more consistent in the parameter ‘sound duration’ which indicates the duration over which sound is present within a given motif ($Ts_1 = 2.1$, $P = 0.03$) and showed a tendency to sing more consistent ‘motif duration’ between motif renditions ($Ts_1 = 1.9$, $P = 0.051$) than tutees from large broods (Table 3). ‘Motif duration’ and ‘sound duration’ were highly correlated with each other (Person $r_{128} = 0.97$, $P < 0.0001$) as well as with ‘element number’ (both $r_{128} > 0.76$, $P < 0.0001$). Although tutees from small and large broods differed in singing consistency, they did not differ significantly in any of the song structure parameters measuring song complexity and singing performance (Tables 3 and 4).

However, when it came to the learning related scores, tutees from small broods learned more often elements that followed each others in the song motif of their tutor than tutees from large broods (‘shared

Table 3. Average values of song structure parameters for tutees from small and large broods and their singing consistency measured by repeatability estimates *R*

	Tutees from small broods			Tutees from large broods			*R* difference†	
	$\overline{X}$ ± 1 SD	$F_{12,52}$	*R* ± 1 SE	$\overline{X}$ ± 1 SD	$F_{12,52}$	*R* ± 1 SE	Ts_1	*P*
Element number	14.3 ± 5.4	32.2****	0.86 ± 0.06	12.4 ± 2.9	6.9****	0.54 ± 0.13	1.6	0.1
Element repertoire††	4.3 ± 0.7	4.1***	0.38 ± 0.14	4.2 ± 0.9	6.1****	0.50 ± 0.14	-0.3	0.7
Motif duration	888 ± 352	33.1****	0.87 ± 0.05	767 ± 157	5.2****	0.45 ± 0.14	1.9	**0.051**
Sound duration	714 ± 274	33.2****	0.87 ± 0.05	596 ± 109	4.0***	0.38 ± 0.14	2.1	**0.03**
Element rate	16.6 ± 3.7	54.4****	0.92 ± 0.04	16.7 ± 4.5	23.6****	0.82 ± 0.07	0.9	0.3
PF average	3171 ± 355	22.5****	0.81 ± 0.07	3195 ± 276	7.0****	0.54 ± 0.13	1.2	0.2
PF maximum	4759 ± 509	11.0****	0.67 ± 0.11	4741 ± 287	2.4*	0.22 ± 0.13	1.3	0.2
PF minimum	1959 ± 271	14.9****	0.74 ± 0.09	1947 ± 390	22.5****	0.81 ± 0.07	-0.4	0.7
FF average	1606 ± 389	66.7****	0.93 ± 0.03	1597 ± 565	121.3****	0.96 ± 0.02	-0.7	0.5
FF maximum	1921 ± 475	65.2****	0.93 ± 0.03	1889 ± 636	93.6****	0.95 ± 0.02	-0.4	0.7
FF minimum	1313 ± 336	72.2****	0.93 ± 0.03	1331 ± 472	133.1****	0.96 ± 0.02	-0.7	0.5
Harmonicity	-2.1 ± 0.3	13.9****	0.72 ± 0.10	-2.1 ± 0.3	18.7****	0.78 ± 0.08	-0.3	0.7

PF: peak frequency; FF: fundamental frequency.
* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

For each of the parameters, shown are grand means $\overline{X}$ for the 13 tutees from small broods and 13 tutees from large broods, which average the means of five motifs per bird.

†The difference in repeatability *R* between treatment groups is tested with homogeneity tests *Ts*.

††'Element repertoire' did not achieve a normal distribution for tutees from both small and large broods even after a log-transformation (both $Z_{64} < 1.95$, $P > 0.001$), but we gave test values for completeness (the non-parametric test equivalent of a one-way ANOVA, i.e. Kruskal-Wallis test, does not give the intra-class correlation coefficient which measures repeatability).

'Element rate' for tutees from small broods and 'harmonicity' for tutees from large broods were log-transformed to achieve normal distribution (both $Z_{64} < 1.35$, $P > 0.06$). We used the absolute values of 'harmonicity' prior to log-transformation since values were all negative. All other parameters were normally distributed (all $Z_{64} < 1.3$, $P > 0.8$).

transitions Tutee/Tutor': $F_{1,24} = 7.3$, $P = 0.01$; Table 5; Fig. 4a) and added fewer new elements between the elements they learned from the song motif of their tutor ('shared transitions Tutor/Tutee': $F_{1,24} = 6.8$, $P = 0.02$; Table 5). This was not because tutees from small broods either learned a higher proportion of elements from the song motif of their tutor than tutees from large broods ('shared elements Tutee/Tutor': $F_{1,24} = 1.9$, $P = 0.2$; Table 6; Fig 4b) or because they added a lower proportion of new elements to their song motif in comparison to the song motif of their tutor ('shared elements Tutor/Tutee': $F_{1,24} = 1.0$, $P = 0.3$; Table 6). Although birds that shared proportionally more element transitions with a tutor also shared proportionally more elements (effect

Table 4. Results of the linear mixed model analyses testing for the effect of brood size and year of treatment on song structure parameters

	Brood size			Year			Brood size x year		
	Effect size ± 1 SE	χ^2_1	P	Effect size ± 1 SE	χ^2_1	P	Effect size ± 1 SE	χ^2_1	P
Element number	0.12 ± 0.12	0.91	0.3	0.05 ± 0.12	0.16	0.7	0.27 ± 0.23	1.18	0.3
Element repertoire	0.02 ± 0.08	0.09	0.8	-0.16 ± 0.08	3.78	0.1	0.14 ± 0.17	0.65	0.4
Motif duration	0.10 ± 0.12	0.62	0.4	0.23 ± 0.11	3.63	0.1	0.28 ± 0.22	1.57	0.2
Sound duration	0.13 ± 0.11	1.33	0.2	0.20 ± 0.11	3.11	0.1	0.25 ± 0.21	1.35	0.2
Element rate	0.01 ± 0.09	0.02	0.9	-0.17 ± 0.09	3.71	0.1	-0.05 ± 0.17	0.08	0.8
PF average	-23 ± 120	0.04	0.8	-80 ± 119	0.45	0.5	81 ± 238	0.12	0.7
PF maximum†	39 ± 171	0.05	0.8	-8.46 ± 198	0.002	0.9	-57 ± 359	0.03	0.9
PF minimum	12 ± 126	0.01	0.9	-240 ± 118	3.84	0.1	70 ± 235	0.09	0.8
FF average	9 ± 183	0.002	0.96	-181 ± 180	0.99	0.3	-94 ± 359	0.07	0.8
FF maximum	32 ± 211	0.02	0.9	-164 ± 210	0.61	0.4	-116 ± 419	0.08	0.8
FF minimum	-18 ± 154	0.01	0.9	-204 ± 150	1.80	0.2	-64 ± 299	0.05	0.8
Harmonicity	0.01 ± 0.12	0.003	0.96	0.02 ± 0.12	0.03	0.9	0.37 ± 0.23	2.48	0.1

PF: peak frequency; FF: fundamental frequency.

Full model: brood size x year + bird identity, with a Poisson distribution for 'element number' and 'element repertoire' and a Gaussian distribution for the remaining song structure parameters. 'Motif duration', 'sound duration' and 'element rate' were log-transformed to achieve Gaussian distribution (all $Z_{129} < 0.85$, $P > 0.45$). All other parameters were normally distributed (all $Z_{129} < 1.35$, $P > 0.059$). We used the absolute values of 'harmonicity' since values were all negative.

†We retained 'birth nest' as a random factor in the model on 'peak frequency maximum' since the model without 'birth nest' differed from the main model: brood size x year + bird identity + birth nest + foster brood ($\chi^2_1 = 4$, $P = 0.047$).

'Brood size' and 'year' were binary variables (SMALL or LARGE and 2004 or 2005 respectively). 'Bird identity' was entered as random effect and always kept in the models to account for the five motifs per individual. The degrees of freedom reflect the comparison of the models with and without the tested parameter following sequential deletion of 'brood size x year', 'year' and 'brood size'.

of 'shared elements Tutee/Tutor' on 'shared transitions Tutee/Tutor': $F_{1,23} = 8.6$, $P = 0.01$; effect of 'shared elements Tutor/Tutee' on 'shared transitions Tutor/Tutee': $F_{1,23} = 7.7$, $P = 0.01$; Table 5), this relationship did not differ between treatments (non significant interactions between 'brood size' and 'shared elements Tutee/Tutor' or 'shared elements Tutor/Tutee'; see Table 5). The significant effect of brood size on the proportions of shared transitions was thus independent of the expected and observed overall positive relationship between proportions of shared elements and shared element transitions (Table 5).

In line with the literature, male tutees learned their song from the male song tutor they were housed with just after independence rather

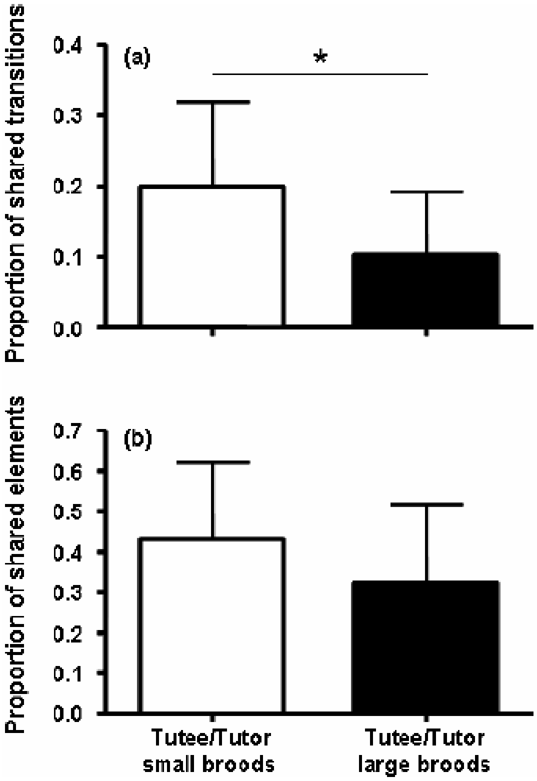

Figure 4. Effects of brood size manipulation on learning from tutor. Shown are (a) ‘shared transitions Tutee/Tutor’ and (b) ‘shared elements Tutee/Tutor’ for birds from small and large broods. Shown are grand means ± 1 SD; for every bars, n = 13.

than from their foster father during rearing (proportions of shared elements: $F_{2.5,70.3} = 19.8$, $P < 0.0001$, with Huyn Feldt correction; proportions of shared element transitions: Friedman test $Fr_4 = 54.1$, $P < 0.0001$; see results of post-hoc tests in Table 7; Fig. 5a,b). Tutees did not share a higher proportion of elements or element transitions with their foster father’s song motif than the observed random patterns of overlap in our study population (Table 7; Fig. 5a,b).

Although tutees did not learn from their foster father’s song motif, the higher the proportion of elements the song motifs of foster father and tutor shared, the higher the proportion of tutee’s elements in the tutor’s song motif was (effect of ‘shared elements Tutor/Foster father’ on ‘shared elements Tutor/Tutee’: $F_{1,24} = 5.0$, $P = 0.03$; Table 6).

Table 5. Results of the generalized linear model analyses testing for the effect of brood size, the proportions of shared elements between tutors and tutees, the scores measuring random overlap between song motifs and year of treatment on the proportions of shared element transitions between tutors and tutees

	Effect size ± 1 SE	*F*	d.f.	*P*
Shared transitions Tutee/Tutor				
Final model				
BS	0.07 ± 0.04	7.3	1,24	**0.01**
Shared elements Tutee/Tutor	0.26 ± 0.09	8.6	1,23	**0.01**
Rejected terms				
Shared transitions Tutor/Foster father	-0.03 ± 0.12	0.05	1,22	0.8
Year	-0.005 ± 0.04	0.01	1,21	0.9
BS x Shared elements Tutee/Tutor	-0.04 ± 0.19	0.04	1,20	0.8
BS x Shared transitions Tutor/Foster father	0.001 ± 0.33	0.0001	1,19	0.99
Shared elements Tutee/Tutor x Shared transitions Tutor/Foster father	-2.17 ± 1.27	2.9	1,18	0.1
BS x Year	-0.14 ± 0.09	2.6	1,17	0.1
Shared elements Tutee/Tutor x Year	-0.09 ± 0.25	0.1	1,16	0.7
Shared transitions Tutor/Foster father x Year	0.28 ± 0.35	0.7	1,15	0.4
BS x Shared elements Tutee/Tutor x Shared transitions Tutor/Foster father	-4.35 ± 3.09	2.0	1,14	0.2
BS x Shared elements Tutee/Tutor x Year	0.29 ± 0.51	0.3	1,13	0.6
BS x Shared transitions Tutor/Foster father x Year	-0.80 ± 0.83	0.9	1,12	0.4
Shared elements Tutee/Tutor x Shared transitions Tutor/Foster father x Year	-1.39 ± 3.72	0.1	1,11	0.7
BS x Shared elements Tutee/Tutor x Shared transitions Tutor/Foster father x Year	-5.95 ± 7.64	0.6	1,10	0.5
Shared transitions Tutor/Tutee				
Final model				
BS	0.06 ± 0.04	6.8	1,24	**0.02**
Shared elements Tutor/Tutee	0.26 ± 0.09	7.7	1,23	**0.01**
Rejected terms				
Shared transitions Tutor/Foster father	-0.06 ± 0.12	0.3	1,22	0.6
Year	-0.01 ± 0.04	0.03	1,21	0.9
BS x Shared elements Tutor/Tutee	-0.08 ± 0.20	0.1	1,20	0.7
BS x Shared transitions Tutor/Foster father	0.16 ± 0.32	0.2	1,19	0.6
Shared elements Tutor/Tutee x Shared transitions Tutor/Foster father	-1.57 ± 1.19	1.7	1,18	0.2
BS x Year	-0.02 ± 0.09	0.1	1,17	0.8
Shared elements Tutor/Tutee x Year	-0.15 ± 0.27	0.3	1,16	0.6
Shared transitions Tutor/Foster father x Year	0.16 ± 0.42	0.2	1,15	0.7
BS x Shared elements Tutor/Tutee x Shared transitions Tutor/Foster father	-2.83 ± 3.09	0.8	1,14	0.4
BS x Shared elements Tutor/Tutee x Year	0.54 ± 0.62	0.8	1,13	0.4
BS x Shared transitions Tutor/Foster father x Year	-1.31 ± 1.00	1.7	1,12	0.2
Shared elements Tutor/Tutee x Shared transitions Tutor/Foster father x Year	1.40 ± 5.26	0.1	1,11	0.8
BS x Shared elements Tutor/Tutee x Shared transitions Tutor/Foster father x Year	15.78 ± 10.29	2.4	1,10	0.2

BS: brood size.

Full model: brood size x proportion of shared elements x score measuring random overlap between song motifs x year, with a Gaussian distribution. The proportions of shared element transitions were arc-sine transformed (both $Z_{25} < 0.6$, $P > 0.8$). ‘Brood size’ and ‘year’ were binary variables (SMALL or LARGE and 2004 or 2005 respectively). We used arc-sin transformation of ‘shared elements Tutee/Tutor’, ‘shared elements Tutor/Tutee’ and ‘shared transitions Tutor/Foster father’ in analyses.

This effect was independent of treatment (non significant interaction between ‘brood size’ and ‘shared elements Tutor/Foster father’; see

Table 6. Results of the generalized linear model analyses testing for the effect of brood size, the scores measuring random overlap between song motifs and year of treatment on the proportions of shared elements between tutors and tutees

	Effect size ± 1 SE	*F*	d.f.	*P*
Shared elements Tutee/Tutor				
Final model				
Brood size	0.46 ± 0.33	1.9	1,24	0.2
Rejected terms				
Shared elements Tutor/Foster father	0.28 ± 1.06	0.1	1,23	0.8
Year	-0.43 ± 0.34	1.6	1,22	0.2
Brood size x Shared elements Tutor/Foster father	3.64 ± 2.41	2.3	1,21	0.1
Brood size x Year	0.66 ± 0.67	1.0	1,20	0.3
Shared elements Tutor/Foster father x Year	2.99 ± 2.61	1.3	1,19	0.3
Brood size x Shared elements Tutor/Foster father x Year	-6.82 ± 5.42	1.6	1,18	0.2
Shared elements Tutor/Tutee				
Final model				
Shared elements Tutor/Foster father	2.44 ± 1.12	5.0	1,24	**0.03**
Rejected terms				
Brood size	0.25 ± 0.30	1.0	1,24	0.3
Year	-0.50 ± 0.30	2.8	1,22	0.1
Brood size x Shared elements Tutor/Foster father	2.70 ± 2.22	1.5	1,21	0.2
Brood size x Year	-0.13 ± 0.61	0.05	1,20	0.8
Shared elements Tutor/Foster father x Year	3.80 ± 2.31	2.8	1,19	0.1
Brood size x Shared elements Tutor/Foster father x Year	-3.75 ± 4.80	0.6	1,18	0.4

Full model: brood size x score measuring random overlap between song motifs x year, with a quasi-binomial distribution. ‘Brood size’ and ‘year’ were binary variables (SMALL or LARGE and 2004 or 2005 respectively). We used arc-sin transformation of ‘shared elements Tutor/Foster father’ in analyses.

Table 6) and was absent on the other learning related scores. The amount of song sharing between foster father and tutor did not affect the proportion of tutee’s element transitions in the song motif of the tutor (no effects of ‘shared transitions Tutor/Foster father’ on ‘shared transitions Tutor/Tutee’) and did not facilitate learning of elements and element transitions from the tutor’s song motif (no effects of ‘shared elements Tutor/Foster father’ and ‘shared transitions Tutor/Foster father’ on ‘shared elements Tutee/Tutor’ and ‘shared transitions Tutee/Tutor’ respectively; Tables 5 and 6).

DISCUSSION

The treatment affected song along several dimensions. Birds that

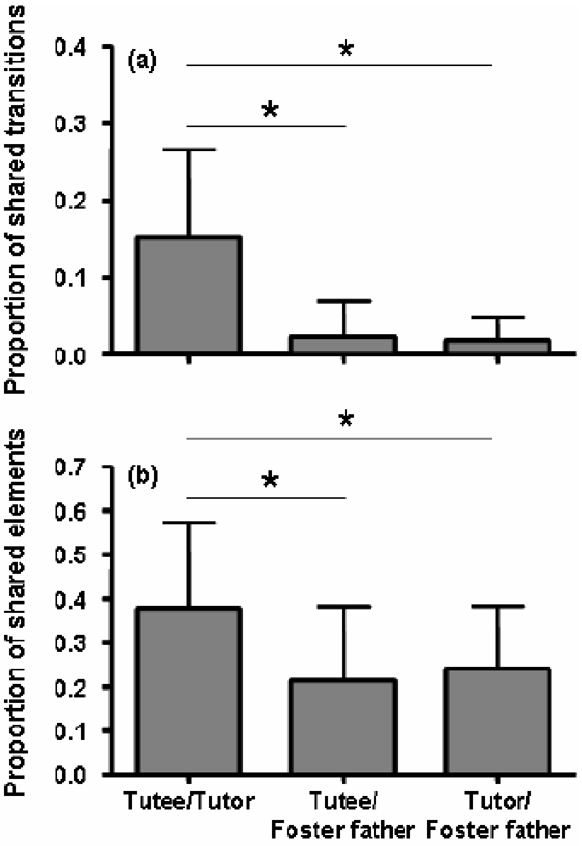

Figure 5. Comparison of the amount of song sharing between tutors and tutees, foster fathers and tutees, and foster fathers and tutors. Shown are (a) 'shared transitions Tutee/Tutor', 'shared transitions Tutee/Foster father' and 'shared transitions Tutor/Foster father' and (b) 'shared elements Tutee/Tutor', 'shared elements Tutee/Foster father' and 'shared elements Tutor/Foster father' for birds of both treatment groups. Shown are grand means ± 1 SD; for every symbols, n = 26.

originated from large broods showed less consistent singing than the birds from small broods as most noticeable in the parameter 'motif sound duration'. Increased variation in this parameter not only indicates that a bird changes the total amount of sound produced per motif delivery, but is also an indirect measure of consistency of song delivery. To arrive at a smaller or larger total in sound, a bird needs to alter the number or duration of elements. In addition to this general

Table 7. Paired comparisons of the song sharing scores for the proportions of shared elements and shared element transitions

Paired comparisons			Shared elements				Shared element transitions	
			t_{25}	P	Adjusted $\alpha^{\dagger}$	Effect size††	Absolute difference†††	Effect size††
Tutee/Foster father	vs.	Tutor/Foster father	0.7	0.5	0.005	0.17	0	0.11
Foster father/Tutee	vs.	Tutee/Foster father	1.3	0.2	0.006	0.16	5	0.03
Tutor/Foster father	vs.	Foster father/Tutee	1.8	0.1	0.006	0.38	5	0.07
Tutee/Tutor	vs.	Tutor/Tutee	2	0.1	0.007	0.24	6	0.04
Tutee/Tutor	vs.	Tutor/Foster father	3.1	0.005	0.008	0.80	53*	1.59
Tutee/Tutor	vs.	Tutee/Foster father	4.8	0.0001	0.01	0.90	48*	1.48
Tutor/Tutee	vs.	Tutor/Foster father	4.9	0.0001	0.013	1.12	47*	1.60
Tutee/Tutor	vs.	Foster father/Tutee	5.7	0.00001	0.017	1.15	53*	1.50
Tutor/Tutee	vs.	Tutee/Foster father	5.7	0.00001	0.025	1.20	42*	1.48
Tutor/Tutee	vs.	Foster father/Tutee	7.6	0.0000001	0.05	1.51	47*	1.51

†The *P*-values of each paired-sample t-tests is compared to the adjusted level of significance following sequential Bonferroni (e.g. in first row, the two song sharing scores do not differ since the *P*-value = 0.5 is higher than the adjusted α-level = 0.005). The paired comparisons between scores in the first four rows are not significant, while all the remaining ones are.

††Calculated as Cohen's d: $0 < d < 2$. The effect size is generally considered to be large when $d \geq 0.8$ (Cohen, 1988).

†††For the post-hoc tests following Friedman test, 32 is the critical difference above which the difference between two scores is significant; $*P < 0.05$.

difference in performance, males from large broods had learned less accurately than males from small broods. In motifs of males from large broods, fewer of the element transitions corresponded to the tutor's than was observed for the males from the small broods. It is important to stress that each of the experimental male-male tutee pairs learned their song from the same tutor (one from a small and large brood each with a random song tutor). Thus the observed differences can not be accounted by differences in song tutoring quality or in the number of tutees learning from the same tutor and thus must be strongly linked with the differences in the early rearing environment. Our results thus support the developmental stress hypothesis (Nowicki et al., 1998; Nowicki et al., 2002a). The natural variation of early nutritional and social environment created through brood size manipulation affected the outcome of song syntax learning and induced condition dependence of song features in males. The brood size manipulation did not affect element learning from the song tutor nor song complexity, as measured by repertoire size, which is consistent with the findings of Gil et al. (2006). Instead, our findings showed that other aspects of song were affected that to date have been little studied in the context of male-male competition and female mate choice. Our results show that singing consistency and the outcome of different syntax learning can act as long-term signals of male past condition. Interestingly, while the variation in singing consistency could be detected by conspecifics without knowledge of the model song, the assessment of variation in the accuracy in syntax learning might need knowledge of the model song.

There is some evidence in several songbird species that consistent vocal performance can be an indicator of male quality (Christie et al., 2004; Lambrechts and Dhondt, 1986). Song production involves complex muscular contractions and the fine coordination of special respiratory mechanisms (Suthers and Zollinger, 2004). This performance is doubtless difficult to sustain in stereotyped fashion over an extended bout of singing, and fatigue may lead to increasing variation between song renditions. The degree to which a bird can sustain consistency may thus reflect levels of stamina and neuromuscular coordination that are intrinsic to its current condition. In zebra finches, we previously found that an aspect of singing consistency, namely motif stereotypy (defined as singing the same set of invariant elements in an invariant order between individually distinctive song motifs), and the proportion of sound within motif positively predicted an important part of the variation in male morphology (between 22 and 51% depending on morphological traits, Chapter 2 of this thesis), suggesting that such song features may depend on body size. Birds reared in large broods in our study population showed reduced size during development and at adulthood (Chapter 5 of this thesis), an effect also found by other studies manipulating brood sizes (de Kogel, 1997; Naguib et al., 2004).

The muscular contractions involved in singing undeniably need energy. In a previous study, we found that the birds from large broods had a higher resting metabolic rate, which means higher energy requirements, than birds from small broods (Chapter 3 of this thesis). Although evidences of between-individual differences in the metabolic cost of song production (e.g. song output and amplitude) in bird species are mixed (Gil and Gahr, 2002), consistent singing could be another song feature limited by energetic constraints, additionally or alternatively to motor constraints. Understanding the exact constraints or costs underlying singing consistency definitely requires further research. But for now, we showed that singing consistency is a condition-dependent trait in zebra finches, which is likely to be a remnant of the past developmental history of the birds.

Such a condition-dependent trait might be used as a signal containing reliable information on male quality in female mate choice (Dawkins and Krebs, 1978). Singing consistency has been suggested to play a role in female mate choice in songbirds. Indeed, highly consistent singing has been positively correlated to male extra-pair success in chestnut-sided warblers *Dendroica pensylvanica* (Byers, 2007). Zebra finch females, when tested with the songs analyzed here, showed assortative song preferences according to their own and the males' rearing background. They were thus able to discriminate between the songs of males from small and large broods (Chapter 5 of this thesis). Spencer et al. (2005b) also reported female preferences to be dependent on male condition (stress treatment during early male development was either administration of corticosterone or restriction of food, Spencer et al., 2003). In both experiments, the songs presented to females were natural songs consisting of several motifs, allowing females to potentially assess difference in consistency in motif sound duration between the songs of non-stressed and stressed males. Motif sound duration is likely to play a role in female mate choice since we previously found the amount of sound per unit of time (of one song relatively to another) to be predictive of female preference strength (Chapter 2 of this thesis). Variation in consistency in motif sound duration among males is therefore a potential signal used by females in their mate choice decisions.

The second marked difference we found between the songs of males from small and large broods was the accuracy in syntax learning (i.e. learned order of elements shared with the model song). This parameter might be hard to detect for females without prior knowledge of the model song unless there were specific types of transitions males failed to learn. This raises the questions of experience dependency of female preferences. Evidence is accumulating that early song exposure influences adult acoustic perception in songbird females (Riebel, 2003a; Riebel, 2003b). Presumably, because they had learned song features typical of their population, female song sparrows, *Melospiza melodia*, were able to discriminate well-learned and poorly learned songs (Nowicki et al., 2002b). However, it is unclear whether such learned preferences for specific songs included learning to prefer specific syntactical arrangements too. At least one study however suggests that early exposure to song might also affect female preference functions for an aspect of phonological syntax. Draganoiu et al. (2002) found that isolate female canaries, *Serinus canaria*, showed stronger preferences for fast trills than experienced females, which showed less pronounced preferences for extreme stimuli.

However, even if learning processes play an important role in the development of song preferences, knowledge of a specific male's model song does not have to be a prerequisite to judging its quality if there are some general rules constraining element order females can also infer from other songs (for instance some transition probabilities between element categories can be less or more frequent than others). Some males might be better in following those rules or in transgressing them, which could signal their quality and/or enhance their attractiveness. In consequence, a song that conforms to or deviates from the most common species-specific syntactical rule might be perceived as such and used in mate choice decisions. In support of this hypothesis, Braaten et al. (2006) showed that zebra finches are capable of discriminating conspecific songs that differ only in element order. Verzijden et al. (2007) also reported that zebra finches can learn to discriminate songs that differed in the position of an odd element in a series of repeated elements. Interestingly, Collins (1999) showed that zebra finch females prefer songs with a different element order between motifs over stereotyped songs with a fixed element order between motifs, suggesting a preference bias for songs deviating from the species-specific syntactical rule.

As Gil et al. (2006), we found no effects of brood size manipulation on element learning. However, birds did not only copy the elements of their tutors, but also the element sequencing and there was an effect of the treatment on this aspect of syntax learning. Earlier studies have described the phenomenon of "chunking" whereby groups of elements seem to be copied in concert (ten Cate and Slater, 1991; Williams and Staples, 1992) and which is highly suggestive of a tight link between a concerted learning of elements' phonology and sequential positions. Moreover, in zebra finches' adult crystallized song, syntactical rules seemed to be based on sequences of elements (Lachlan et al., unpublished). But there are also observations that the element sequences learned from the tutor's song can be rearranged differently among birds tutored by the same adult singer (Williams, 2004). Clearly, the issue of how tightly element transition and element learning are linked deserves further study. The observation that more element learning meant more transition learning provides support that the two are linked, but controlling for this in our analysis we still found an additional effect of the brood size on the proportion of shared element transitions. Thus although birds from small and large broods did not differ in the accuracy of element learning, they differed in the way they rearranged learned element sequences, which suggests syntax learning differences. Clearly, further research is needed if we are to understand both the mechanisms underlying these differences and their function in communication.

However, if events associated with the development of song can have a continuing impact on song performance in adults, notably on the ordering of elements or song types (reviewed in Williams, 2004), the interesting question arises, namely which inaccuracies derive from constraints operating during the memorization or during the motor phase of song learning (Slater, 1989). In zebra finches, these two phases overlap (Jones et al., 1996; Slater et al., 1988) making difficult to delineate the exact nature of these constraints. Neural, physiological, physical and social constraints have been suggested as, probably non-mutually exclusive, constraints for the production and maintenance of the male song signal (reviewed in Gil and Gahr, 2002). A stress during development can affect the size of song control brain nuclei (Buchanan et al., 2004; Nowicki et al., 2002a; Spencer et al., 2005a), but the relationship between this effect and the effect we report on syntax learning remains to be established. The syntax of zebra finch songs can be affected by perturbation of the auditory feedback (Funabiki and Konishi, 2003) necessary to achieve a good copy of the tutor song during the motor phase of song learning (Brainard and Doupe, 2002). Auditory feedback could be perturbed by limits on vocal performance capacities, for instance owing to physical constraints, and/or by limits on song practice owing to social aggressive interactions (Gil and Gahr, 2002). Similar constraints could also act up to adulthood in perturbing the continuous auditory feedback necessary to produce species-specific song syntax (Okanoya and Yamaguchi, 1997).

Against this background, the finding that birds from large broods showed higher inaccuracy in syntax learning than birds from small broods might suggest that the syntax of the model song we had made available to tutees in a pair-wise design was more difficult to learn or to produce for the birds from large broods. Both the element structure and the organization of the elements within the song may influence the choice of model to be copied (Marler and Peters, 1977; Marler and Peters, 1988). We can not refute that the birds from large broods if they had had the choice might have selected an ‘easier’ song to learn from (calibration hypothesis, Podos et al., 2004) and that song tutor selection would have been guided by the overlap between the tutor and foster father songs (this study, Clayton, 1987; Mann and Slater, 1994; Slater and Mann, 1990). Consistent with this idea, Podos (1996) showed that an experimental increase of the rates of element repetition above normal rates make song syntax difficult to learn for swamp sparrow males, resulting in ‘broken syntax’.

While it has been suggested that brood size manipulation in the zebra finch could be a stressor too weak to drastically affect song development, thus resulting at best in subtle differences in song learning and song features (Gil et al., 2006), we here provide evidence that this manipulation induced condition dependence concomitantly in an aspect of song learning and a song feature not routinely analyzed. It remains to be investigated whether those song features we found to be long-term signals of condition in zebra finches are used in female mate choice or whether females use other aspects of song not covered by our analysis (Chapter 5 of this thesis, Nowicki et al., 2002b; Spencer et al., 2003).

A full understanding of how song may act as a reliable indicator of male quality requires more investigations into the multiple constraints and mechanisms controlling song development, production and maintenance. But for now, this experiment has shown that certain aspects of song production lastingly reflect male past condition. A trade-off between the quantity and the quality of offspring could thus provide an explanation for the evolution of reliable signals, which have to date, eluded satisfactory explanation (Gil and Gahr, 2002). These questions will need to be addressed in more detail if we are to understand condition dependence of mating signals in those taxa where cultural transmission and preference learning of the mating signal coexist.

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APPENDIX

Appendix 1. Standard settings used in Luscinia software (http://luscinia.sourceforge.net)

1a. Parameter settings for maximizing the estimated fundamental frequency of elements.
We digitally varied 'fundamental frequency bias' between 0.5 and 2.0 to obtain the best estimation of the fundamental frequency of each specific element. 'Fundamental frequency bias' simply changes the way that different hypotheses about the fundamental frequency are weighted: a high value will tend to decrease the measured fundamental frequency.

1b. Parameter settings for maximizing the signal to noise ratio of noisy elements.
The estimation of the fundamental frequency was sometimes difficult to render accurately especially for noisy elements despite the 'fundamental frequency bias' feature implemented in Luscinia (see Appendix 1a). We could achieve a better estimation of the fundamental frequency of these elements while reducing reverberation on the spectrograph in varying the 'echo reduction' and dynamic range within a range of 0-50 % (track-back set at 50 ms) and 50-90 dB respectively.

1c. Parameter weightings for calculation of distance measures between each pair of elements of two song motifs.
We digitally set the weighting of element duration and fundamental frequency at 1, peak frequency and harmonicity at 0.2. We weighted each parameter value time point by its corresponding amplitude, in effect lowering the importance of soft sounds and noisy parts of an element.

Appendix 2. Algorithm used to calculate the number of shared element transitions between two motifs

$$T_{ab} = \sum_{i=1}^{m-1} \left(\frac{2 \cdot \sum_{j=1}^{n-1} p_{ij}^{T}}{\sum_{j=1}^{n} p_{ij}^{S} + \sum_{j=1}^{n} p_{(i+1)j}^{s}} \right)$$

where T_{ab} = shared element transitions between motifs a and b;
a has m elements; b has n elements;
p^{T}_{ij} = transition sharing between transition i in a and j in b;
p^{T}_{ij} = 1 if transition is shared; p^{T}_{ij} =0 if not;
p^{S}_{ij} = element sharing between element i in a and j in b.

The algorithm corrects for the probability that transitions could be shared by accident, simply because the elements themselves were shared multiple times in the two motifs. In other words, if the elements in a transition are shared multiple times within the motif, the overall transition score is lower, because it "means less" that the transition is there.