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

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General introduction, thesis overview and general conclusions

Marie-Jeanne Holveck

Chapter 1

GENERAL INTRODUCTION

Sexual selection and between-individual variation

Sexual selection is an evolutionary force which allows explaining the origin and maintenance of extravagant traits like elaborate songs, conspicuous courtship displays or bright coloration (Darwin, 1871). Such conspicuous traits seem *a priori* to reduce rather than to enhance the survival chance of their bearer, for instance through increased predation risks. However, these traits can evolve because their bearers are more competitive over access to mates and/or attractive towards members of the opposite sex, and therefore have a reproductive advantage. Numerous correlational and experimental studies from many taxa have now confirmed that males with enhanced ornamentation or other preferred attributes, have a mating advantage arising from female mate choice (Andersson, 1994). In accordance with the handicap principle of signaling (Grafen, 1990; Zahavi, 1975), variation in male quality determines the costs incurred with increased investment in ornaments, leading to condition-dependent expression of sexual traits, condition (or quality) in this case being 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). However, not only advertising for mates but also choosing mates is likely to incur costs. Therefore, one might also expect that variation in female quality should determine how investment in mate choice, a costly behaviour in many species (Pomiankowski, 1987), is optimized, leading to condition-dependent expression of preferences (Bakker et al., 1999; Jennions and Petrie, 1997). However, while between-male variation in sexually selected traits and in mating success is well-documented, variation in female mating preferences and in reproductive decisions has received relatively less attention from theorists and empiricists alike (Jennions and Petrie, 1997; Widemo and Saether, 1999).Why is it so?

The fact that female mating preferences are not as conspicuous as male ornamentation is probably not the only reason why there is so little documentation on variation in female choice as yet. Mate choice is a complex behaviour. The outcome of mate choice (i.e. mating pattern) not only depends on individual mating preferences but also on the extent to which they can be expressed (e.g. availability of mates), which makes difficult to determine mating preferences solely from the outcome of mate choice (Jennions and Petrie, 1997; Widemo and Saether, 1999). In addition, until recently most studies of sexual selection worked with the assumption that individuals should aim for the highest-quality males available in order to maximize their fitness and tended to discard the variation in female preferences within populations as noise in the data. However, females within a population often show marked variation in mating preferences (Jennions and Petrie, 1997; Widemo and Saether, 1999). It is this variation in female mating preferences and reproductive decisions that will affect the strength and direction of sexual selection and consequently the evolution of preferred male traits (Jennions and Petrie, 1997; Widemo and Saether, 1999). Sexual selection is a co-evolutionary process between females and males so ignoring the presence of variation in females overlooks a key aspect of this process.

Despite the recent increase of studies reporting evidence of between-female variation in their preferences, very little is known about what causes and maintains this variation (Badyaev and Qvarnström, 2002; Cotton et al., 2006; Jennions and Petrie, 1997). Several factors have nevertheless been proposed to account for the observed variation in female preferences like genetic factors, developmental trajectories and environmental factors (for an extensive and detailed list, see Badyaev and Qvarnström, 2002; Cotton et al., 2006; Jennions and Petrie, 1997; Ryan et al., 2007; Widemo and Saether, 1999). Ryan et al. (2007) suggested that these factors might influence the ability to accurately assess potential mates (e.g. decoding of mating signals) and introduce variability to preferences (e.g. state or condition, context and past experiences via learning).

Female mating decisions commonly involve the assessment of multidimensional signals providing a wide range of messages and involving different sensory modalities like acoustic and visual ones (Candolin, 2003; Hebets and Papaj, 2005). Which signals females will pay more attention to may depend on whether females are predominantly looking for direct or indirect benefits (e.g. resource holding potential versus genetic benefits, Candolin, 2003) as well as on the information content and reliability of different signals. It has specifically been proposed that multiple signals provide information on male condition over different time-scales (multiple message hypothesis, Møller and Pomiankowski, 1993). Short-term signals will respond rapidly to momentary variations in condition, while long-term signals lastingly reflect past condition, for instance during the period of juvenile growth and development. If females differ in how they use or rank such signal arrays, this could be one reason for why they show different mating preferences. Several factors could contribute to such inter-individual differences. Next to a genetic base to differences in female preferences, several non-genetic factors can also affect female preferences for different signal variants: e.g. their physiological state and condition (Cotton et al., 2006) and past and present ecological and social contexts, which may also influence which signals females pay more attention to (Candolin, 2003; Jennions and Petrie, 1997; Wagner, 1998). Past experiences can strongly influence subsequent preferences on a short and a long time scale. Female preferences can change based on recent experience gained from previous interactions with males (Collins, 1995; Marler et al., 1997), or due to age (rather than experience *per se*, e.g. Kodric-Brown and Nicoletto, 2001). On a longer time scale, early sensory learning has been well demonstrated as an important source of variation in adult preferences in the context of sexual imprinting in the olfactory domain in mammals (Owens et al., 1999) and in the visual or auditory domain in birds (Riebel, 2003a; Riebel, 2003b; ten Cate and Vos, 1999).

Against this background, the aim of this thesis is to address the role of context and developmental condition as two poorly understood factors in causing variation in female preferences. In particular, I experimentally investigated whether, how and to what extent female preferences and their ensuing mating decisions covaried with i) the quantity (i.e. single vs. multiple) and quality of mating signals in combination with the context in which these signals were presented (Chapter 2) and with ii) females' and males' early ecological and social experiences i.e. the interplay between early condition and learning (Chapters 3 to 5). I addressed these questions in songbirds, a model which is particularly interesting to study in this context since learning in the vocal domain occurs both for song production and perception (Riebel, 2003a; Riebel, 2003b; ten Cate, 2000) and preferences for the song are commonly recognized as a strong selection factor (Andersson, 1994; Searcy and Yasukawa, 1996). Therefore in addition to genetic, condition and cultural-transmission dependency of song variation (Catchpole and Slater, 1995), the developmental trajectory and learning process underlying the acquisition of song preferences are likely to influence mating decisions and thus select for specific variants of learned songs.

Song, preference, learning and developmental stress in songbirds

Songbirds show many morphological and behavioural secondary sexual traits, and feature prominently in studies of sexual selection. Although male song undoubtedly plays an important role in female choice (Catchpole and Slater, 1995; Searcy and Yasukawa, 1996), the relative weighting of song versus other male phenotypic features such as morphological traits and display intensity remains poorly understood (Collins et al., 1994; Patricelli et al., 2003). Moreover, surprisingly little is known about which structural features of a song determine its attractiveness to females, and how such features might relate to male quality. The likely reason for this lack of knowledge is that birdsong , like other complex mating displays, varies along many dimensions and each of its features may signal different aspect of male quality (Gil and Gahr, 2002).

Many songbirds have a sensitive period early in life during which exposure to song influences the details of song that a male later produces (Catchpole and Slater, 1995) and that a female later prefers (Riebel, 2003a; Riebel, 2003b). Since variation in male song is mostly culturally inherited, this has raised the question of how this trait may reliably signal male quality. The developmental stress hypothesis (Buchanan et al., 2003; Nowicki et al., 1998; Nowicki et al., 2002a) proposed that learned song can indicate male quality because the development of brain structures mediating song learning and production occurs during the period of fastest development, i.e. when young birds are most vulnerable. Therefore, male learned song could act as a long-term signal of condition and females may gain reliable information about how well males fared in the face of an early developmental stress.

Several empirical studies have now demonstrated effects of various 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). Supporting the function of song as an indicator of male past developmental history, females have been shown to prefer well-learned songs to poorly-learned songs in song sparrows (Nowicki et al., 2002b) and the songs of early non-stressed males to the ones of early stressed males in zebra finches (Spencer et al., 2005b). However, there is limited evidence that developmental stress causes differences in male song learning in songbirds although it is a fundamental prerequisite of the hypothesis. To date, there have been only two studies that empirically examined the effect of developmental stress on song learning (Gil et al., 2006; Nowicki et al., 2002a), and only one could reported such an effect (Nowicki et al., 2002a). In addition, it is remarkable that both the original hypothesis and empirical tests so far neglected the development of female song preferences, which like male song have a strong learned component (Riebel, 2003a; Riebel, 2003b). Thus, very little is known on whether socially learned preferences are sensitive to early non-social environmental factors.

The zebra finch, *Taeniopygia guttata*, as model species and brood size manipulation as an experimental tool to induce developmental stress

I decided to address the question(s) of effects of the early environment on male traits and females preferences in the zebra finch. The zebra finch is a well established avian model system in current studies on sexual selection, song learning and sensory development. Zebra finches undergo a rapid development, which makes it easy to follow a bird from hatching to maturation and mating. Zebra finches fledge at about 20 days post-hatching, reach nutritional independence at about 35 days, and are sexually mature at around 100 days (Zann, 1996). In zebra finches, only males sing, but both males and females show vocal learning. Male song learning takes place between 25-90 days of age, but the most sensitive period for song acquisition in males ranges mainly from days 35 to 65 (for reviews of male song learning see Jones et al., 1996; Slater et al., 1988). Female song preference learning seems to parallel the time course for song acquisition in males since it ranges mainly between 25 and 70 days of age (Riebel, 2003a). Adult females show repeatable preferences for the song to which they were exposed between 35-65 days of age (Riebel, 2000).

The current literature on mating preferences in zebra finches, despite intensive experimental studies and ample examples of how several male morphological and behavioural traits are involved in mate choice, for example song rate and beak colour (Collins, 1994; Collins and ten Cate, 1996; Forstmeier and Birkhead, 2004; ten Cate and Mug, 1984; Zann, 1996), also illustrates how difficult it is to test the relative importance of acoustic and visual signals provided by males in female mate choice. However, because of the general good background information on mate choice, zebra finches therefore provide a good model system with which to systematically examine the context dependency of different signal dimensions as well as what attributes make songs attractive to females and what the preferred song features say about the singer.

A neglected aspect in this context is the possible interactions between ecological and social factors during development on male song, and on female song preference learning as well as on male and female reproductive decisions. In this thesis I addressed these questions. To manipulate early (nestling) condition I chose brood size manipulations. Among the different methods that have been reported to be efficient in varying nestling condition (i.e. brood size manipulation, direct manipulation of food availability, corticosterone administration or parasite infection), the reduction and enlargement of brood sizes present the advantage to manipulate early condition within an ecological meaningful range. Zebra finches were raised in brood sizes within the natural species-specific range (Zann, 1996), namely in either small (2-3 chicks) or large (5-6 chicks) broods. Brood size manipulation indirectly manipulates food intake during development. An enlargement of brood size induces an increase in nestling competition for access to food (e.g. Neuenschwander et al., 2003) and / or an absence of full parental compensation in food provisioning (Stearns, 1992), which result in a deterioration of nestling body condition. Brood size manipulation has also been shown to affect adult morphology (de Kogel, 1997; Naguib et al., 2004), male secondary sexual traits (de Kogel and Prijs, 1996), survival (de Kogel, 1997) and fitness, for instance the age of the first reproduction (Alonso-Alvarez et al., 2006). In this thesis, I explored the long-term consequences of this phenotypic manipulation early in life on male learned song and female learned song preferences.

THESIS OVERVIEW

The aim of this thesis was to examine the causes of variation in male mating signals and female mating preferences and decisions, using the zebra finch as a model system. I investigated whether female weighing of different mating signals (i.e. acoustic vs. visual) depended upon the context in which they were presented (Chapter 2), whether females could judge male quality based on the information content of the acoustic signal only (Chapters 2 and 5) and whether the information content of the signal as well as the decoding of mating signals was dependent upon males' and females' early social learning and condition

(Chapters 3 to 5), which was manipulated through varying ecological and social factors during development.

In Chapter 2, I examined the relative importance of different signal modalities and their context dependency in female mating preferences. Song is well established to play an important role in mate choice in zebra finch, but its relative role with regard to visual cues and display intensity is unclear as different studies used different test paradigms. Female mating preferences were thus tested across three different widely used testing paradigms, presenting male song only (operant conditioning tests and phonotaxis tests) or full courtship displays (association tests with live males). Other than in classic discrimination task where positive and negative reinforcers like food reward and time out periods are used to test subject discrimination abilities, operant conditioning tests use the song itself as a reinforcer. Females can thus actively control their exposure to songs by pecking keys. Preferences were assessed by comparing the relative frequencies with which one stimulus was chosen over another. In phonotaxis tests and association tests with live males, the number and total duration of approaches to either stimulus (songs or males respectively) were taken as measure of preference. Females showed significant and consistent preferences either for live males or their songs across the three tests. The song structure parameters that predicted female preferences best were context-independent and also predicted male morphology. Besides validating the different designs to assess preferences, these results show that song structure independent of total song output must contain sufficient information on the singer for female mate choice.

In Chapters 3 to 5, I investigated the combined effect of prior ecological and social experiences on individual phenotypes, song learning, song preferences and reproductive decisions in adulthood. Bird nestling and adult condition was experimentally varied by brood size manipulations, which indirectly manipulates food intake during development. After nutritional independence, birds were placed in mixed-treatment, mixed-sex groups of four individuals with an unfamiliar, unrelated, mated adult male that acted as ‘song tutor’ during the song acquisition phase.

Chapter 3 is concerned with investigating the potential metabolic constraints underlying the long-term effects of developmental condition on survival and fitness prospects. In a collaborative project (with Simon Verhulst from Groningen University), I found that the metabolic rate of 1-year-old zebra finches was higher when they had been reared in large broods. In combination with earlier observations in these species (de Kogel, 1997), that birds reared in large broods live for a shorter time, our finding suggests that metabolic efficiency may play a role in mediating the long-term survival and fitness consequences of rearing conditions.

In Chapter 4, I examined whether prior ecological and social experiences affected male song learning and song features, while including multiple song parameters to account for songs' multidimensionality. I recorded all adult males from the brood size manipulation experiment once they reached sexual maturity and analyzed several song features and the amount of elements and element transitions learned from the song tutor with computer-based acoustic analysis. I found that the birds from large broods learned fewer element transitions as they appear in the song of their tutor than the birds from small broods. In addition, the birds from large broods showed less consistent singing than the birds from small broods. These results therefore support the developmental stress hypothesis (Buchanan et al., 2003; Nowicki et al., 1998; Nowicki et al., 2002a): the natural variation of early nutritional and social environment created through brood size manipulation affected the accuracy with which the syntactical structure of the song is learned and induced condition dependence in male singing consistency.

In Chapter 5, I examined how females' early social learning and condition contributed to variation in their mating preferences and reproductive decisions. Using the well-established operant conditioning set-up (Chapter 2), I tested preferences of adult females reared in either small or large broods for songs of males reared in either small or large broods. Females showed condition-dependent preferences: females from small broods (i.e. high condition females) preferred males from small broods, but females from large broods (i.e. low condition females) preferred males from large broods over males from small broods. In a breeding experiment, females paired up with males assortatively with respect to rearing conditions had shorter egg laying latency than females in disassortative pairs, which is consistent with the song choice results. It also suggests that males and females with a similar developmental background accepted each others faster as partners, which can increase their lifetime reproductive success (Alonso-Alvarez et al., 2006). Importantly, the perception that females from large broods had of male condition was not impaired since all females invested more in egg mass when paired with males from small broods. Thus females from both rearing conditions agreed on the phenotypic quality of the males they were paired to, despite showing different preferences. These results provide evidence that the early environmental variation induced variation in individual condition, which translated to substantial phenotypic plasticity, notably in the direction of female mating preferences.

GENERAL CONCLUSIONS

This thesis shows that female mating preferences and their reproductive decisions but not their perception of male condition vary substantially according to variation in early social learning and condition they experienced. Therefore, developmental condition is an important source of variation in female mating preferences and reproductive decisions. The effects could go as far as females actively preferring low quality males. This condition-dependency of the direction of female preferences indicates that not all females aimed for the highest-quality males available. Such condition-dependent preferences are likely to result into assortative mating by condition, which, as reported, can be beneficial in term of an increase of lifetime reproductive success for both individuals in the pair. This is at first counter-intuitive as sexual selection theory predicts that females should always prefer the highest-quality males when they are given the choice, but recent theoretical modelling work suggests that a preference for low-quality individuals could be an adaptive strategy under certain selection pressures. When the competition over mate access is high or when mutual mate choice exists, low-quality females are likely to be out-competed by higher-quality females or to be avoided or deserted by males in favour of high-quality females. Thus in those cases, an active preference for low- instead of high-quality males could allow females to save time and energy and thus increase their fitness.

Male song appeared to play a major role in mediating the observed variation in female preferences. The difference in preference direction between females from small and large broods occurred while females had access to the song only and this variation in their song preferences was confirmed by the timing of their reproductive decisions. Furthermore, preferences for song were consistent across different test contexts and translated into identical preferences for the singer. Taken together, these findings strongly suggest that the song contains sufficient information on the singer for female mate choice. In support of this, I showed evidence that some song features reflected male morphology and/or male past developmental history. Thus, males' learned song can act as long-term signal of their past condition thereby providing reliable information to females about how well males fared in the face of an early developmental stress.

To conclude, this thesis provides evidence that both male song learning and female socially learned preferences are co-dependent on variation in early social and non-social environmental factors. Both mating preferences and aspects of song production lastingly reflect individual past condition. Therefore, an approach that takes into account past experiences and state-dependent life-history traits might prove extremely fruitful to further our understanding of sexual selection and of the evolutionary dynamics between preferences and sexually selected, culturally transmitted traits.

Chapter 2

Preferred songs predict preferred males: consistency and repeatability of zebra finch females across three test contexts

Marie-Jeanne Holveck and Katharina Riebel
In *Animal Behaviour*, 2007, 74, 297-309

Chapter 2

ABSTRACT

Male mating signals are often multidimensional, potentially providing multiple messages to females. However, the relative importance of different signal dimensions and their context dependency are poorly understood. Even in a well-studied species such as the zebra finch, *Taeniopygia guttata*, an important avian model for the study of mate choice, there is little consensus on the relative weighting of visual versus acoustic signals in mate choice. We therefore tested the consistency and repeatability of female mating preferences across different test contexts, presenting male song only or full courtship displays. We concurrently conducted a detailed analysis of male song characteristics and morphological traits. Females' individual preferences were consistent across three commonly used binary test paradigms (operant and phonotaxis tests with songs and association tests with live males). Preference direction was thus independent of test contexts. Preference strength was repeatable only between the operant and live male tests, possibly because these two tests allowed active interaction with songs or males whereas exposure to songs in the phonotaxis test was passive. The song structure parameters that predicted female preferences best were context independent and also predicted male morphology. We conclude from the combined results that song structure (in addition to song rate or absolute output as previously suggested) does contain sufficient information on the singer for female mate choice. We suggest that the earlier focus on song rate rather than song content might partly account for the differences between studies in the importance attributed to acoustic versus visual signals.

KEYWORDS: context-repeatable mating preference, male choice test, mate choice, multiple signals, operant test, phonotaxis test, song structure, *Taeniopygia guttata*, zebra finch.

Despite intensive research on female mate choice and the evolution of secondary sexual traits over the past few decades (Andersson, 1994), surprisingly little is known about within-population variation in female preference (Jennions and Petrie, 1997; Widemo and Saether, 1999). Females' mating decisions are often based on multidimensional signals providing a wide range of messages and involving different sensory modalities (e.g. acoustic and visual) propagated on different temporal and spatial scales (Candolin, 2003; Hebets and Papaj, 2005). Two types of not mutually exclusive functional hypotheses have been suggested to account for multiple ornaments and mating signals: they could provide (1) back-up messages or (2) multiple messages on different aspects of male quality. Within-population variation in female mating preferences (i.e. the response to sample stimuli) can arise through variation in condition or context and/or genetic, cultural or phenotypic compatibility (Jennions and Petrie, 1997; Qvarnström, 2001; Widemo and Saether, 1999). If females within a population differ in whether they predominantly look for direct or indirect benefits (e.g. resource-holding potential versus genetic benefits), they may pay attention to different signals to choose the most suitable male (Candolin, 2003). Differences in female mating preferences are also likely to be influenced by social factors such as the intensity of male-male or female-female competition (Jennions and Petrie, 1997; Widemo and Saether, 1999). For instance, mate density may affect female choosiness (i.e. the time and effort the female is prepared to invest in finding and assessing mates), the cost of sampling and sampling strategies (i.e. the decision rule adopted in mate assessment).

Both the multiple message and back-up signal scenarios might lead to context dependency of the weighting of particular signals (Candolin, 2003; Wagner, 1998), a poorly understood issue (Candolin, 2003; Jennions and Petrie, 1997). It has rightly been pointed out that different test methods might inadvertently lead to context-specific weighting of different signals. For example, they may allow different levels of interaction between males and females (Waas and Wordsworth, 1999), which may influence which traits females pay more attention to. Hence, some of the documented variation in female preferences might not arise from differences between females or populations but might be an artefact arising from the wide range of different methods used to measure female mating preferences (Wagner, 1998). One of our aims in this study was to examine the impact of the test method on estimated female preferences in zebra finches, *Taeniopygia guttata*.

In songbirds, one of the most intensively studied taxa in current studies of mate choice behaviour, there is ample evidence that male song is important for female choice (Catchpole and Slater, 1995; Searcy and Yasukawa, 1996). However, the relative weighting of song versus other phenotypic features of males such as morphological traits and display intensity remains poorly understood (Collins et al., 1994; Patricelli et al., 2003). Furthermore, surprisingly little is known about which structural features of a song determine its attractiveness to females, and how such features might relate to male quality. The current literature on mating preference in the zebra finch, a species that has been intensively studied experimentally in the laboratory, illustrates rather well how difficult it is to test the relative importance in female mate choice of acoustic and visual signals provided by male courtship displays such as song rate and beak colour (Collins, 1994; Collins and ten Cate, 1996; Forstmeier and Birkhead, 2004; ten Cate and Mug, 1984; Zann, 1996). Zebra finches therefore provide a good model with which to compare the consistency and the repeatability of female mating preferences as well as to examine what exact attributes make songs attractive to females and what the preferred song features say about the singer. To this end we tested females across three different commonly used test paradigms involving single or multiple sensory modalities. An operant test with song as reinforcer (Houx and ten Cate, 1999; Leadbeater et al., 2005; Riebel, 2000; Riebel et al., 2002) and a phonotaxis test both tested preferences for the acoustic signal alone (Clayton, 1988; Miller, 1979a; Miller, 1979b; Neubauer, 1999). A spatial association test with a choice between two live males presented a test situation where the acoustic signal was combined with additional static and dynamic visual and behavioural signals. The latter is by far the most commonly used mate choice test type (reviewed in Forstmeier and Birkhead, 2004). We assessed both preference consistency (identical direction) and repeatability (identical strength) of the within-individual preference for a specific stimulus in different test contexts. Subsequently, we compared the outcome of an in-depth song analysis with female song preferences and male morphological traits to test whether structural song parameters can predict female preferences on the one hand and male quality on the other.

If multiple signals act as multiple messages then we expect more consistency and repeatability in female preferences when comparing the two tests involving song only (operant and phonotaxis tests) than when comparing tests involving single versus multiple sensory modalities (i.e. phonotaxis or operant versus live male tests). However, if multiple signals act as back-up signals, females should not differ in their preference direction between the tests involving song only and the live male tests.

SUBJECTS AND REARING CONDITIONS

We used wild-morph domesticated zebra finches (*N* = 35, 17 males and 18 females) from an outbred breeding colony at Leiden University, The Netherlands. The birds were kept on a 13.5:10.5 h light:dark schedule (lights on between 0700 and 2030 hours CET) at 20-22ºC and 35-50% humidity. Birds had ad libitum access to a commercial tropical seed mixture enriched with GistoCal mineral and vitamin powder (Beaphar B.V., Raalte, The Netherlands), drinking water and cuttlebone. This basic diet was supplemented three times a week with 3-4 g of 'egg food' (Witte Molen, B.V., Meeuwen, The Netherlands) per bird, twice a week with branches of millet and once a week with germinated tropical seeds. All subjects had been reared by their parents in standard laboratory cages (80 x 40 cm and 40 cm high) equipped with a nestbox. At 65 days (i.e. after the peak of the sensitive period for song learning, Slater et al., 1988), they were moved to be housed in single-sex groups with eight to nine individuals per cage (100 x 60 cm and 60 cm high). All subjects were about 2 years old (20 ± 5 months, *N* = 35) and had no breeding experience when the experiments started. Subjects that encountered each other in preference tests were unfamiliar to each other and had a coefficient of relatedness less than 0.125.

PREFERENCE CONSISTENCY AND REPEATABILITY

Methods

Stimulus preparation

Following Sossinka and Böhner (1980), we call an individual's specific syllable sequence 'the motif'. A 'song' consists of a series of introductory syllables followed by several repetitions of the motif (range 1-10 for nondirected songs). For the preparation of the stimulus songs, we recorded nondirected songs of 17 males. Males were placed singly in a cage (70 x 30 cm and 45 cm high) on a wooden shelf (100 x 55 cm) at a height of 120 cm in a sound attenuation chamber (100 x 200 cm and 220 cm high). Songs were recorded at a distance of 75 cm from the cage (Sennheiser MKH40 microphone, Wedemark, Germany and Sony TCD5 Pro II cassette recorder, Tokyo, Japan). Songs were digitized (25 000-Hz sample rate) using Signal/Rts software

(Engineering Design, Belmont, MA, U.S.A.) and a lowpass filter (cutoff frequency 10 000 Hz; Frequency Devices 900C/9L8B, Haverhill, MA, U.S.A.). We chose one natural song per male and digitally deleted those introductory syllables at the beginning that were additional to the number of introductory syllables that occurred as part of the motif within songs. All chosen songs had four motifs. Using the Praat sound analysis software (version 4.2.07 forWindows, freely available from http://www.praat.org) songs were highpass filtered at 500 Hz (smoothing = 100 Hz) to remove low-frequency background noise. Amplitudes were root mean-square equalized (peak digitally scaled to 1 with Praat software).We formed 18 unique stimulus dyads without combining the same two males twice. However, we assigned each male two ($N = 15$) or three times ($N = 2$) to a different stimulus dyad by matching song duration as much as possible (average difference in song duration between dyads ± SD = 0.41 ± 0.3 s).

Preference tests

Each of the 18 females was tested with one of the 18 unique stimulus dyads in the operant test, the phonotaxis test and the live male test. The test order was fully balanced with regard to test type. With a total of 18 females and six possible combinations, there were three females per possible test order. At least 7 days of rest separated two consecutive tests (time between tests 1 and 2 ± SD = 10.1 ± 3.8 days; time between tests 2 and 3 = 13.7 ± 6.8 days).

The experimental set-up of the operant test has been described in detail elsewhere (Houx and ten Cate, 1999; Riebel, 2000; Riebel and Smallegange, 2003; Riebel et al., 2002). Briefly, there were two different songs associated with two different response keys (Fig. 1a). Pecking either of the two keys triggered a playback of a song via a loudspeaker (Blaupunkt CB 4500, Hildesheim, Germany) with a maximum amplitude of 70 dB at 30 cm from the speaker (re 20 μPa; CEL-231 sound level meter, fast response F and low range A LO settings, Lucas CEL Instruments Ltd, Hitchin, Herts, U.K.). Experimental cages were placed singly in sound attenuation chambers (100 x 200 cm and 220 cm high) on a shelf (100 x 55 cm) at a height of 70 cm. Observation was possible through a one-way mirror. A custom-built minicomputer with an Oki MSM6388 (Tokyo, Japan) sound chip controlled the playback, automatically swapped the stimuli between the two keys each night and kept a data log. The tested females were transferred to the operant cages between 0830 and 0930 hours. The red

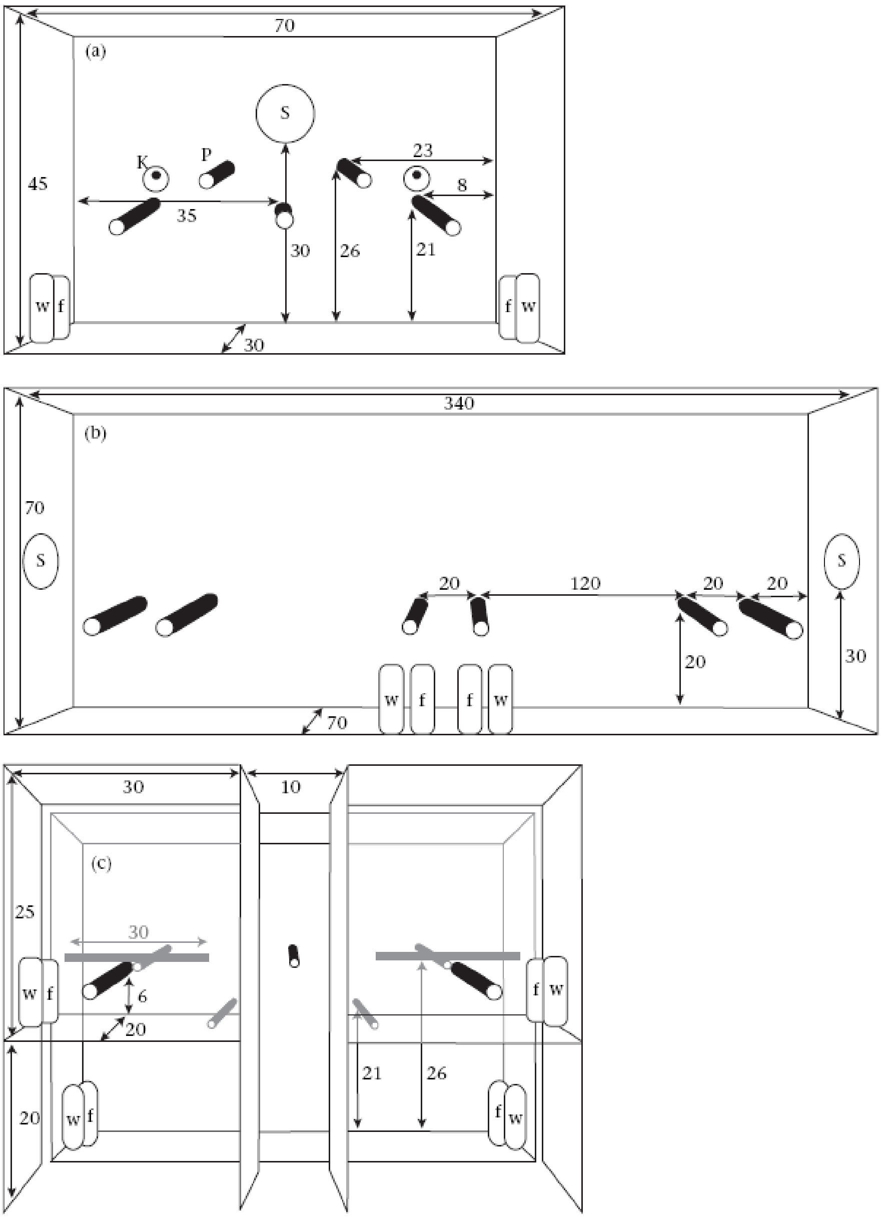


Figure 1. Schematic front views of the test designs used for (a) operant, (b) phonotaxis and (c) live male tests; (c) shows two side cages in front of a central test cage with the same dimensions as in the cage used for the operant test (except for perch height). Cages were made from plywood but with the long front side made from wire mesh. S: speaker; K: pecking keys; P: perch; w: water; f: food. All measures in cm.

LED lights of the keys were switched on only during the days of the training phase. Four of 18 females learned to peck the keys by autoshaping after they accidentally pecked the keys. Those that had not started to do so after 2 days underwent a training procedure (one or two daily sessions of about 20 min each for 1-8 days, $\overline{X}$ ± SE = 3.6 ± 2.3 days, *N* =13, one female did not learn to perform the operant task). During training, we first drew the attention of the birds to the keys by flashing the LED lights. We rewarded birds with playback (same songs as stimulus songs) when they perched near the key, then when exploring the plastic disk surrounding the key, until they finally started to peck the keys proper. Once a female pecked both keys regularly on the same day after the training session, the preference test started the next day and lasted 2 full days. As before, the stimuli were swapped between the two sides daily but the red LED lights were now switched off.

For the phonotaxis test, a long test cage (340 x 70 cm and 70 cm high; Fig. 1b) was placed at the far end of a rectangular experimental room. The size of the cage allowed the female to approach or evade the stimulus. All sides of the cage were solid plywood and padded with foam for sound attenuation, except the long front side which had wire mesh. Loudspeakers were attached to each end wall behind a central opening. The cage had six perches. Perching on the two outermost perches was counted as an approach (i.e. within 40 cm of the loudspeaker); the remainder of the cage including the floor was defined as neutral. Parallel to the long side of the test cage at a distance of 170 cm, a dark plastic partition with a small central opening (20 x 5 cm) divided the room and hid the observer and the playback equipment from the cage. We edited stimuli with Cool edit 2000 software version 1.0 for Windows (Syntrillium Software Corporation, Scottsdale, AZ, U.S.A.). The playback stimuli consisted of a 1-min sequence of four repetitions of the same song with 5-s silent intersong intervals. This song rate is within the naturally observed range for zebra finches (Sossinka and Böhner, 1980; Zann, 1996). The two songs assigned to a stimulus dyad were edited as one 2-min stereo file with one stimulus on the left channel (for the first minute) and the other one on the right (for the second minute). This allowed continuous alternating playback of the two stimuli via the two loudspeakers (same speaker and settings as in the operant test) during the 14-min test (see below) using the loop mode of the CD-player (Venturer DM8802-00, Venturer Electronics Inc, Markham, Ontario, Canada, U.K. and JVC AXR562BK stereo amplifier, JVC, Yokohama, Japan) We randomly assigned both the cage side for the first playback and the first stimulus used at the beginning of each test.

For the live male test, the test cage holding the female was situated in one of the sound attenuation chambers also used for the operant tests. Stimulus males were placed into two small cages in front of the long front side of the test cage (Fig. 1c) so that they could not see each other. The tested female could perch near either male or avoid associating with them altogether when perching in the centre or on the floor of her cage. The two small cages with the stimulus males could be obstructed from the female's view by a solid grey plastic panel that we could pull up by a string from outside the experimental chamber.

In both phonotaxis and live male tests, we moved the birds to their respective test cages to acclimatize the day before the preference test. We conducted test the following day between 0830 and 1230 hours. Each test started with a baseline observation period (range 15-17 min) without stimulus exposure. At this stage, the plastic panels were still lowered between side cages and test cage in the live male test (Fig. 1c). This was followed by 14 min with the plastic panels raised in the live male tests or with 14 alternating song playbacks (i.e. seven per stimulus) in the phonotaxis test. After a second stimulus-free period (range 15-17 min; lowered screen in the live male test) during which the stimuli were reversed between sides, another 14 min of stimulus exposure followed. Every 14 min, stimulus exposure started when the tested female was in the centre of the cage. Females had to visit at least one of the two stimuli in each 14-min test session and had to spend at least 10% of the total test time in front of either stimulus for the data to be included in the analyses. Of 18 females, 17 fulfilled these criteria.

The study was approved by the local Ethical Committee (Dierexperimentencommissie Universiteit Leiden).

Statistical analyses

We defined the preferred stimulus as the stimulus that was preferred in at least two of the three tests. Preference strength was set equal to the preference ratios (choices for the preferred stimulus divided by the total number of choices). Preference ratios were either the relative number of keypecks for the preferred song (operant test) or (for comparison with earlier studies) the relative time spent close to the preferred stimulus in the phonotaxis and live male tests. However, we were interested in seeing whether preferences in these two tests were also reflected by the number of times a female approached a specific stimulus. Thus for the phonotaxis and live male tests, we also calculated visit ratios (number of visits to the preferred stimulus divided by the total number of visits). Female activity level was defined as the absolute number of keypecks (operant test) or visits (phonotaxis and live male tests).We log-transformed activity level for statistical analyses. Visit intensity was expressed as the percentage change from the previous event, i.e. the number of switches between stimuli (N_s) in relation to the total number of visits (V) minus two to correct for the fact that the last visit of both test sessions (2 days for the operant test, two times 14 min for the phonotaxis and live male tests) could not be followed by a switch (visit intensity = $(100 \times N_s)/(V - 2)$). Visit intensity can range from 0% (only one stimulus received repeated visits within each session) to 100% (the visited stimulus changed at each visit). Visit time was calculated as the time spent near both stimuli as a percentage of the total test time (applicable only for the phonotaxis and live male tests). For statistical comparisons of the three preference tests, all proportion and percentage data (i.e. preference ratios, visit ratios, visit intensity and visit time) were normalized (calculating Z scores) to account for differences in mean and variance.

In our comparison of female preference across the three tests, we made a distinction between the consistency of preference direction (a nonparametric measure with binary scoring 0 or 1: the stimulus with the highest preference ratio was defined as preferred) and the repeatability of preference strength (quantitative measure of preference ratios). To test the consistency of preferences across the three tests we used Cochran Q test. The repeatability of preference ratios across the three tests was calculated following Lessells and Boag (1987) using a one-way ANOVA with preference ratios as the dependent variable and female identity as the between-subjects factor. The standard error of the repeatability estimate R was calculated as the square root of the sampling variance of the intraclass correlation (Becker, 1984). We also estimated repeatability between the three preference ratio sets two at a time resulting in a significance level of $\alpha = 0.025$ after a correction for multiple tests and compared them with the repeatability estimates of the other measures of female choice behaviour. Effects of test order (between-subjects factor) and type (within-subject variables) were tested on all measures of female choice behaviour with two-factor mixed ANOVAs. All statistical analyses were two tailed and calculated using SPSS statistical software, release 10.0.7 (SPSS Inc., Chicago, IL, U.S.A.); means are given ± 1 SD.

Results

Only one female did not respond in both the operant and phonotaxis tests and was excluded from further analyses. Female preferences were consistent across the three tests (Cochran Q test: $Q_2 = 6$, $N = 17$, exact $P = 0.11$; Table 1). Across all tests ($N = 51$, 17 females x 3 tests), there were only three instances where females changed the direction of their preferences. It was always the preference in the live male test that differed from the preferences in the operant and phonotaxis tests (i.e. the preference ratio changed from >0.5 to <0.5 for females 2, 5 and 12; Fig. 2, Table 1). Preference ratios were independent of test type and order and significantly repeatable between operant and live male tests ($R = 0.56$, Table 2).

Table 3 gives the means of all measures of female choice behaviour per test. Preference ratios were highly correlated with visit ratios both in the phonotaxis (Pearson correlation: $r_{15} = 0.71$, $P < 0.01$) and live male tests ($r_{15} = 0.75$, $P < 0.001$). None of the measures was affected by test order, except for visit ratio (calculated only for the phonotaxis and live male tests, see Methods and Table 2). Visit ratios increased with test order suggesting that a previous exposure to the stimuli (either to songs only or to males) subsequently reinforced the number of visits to the preferred stimulus; but note that for this comparison the operant test was not taken into account, which means that, depending on test order position of the operant test, comparisons had to be made between first and second, second and third or first and third test. Test type significantly affected activity levels with the highest value for the operant test (Table 2), but preference ratios were independent of activity levels in all three tests. Preference ratios were not correlated with number of keypecks (operant test: $r_{15} = -0.29$, $P = 0.26$) or number of visits (phonotaxis test: $r_{15} = -0.42$, $P = 0.10$; live male test: $r_{15} = -0.38$, $P = 0.14$).

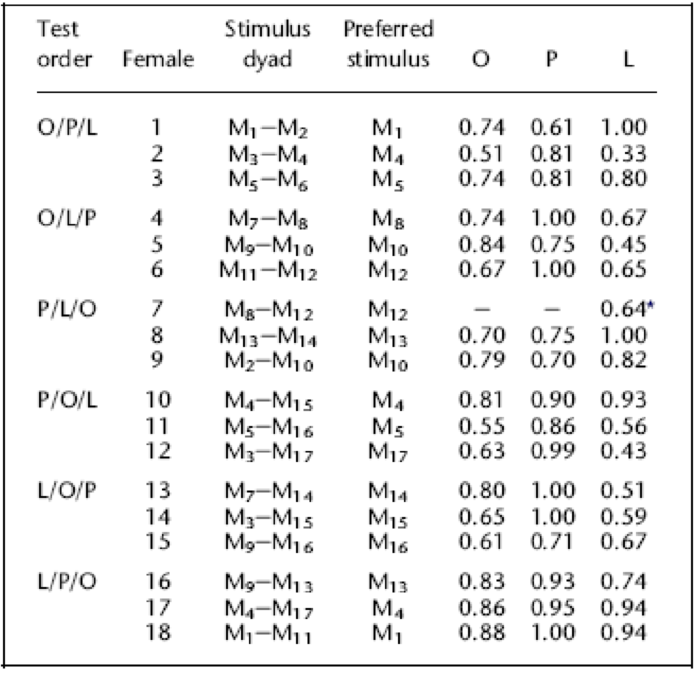
PREFERENCES AND MALE TRAITS

Methods

Song analysis

All songs were analysed blind to male identity by M.J.H. Table 4 and Fig. 3 explain the catalogue of song measures in detail. Sound density was assessed with a gating function (Signal/Rts software) that

Table 1. Preferred stimulus within dyads (i.e. stimulus preferred in at least two tests out of three) and associated preference ratios in the three test designs for the 18 tested females



Test order	Female	Stimulus dyad	Preferred stimulus	O	P	L
O/P/L	1	M_1–M_2	M_1	0.74	0.61	1.00
	2	M_3–M_4	M_4	0.51	0.81	0.33
	3	M_5–M_6	M_5	0.74	0.81	0.80
O/L/P	4	M_7–M_8	M_8	0.74	1.00	0.67
	5	M_9–M_{10}	M_{10}	0.84	0.75	0.45
	6	M_{11}–M_{12}	M_{12}	0.67	1.00	0.65
P/L/O	7	M_8–M_{12}	M_{12}	–	–	0.64*
	8	M_{13}–M_{14}	M_{13}	0.70	0.75	1.00
	9	M_2–M_{10}	M_{10}	0.79	0.70	0.82
P/O/L	10	M_4–M_{15}	M_4	0.81	0.90	0.93
	11	M_5–M_{16}	M_5	0.55	0.86	0.56
	12	M_3–M_{17}	M_{17}	0.63	0.99	0.43
L/O/P	13	M_7–M_{14}	M_{14}	0.80	1.00	0.51
	14	M_3–M_{15}	M_{15}	0.65	1.00	0.59
	15	M_9–M_{16}	M_{16}	0.61	0.71	0.67
L/P/O	16	M_9–M_{13}	M_{13}	0.83	0.93	0.74
	17	M_4–M_{17}	M_4	0.86	0.95	0.94
	18	M_1–M_{11}	M_1	0.88	1.00	0.94

O: operant test; P: phonotaxis test; L: live male test. M_1-M_{17}: 17 males used for song or live stimuli.

*This female is listed for completeness, but as the only nonresponder in two tests was excluded from the statistical analyses.

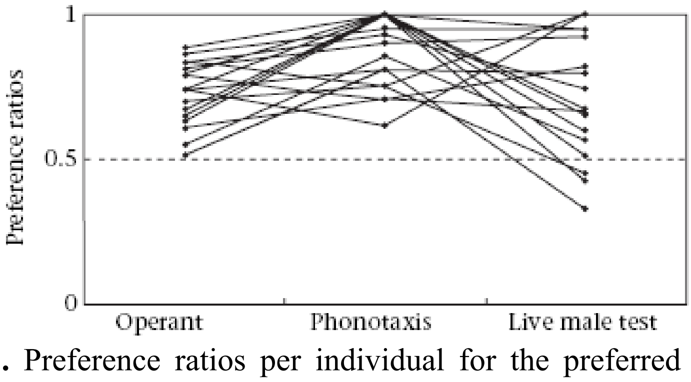
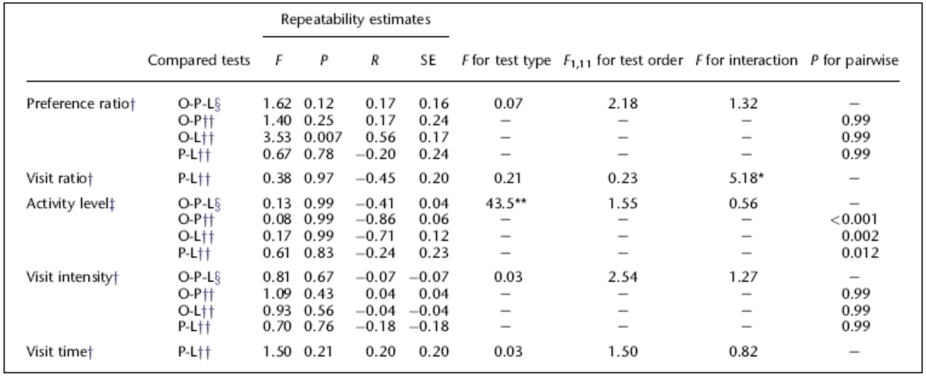


Figure 2. Preference ratios per individual for the preferred stimulus (i.e. stimulus preferred in at least two of the three tests) across the three test designs for all females (for test order see Table 1).

Table 2. Repeatability estimates and effect of test type and test order on all measures of female choice behaviour across the three tests and/or in pairwise comparisons (for test type effect with Bonferroni adjustment for multiple comparisons)



		Repeatability estimates							
	Compared tests	F	P	R	SE	F for test type	$F_{1,11}$ for test order	F for interaction	P for pairwise
Preference ratio†	O-P-L§	1.62	0.12	0.17	0.16	0.07	2.18	1.32	–
	O-P††	1.40	0.25	0.17	0.24	–	–	–	0.99
	O-L††	3.53	0.007	0.56	0.17	–	–	–	0.99
	P-L††	0.67	0.78	−0.20	0.24	–	–	–	0.99
Visit ratio†	P-L††	0.38	0.97	−0.45	0.20	0.21	0.23	5.18*	–
Activity level‡	O-P-L§	0.13	0.99	−0.41	0.04	43.5**	1.55	0.56	–
	O-P††	0.08	0.99	−0.86	0.06	–	–	–	<0.001
	O-L††	0.17	0.99	−0.71	0.12	–	–	–	0.002
	P-L††	0.61	0.83	−0.24	0.23	–	–	–	0.012
Visit intensity†	O-P-L§	0.81	0.67	−0.07	−0.07	0.03	2.54	1.27	–
	O-P††	1.09	0.43	0.04	0.04	–	–	–	0.99
	O-L††	0.93	0.56	−0.04	−0.04	–	–	–	0.99
	P-L††	0.70	0.76	−0.18	−0.18	–	–	–	0.99
Visit time†	P-L††	1.50	0.21	0.20	0.20	0.03	1.50	0.82	–

O: operant test; P: phonotaxis test; L: live male test.

*P < 0.05; **P < 0.001; two-factor mixed ANOVAs with assumed sphericity values for preference ratio, activity level and visit intensity and Greenhouse-Geisser values for visit ratio and visit time.

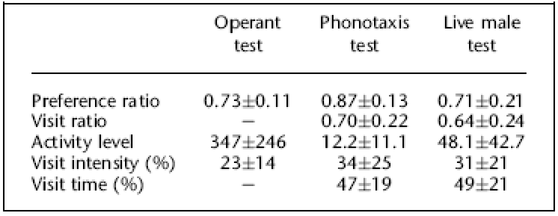
†Z score-transformed data.

‡Log-transformed data.

§$F_{16,34}$ for repeatability estimates, $F_{2,22}$ for test type and $F_{10,22}$ for the interaction 'test type x test order'.

††$F_{16,17}$ for repeatability estimates, $F_{1,11}$ for test type and $F_{5,11}$ for the interaction 'test type x test order'.

Table 3. Means ± 1 SD of all measures of female choice behaviour in the three tests ($N = 17$)



	Operant test	Phonotaxis test	Live male test
Preference ratio	0.73±0.11	0.87±0.13	0.71±0.21
Visit ratio	–	0.70±0.22	0.64±0.24
Activity level	347±246	12.2±11.1	48.1±42.7
Visit intensity (%)	23±14	34±25	31±21
Visit time (%)	–	47±19	49±21

identified all time points where the sound level exceeded a power output of 0.05 V (for at least 10 successive ms) as a sound and the rest as silence (Leadbeater et al., 2005). We used the amplitude contour of the song (Fig. 3a) as an automatic measure of the number of syllables per song (also checked by visual analysis of spectrograms). This provided an objective criterion for separating syllables by eliminating

Table 4. Definition of the nine investigated song structure parameters

Parameter list	Definition and/or equation
Song repertoire	
Syllable number	Total number of syllables per song
Stereotypy coefficient, SC*	$\mathrm{SC} = 1 - ((g(\mathrm{I},\mathrm{II}) + \ldots + g(\mathrm{III},\mathrm{IV}))/(3 \ldots N_{\mathrm{s,i}}))$ where $g(\mathrm{I},\mathrm{II})$, $g(\mathrm{I},\mathrm{III}), \ldots$ are the number of changes between all 6 possible pairs of motifs in number of syllables and syllable types (i.e. the sum of numbers of syllable deletion, repetition and new appearances); $N_{s,i}$ is the total number of syllables of the *i*th motif of the song. SC = 0 when there is no similarity between motifs and 1 when all motifs are identical
Syllable types	Proportion of unique syllable types per song = $N_{\mathrm{st}}/N_{\mathrm{s}}$ where N_{st} is the number of different syllable types and N_{s} the total number of syllables in a song
Intramotif repeated syllables	Proportion of repeated syllables within motifs per song $= (\sum_{i=1}^{4} \sum_{j=1}^{N_{\mathrm{st}i}} p_{j,i})/N_{\mathrm{s}}$ where *i* sums over the four motifs, $N_{\mathrm{st},i}$ is the number of syllable types in motif *i* and $p_{j,i}$ is the number of repetitions of syllable type *j* in motif *i*
Temporal parameters	
Song duration (s)	$d_{\mathrm{song}} = \sum_{i=1}^{4} d_{\mathrm{motif},i} + \sum_{j=1}^{3} d_{\mathrm{IMS},j}$ where $d_{\mathrm{motif},i}$ is the duration of the *i*th motif of the song and $d_{\mathrm{IMS},j}$ is the duration of the *j*th intermotif silence
Syllable rate	Total number of syllables/s $= N_{\mathrm{e}}/d_{\mathrm{song}}$
Proportion of motif duration	Proportion of motif duration per song $= (\sum_{i=1}^{4} d_{\mathrm{motif},i})/d_{\mathrm{song}}$; identical to the reciprocal of the proportion of intermotif silences per song
Sound density	
Global sound density (identical to 'acoustic density' in Leadbeater et al. 2005)	Proportion of sound density per song (i.e. proportion of song over which sound was present) $= (\sum_{i=1}^{N_{\mathrm{s}}} d_{\mathrm{s,i}})/d_{\mathrm{song}}$ where $d_{\mathrm{s,i}}$ is the duration of the *i*th syllable of the song.
Intramotif sound density	Proportion of sound density in the 4 motifs of a song (excluding intermotif silences) $= (\sum_{i=1}^{N_{\mathrm{s}}} d_{\mathrm{s,i}})/(\sum_{j=1}^{4} d_{\mathrm{motif},j})$

See Fig. 3 for abbreviations.

*To measure the stereotypy of syllable sequencing between the four motifs of each stimulus song, we introduced a new measure we termed 'stereotypy coefficient', SC, which is slightly different but more detailed than the stereotypy score (SS) proposed by Scharff and Nottebohm (1991). When applying both formulae to our data we found the values from SS and SC to be significantly correlated with each other (Pearson correlation: $r_{15} = 0.59$, $P = 0.013$).

the substantial variation caused by human decision (Jones et al., 2001a). However, this method yielded several composite syllables that authors applying the 'sudden change in frequency' criterion would have split (Williams and Staples, 1992). We accepted this since little is known about whether zebra finches perceive composite syllables as one or a quick succession of elements (but see Franz and Goller, 2002). However, one should be aware that the average syllable/element repertoire might differ between published studies because of the criteria used and not because of differences in repertoire size between colonies (which, however, might also exist, Slater and Clayton, 1991).

Morphometry analysis

Immediately after the live male test (between 0930 and 1200 hours), we weighed each male (±0.1 g) on a Sartorius BL600 scale. The following morphometric measures (Baumel et al., 1979) were taken with callipers (±0.05 mm): tarsus length (distance from the right tibiotarsus-tarsometatarsal joint to the point of the tarsometatarsal joint at the base

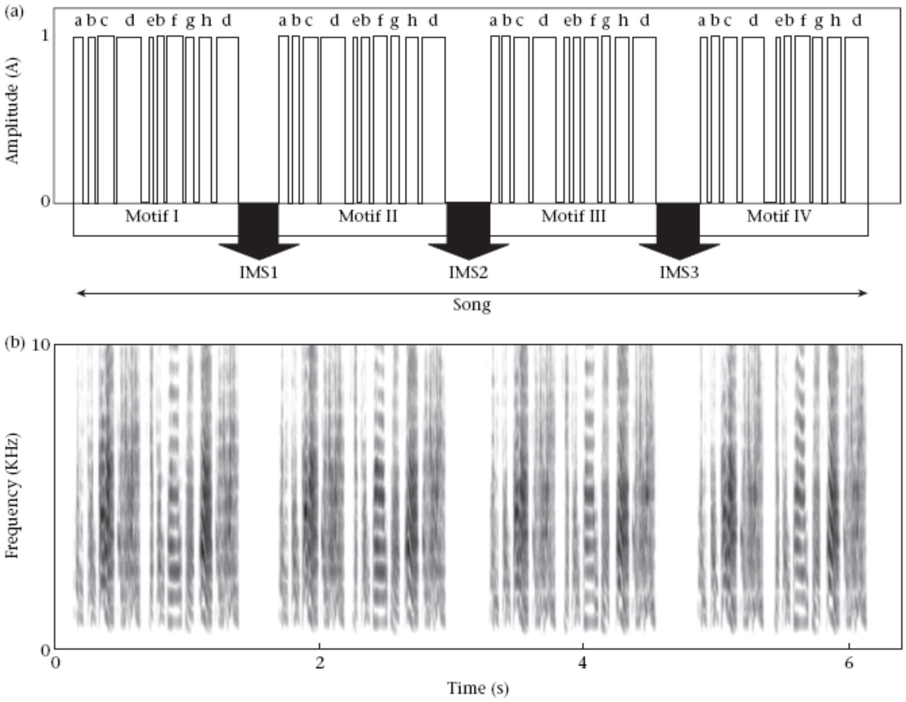


Figure 3. Example of (a) the output of the gating procedure used to measure sound density for a zebra finch song with (b) the relevant spectrogram. In this example, the song is composed of eight syllable types with a total of 10 syllables, i.e. there are repeated syllables within a motif. a, b, c, d, e, f, g, h: Syllable types. IMS: intermotif silence.

Table 5. Morphological traits of the 17 males used to test female preferences and repeatability estimates of the measures

				Repeatability estimates			
Morphological traits	Mean±SD	Range	N	$F_{16,34}$	P	R	SE
Mass (g)	16.3±3.2	13.0–24.5	17	–	–	–	–
Tarsus length (mm)	15.6±0.7	14.6–17.6	17	49.2	<0.001	0.94	0.02
Wing chord (mm)*	57.5±1.1	55.3–58.8	16	7.7	<0.001	0.69	0.11
Beak length (mm)	10.8±0.4	10.2–11.8	17	80.0	<0.001	0.96	0.02
Beak width (mm)	7.0±0.3	6.7–7.6	17	75.2	<0.001	0.96	0.02
Body condition index*	0.0±1.0	−2.1–1.6	16	–	–	–	–
Beak upper area (mm^2)	38.0±2.0	34.8–42.1	17	–	–	–	–

*$F_{15,32}$ since one male with broken primary feathers was excluded from the analyses.

of the right middle anterior toe); wing chord (from the bend of the flattened right wing (wrist) to the tip of the longest primary feather); beak length (tip of the upper mandible to the end of the culmen at its intersection with the forehead); beak width (between the two lateral sides of the upper mandible above the nostrils). All measures were taken three times, were highly repeatable (Table 5) and involved brief handling of 2-3 min per bird.

We calculated body condition index (BCI) as the standard residual index of the linear regression of body mass on structural size (Jakob et al., 1996). All morphometric measures were ln transformed to meet the homoscedasticity assumption of the regression analyses described below. The proportion of variance in ln body mass explained by the four size variables was determined by simple linear regressions (Gosler et al., 1998). We found that ln tarsus length, ln beak width, ln wing chord and ln beak length explained 67, 65, 50 and 10% of the variance in ln body mass, respectively. We then tried to improve the explained variance in ln body mass by using the factors extracted with principal components analyses (PCA; orthogonal rotation: varimax with Kaiser normalization) including different combinations of variables (with always at least tarsus length and wing chord). The first principal component (PC) of size (PC1; 74% of explained variance after rotation) based on tarsus length and wing chord explained more variance in ln body mass (80%) than any of the other structural size parameters alone. Our BCI thus refers to the standard residuals of the simple linear regression of ln body mass on the first PC based on tarsus length and wing chord (Table 5). Adding beak width to the PCA only slightly improved the explained variance in mass (82%) whereas adding beak length weakened it (74%). Beak width and length could not be entered at the same time in the PCA because they were too weakly correlated (Pearson correlation: $r_{15} = -0.05$, NS). We therefore decided to use a combined measure of beak length and width to approximate beak surface (mm^2) of the upper mandible area (beak upper area = beak length x width/2; Table 5).

Statistical analyses

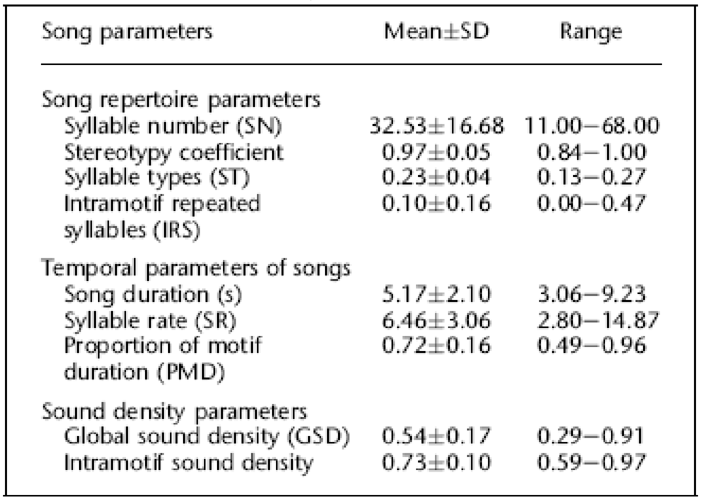
We did our analysis of song as a predictor of female preference in the two song preference tests (operant and phonotaxis tests), as female choice in the live male test could have been based on any male phenotypic trait (e.g. male morphology and display intensity, Collins and ten Cate, 1996). All 17 successfully tested females showed perfect consistency in preference direction between the operant and phonotaxis tests (Table 1). Nevertheless, test context differed and none of the measures of female choice behaviour was repeatable between these two tests (Table 2). Thus we analysed for each test separately which of the measured song structure parameters predicted relative preference strength (i.e. preference ratio). For this analysis, the use of absolute values for the song parameters was inappropriate as each female could choose between two stimuli only rather than the whole set. We therefore calculated relative differences in song parameters between the two stimuli of a dyad (value of preferred stimulus - value of nonpreferred stimulus divided by the sum of both stimuli) to test whether song structure predicted female preferences, but worked with the absolute measurement values when analysing whether variation in song parameters predicted variation in male morphological traits. To avoid multiple testing, we reduced song structure parameters with principal components analyses (orthogonal rotation: varimax with Kaiser normalization) after removing highly intercorrelated parameters (Table 6). To ensure that the scores of the principal components were uncorrelated we used the Anderson-Rubin method (Field, 2000), a necessary precondition for the subsequent stepwise linear regression analyses, which aimed to test whether the principal components obtained from the two separate PCAs could predict (1) female preference ratios (arcsine transformed to meet regression assumptions) in the operant and phonotaxis tests and (2) male morphology.

Results

Song preferences and relative differences in song structure

The PCA of the song structure parameters led to the extraction of three principal components (PCs) with eigenvalues >0.9 that we labelled 'relative performance', 'relative sound density' and 'relative proportion of different syllables', according to the relative load of the six entered parameters (Table 7). The PC1 'relative performance' accounted for 34% of the variation in female preference ratios in the operant test ($F_{1,15} = 7.7$, $R^2 = 0.34$, $P = 0.014$) and for 28% in the phonotaxis test ($F_{1,15} = 5.8$, $R^2 = 0.28$, $P = 0.03$). The preferred songs within dyads had a higher proportion of motif duration per song (i.e. lower proportion of intermotif silences) and more syllables than the less preferred songs in both preference tests (Fig. 4a). The predictive value of the PC1 'relative performance' for female preference ratios in the operant test was reinforced by the PC2 'relative sound density' when included in the stepwise regression in the model at step 2 ($F_{1,15} = 8.7$, $R^2 = 0.74$, $P = 0.004$). Thus, in the operant test, the preferred songs within dyads also had a higher intramotif sound density than the less preferred songs (Fig.

Table 6. Song structure parameters (see Table 4 for definition) of the 17 males used to test female preferences



Song parameters	Mean±SD	Range
Song repertoire parameters		
Syllable number (SN)	32.53±16.68	11.00–68.00
Stereotypy coefficient	0.97±0.05	0.84–1.00
Syllable types (ST)	0.23±0.04	0.13–0.27
Intramotif repeated syllables (IRS)	0.10±0.16	0.00–0.47
Temporal parameters of songs		
Song duration (s)	5.17±2.10	3.06–9.23
Syllable rate (SR)	6.46±3.06	2.80–14.87
Proportion of motif duration (PMD)	0.72±0.16	0.49–0.96
Sound density parameters		
Global sound density (GSD)	0.54±0.17	0.29–0.91
Intramotif sound density	0.73±0.10	0.59–0.97

SN/SR, ST/IRS, PMD/GSD: The two parameters of each pair were highly intercorrelated (i.e. Pearson $-0.9 > r > 0.9$) and could not be entered at the same time into a principal components analysis (PCA). For the PCA testing whether relative differences in song structure parameters predicted female preference ratios, SR, IRS and GSD were not entered (SN/SR: $r_{15} = 0.98$; ST/IRS: $r_{15} = -0.96$; PMD/GSD: $r_{15} = 0.92$); for the PCA testing whether absolute measures of song structure parameters predicted male morphology, ST and GSD were not entered (ST/IRS: $r_{15} = -0.98$; PMD/GSD: $r_{15} = 0.92$; all $P < 0.001$). The parameters entered in the two independent PCAs differed because the relative difference for IRS and the absolute measure for ST could not achieve a normal distribution even after appropriate transformation. However, we are confident that it did not affect our interpretation of results since ST and IRS were highly correlated for both relative differences and absolute measures of song structure parameters (both $r_{15} < -0.96$, $P < 0.001$). All other relative differences and absolute measures of the song structure parameters were normally distributed (one-sample Kolmogorov-Smirnov test: for relative differences: all $Z < 1.18$, $N = 17$, $P > 0.12$; for absolute measures: all $Z < 1.32$, $N = 17$, $P > 0.06$).

4b).

Male morphology and song structure

This second PCA led again to the extraction of three principal components with eigenvalues >0.8, but the loading of these had changed. They were thus now labelled differently: ‘proportion of identical syllables’, ‘performance and sound density’ and ‘motif stereotypy’ (Table 7). PC2 ‘performance and sound density’ accounted for 32% of the variation in male beak length ($F_{1,15} = 7.1$, $R^2 = 0.32$, $P =$

Table 7. Rotated component matrices and percentages of explained variance (after rotation) of the two principal components analyses (PCA) of song structure parameters

	PC1	PC2	PC3
PCA on relative differences within dyad			
Label of principal components	Relative performance	Relative sound density	Relative proportion of different syllables
Parameters			
RD proportion of motif duration	**0.88**	0.17	−0.06
RD syllable number	**0.80**	0.15	−0.19
RD intramotif sound density	0.30	**0.86**	0.13
RD song duration	−0.07	**−0.58**	0.10
RD syllable types	−0.37	0.24	**0.84**
RD stereotypy coefficient	−0.05	0.53	**−0.76**
% Explained variance	32.89	24.43	22.43
PCA on individual absolute measures			
Label of principal components	Proportion of identical syllables	Performance and sound density	Motif stereotypy
Parameters			
Intramotif repeated syllables	**0.93**	−0.02	−0.14
Song duration	**0.87**	−0.22	−0.25
Syllable number	**0.87**	0.46	−0.02
Syllable rate	0.17	**0.90**	0.19
Proportion of motif duration	0.09	**0.86**	−0.26
Intramotif sound density	−0.32	**0.82**	0.23
Stereotypy coefficient	−0.21	0.05	**0.95**
% of Explained variance	36.75	35.57	16.33

Analyses were based on relative differences (RD) within dyads (principal components extracted when initial eigenvalues >0.9; rotation converged in 8 iterations) and on individual absolute measures (principal components extracted when initial eigenvalues >0.8; rotation converged in 4 iterations). Some of the parameters entered in the PCA on relative differences are different from those entered in the PCA on absolute measures because of different intercorrelations between song parameters in the two PCAs (see footnotes of Table 6). Values in bold indicate those variables that contributed most to a particular principal component.

0.018), 29% in beak upper area ($F_{1,15} = 6.0$, $R^2 = 0.29$, $P = 0.027$), 25% in mass ($F_{1,15} = 5.0$, $R^2 = 0.25$, $P = 0.040$) and 22% in tarsus length ($F_{1,15} = 4.2$, $R^2 = 0.22$, $P = 0.059$). Songs with a higher syllable rate, a higher proportion of motif duration per song (i.e. lower proportion of intermotif silences) and a higher intramotif sound density predicted larger beak length, beak upper area, mass and, almost significantly, tarsus length. The predictive value of the PC2 ‘performance and sound density’ for beak length was reinforced by the PC3 ‘motif stereotypy’ when included in the stepwise regression in the model at step 2 ($F_{1,15} = 9.3$, $R^2 = 0.57$, $P = 0.003$). The PC3 ‘motif stereotypy’ also accounted for 51% of the variation in beak width ($F_{1,15} = 15.4$, $R^2 = 0.51$, $P = 0.001$). Songs with higher stereotypy were associated with longer and larger beaks. None of the included parameters predicted wing chord or BCI.

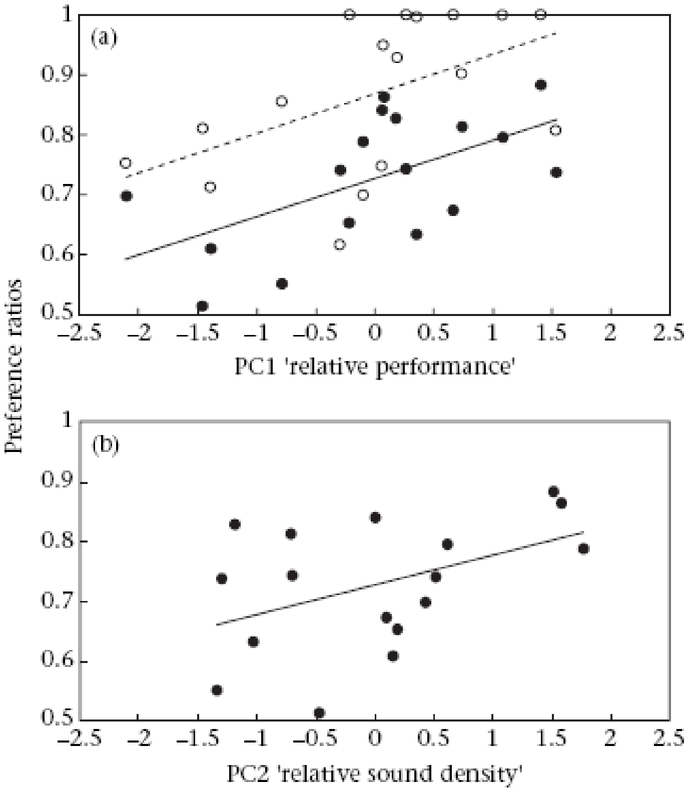


Figure 4. Relation between preference ratios and (a) the first principal component (PC1 ‘relative performance’) in the operant test (●, —) and in the phonotaxis test (○, - - -) and (b) the second principal component (PC2 ‘relative sound density’) in the operant test (linear regression lines are shown).

DISCUSSION

Females preferred the same song or the singer of this song in all three tests. As the preference was consistent independent of whether females could choose only between songs or between songs and a live male, male zebra finch song must contain sufficient information for females to judge male quality. The outcome of the multiple regression analyses further confirmed this: song characteristics predicted both variation in female preferences and male traits. The three commonly used binary choice designs not only yielded concordant results on the direction of female preference, but in two of them, the operant test and the live male test, the magnitude of relative preference strength was repeatable. This was highly surprising: females were tested only once in each test paradigm and in different orders, which left substantial potential scope for the outcome to be influenced not only by the test context but also by short-term fluctuation in female motivation. Furthermore, the operant test might be considered a highly artificial context (key pecking for song reward) whereas the live male test provided the opportunity to see, hear and interact with two live males. Why was the preference strength for males or their songs repeatable between these two tests, but not between the two tests involving song only (i.e. operant and phonotaxis tests)?

A number of aspects differed between these tests. In the much larger phonotaxis cage females had to travel further to be close to the stimuli than in the operant test and in the live male test cage. Therefore the phonotaxis test could have demanded more effort to approach the stimuli than the other two tests, which may have influenced female preference strength. Perhaps more importantly though, the operant test was an active choice design that allowed *ad libitum*, repeated sampling and active control of exposure to the stimulus by females. In the phonotaxis test, females could not actively control the playback or interact with it. The significant repeatability of preference strength between operant and live male tests further supports the notion that active operant-conditioning techniques are a highly suitable means for assessing direction and strength of female song preferences (for discussion see Riebel and Slater, 1998). Reassuringly, given the large existing body of literature on phonotaxis tests, although preference strength differed, the actual direction of females' preferences was consistent with the other two tests.

A low activity level, i.e. a low motivation in assessing stimuli (as in the phonotaxis test), and sequential rather than simultaneous presentation of stimuli may yield fewer opportunities to compare stimuli, which in turn might directly affect the expressed preference (Brooks and Endler, 2001; Rowland et al., 1995; Wagner, 1998). However, females in the phonotaxis test could hear both songs without having to perch close to the speakers. Furthermore, in all three tests, preference strength was independent of the motivation to sample stimuli (preference strength and activity level were not correlated).

Females could also have been affected by the variation in time between tests (Johnsen and Zuk, 1996; Morris et al., 2003) or learning opportunities between repeated tests (Hager and Teale, 1994) arising from the different test situations. However, we found that the measures of female choice behaviour were independent of the test order making these explanations unlikely.

Females are known to prefer males with higher song rates (Collins et al., 1994; de Kogel and Prijs, 1996; Forstmeier, 2004; Houtman, 1992), so could differences in song rate explain the differences in preference strength between the tests? In the operant test, female activity level determined the song rate. However, song rate was not measured in the live male test. Although it was obvious from observations during the tests that males sang at different rates, we do not know whether the repeatable preference strength between the operant and live male tests was associated with song rates. However, we do know that motif rate per song was identical in both playback tests but did not lead to repeatable preference strength. Furthermore, the different song rates between the three tests did not alter the direction of preference either. Therefore, song rate is unlikely to be an important factor in explaining our results, suggesting that song structure per se contains sufficient information for females to base their decisions on. Hence, song structure must either contribute disproportionately to female choice or is highly correlated with other male features relating to female choice.

Some song structure parameters did indeed predict variation in female preference strength. Females had stronger preferences for songs with lower proportions of intermotif silences and more syllables than the alternative stimulus songs (PC1 ‘relative performance’) in both operant and phonotaxis tests. This finding is consistent with the idea that female attention may depend on sound continuity in the song (Goller and Daley, 2001) and with the finding that females prefer larger syllable repertoires (Neubauer, 1999). However, there are two caveats. First, we measured syllable repertoire size and not element repertoire size for which the outcome may be different. Second, repertoire size in the zebra finch is perhaps better defined by the number of syllable or element categories (Nowicki et al., 2002a) rather than by the total number of syllables. In the operant test, preference strength was also predicted by a higher intramotif sound density (PC2 ‘relative sound density’). Sound density within motifs was not found to be a good predictor of female preferences by Leadbeater et al. (2005), but they tested females with single-motif songs whereas we used four-motif songs. Furthermore, we found that sound density reinforced the predictive value of the PC1 ‘relative performance’, not that it was a significant predictor of female preference on its own. Furthermore, females might not necessarily perceive the proportion of intermotif silences (parameter of highest load in PC1 ‘relative performance’) and the intramotif sound density (PC2 ‘relative sound density’) separately, as both parameters measure the amount of sound per unit of time, albeit different aspects of this.

Overall, the weighting of song structure parameters of the PC1 ‘relative performance’ was not dependent on the test design. Preference functions are probably less affected by context than choosiness (i.e. the effort and time that an individual is prepared to invest in finding and assessing mates) and sampling strategies (i.e. the decision rule adopted in mate assessment). This suggestion is reinforced by the high repeatability of female song preferences across substantial time spans (Riebel, 2000).

The main predictors of song preference strength were also predictors of a number of male phenotypic aspects. Therefore our results suggest that some structural song parameters that are thought to signal male quality are highly correlated with other preferred traits, which supports the idea that different characteristics are back-up signals (Johnstone, 1996). Songs with higher syllable rate, a lower proportion of intermotif silences and higher intramotif sound density (i.e. PC2 ‘performance and sound density’) predicted larger male beak length, beak upper area and mass, but not current male body condition (BC1). High syllable rates are associated with increased male attractiveness (reviewed in Gil and Gahr, 2002) and may signal male quality since fast singing patterns are hypothesized to require more vocal-respiratory coordination (Gil and Gahr, 2002; Goller and Daley, 2001) and fast syllable delivery is more demanding on the motor system (Franz and Goller, 2003). In addition, higher sound intensity songs may stimulate the female sensory system more than low-intensity ones (e.g. Basolo, 1990; Endler, 1992; Kirkpatrick and Ryan, 1991).

Our results could be taken to suggest great redundancy in male courtship display, at least over the modest time of our measurements, and an overall population-level preference for some song characteristics. However, the switching of preference by the three females in the live male test suggests that some females might rank signals differently or that no single trait might signal absolute quality. Different ornaments may indicate condition over different timescales, with some reflecting condition during development while others are more dynamic and respond to changes in condition at adulthood (e.g. Hill et al., 1999; Møller et al., 1998; Scheuber et al., 2003a; Scheuber et al., 2003b; Sorenson and Derrickson, 1994). In close-ended learners such as zebra finches, song structure may reflect how well a male fared during posthatch development and thus provide a condition-dependent signal of males' early development (Nowicki et al., 1998; Spencer et al., 2003; Spencer et al., 2005b, but see Gil et al., 2006). Such long-term effects of early experience may also be seen in other male sexually selected traits (Birkhead et al., 1999; de Kogel and Prijs, 1996; but see Blount et al., 2003). However, while song structure is likely to remain stable in close-ended learners, other sexually selected traits such as song rate and beak colour can change after a relatively short period of manipulation of food and exercise in the laboratory (Birkhead et al., 1998) or after an immune challenge (Faivre et al., 2003). Earlier studies had found the two traits to be correlated when the differences between past and present condition were smaller (Collins et al., 1994; Houtman, 1992). This could explain why contradictory results have been found on the weighting of these condition-dependent traits in mate choice (Collins, 1994; Collins and ten Cate, 1996; Forstmeier and Birkhead, 2004). This also suggests that some condition-dependent signals, because of their dynamic character, may switch between being back-up and multiple message signals (within and between individuals). Our data, together with the literature on the zebra finch, support the view that the 'multiple messages' and 'back-up signal' hypotheses should not be seen as mutually exclusive (Candolin, 2003; van Doorn and Weissing, 2004).

ACKNOWLEDGMENTS

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Chapter 3

Long-term effects of manipulated natal brood size on metabolic rate in zebra finches

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ABSTRACT

Long-term effects of developmental conditions on health, longevity and other fitness components in humans are drawing increasing attention. In evolutionary ecology, such effects are of similar importance because of their role in the trade-off between quantity and quality of offspring. The central role of energy consumption is well documented for some long-term health effects in humans (e.g. obesity), but little is known of the long-term effects of rearing conditions on energy requirements later in life. We manipulated the rearing conditions in zebra finches (*Taeniopygia guttata*) using brood size manipulation and cross-fostering. It has previously been shown in this species that being reared in a large brood has negative fitness consequences, and that such effects are stronger in daughters than in sons. We show that, independent of mass, standard metabolic rate of 1-year-old birds was higher when they had been reared in a large brood, and this is to our knowledge the first demonstration of such an effect. Furthermore, the brood size effect was stronger in daughters than in sons. This suggests that metabolic efficiency may play a role in mediating the long-term fitness consequences of rearing conditions.

KEYWORDS: metabolic programming, metabolic syndrome, brood size manipulation, developmental stress, *Taeniopygia guttata*.

In many species, including humans, offspring with low birth weight, low growth rate and/or low mass at independence have lower fitness prospects, and effects of developmental conditions can persist well into adulthood (Gebhardt-Henrich and Richner, 1998; Lindström, 1999; Lummaa and Clutton-Brock, 2002). This well-documented phenomenon is the cause of the trade-off between quantity and quality of offspring, which is one of the cornerstones of life-history theory (Lessells, 1991). However, despite its importance in life-history evolution, the mechanisms underlying growth effects on fitness prospects are not well known.

The importance of long-term consequences of developmental conditions has increasingly been recognized in the medical and biological sciences in recent years (e.g. Bateson et al., 2004; Gluckman et al., 2005). Although human studies on the physiological consequences of perinatal conditions revolve around energy metabolism (Desai and Hales, 1997), the long-term effects of nutritional stress during development on metabolic rate and energy requirements later in life have been less studied in both humans and animals. However, individuals with higher metabolic rates will have to invest more into foraging and are potentially more vulnerable to fluctuations in food availability and environmentally triggered increased energy demands (e.g. climatic or social challenges). Thus, long-term effects of rearing conditions on energy requirements could substantially affect fitness.

We investigated the long-term effect of the rearing environment on standard metabolic rate (SMR) in zebra finches (*Taeniopygia guttata*). We measured SMR in resting post-absorptive birds at night, but at a temperature below the lower critical temperature. SMR constitutes a large part of total energy consumption, and is highly variable within populations (Speakman et al., 2003). What causes this variation is poorly understood, but SMR is a repeatable trait (e.g. Rønning et al., 2005), indicating that SMR is an individual characteristic that may be susceptible to genetic and environmental effects. To study the effects of developmental conditions, we manipulated brood size, which has previously been shown to modify growth in the nest and lifespan of offspring after independence (de Kogel, 1997; Deerenberg et al., 1996). In zebra finches, daughters are more susceptible to nutritional stress than sons, either when induced using brood size manipulation (de Kogel, 1997), or through food rationing (Martins, 2004), and we therefore also compared the brood size effects on SMR between the sexes.

MATERIAL AND METHODS

We used wild-type outbred zebra finches, housed in 80 x 40 x 40 cm cages on a 13.30 : 10.30 h L:D (lights on at 7:00 h) schedule at 20–22°C and 35–50% humidity. All birds had *ad libitum* access to commercial tropical seed mixture, drinking water and cuttlebone supplemented three times a week with 3–4 g of egg food per bird, twice a week with branches of millet, and once a week with germinated tropical seeds. Chicks of 14 breeding pairs were cross-fostered when they were 3.9 ± 1.6 days old to form eight small (2 or 3 chicks) and six large (5 or 6 chicks) broods. All chicks were cross-fostered. There was some chick mortality before day 10 (two from small and one from large broods), and we used the brood size at day 10 to characterize the rearing conditions. Chicks were housed with their foster parents until independence (age 34.2 ± 3.4 days), then in mixed-sex groups of four birds together with an adult male until age 70.2 ± 3.4 days. Subsequently, the birds were housed in single-sex groups of 4 or 5 birds per cage. The 43 subjects (19 from small broods; 24 from large broods) were 13 months old when SMR was measured. Birds were reared in Leiden (The Netherlands) and moved to Groningen (The Netherlands) for the respirometry measurements, where they were housed under similar conditions and in the same social groups as in Leiden. Metabolic measurements started one week after the birds arrived in Groningen.

Metabolic rate was measured as described by Verhulst et al. (2005). SMR was defined as the lowest hourly running mean. Ambient temperature in the metabolic chambers was 22.16°C (s.d. = 0.30), which was within the range normally experienced in the holding cages. Birds were weighed when moved into and out of the metabolic chambers, and we used the mean of these values to indicate mass. Birds were placed in the (dark) metabolic chamber at 17:50 ± 84 min. Metabolic rate was measured for two nights in each bird; the interval between successive measurements was 5.1 days (±1.0 day, range 4–7 days). SMR was independent of ambient temperature, the time birds were placed in the metabolic chamber, and (for the second measurement) the number of days elapsed between successive measurements. These variables were ignored in the analyses.

SMR depends on mass, which in turn is determined by body size and nutritional stores. However, effects of size (as estimated using tarsus length) and residual mass (residuals of regression of mass on size) on SMR were indistinguishable (analysis not shown). For simplicity, we therefore did not separate between size and ‘condition’ effects but simply corrected SMR for mass. Other measures of body size, such as wing length and beak dimensions, did not explain additional variation in mass (or SMR), and were therefore ignored.

Data were analysed using general linear mixed models (GLMMs), with ‘individual’ as random effect to accommodate the fact that the two measurements made on each individual cannot be considered statistically independent samples. For the same reason, we tested foster nest and nest of origin as random effect in the analyses. Repeatability was calculated following Lessells and Boag (1987), and the standard error (s.e.) of the repeatability was calculated following Becker (1984).

RESULTS

Repeatability (R) of SMR was high at 0.77 (s.e. = 0.06, $F_{42,43} = 7.82$, $p < 0.001$). SMR depends on mass, which was highly repeatable, but repeatability remained high when SMR was corrected for mass (residuals of a regression of SMR on mass: $R = 0.55$, s.e. = 0.11, $F_{42,43} = 3.48$, $p < 0.001$). This confirms that individuals used in this study could be characterized by their SMR, at least over the modest timescale of our measurements.

SMR was higher in birds reared in large broods when compared to birds reared in small broods ($F_{1,43} = 7.7$, $p < 0.01$, GLMM including individual as random effect). Expansion of this model with other potentially relevant parameters did not change the results (Table 1). SMR was significantly lower during the second measurement, but the brood size effect was independent of measurement number (i.e. there was no significant brood size x measurement number interaction). SMR was strongly dependent on mass, but the brood size effect on SMR cannot be explained by effects of mass. Firstly, mass during the SMR measurements was independent of manipulated brood size ($F_{1,43} = 1.0$, $p = 0.3$). Body composition could depend on manipulated brood size, e.g. when birds reared in large broods were small but were fat as adults, but tarsus length at adulthood, a reliable measure of body size, was independent of brood size ($F_{1,41} = 1.5$, $p = 0.2$). Secondly, the effect of brood size on SMR remained significant when mass was included in the mixed model (Figure 1), and there was no interaction between

Table 1. Results of the general linear mixed model analysis of SMR (*W*). (Series indicates first/second measurement (0 and 1, respectively). Sex is a dummy variable (0, female; 1, male). Top panel shows the model with all significant parameters, bottom panel shows the parameters that were not significant when added to the final model. Note that the significance of the effect of sex changes when the brood size x sex interaction is added together with sex (see text for details)

	coefficient (s.e.)	*F* (d.f.)	*p*
parameter			
constant	0.059 (0.044)		
individual[a]		2.41 (41, 41)	<0.005
series	−0.013 (0.005)	7.29 (1, 41)	0.01
mass (g)	0.019 (0.003)	54.0 (1, 41)	<0.001
brood size	0.012 (0.004)	8.89 (1, 41)	<0.005
rejected terms			
sex	−0.01 (0.01)	0.89 (1, 41)	0.4
brood size×mass	0.002 (0.002)	1.3 (1, 40)	0.3
brood size×series	0.003 (0.004)	0.50 (1, 40)	0.5
foster brood[a]		1.2 (12, 41)	0.3
birth nest[a]		1.0 (12, 41)	0.4

[a] Entered as random effect.

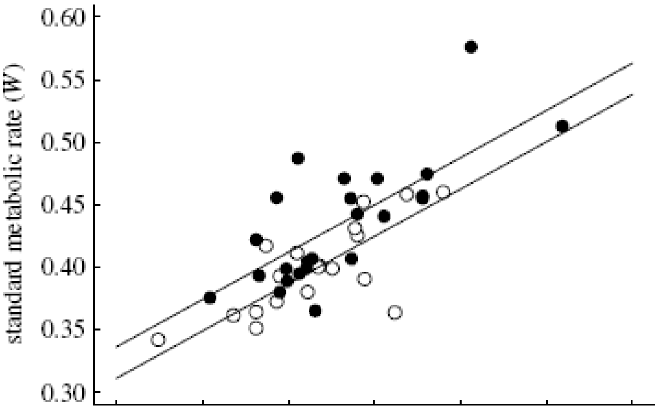

Figure 1. SMR (*W*) in relation to mass and manipulated natal brood size (open circles: small broods, 2 or 3 chicks; filled circles: large broods, 5 or 6 chicks). Data shown are the averages per individual over the first and second measurement.

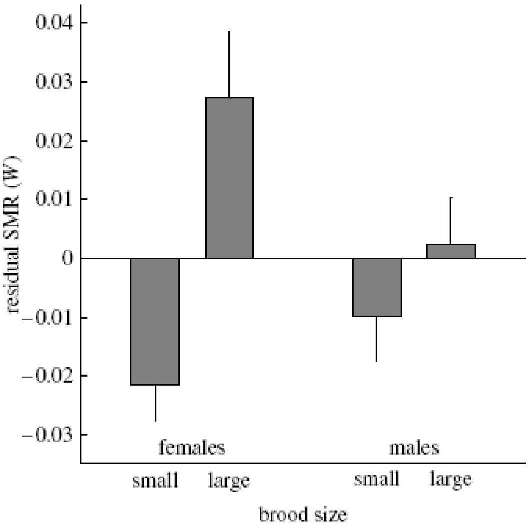

Figure 2. Residual SMR (*W*, ± s.e.) in relation to sex and manipulated brood size. Statistical analysis was based on raw data, but the averages shown here were calculated as follows: (i) residuals of regressions of SMR on mass were calculated separately for the first and second measurement, (ii) the mean residual was calculated per individual, and (iii) these means were averaged per group. From left to right, $n = 6$, 8, 13 and 16.

brood size and mass (Table 1). There was no sex difference in mass or size (tarsus length), and no significant effect of sex on SMR when added to the model (Table 1). However, the brood size effect on SMR was stronger in daughters than in sons (Figure 2; when added to the model in Table 1, sex: $F_{1,41} = 2.95$, $p = 0.09$, sex x brood size interaction: $F_{1,41} = 4.09$, $p < 0.05$).

DISCUSSION

In adult birds of 1 year of age, SMR was approximately 9% higher in birds that had been reared in large broods when compared to birds reared in small broods (Figure 1). To our knowledge, this is the first study to demonstrate a long-term effect of rearing conditions on metabolic efficiency independent of mass. (Vickers et al. (2000) reported a comparable effect using rodents, but their finding may be explained by the long-term effects of rearing conditions on mass that they also found.). The fact that effects on SMR persisted into adulthood suggests that early post-natal conditions can induce different metabolic phenotypes. The nestling phase seems to be a critical period during development when arising effects of rearing conditions are (at least partly) irreversible. Fitness consequences of rearing conditions may differ between the sexes (Råberg et al., 2005; Trivers and Willard, 1973), and in the zebra finch it has been found that daughters are more susceptible to nutritional stress than sons (de Kogel, 1997; Martins, 2004). In line with these findings, we show that the brood size effect on SMR was significantly stronger in daughters (Figure 2). Thus, our results indicate that long-term brood size effects on metabolic rate may be part of the mechanism causing the effects of brood size on fitness prospects.

Development of a functional explanation (Cuthill, 2005) for the brood size effect on SMR is hampered by our lack of knowledge of the functional consequences of SMR variation (Speakman et al., 2004a; Williams and Vézina, 2001). However, earlier data showed that birds reared in large broods (that we found to have a high SMR) live for a shorter time (de Kogel, 1997). Furthermore, the brood size effect on SMR was found to be significantly stronger in daughters, in agreement with the sexual difference in brood size effect on lifespan (de Kogel, 1997). However, our finding that a high SMR is associated with low-fitness prospects is inferred from the combination of different brood size manipulation studies, and there is a need for data that enable a direct test for an association between SMR and longevity. The negative association between SMR and longevity that emerges from our study in combination with the results of de Kogel (1997) contrasts with recent work on mice, where a positive association between SMR and lifespan was found (Speakman et al., 2004b). Understanding the causes of the taxonomic variation in these associations poses a challenge that could be an important step towards a better understanding of the relationship between lifespan and energy consumption.

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Chapter 4

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

Marie-Jeanne Holveck, Katharina Riebel, Robert Lachlan, Ana Catarina Goncalves Vieira de Castro and Carel ten Cate

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)

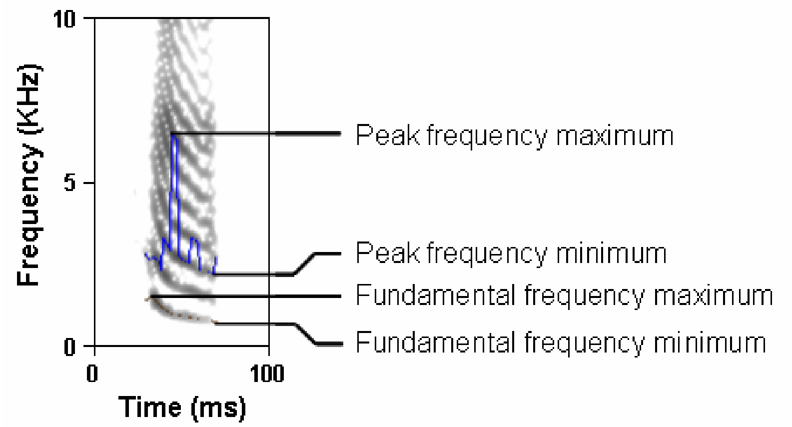

Figure 1. Spectrograms of a song element showing the automated measures of frequency parameters by the Luscinia software (see Table 1 for definitions).

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.

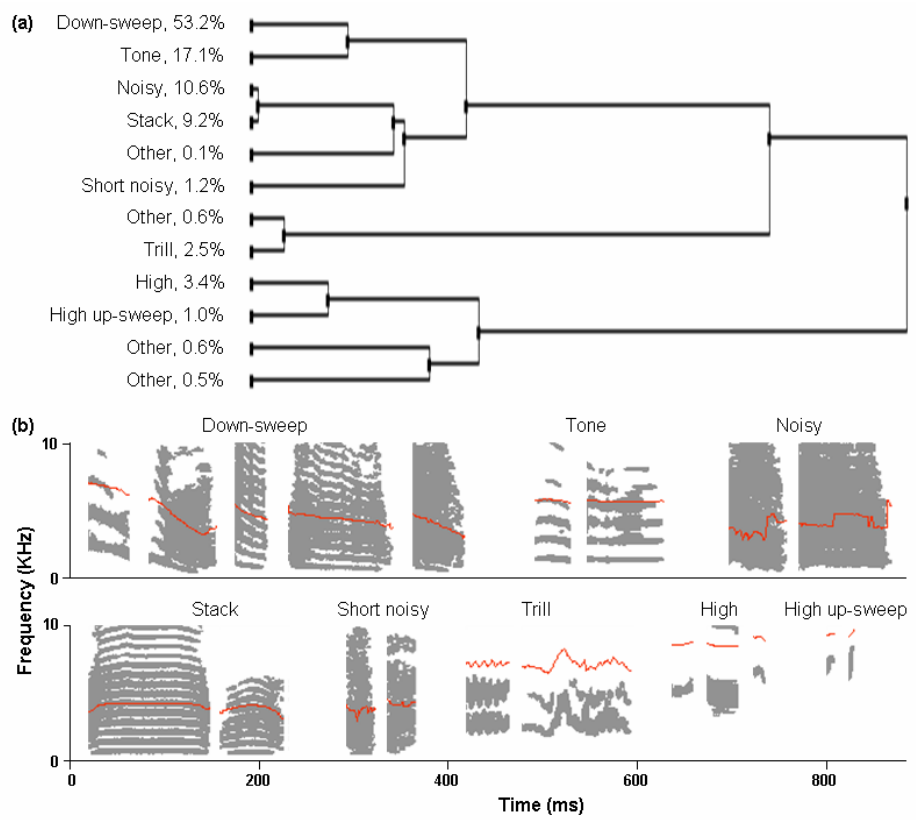

Figure 2. The UPGMA-tree clustering the 3736 elements from the 265 motifs of the 53 tutees, tutors and foster fathers and examples of elements for each of the 8 most represented categories (see Table 2 for phonology of each element category). Percentages of each element category are based on the 1730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

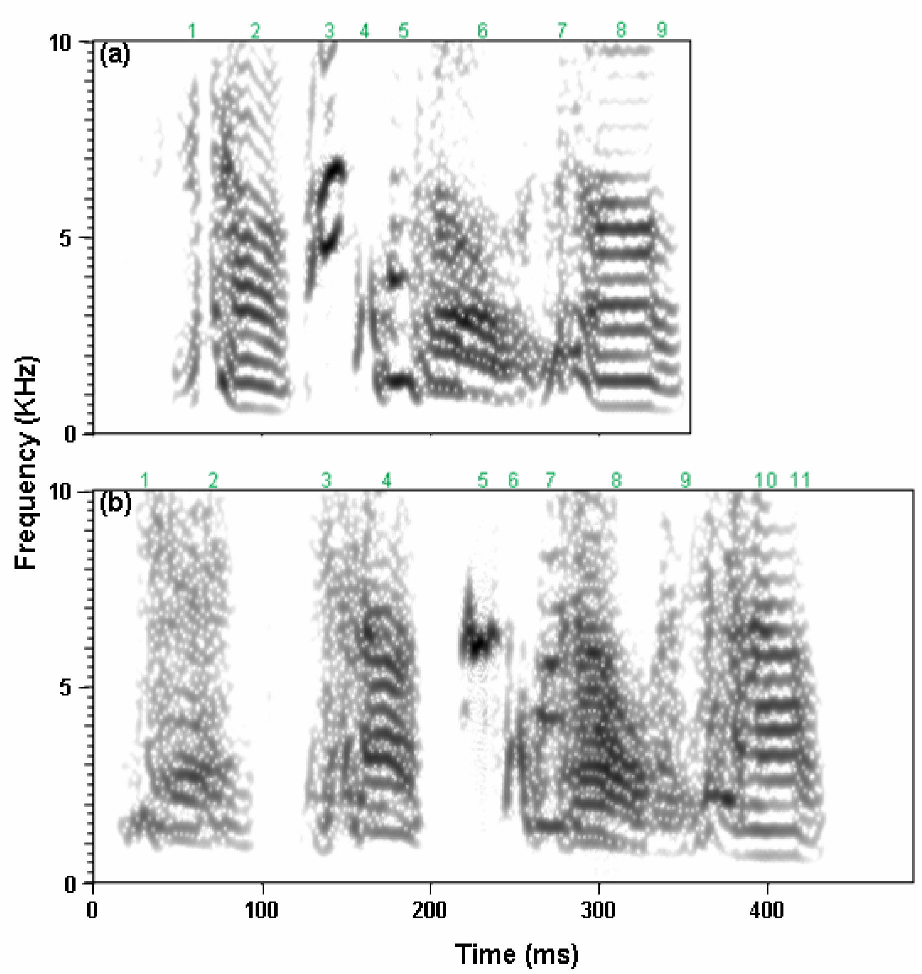

Figure 3. Spectrograms showing the song motifs of a tutor (a) and one of one of its tutees (b) to illustrate their numbers of shared elements and shared element transitions. Elements 1, 2, 5, 6, 7, 8 and 9 in the tutor motif are respectively shared with elements 3, 4, 7, 8, 9, 10 and 11 in the tutee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

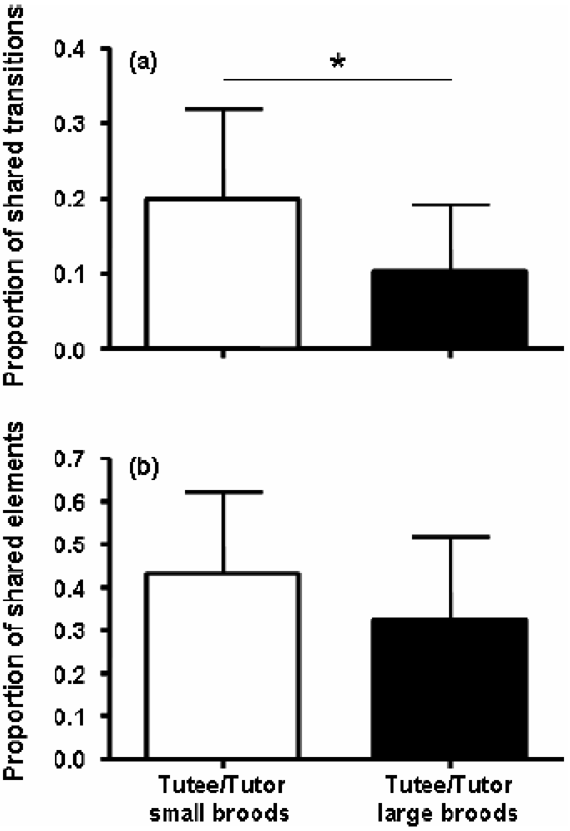


Figure 4. Effects of brood size manipulation on learning from tutor. Shown are (a) 'shared transitions Tutee/Tutor' and (b) 'shared elements Tutee/Tutor' for birds from small and large broods. Shown are grand means ± 1 SD; for every bars, n = 13.

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

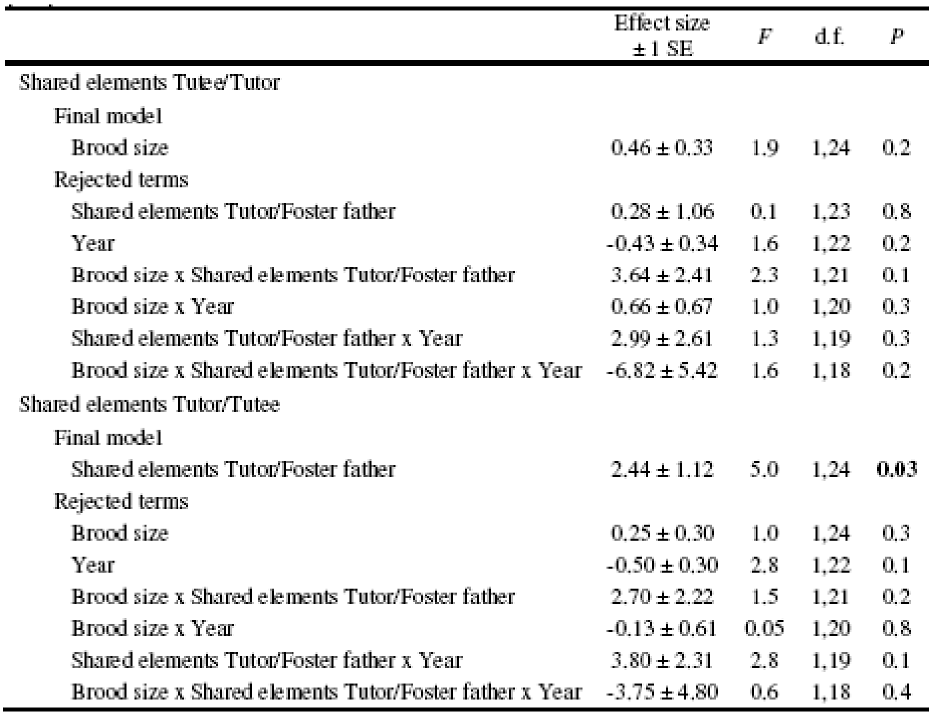
	Effect size ± 1 SE	*F*	d.f.	*P*
Shared transitions Tutee/Tutor				
Final model				
BS	0.07 ± 0.04	7.3	1,24	**0.01**
Shared elements Tutee/Tutor	0.26 ± 0.09	8.6	1,23	**0.01**
Rejected terms				
Shared transitions Tutor/Foster father	-0.03 ± 0.12	0.05	1,22	0.8
Year	-0.005 ± 0.04	0.01	1,21	0.9
BS x Shared 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

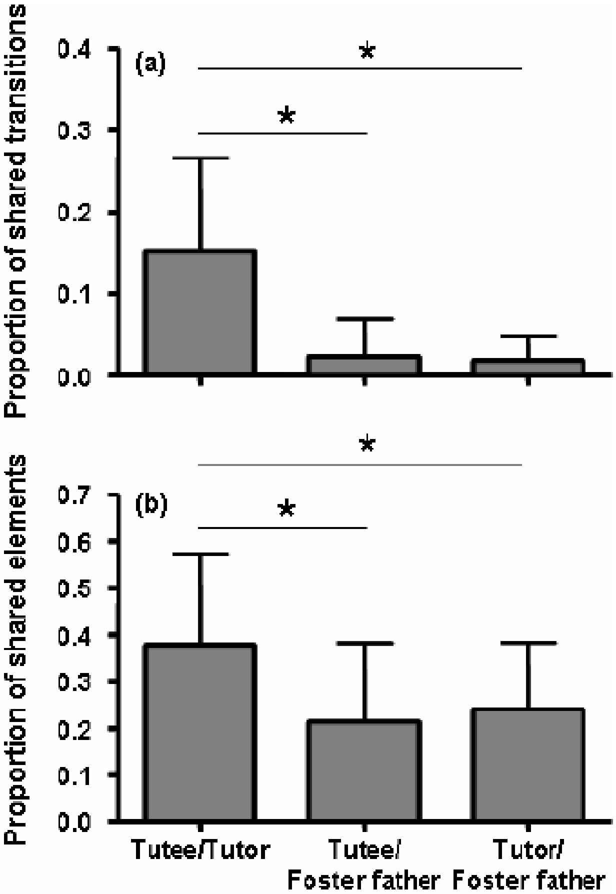


Figure 5. Comparison of the amount of song sharing between tutors and tutees, foster fathers and tutees, and foster fathers and tutors. Shown are (a) 'shared transitions Tutee/Tutor', 'shared transitions Tutee/Foster father' and 'shared transitions Tutor/Foster father' and (b) 'shared elements Tutee/Tutor', '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.

Chapter 5

Low quality females avoid high quality males when choosing a mate

Marie-Jeanne Holveck and Katharina Riebel

ABSTRACT

Sexual selection studies generally assume that all females prefer to mate with the highest-quality male. However, female sexual preferences vary in many species including humans but what causes and maintains this variation remains an enigma (Badyaev and Qvarnström, 2002; Cotton et al., 2006; Jennions and Petrie, 1997). Here we show experimentally in a songbird that unfavourable developmental conditions modify partner preference such that females actively avoid high-quality males in favour of low-quality males. We manipulated brood size of captive zebra finches *Taeniopygia guttata* to rear low- and high-quality individuals, those from large and small brood sizes respectively (Alonso-Alvarez et al., 2006; de Kogel, 1997; de Kogel and Prijs, 1996; Naguib et al., 2004). When choosing between male songs (Chapter 2 of this thesis), high-quality females preferred the songs of high-quality males, in agreement with general expectations. In contrast, low-quality females significantly preferred songs of low-quality males over high-quality males. This pattern was confirmed during reproduction: latency until laying the first egg was shorter when birds were paired assortatively with respect to rearing brood size (quality). This suggests males with similar developmental background were accepted faster as partners, in agreement with the song choice results. Females invested more in egg mass if paired with high-quality males, regardless of their own quality. This form of differential allocation (Burley, 1986) indicates that female perception of male quality (other than their choices) was independent of their own quality. These results demonstrate that the direction of female mating preferences can be based on self-assessed phenotypic quality, a phenomenon previously only reported for humans (Little et al., 2001; Todd et al., 2007). We suggest that the assortative mating preferences of low-quality females may be an adaptive strategy (Fawcett and Johnstone, 2003; Härdling and Kokko, 2005; Johnstone, 1997; McNamara et al., 1999; Real, 1991) to avoid costs of intra-sexual competition over high-quality males, and increased risk of desertion by higher-quality males.

Studies of sexual selection generally assume that females prefer to mate with the highest-quality male available. Consequently, variation in female preferences has often been considered an empirical nuisance rather than a salient feature of sexual selection. However, rather than being entirely stochastic, the variation in female preference is often stable (Bakker et al., 1999; Qvarnström et al., 2000; Riebel, 2000), and a full understanding of sexual selection has to take the causes and consequences of such variation into account.

In addition to genetic influences on female preferences, phenotypic aspects such as females' condition (Cotton et al., 2006) or learned preferences (Riebel, 2000; Riebel, 2003a) are potential sources of preference variation. Female songbirds, for example, display large and repeatable variation in song preference that are in part the result of their early experiences (Riebel, 2000). The developmental stress hypothesis (Buchanan et al., 2003; Nowicki et al., 2002a) proposed that nutrition and social environment during early development affect male song learning and, as a consequence, song quality (Spencer et al., 2003; Spencer et al., 2005b, but see Gil et al., 2006). We extend this hypothesis, and investigated whether female song preference learning and mating behaviour are also modulated by developmental conditions. We used brood size manipulation, to manipulate the early social environment and to induce long-term phenotypic changes within an ecological meaningful range (Alonso-Alvarez et al., 2006; de Kogel, 1997; de Kogel and Prijs, 1996; Naguib et al., 2004).

In our experiment, subjects were raised by unrelated foster parents in either small or large broods (Fig. 1). We refer to these as HIGH and LOW condition treatments respectively. As expected (Alonso-Alvarez et al., 2006; de Kogel, 1997; de Kogel and Prijs, 1996; Naguib et al., 2004), the brood size manipulations affected offspring condition. As fledglings, birds from small broods had larger body sizes than those from large broods (day 35: mean tarsus length ± 1 s.e. small broods = 15.48 ± 0.07 mm, $n = 48$; large broods = 15.15 ± 0.07 mm, $n = 57$; $F_{1,27} = 10.92$, $P = 0.0015$), and this effect persisted into adulthood (day 180: mean ± 1 s.e. small broods = 15.50 ± 0.05 mm, $n = 47$; large broods = 15.13 ± 0.07 mm, $n = 53$; $F_{1,27} = 20.60$, $P < 0.0001$). As juveniles during the sensitive phase for song learning (Slater et al., 1988) birds were in mixed-treatment mixed-sex groups of four individuals with an unfamiliar unrelated mated adult male that acted as 'song tutor' (Gil et al., 2006, Fig. 1).

After females reached adulthood their song preferences were tested

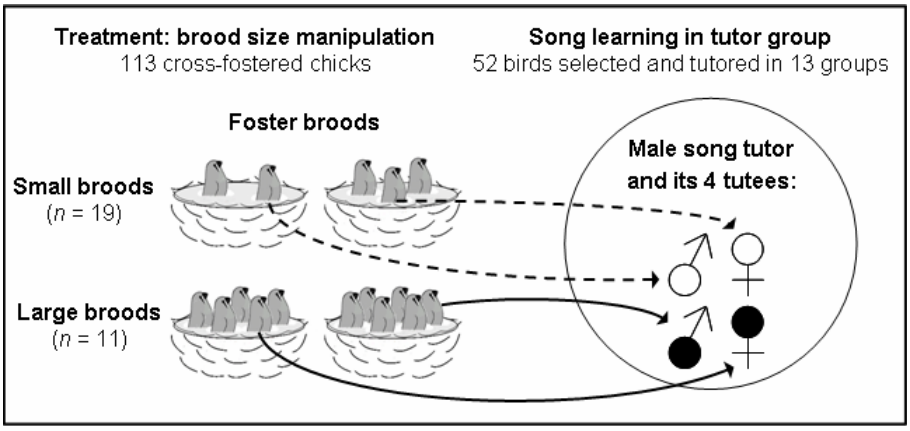

Figure 1. Experimental procedure. Chicks (*n* = 113) of 30 pairs (14 in 2004; 16 in 2005; Table S1) were cross-fostered at 3 ± 1.7 days post-hatching in either small (2-3 chicks, *n* = 19 broods) or large broods (5-6 chicks, *n* = 11 broods), until nutritional independence. During the subsequent sensitive period for song learning, 13 groups (6 in 2004, 7 in 2005) of four genetically and fosterly unrelated chicks (one of each sex and treatment) were housed each with an unrelated tutor and his mate from 33.5 ± 3.3 to 69.4 ± 3.3 days post-hatching (*n* = 52 tutees). Afterwards and between experiments, tutees were housed in single-sex groups of 4-5 birds.

in an operant conditioning set-up (Chapter 2 of this thesis). Preference measured in this way predicts real mate choice in zebra finches (Chapter 2 of this thesis). In the tests the females were offered the choice between the songs of a HIGH and a LOW condition male that had learned their songs simultaneously in one of the other tutor groups (unfamiliar to the choosing female). Females were tested twice with different song pairs, and showed strong assortative preferences by rearing environment in both tests (Fig. 2; $F_{1,22} = 140.9$, $P < 0.0001$), despite having no prior experience with the test songs and in absence of other cues on male quality. They thus preferred the song of males that were reared in the same environment as themselves. This effect was symmetrical, in the sense that HIGH condition females significantly preferred songs of HIGH condition males (one-sample $t_{11} = 10.8$, $P < 0.0001$), and LOW condition females significantly preferred the songs of LOW condition males ($t_{11} = -5.7$, $P < 0.001$). Every single female preferred a male of matching background. This effect was absent when

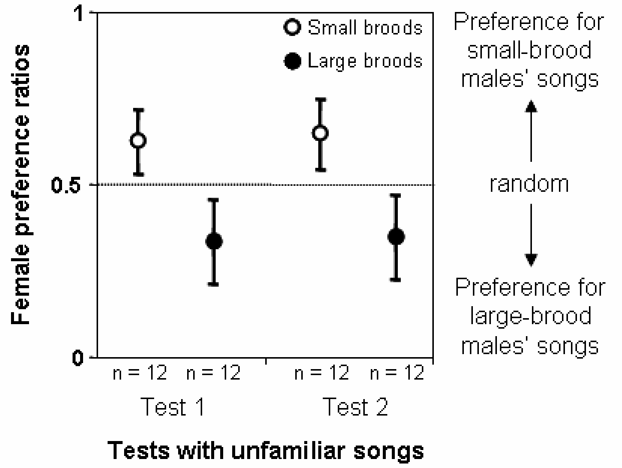

Figure 2. Preference ratios for unfamiliar songs of small- versus large-brood males. Females (12 from small broods and 12 from large broods; one female died eliminating a set) were tested twice with two different sets of unfamiliar songs (Tests 1 and 2). Male songs were considered unfamiliar to females when individuals were not from the same birth nest, foster brood or tutor group. Shown are means ± 1 s.d..

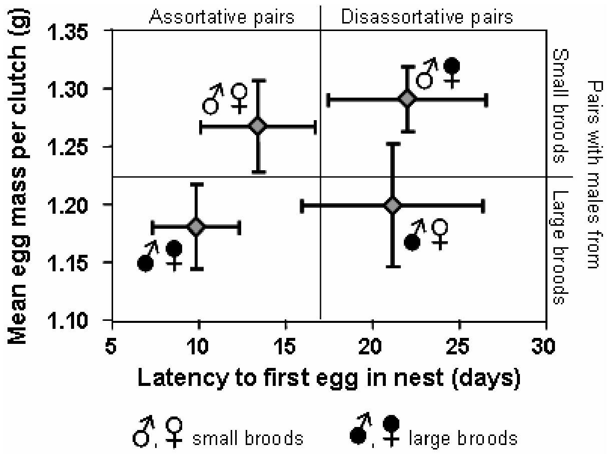

Figure 3. Mean egg mass per clutch in relation to latency to first egg in nest for females paired assortatively or disassortatively with respect to brood size. Females had not been exposed to the song of their mate in the earlier preference tests. Shown are means ± 1 s.e.m.; *n* = 6 for every pair category except for the one with birds from small broods where *n* = 5 (1 female had still not laid any eggs after 67 days). Clutch size did not differ significantly between groups (Table S5).

females chose between the songs of the HIGH and LOW condition males that were their social companions during the tutoring phase (HIGH-females: $t_{11} = 0.05$, $P = 0.97$; LOW-females: $t_{11} = -0.15$, $P = 0.9$; $F_{1,22} = 0.0001$, $P = 0.99$). Thus, early familiarity appears to interfere with the judgement of male quality (Riebel, 2000).

To investigate the reproductive consequences of the assortative mating preferences we paired females with unfamiliar males either assortatively (HIGH/HIGH or LOW/LOW) or disassortively (HIGH/LOW or LOW/HIGH) and allowed the birds to breed. Females in assortative pairs advanced the time of their first breeding in comparison to females in disassortative pairs (Fig.3; $F_{1,21} = 8.08$, $P < 0.01$), while there was no effect of the developmental background of the female per se ($F_{1,20} = 0.12$, $P = 0.7$). This indicates that females accepted males sooner as a mate when he had the same developmental background, which is consistent with the song preferences. However, the perception that LOW condition females had of male condition was not impaired since females made a larger reproductive investment, as measured by the mean egg mass per clutch, when mated to a HIGH condition male (Fig.3; $F_{1,21} = 6.64$, $P < 0.02$), regardless of their own developmental background ($F_{1,15} = 1.15$, $P = 0.7$). This result is important, because it demonstrates that females from HIGH and LOW condition treatments did in fact agree on the phenotypic quality of the males they were paired to, despite showing different preferences. Thus the results of the mate choice and breeding experiments both revealed rearing conditions as a source of variation in female mating behaviour.

To our knowledge, we provide the first demonstration that rearing conditions can change the *direction* of female mating preferences towards low-quality males (Cotton et al., 2006) in the absence of competition over access to mates (Fawcett and Johnstone, 2003; Härdling and Kokko, 2005) and without any evidence that low condition affected female choosiness (Burley and Foster, 2006; Cotton et al., 2006). Female motivation to hear songs was independent of the rearing treatment (comparing total number of key pecks; Table S3) and they showed striking discrimination abilities. They were able to extract information on unfamiliar males' developmental background from differences in song quality only (Fig. 2).

This begs the question of why low-quality females opted for low-quality males. Such a preference for low-quality individuals could arise 1) in competition over access to mates: females in lower condition are likely to loose out (Fawcett and Johnstone, 2003; Härdling and Kokko, 2005), thus they will save time and energy by preferring lower-quality mates; 2) in species with mutual mate choice: if low-condition individuals face higher rejection or divorce rates (Johnstone, 1997; McNamara et al., 1999; Real, 1991), it could pay them to go for lower-quality individuals straight away. These theoretical assumptions are met: Zebra finches show mutual mate choice and biparental care (Jones et al., 2001b). Advancing the time of first breeding increases lifetime reproductive success in zebra finches (Alonso-Alvarez et al., 2006) and producing heavier eggs increases hatchling survival (Christians, 2002). Low-quality females will thus increase their fitness if they save time (Alonso-Alvarez et al., 2006) by actively preferring low- instead of high-quality individuals for which they are likely to be out-competed by higher-quality females (Jones et al., 2001b) or because males might desert them for high-quality females once the opportunity arises (McNamara et al., 1999). This illustrates how and why the assortative mating preferences of low-quality females might be an adaptive strategy. However it remains to be demonstrated that a preference for low-quality males indeed allows low-quality females to avoid the costs of female-female competition over mate access and/or guarding and to improve their fitness.

Our experiments clearly revealed state-dependent behaviour: Both low- and high-quality females determined their level of investment (the timing and mass of the laid eggs, two important fitness related parameters: Christians, 2002) strategically based on whether a male matched their (condition-dependent) preferences but also on his absolute quality. Whereas earlier studies reported as foremost effect of low condition reduced female choosiness in zebra finches (Burley and Foster, 2006) and other animal species (Cotton et al., 2006), the finding that females prefer males who match their own quality is highly reminiscent of what so far has only been reported for human mating preferences where self-perceived attractiveness has been found to influence a woman’s preference function (Little et al., 2001; Todd et al., 2007). Our experiment has demonstrated that developmental conditions can be at the heart of such rightly so perceived differences. This lends empirical support to the increasing theoretical appeals to investigate mate choice as state-dependent behaviour and to integrate individual developmental trajectories (Badyaev and Qvarnström, 2002; Cotton et al., 2006; McNamara and Houston, 1996) into sexual selection studies. As demonstrated here it has been rightly advocated earlier that this might prove extremely fruitful in addressing as yet unexplained variation in female mating preferences (Badyaev and Qvarnström, 2002; Cotton et al., 2006; Jennions and Petrie, 1997).

METHODS

Housing

Birds were housed in standard laboratory cages (80 x 40 x 40 cm) on a 13.5:10.5 hour light:dark schedule (lights on: 07:00 C.E.T.) at 20-22°C and 35–50% humidity with *ad libitum* access to a commercial tropical seed mixture (Tijssen goed voor dieren, Hazerswoude, Holland), drinking water and cuttlebone supplemented thrice weekly with egg food (Witte Molen, B.V., Meeuwen, Holland), twice with millet branches and once with germinated seeds.

Song preference tests

Starting at 164 ± 15 days post-hatching, the three tests per female each lasted two days with 8.1 ± 1.4 intermittent days in the home cage. Tests, one with songs of familiar males from females' own tutor groups and two with unfamiliar males' songs, were conducted blind with regard to treatment in eight identical binary-choice operant set-ups (Chapter 2 of this thesis). Presentation of stimulus songs was fully balanced with regard to potential side preference effects (Chapter 2 of this thesis). Preference ratios were the number of key pecks for HIGH song divided by total number of key pecks.

Stimulus songs

We recorded non-directed song of each male tutee (*n* = 26) at 141 ± 13 days post-hatching in a cage (70 x 30 x 45 cm) in a sound attenuation chamber (Sennheiser MKH40 microphone 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). Stimulus preparation was conducted blind with regard to male treatment. For each song stimulus, we chose one four-motif song per male, digitally deleted introductory elements, highpass filtered the songs at 500 Hz (smoothing = 100 Hz) to remove low-frequency background noise and RMS-equalized amplitudes (peak digitally scaled to 1) with Praat software (4.2.07 for Windows, http://www.praat.org).

Body size measurements

Tarsus length, a good correlate of structural size in birds, is the distance from the right tibiotarsus-tarsometatarsal joint to the point of the tarsometatarsal joint at the base of the right middle anterior toe (Baumel et al., 1979). It was measured with callipers to the nearest 0.05 mm at days 35 and 180 (mean age of brood). Measures taken three times were highly repeatable (Becker, 1984; Lessells and Boag, 1987): day 35: $F_{(104,210)} = 96.7$, $P < 0.001$, $R = 0.97 \pm 0.005$; day 180: $F_{(99,200)} = 142.5$, $P < 0.001$, $R = 0.98 \pm 0.004$.

Breeding experiment

For their first breeding attempt (one female died and was replaced by a tape-tutored, instead of live-tutored, one reared in a corresponding manipulated brood), six pairs for each of the four possible brood size*sex combinations were formed between unfamiliar and genetically unrelated birds, resulting in 12 assortative or disassortative pairs (2004: 554 ± 12 days, 11 pairs; 2005: 431 ± 11 days, 13 pairs). Nest boxes (20 x 10 x 10 cm) were provided with hay as nesting material and checked once daily after 10:00 hours. New eggs were weighed to the nearest 0.1g (Sartorius BL600 scale) and marked with indelible pen on the day of laying. A clutch was considered complete if no new eggs were laid over four days.

All procedures followed Dutch laws and were approved by Leiden University committee for animal experimentation.

Statistical analysis

Data were analyzed with linear models (generalized and/or mixed; two-tailed, $\alpha = 0.05$) in R software (2.4.1 for Windows, http://www.r-project.org). Birth nest and foster brood were crossed random effects in models with the response variables preference ratios, key pecks and tarsus size at day 35 and 180. We sequentially deleted random factors explaining less than 0.2 % variance, higher order interactions and then factors with $P > 0.05$ until reaching the minimal adequate model. The analyses of the breeding experiment (on latency to first egg in the nest, clutch size and mean egg mass per clutch) gave identical outcomes if run with or without the pair with the replacement female. For full details see Tables S2-S5. All means are quoted ± 1 s.d. except where stated otherwise.

ACKNOWLEDGMENTS

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SUPPLEMENTARY INFORMATION

Table S1. Age (days ± 1 s.d.) and sample sizes of birds

	Both years	Year 2004	Year 2005	Difference
Cross-fostering	3.0 ± 1.7 (113 chicks)	3.9 ± 1.6 (56 chicks[†])	2.1 ± 1.4 (57 chicks[††])	1.8
Start of song tutoring	33.5 ± 3.3 (105 birds)	34 ± 3 (53 birds)	33 ± 3 (52 birds)	1
End of song tutoring	69.4 ± 3.3 (104 birds)	70 ± 3 (53 birds)	69 ± 3 (51 birds)	1
Song recording[†††]	141 ± 13 (26 males)	141 ± 7 (12 males)	141 ± 16 (14 males)	0
Start of song preference tests	163 ± 15 (24 females)	159 ± 16 (12 females)	167 ± 15 (12 females)	8
Breeding pairs[†††]	487 ± 63 (48 birds)	554 ± 12 (22 birds)	431 ± 11 (26 birds)	123

8 birds died before the start of song tutoring (6 HIGH and 2 LOW); 1 (LOW) died between the start and the end of the song tutoring.

[†] 24 chicks from 8 small broods (HIGH condition treatment) and 32 chicks from 6 large broods (LOW condition treatment).

[††] 30 chicks from 11 small broods and 27 chicks from 5 large broods.

[†††] In each year, 1 female (HIGH) died before the pair formation. To replace the missing pair, we formed an extra pair in 2005 with a tape-tutored, instead of live-tutored, female (HIGH). We therefore formed 11 pairs in 2004 (male/female: 2 HIGH/HIGH, 3 LOW/LOW, 3 HIGH/LOW and 3 LOW/HIGH) and 13 pairs in 2005 (male/female: 4 HIGH/HIGH, 3 LOW/LOW, 3 HIGH/LOW and 3 LOW/HIGH).

Table S2. Results of the two linear mixed-model analyses of tarsus size (mm) of the study population

	Day 35			Day 180		
	Effect size (s.e.)	*F* (d.f.)	*P*	Effect size (s.e.)	*F* (d.f.)	*P*
Final model						
BS	-0.14 (0.04)	10.92 (1,27)	**0.0015**	-0.18 (0.04)	20.60 (1,27)	**<0.0001**
Rejected terms						
Year	-0.00004 (0.13)	0.00 (1,26)	0.99	-0.07 (0.12)	0.42 (1,26)	0.5
Sex	-0.05 (0.10)	0.32 (1,25)	0.6	-0.05 (0.09)	0.38 (1,25)	0.5
Age	0.01 (0.03)	0.04 (1,24)	0.8	-0.01 (0.05)	0.05 (1,24)	0.8
BS x year	-0.05 (0.09)	0.34 (1,23)	0.6	0.03 (0.08)	0.13 (1,23)	0.7
BS x sex	0.06 (0.07)	0.70 (1,22)	0.4	0.06 (0.07)	0.74 (1,22)	0.4
Year x sex	-0.03 (0.20)	0.03 (1,21)	0.9	-0.23 (0.17)	1.83 (1,21)	0.2
BS x age	0.01 (0.02)	0.24 (1,20)	0.6	0.03 (0.04)	0.52 (1,20)	0.5
Year x age	-0.08 (0.10)	0.68 (1,19)	0.4	-0.05 (0.09)	0.27 (1,19)	0.6
Sex x age	0.004 (0.05)	0.10 (1,18)	0.9	0.03 (0.09)	0.14 (1,18)	0.7
BS x year x sex	-0.26 (0.16)	2.81 (1,17)	0.1	-0.07 (0.14)	0.27 (1,17)	0.6
BS x year x age	0.07 (0.07)	1.00 (1,16)	0.3	0.02 (0.07)	0.10 (1,16)	0.7
BS x sex x age	-0.04 (0.05)	0.06 (1,15)	0.4	0.02 (0.07)	0.08 (1,15)	0.8
Year x sex x age	-0.05 (0.18)	0.06 (1,14)	0.8	-0.07 (0.19)	0.15 (1,14)	0.7
BS x year x sex x age	-0.10 (0.15)	0.43 (1,13)	0.5	-0.18 (0.16)	1.37 (1,13)	0.2
$n^{\dagger}$		105			100	

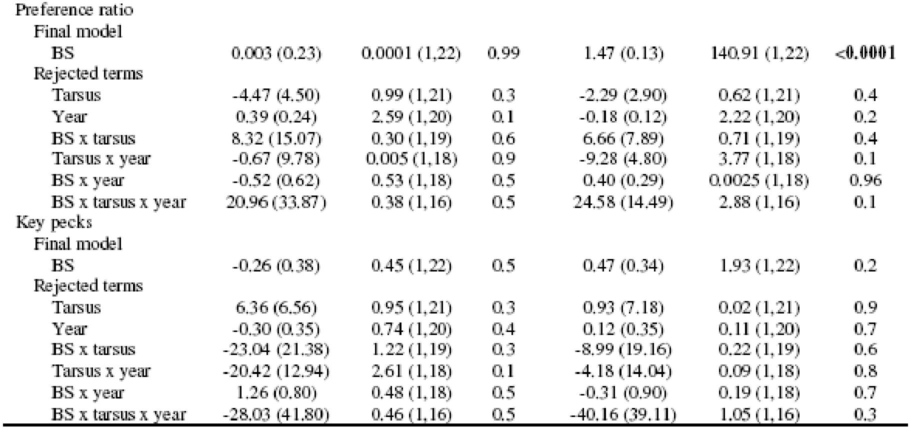
BS: brood size.

Five chicks (3 HIGH, 2 LOW) died before day 10 (mean age of brood). Therefore, we used brood size at day 10 to characterize the rearing conditions. “Brood size” was 2, 3, 5 or 6. “Year” is a binary variable (2004 or 2005) as is “sex” (male or female). “Age” is the individual age in days at the time of measurement (individual age at day 180 is included in the analyses of the study population for comparability although tarsus does not grow any more at that time).

Full model: brood size x year x sex x age + birth nest + foster brood, with a Gaussian distribution. “Rejected terms” are the parameters that were not significant when added to the final model. The random factor “foster brood” was not retained in either of the two models (its explained variance in full models was less than 0.2 %). The random factor “birth nest” was retained in the two models (its explained variance in full models was 25.6% for day 35 and 27.9% for day 180). The degrees of freedom (d.f.) for linear mixed models in R 2.4.1 are inherently approximated. We could deduce the d.f. values given here since the models did not have crossed random effects, but had a single random factor “birth nest” of sample size 29.

†Sample sizes were different for different ages because of mortality: five birds died between day 35 and day 180 (1 HIGH, 4 LOW).

Table S3. Results of the generalized linear model analyses of preference ratio and total number of key pecks



	Test 1			Tests 2 and 3†		
	Effect size (s.e.)	*F* (d.f.)	*P*	Effect size (s.e.)	*F* (d.f.)	*P*
Preference ratio						
Final model						
BS	0.003 (0.23)	0.0001 (1,22)	0.99	1.47 (0.13)	140.91 (1,22)	**<0.0001**
Rejected terms						
Tarsus	-4.47 (4.50)	0.99 (1,21)	0.3	-2.29 (2.90)	0.62 (1,21)	0.4
Year	0.39 (0.24)	2.59 (1,20)	0.1	-0.18 (0.12)	2.22 (1,20)	0.2
BS x tarsus	8.32 (15.07)	0.30 (1,19)	0.6	6.66 (7.89)	0.71 (1,19)	0.4
Tarsus x year	-0.67 (9.78)	0.005 (1,18)	0.9	-9.28 (4.80)	3.77 (1,18)	0.1
BS x year	-0.52 (0.62)	0.53 (1,18)	0.5	0.40 (0.29)	0.0025 (1,18)	0.96
BS x tarsus x year	20.96 (33.87)	0.38 (1,16)	0.5	24.58 (14.49)	2.88 (1,16)	0.1
Key pecks						
Final model						
BS	-0.26 (0.38)	0.45 (1,22)	0.5	0.47 (0.34)	1.93 (1,22)	0.2
Rejected terms						
Tarsus	6.36 (6.56)	0.95 (1,21)	0.3	0.93 (7.18)	0.02 (1,21)	0.9
Year	-0.30 (0.35)	0.74 (1,20)	0.4	0.12 (0.35)	0.11 (1,20)	0.7
BS x tarsus	-23.04 (21.38)	1.22 (1,19)	0.3	-8.99 (19.16)	0.22 (1,19)	0.6
Tarsus x year	-20.42 (12.94)	2.61 (1,18)	0.1	-4.18 (14.04)	0.09 (1,18)	0.8
BS x year	1.26 (0.80)	0.48 (1,18)	0.5	-0.31 (0.90)	0.19 (1,18)	0.7
BS x tarsus x year	-28.03 (41.80)	0.46 (1,16)	0.5	-40.16 (39.11)	1.05 (1,16)	0.3

BS: brood size.

“Brood size” and “year” are binary variables (HIGH or LOW and 2004 or 2005 respectively). Here, brood size at day 10 could not be used to characterize the rearing conditions due to low sample size. “Tarsus” is the adult tarsus size measured after the preference tests at day 180 (mean age of brood). We used its log transformation in the model analyses.

Full model: brood size x tarsus x year + birth nest + foster brood, with a quasi-binomial distribution for “preference ratio” and a quasi-Poisson distribution for “key pecks”. “Rejected terms” are the parameters that were not significant when added to the final model. The random factors “birth nest” and “foster brood” were not retained in any of the 4 models (their explained variances in full models were less than 0.2%).

†Tests 2 and 3 were repeated measures of the same females and were combined in a single analysis by summing the number of pecks over the two tests. We obtained identical outcomes with generalized linear mixed models where female identity was entered as random effect and always kept in the models to account for repeated measures per individual in Tests 2 and 3.

Table S4. Result of the generalized linear model analysis of latency to first egg in the nest (i.e. time between pairing and laying)

	Effect size (s.e.)	*F* (d.f.)	*P*
Final model			
Pair assortativeness	0.66 (0.23)	8.08 (1,21)	**0.01**
Year	0.52 (0.22)	5.64 (1,20)	**0.028**
Rejected terms			
Brood size F	0.06 (0.22)	0.12 (1,20)	0.7
Tarsus F	1.42 (4.24)	0.11 (1,18)	0.7
Tarsus M	0.19 (4.51)	0.002 (1,17)	0.97
Tarsus F x tarsus M	-224.1 (140.0)	2.49 (1,16)	0.1
Pair assortativeness x brood size F	-1.06 (0.63)	0.93 (1,16)	0.3
Pair assortativeness x year	0.07 (0.50)	0.03 (1,15)	0.9
Brood size F x year	-0.31 (0.48)	0.14 (1,14)	0.7
Pair assortativeness x brood size F x year	-0.10 (1.29)	0.72 (1,12)	0.4
n[†]		23	

"F" stands for female and "M" for male. "Pair assortativeness", "brood size F" and "year" are binary variables (assortative or disassortative, HIGH or LOW and 2004 or 2005 respectively). Here, brood size at day 10 could not be used to characterize the rearing conditions due to low sample size. "Tarsus" is the adult tarsus size measured after the preference tests at day 180 (mean age of brood). We used its log transformation in the model analyses.

Full model: pair assortativeness x brood size F x year + tarsus F x tarsus M, with a quasi-Poisson distribution. "Rejected terms" are the parameters that were not significant when added to the final model. We could not test for the random effects "birth nest" and "foster brood" since they were different for the two mates of each pair. A significant effect of "brood size M" would have appeared as a significant effect of the interaction "pair assortativeness x brood size F".

[†] Sample size is not 24 because one female from a HIGH/HIGH pair had still not laid an egg in the nest 67 days after pairing (the last female to lay her first egg in the nest did so 36 days after pairing).

Table S5. Results of the generalized linear model analysis of clutch size and linear model analysis of mean egg mass of an individual clutch

	Clutch size[†]			Mean egg mass within clutch[††]		
	Effect size (s.e.)	χ^2_1	*P*	Effect size (s.e.)	*F* (d.f.)	*P*
Final model						
BS	-	-	-	0.08 (0.04)	6.64 (1,21)	**0.018**
Latency to first egg	-0.009 (0.009)	1.08	0.3	0.004 (0.002)	5.86 (1,20)	**0.025**
Rejected terms						
BS	-0.08 (0.18)	1.02	0.6	-	-	-
PA	0.19 (0.20)	1.09	0.3	-0.03 (0.04)	0.49 (1,21)	0.5
Year	-0.20 (0.20)	1.11	0.3	0.05 (0.04)	1.63 (1,18)	0.2
Tarsus F	-2.21 (2.97)	1.06	0.5	0.55 (0.57)	0.94 (1,17)	0.3
Tarsus M	-1.01 (3.01)	1.01	0.7	0.68 (0.73)	0.87 (1,16)	0.4
PA x BS	0.23 (0.46)	1.03	0.6	0.03 (0.08)	0.15 (1,15)	0.7
PA x year	0.08 (0.39)	1.00	0.8	-0.11 (0.08)	2.10 (1,14)	0.2
Latency to first egg x year	-0.02 (0.03)	1.11	0.4	0.008 (0.005)	2.92 (1,13)	0.1
PA x latency to first egg	0.01 (0.03)	1.00	0.7	0.01 (0.005)	0.13 (1,14)	0.7
BS x latency to first egg	-0.01 (0.02)	1.05	0.6	0.001 (0.004)	0.24 (1,13)	0.6
BS x year	-0.21 (0.40)	1.04	0.6	-0.12 (0.11)	0.20 (1,11)	0.7
Tarsus F x tarsus M	-132.0 (204.2)	1.06	0.5	-68.41 (34.91)	3.84 (1,9)	0.1
PA x BS x latency to first egg	0.09 (0.06)	1.64	0.1	-0.003 (0.008)	0.16 (1,8)	0.7
PA x BS x year	1.03 (1.45)	1.14	0.5	0.11 (0.14)	0.70 (1,7)	0.4
PA x latency to first egg x year	0.13 (0.12)	1.47	0.3	0.03 (0.02)	2.41 (1,6)	0.2
BS x latency to first egg x year	-0.03 (0.07)	1.10	0.6	-0.01 (0.01)	1.16 (1,5)	0.3
PA x BS x latency to first egg x year	0.11 (0.19)	1.18	0.6	0.001 (0.03)	0.002 (1,4)	0.97

BS: brood size; PA: pair assortativeness.

“Pair assortativeness”, “brood size” and “year” are binary variables (assortative or disassortative, HIGH or LOW and 2004 or 2005 respectively). Here, brood size at day 10 could not be used to characterize the rearing conditions due to low sample size. “Latency to first egg” refers to the time between pairing and the first egg laid in the nest. “Tarsus” is the adult tarsus size measured after the preference tests at day 180 (mean age of brood). We used its log transformation in the model analyses.

Full model: pair assortativeness x brood size x latency to first egg x year + tarsus F x tarsus M, with a Poisson distribution for “clutch size” and a Gaussian distribution for “mean egg mass”. “Rejected terms” are the parameters that were not significant when added to the final model. We could not test for the random effects “birth nest” and “foster brood” since they were different for the two mates of each pair.

[†] “Brood size” is the female brood size. A significant effect of male brood size would have appeared as a significant effect of the interaction “pair assortativeness x brood size”. Since *F* tests are inappropriate with a Poisson distribution, we used chi-square tests for which the values were calculated by dividing the residual deviance of the model omitting the tested parameter by the one of the model including the tested parameter. The degrees of freedom therefore reflect the comparison of the models with and without the tested parameter.

[††] “Brood size” is the male brood size. A significant effect of female brood size would have appeared as a significant effect of the interaction “pair assortativeness x brood size”. We safely discarded the factor “clutch size” after checking for its non-significant effect on “mean egg mass”. This was done in a previous analysis since we could not add another factor to the full model due to the loss of degrees of freedom. We did not find an effect of “clutch size” nor of its interaction with other factors on “mean egg mass” in the following model: clutch size x brood size x latency to first egg + tarsus F x tarsus M. The effect of “clutch size” was also not significant when entered as a single factor ($F_{(1,21)}$=2.68, *P*=0.12).

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Nederlandse samenvatting (Dutch summary)

Het effect van de opgroei conditie op de seksuele kenmerken en voorkeuren in zebravinken

In seksueel voortplantende soorten worden alleen de genen van individuen die zich met succes voortplanten geselecteerd. Een succesvolle voortplanting, waarbij de nakomelingen zich ook weer voortplanten, hangt af van of het individu overleeft tot het seksueel volwassen is en of het in staat is om een partner te vinden of daarvoor te strijden. De morfologische en gedragskenmerken van een dier, ook wel het fenotype genoemd, zijn dus niet alleen aangepast aan het overleven, maar aan het aantrekken van zoveel mogelijk goede partners.

Binnen een soort, echter, zien we vaak een grote variatie in de fenotypes, en individuen verschillen enorm in hun succes in het vinden van partners en hoeveel nakomelingen ze produceren. Begrip over de oorzaken en consequenties van de variatie in fenotypes tussen individuen binnen een soort is van centraal belang om vragen over life-history strategieën van individuen en over de evolutie van hun fenotypes te kunnen beantwoorden. De studies in dit proefschrift hebben tot doel bij te dragen aan het onderzoek aan de potentiële oorzaken van de variatie in mannelijke secundaire seksuele kenmerken en in vrouwelijke partner voorkeuren en keuzes in voortplanting.

Veel onderzoeken hebben het bestaan van grote variatie in mannelijke secundaire seksuele kenmerken, de vaak overdreven ornamenten (zoals de staart van de pauw, of uitgebreide zang), laten zien. De gedachte is dat deze grote variatie bestaat omdat het kostbaar is zulke overdreven kenmerken te krijgen en te onderhouden. Als gevolg hiervan kunnen alleen de mannetjes in goede conditie zich zulke vergrootte ornamenten veroorloven. Zulke kenmerken kunnen dus een betrouwbare indicatie zijn van de kwaliteit van een man. Inderdaad hebben diverse correlationele en experimentele onderzoeken in diverse diersoorten laten zien dat mannetjes met overdreven ornamenten een groter aantal nakomelingen hebben doordat ze vaker gekozen worden door een vrouwtje.

De kenmerken van vrouwtjes vertonen ook variatie, en kunnen, net als de kenmerken van mannetjes, fenotypische plasticiteit vertonen. In tegenstelling tot het grote aantal studies dat is geweid aan de variatie in secundaire seksuele kenmerken van mannetjes, zijn er weinig studies die de variatie in de kenmerken van vrouwtjes proberen te verklaren, en deze in relatie te brengen met het reproductief succes van de vrouwtjes. Niet alleen het adverteren voor partners, maar ook het kiezen van een partner kan kostbaar zijn. De variatie in de kwaliteit van de vrouwtjes zou dan dus van invloed moeten zijn op hoe de partnerkeuze geoptimaliseerd is, wat betekend dat de voorkeur van vrouwtjes afhankelijk is van de conditie van het vrouwtje. De theorie van seksuele selectie is echter grotendeels gebaseerd op de aanname dat alle vrouwtjes er de voorkeur aan geven te paren met de man met de beste kwaliteiten en in zulke broedsels ook relatief meer zullen investeren. Dit zou hun reproductief succes maximaliseren. Veel studies negeren daarom de partner voorkeuren van vrouwtjes die afwijken van de norm als ruis in de data. Hierdoor is er dus *erg* weinig bekend over hoe de variatie in partner voorkeuren en reproductieve beslissingen ontstaan en blijven bestaan. Toch zijn er een aantal factoren geïndiceerd die zulke variatie kunnen verklaren. Deze variatie zou genetisch van aard kunnen zijn, vrouwtjes kunnen leren, en de ecologische condities in het verleden en heden van het individu kunnen van invloed zijn. Deze factoren kunnen variatie in de partnervoorkeur van vrouwtjes veroorzaken, doordat ze bijvoorbeeld het inschattingsvermogen van potentiële partners beïnvloeden (het decoderen van broedsignalen), of aan welke signalen vrouwtjes meer aandacht besteden (zijn akoestische signalen belangrijker dan visuele?).

Tegen deze achtergrond wordt in dit proefschrift de rol onderzocht van de context in welke de broedsignalen worden gegeven (Hoofdstuk 2) en de conditie tijdens de ontwikkeling van het mannetje en het vrouwtje (Hoofdstukken 3 t/m 5). Het is tot nu toe niet goed bekend hoe deze twee factoren (context en ontwikkeling) de variatie in hoe vrouwtjes verschillende broed signalen wegen en decoderen (akoestisch en visueel) beïnvloed, en hoe vrouwtjes de kwaliteit van mannetjes beoordelen op basis van de informatie in het signaal.

Deze vragen heb ik gesteld in mijn onderzoek aan een zangvogelsoort, de zebravink: *Taeniopygia guttata*. In deze soort speelt de zang van het mannetje (akoestisch signaal) een belangrijke rol in partnerkeuze. Maar de relatieve rol van de zang in relatie tot de visuele signalen en de intensiviteit van het baltsgedrag is onbekend, omdat verschillende studies verschillende test opstellingen gebruikt hebben. Bovendien is er verrassend weinig bekend over welke structurele kenmerken van een liedje de aantrekkelijkheid ervan bepalen voor een vrouwtje, en hoe zulke kenmerken gerelateerd zijn aan de kwaliteit van een mannetje. Zebravinken zijn daardoor een goed model systeem om systematisch zowel de afhankelijkheid van verschillende signaal dimensies te onderzoeken, als ook welke kenmerken de zang aantrekkelijk maken voor vrouwtjes en wat de liedjes zeggen over de zanger.

Een verwaarloosd aspect in deze context is de mogelijke interactie tussen ecologische (het voeden van de jongen in het nest) en sociale (het aantal jongen in het nest) factoren tijdens de ontwikkeling op de zang van het mannetje, de aangeleerde voorkeur van het vrouwtje voor de zang en op hun reproductieve beslissingen. Zebravinken, zoals zovele andere zangvogels, hebben een gevoelige periode gedurende welke de blootstelling aan zang de details van het liedje dat een mannetje later zingt en het vrouwtje later verkiest, beïnvloed. Omdat variatie in de mannelijke zang hoofdzakelijk cultureel overgedragen wordt, is het de vraag hoe dit kenmerk betrouwbaar de kwaliteit van het mannetje kan aangeven. De ‘ontwikkelingsstress hypothese’ stelt dat de aangeleerde zang de kwaliteit van een mannetje kan aangeven, omdat hersenstructuren die het zangleren en het zingen zelf mogelijk maken zich vormen (ontwikkelen) tijdens de periode van de snelste groei, dat is dus wanneer de jonge vogels het meest kwetsbaar zijn. De mannelijke, aangeleerde zang kan dus een lange termijn signaal zijn van conditie, en vrouwtjes zouden zo dus betrouwbare informatie kunnen krijgen over hoe goed het mannetje de kwetsbare vroege periode heeft doorstaan. Op het moment is er weinig, en evenveel bewijs voor als tegen, de hypothese dat ontwikkelingsstress verschillen veroorzaakt in het zangleren van mannetjes in zangvogels, en tot zover is er nog geen bewijs aan zebravinken. Bovendien is er weinig bekend over de gevoeligheid van de aangeleerde vrouwelijke voorkeuren voor de conditie tijdens de ontwikkeling. De zebravink is extra interessant om te bestuderen in deze context omdat, hoewel alleen mannetjes zingen, zowel mannetjes als vrouwtjes de kenmerken van de zang leren. In deze soort ondergaan de individuen een snelle ontwikkeling naar seksuele volwassenheid, wat hen nog kwetsbaarder maakt voor de effecten van stress tijdens de ontwikkeling, wat repercussies heeft voor hun volwassen fenotype. Zebravinken zijn daardoor een goed modelsysteem om de lange termijn effecten van variaties in de ontwikkeling op de mannelijke aangeleerde zang, de vrouwelijke aangeleerde zangvoorkeur en hun reproductieve beslissingen.

Hoofdstuk 2 onderzoekt het relatieve belang van akoestische (zang) en visuele signalen van de manntjes in verschillende contexten voor de voorkeur van vrouwtjes. Vrouwelijke partner voorkeuren werden getest in drie verschillende, veel gebruikte, opstellingen : operant conditioneren (vrouwtjes wordt geleerd om op knopjes te pikken, zodat ze een liedje horen elke keer dat ze een knopje indrukken en ze kunnen zo zelf hun blootstelling aan de liedjes controleren), phonotaxis (waarbij gemeten wordt hoe dichtbij een vrouwtje naar de bron van het geluid toenadert) en associatie testen met levende mannetjes. In deze drie test opstellingen konden vrouwtjes steeds kiezen tussen twee mannetjes, of de liedjes van twee mannetjes. De operant conditionering en de phonotaxis testen gaven de vrouwtjes de slechts de keuze tussen twee liedjes, terwijl de associatie test met levende mannetjes de vrouwtjes de kans gaf de volledige balts te zien en te horen. Hun voorkeuren werden gemeten door de relatieve frequentie van de keuze voor een stimulus ten opzichte van de tweede stimulus. In de operant conditionering opstelling heb ik het aantal pikken op de knopjes genomen als keuze voor een stimulus. In de phonotaxis test en de associatie test heb ik het aantal keren en de duur van de toenadering naar beide stimuli genomen. Vrouwtjes lieten significante en consistente voorkeuren voor de levende mannetjes en hun liedjes zien in de drie verschillende test opstellingen. Structurele kenmerken aan de zang die de voorkeuren van de vrouwtjes het beste voorspelden (deze kenmerken maten de uitvoering van de zang) waren onafhankelijk van de context van de test en voorspelden ook de lichaamskenmerken van het mannetje (de grootte van de snavel en het gewicht). Behalve dat dit de verschillende test opstellingen om voorkeuren te meten valideert, laten deze resultaten ook zien dat de zang genoeg informatie bevat over de zanger dat het vrouwtje om een keuze te kunnen maken.

Hoofdstukken 3 tot 5 onderzoeken het gecombineerde effect van ecologische (voeden van jongen in nesten) en sociale (aantal jongen per nest) factoren gedurende de ontwikkeling op hun individuele fenotypes, zang leren, zang voorkeuren en de reproductieve beslissingen als volwassenen. De conditie van de jongen en de ouders werd experimenteel beïnvloed door manipulatie van de broedsel grootte. Dit is een gevestigde methode om indirect de voedsel inname van de jongen te manipuleren. Dit gebeurt eigenlijk voornamelijk door de competitie van de nestgenoten voor het voedsel ; deze competitie is groter in grotere broedsels. De zebravinken werden grootgebracht door adoptie ouders in legsel groottes die varieerden binnen de normale grenzen, namelijk ofwel in kleine broedsels van 2-3 jongen, of in grote broedsels van 5-6 jongen. Nadat de jongen voor voedsel van hun ouders onafhankelijk waren (leeftijd 1 maand), werden ze in een groep van gemengde behandeling en gemengde sekse gezet van 4 individuen met een onbekende, niet verwante volwassen man met partner, die de ‘zang tutor’ was tijdens de zang acquisitie fase (van 1 tot 2 maanden oud).

Hoofdstuk 3 laat zien dat de conditie tijdens de ontwikkeling ten minste gedeeltelijk de metabole verbrandingsgraad bepaald in zebravinken: 1 jaar oude vogels die opgegroeid waren in grote broedsels hadden een hogere verbranding dan vogels uit kleine broedsels, wat betekent dat ze meer energie nodig hebben dan vogels in kleine broedsels (zebravinken zijn volwassen als ze 3 maanden oud zijn). Bovendien is dit effect sterker in vrouwtjes dan in mannetjes. Eerder onderzoek had al laten zien dat zebravinken die opgroeien in grote broedsels minder lang leven, en dit effect is ook sterker in vrouwtjes dan in mannetjes. Deze resultaten suggereren dat de metabole efficiëntie een rol kan spelen in de overleving op de lange termijn, en dat dit een van de consequenties is van de conditie tijdens de ontwikkeling.

In Hoofdstuk 4 wordt onderzocht of de ontwikkelingsconditie het leren van de zang en de kenmerken van de zang beïnvloed. Ik heb de zang van volwassen mannetjes die uit het broedsel manipulatie experiment kwamen opgenomen zodra ze seksueel volwassen waren (3 maanden oud). Met de computer heb ik verscheidene zang kenmerken geanalyseerd (zang complexiteit, de uitvoering van de zang en de consistentie van de zang) en het aantal elementen en de transities tussen elementen die geleerd zijn van de tutor. Ik heb gevonden dat de vogels uit grote broedsels minder element transities leren zoals ze in de zang van de tutor voorkomen, dan de vogels uit kleine broedsels. Bovendien vertoonden de vogels uit de grote broedsels minder consistente zang dan de vogels uit kleine broedsels. Deze resultaten ondersteunen dus de ontwikkelingsstress hypothese : de conditie tijdens de ontwikkeling beïnvloedt de precisie waarmee de syntactische structuur van de zang geleerd wordt en introduceert conditie afhankelijkheid in de zang consistentie.

Hoofdstuk 5 onderzoekt of de conditie tijdens ontwikkeling (de broedsel grootte) bijdraagt aan de variatie in de vrouwelijke partnervoorkeuren en reproductieve beslissingen. Gebruik makende van een welbekende operant conditionering opstelling (Hoofdstuk 2), heb ik de voorkeuren van volwassen vrouwtjes getest, opgegroeid in een klein dan wel een groot broedsel, voor de zang van mannetjes uit een groot of een klein broedsel (andere broedsels dan die van het te testen vrouwtje). Vrouwtjes vertoonden conditie afhankelijke voorkeuren : vrouwtjes uit kleine broedsels (dus een goede conditie) hadden een voorkeur voor mannetjes uit kleine broedsels. Echter, vrouwtjes uit grote broedsels (dus een slechtere conditie) hadden een voorkeur voor mannetjes uit grote broedsels ten opzichte van mannetjes uit kleine broedsels. Deze partnervoorkeuren werden nog eens bevestigd tijdens de voortplanting: vrouwtjes die gepaard werden met mannetjes uit een zelfde broedsel grootte (groot met groot en klein met klein) broedden eerder dan vrouwtjes die gepaard werden met een mannetje uit een andere broedsel grootte (groot met klein en klein met groot). Dit suggereert dat mannetjes met een vergelijkbare conditie tijdens de ontwikkeling als het vrouwtje sneller geaccepteerd werden als partner. Het is hierbij belangrijk te vermelden dat de perceptie van de vrouwtjes over de conditie van het mannetje niet verstoord was omdat alle vrouwtjes meer investeerden in het gewicht van de eieren wanneer ze gepaard waren met een mannetje uit een klein broedsel. De vrouwtjes waren dus eensgezind over de beoordeling van de conditie van het mannetje, hoewel ze wel verschillende voorkeuren hadden. Deze resultaten leveren bewijs dat de conditie tijdens de ontwikkeling variatie kan veroorzaken in de individuele conditie, en dat deze zich vertaalt in substantiële fenotypische plasiticiteit, met name in de richting van de partnervoorkeuren van de vrouwen.

Heeft het werk in dit proefschrift ons begrip verbeterd van de oorzaken en consequenties van de variatie in mannelijke secundaire seksuele kenmerken en in vrouwelijke partnervoorkeuren en reproductieve beslissingen? Hoofdstuk 5 laat zien dat de conditie tijdens de ontwikkeling een belangrijke bron is voor variatie in de vrouwelijke partner voorkeuren en reproductieve beslissingen. Vrouwtjes die een goede of een slechte conditie ondervonden tijdens de ontwikkeling vertoonden tegenovergestelde voorkeuren. Dit suggereert dat er een verandering plaats vindt in hoe ze informatie verwerken, maar dit impliceert niet dat de cognitieve capaciteit verminderd door de conditie tijdens de ontwikkeling. Het is interessant dat dit effect zo ver gaat dat de vrouwtjes actief kiezen voor mannetjes die ook een slechte conditie hadden. Dit is op het eerste gezicht tegen-intuïtief, omdat de theorie van de seksuele selectie voorspelt dat vrouwtjes altijd een voorkeur zouden moeten hebben voor de mannetjes van de hoogste kwaliteit wanneer ze de keuze hebben. Echter, recente theoretische modellen laten zien dat een voorkeur voor mannetjes van mindere kwaliteit een adaptieve strategie kan zijn als die geëvolueerd is onder bepaalde selectie drukken. Wanneer de competitie voor de toegang tot partners hoog is, of wanneer beide partners kiezen, is het waarschijnlijk dat de vrouwtjes van mindere kwaliteit eruit geconcurreerd worden door de vrouwtjes van hogere kwaliteit, of dat mannetjes ze links laten liggen, of ze verlaten ze om verder te gaan met een vrouwtje van hogere kwaliteit. Door meteen een partner te kiezen die van een relatief lagere kwaliteit is, verlagen die vrouwtjes zulke risico's en besparen ze tijd en energie, wat blijkbaar erg kostbaar voor ze is, gezien de effecten van de conditie tijdens de ontwikkeling op hun metabole verbrandingsgraad (Hoofdstuk 3) en overleving kansen. Uiteindelijk kan een actieve voorkeur voor lagere, in plaats van hogere kwaliteit mannetjes ervoor zorgen dat de vrouwtjes van lagere kwaliteit hun reproductieve succes optimaliseren (bijvoorbeeld door eerder te kunnen broeden, zoals in Hoofdstuk 5).

De mannelijke zang bleek een belangrijke rol te spelen in de gevonden variatie in de voorkeuren van vrouwtjes. Het verschil in voorkeur tussen de vrouwtjes uit kleine en grote nesten was duidelijk terwijl ze alleen toegang hadden tot de zang van de mannetjes, en deze variatie werd later bevestigd in de timing van hun reproductieve beslissingen (Hoofdstuk 5). Verder was het zo dat de voorkeur voor de zang consistent was in verschillende contexten en deze zich vertaalde in een vergelijkbare voorkeur voor de zanger (Hoofdstuk 2). Alles bij elkaar genomen wekken deze resultaten sterk de suggestie dat de zang genoeg informatie geeft over de zanger voor de vrouwtjes om een keuze te kunnen maken. Verdere ondersteuning hiervoor is dat sommige kenmerken aan de zang een relatie hebben met de morfologie van het mannetje (Hoofdstuk 2) en/of de ontwikkelingsgeschiedenis van het mannetje (Hoofdstuk 4). De geleerde zang van het mannetje kan dus optreden als een lange termijn signaal van zijn conditie in het verleden, en dit geeft dus betrouwbare informatie aan de vrouwtjes over hoe goed het de mannetjes is vergaan tijdens hun ontwikkeling.

In conclusie levert dit proefschrift bewijs dat zowel de mannelijke aangeleerde zang en de vrouwelijke sociaal geleerde voorkeuren afhankelijk zijn van vroege ecologische en sociale factoren. Zowel partnervoorkeuren als aspecten van de zangproductie reflecteren lang de conditie tijdens de ontwikkeling van een individu. Een aanpak die zowel de ervaringen in het verleden, als state-dependent life history kenmerken in ogenschouw neemt kan erg vruchtbaar zijn om ons begrip te vergroten van seksuele selectie en de evolutionaire dynamiek tussen voorkeuren en de seksueel geselecteerde, cultureel overdraagbare kenmerken.

Résumé en français (French summary)

Les effets des conditions développementales sur les traits et préférences sexuels des diamants mandarins

Chez les espèces à reproduction sexuelle, seuls les gènes des individus qui réussissent à se reproduirent peuvent être sélectionnés. Une reproduction réussie, c'est-à-dire qui donne une descendance qui se reproduit à son tour, dépend à la fois de la capacité de l'individu à survivre jusqu'à sa maturité sexuelle et de sa capacité à attirer un partenaire et/ou rivaliser pour un partenaire. Ainsi, l'aspect et le comportement d'un animal, c'est-à-dire son phénotype, devraient être adaptés non seulement pour l'aider à survivre, mais également pour l'aider à acquérir le plus grand nombre de partenaires ou les partenaires de qualité supérieure.

Cependant, les individus au sein d'une espèce présentent souvent une grande variation phénotypique et diffèrent considérablement dans leur succès d'appariement et reproducteur. Comprendre les causes et les conséquences de la variation phénotypique entre individus d'une espèce est fondamental pour répondre à des questions sur les stratégies d'histoire de vie des individus et sur l'évolution de leurs phénotypes. Les études présentées dans cette thèse ont pour but d'explorer certaines des causes potentielles de la variation des traits sexuels secondaires entre les mâles et des préférences sexuelles et décisions reproductives entre les femelles.

De nombreuses études chez les mâles ont rapporté l'existence d'une variation importante dans leurs traits sexuels secondaires, qui sont souvent des ornements extravagants (comme la queue du paon ou les chants élaborés de certains oiseaux). Cette grande variation existerait parce que les ornements extravagants sont coûteux à produire ou à maintenir. En conséquence, seuls les mâles en bonne condition pourraient se permettre de posséder les ornements les plus extravagants, qui ainsi signaleraient de manière fiable la qualité des mâles. En effet, de nombreuses études corrélationnelles et expérimentales chez de nombreux groupes taxonomiques animaux ont montré que les mâles possédant des ornements plus extravagants, ou des attributs particuliers, ont un succès reproducteur plus élevé car ils sont plus souvent choisi par les femelles pour la reproduction.

En revanche, beaucoup moins d'études se sont attachées à expliquer la variation des traits des femelles, qui a priori comme les traits des mâles sont susceptibles de montrer une plasticité phénotypique, et de relier cette variation au succès reproducteur des femelles. Choisir un partenaire, tout comme s'employer à l'attirer, est susceptible d'entraîner des coûts. Par conséquent, la variation de qualité entre les femelles devrait déterminer comment l'investissement dans le choix du partenaire est optimisé. Ceci pourrait conduire à une expression "condition-dépendante" des préférences sexuelles des femelles. Cependant, la théorie de la sélection sexuelle est en grande partie fondée sur l'idée que toutes les femelles devraient préférer s'accoupler et investir plus avec les mâles de meilleure qualité afin d'optimiser leur succès reproducteur. Ainsi, beaucoup d'études ne tiennent pas compte des préférences déviant de la "norme" ce qui a pour conséquence de limiter notre compréhension de ce qui cause et maintient une variation de préférences sexuelles et de décisions reproductives entre les femelles. Plusieurs facteurs ont néanmoins été proposés pour essayer de rendre compte de l'observation de variation des préférences entre les femelles, comme les facteurs génétiques, les processus d'apprentissage ou le contexte écologique et social passé et présent. Ces facteurs pourraient introduire une variabilité dans les préférences des femelles, par exemple en influençant leur capacité à évaluer précisément des partenaires potentiels (par exemple quant à l'interprétation des signaux), ou les signaux auxquels les femelles prêtent le plus d'attention (par exemple l'importance relative des signaux acoustique et visuel), ou leur condition physiologique ou générale, ou encore la quantité et qualité de l'expérience acquise (via l'apprentissage).

Dans ce contexte général, cette thèse explore le rôle du contexte dans lequel les signaux sont présentés (Chapitre 2) et des conditions développementales des femelles et des mâles (Chapitres 3 à 5) sur la variation dans la façon dont les femelles évaluent et décodent différents signaux (acoustique et visuel) et jugent la qualité des mâles en se basant sur l'information que contient le signal.

J'ai abordé ces questions chez une espèce d'oiseau chanteur, le diamant mandarin *Taeniopygia guttata*. Chez cette espèce, le chant des mâles (signal acoustique) joue un rôle important dans le choix du partenaire chez les femelles. Cependant, le rôle relatif du chant par rapport aux signaux visuels et à l'intensité de la parade sexuelle reste peu compris. Ceci est en grande partie dû au fait que différentes études utilisent différents paradigmes pour tester les préférences des femelles. De plus, nous avons une connaissance très limitée des caractéristiques structurelles du chant qui déterminent l'attractivité des mâles et sur ce que révèlent ces caractéristiques sur la qualité des mâles. En ce sens, les diamants mandarins constituent un bon modèle biologique pour examiner de manière systématique si les préférences pour différentes dimensions du signal dépendent du contexte expérimental dans lequel le signal est présenté, quels sont exactement les attributs qui rendent un chant attractif et ce que les caractéristiques préférées dans le chant révèlent sur le chanteur.

Un aspect négligé dans ce contexte est l'effet potentiel des interactions entre facteurs écologiques (par exemple nourrissage des jeunes dans la nichée) et sociaux (par exemple nombre de jeunes dans la nichée) pendant le développement sur l'apprentissage du chant chez les mâles et des préférences chez les femelles ainsi que sur leurs décisions reproductives. En effet, les diamants mandarins, comme un grand nombre d'oiseaux chanteurs, ont une période sensible précoce pendant laquelle l'exposition à un chant influence les détails du chant qui est par la suite produit par un mâle et préféré par une femelle. La variation de chant entre les mâles étant en grande partie héritée culturellement, des chercheurs se sont demandés comment ce trait pouvait signaler de façon fiable la qualité des mâles. Ainsi l'hypothèse du stress développemental propose que le chant appris par les mâles peut indiquer leur qualité parce que le développement des structures cérébrales nécessaires à l'apprentissage et à la production du chant a lieu pendant la période de croissance la plus rapide, c'est-à-dire lorsque les oisillons sont le plus vulnérable. Par conséquent, le chant appris par les mâles pourrait signaler à long terme leur condition pour les femelles en reflétant comment les mâles se portent après avoir connu un stress développemental. Jusqu'à présent, les effets d'un stress développemental sur des variations dans l'apprentissage du chant entre les mâles restent équivoques chez les oiseaux chanteurs en général, et n'ont jamais été montrés chez les diamants mandarins. Notre connaissance est encore plus limitée en ce qui concerne la sensibilité des préférences apprises par les femelles aux conditions développementales. Le diamant mandarin est particulièrement intéressant à étudier dans ce contexte puisque, même si seuls les mâles chantent chez cette espèce, les femelles comme les mâles font un apprentissage vocal. De plus, les individus ont un développement très rapide jusqu'à leur maturité sexuelle, ce qui peut les rendre d'autant plus vulnérables aux effets d'un stress développemental avec des répercutions sur leur phénotype adulte. Les diamants mandarins constituent donc un bon modèle biologique pour étudier les conséquences à long terme de variations développementales sur les phénotypes des individus, sur le chant appris par les mâles, sur les préférences pour le chant apprises par les femelles et sur leurs décisions reproductives.

Le Chapitre 2 explore l'importance relative des signaux acoustique (chant du mâle) et visuel (le mâle) sur les préférences sexuelles des femelles et explore si ces préférences dépendent du contexte expérimental dans lequel les signaux sont présentés. Les préférences des femelles ont été testées dans trois dispositifs expérimentaux différents et fréquemment utilisés dans les études de préférence : conditionnement opérant (les femelles sont conditionnées à presser sur des boutons, chaque fois qu'elles pressent elles entendent un chant associé au bouton pressé, et peuvent donc contrôler de manière active leur exposition aux chants), phonotaxie (mesure l'orientation en fonction d'une source sonore) et tests d'association spatiale avec des mâles. Ces trois dispositifs permettaient aux femelles de choisir soit entre deux mâles soit entre leurs deux chants. Les tests de choix de conditionnement opérant et de phonotaxie présentaient le chant du mâle seul alors que les tests d'association avec des mâles présentaient la parade sexuelle complète. J'ai évalué les préférences en comparant la fréquence relative avec laquelle un stimulus était choisi sur l'autre. Dans les tests de conditionnement opérant, j'ai utilisé le nombre de fois que les femelles ont pressé sur les boutons. Dans les tests de phonotaxie et les tests d'association avec les mâles, j'ai utilisé le nombre et la durée totale des approches vers l'un ou l'autre des deux stimuli (respectivement les chants ou les mâles). Un nombre significatif de femelles testées a fidèlement préféré un mâle ou son chant et ce dans les trois dispositifs. Les caractéristiques structurelles du chant qui prédisaient le mieux les préférences des femelles (ces caractéristiques mesuraient la performance du chant) étaient indépendantes du contexte et prédisaient également la morphologie des mâles (à savoir la taille du bec et la masse). Non seulement ces résultats valident les différents dispositifs utilisés pour tester les préférences, mais montrent également que le chant doit contenir une information suffisante sur le chanteur lors du choix d'un partenaire par les femelles.

Les Chapitres 3 à 5 explorent l'effet combiné des facteurs écologiques (nourrissage des jeunes dans la nichée) et sociaux (nombre de jeunes dans la nichée) au cours du développement sur les phénotypes des individus, sur l'apprentissage du chant, sur les préférences pour le chant et sur les décisions reproductives chez les oiseaux adultes. J’ai expérimentalement modifié la condition des oiseaux (de oisillons à adultes) par une manipulation de la taille des nichées. Cette méthode est bien établie chez le diamant mandarin et permet de manipuler indirectement la prise de nourriture pendant le développement, ce principalement grâce à la compétition entre oisillons pour l’accès à la nourriture, compétition qui est plus élevée dans les nichées dont la taille est plus grande. Les diamants mandarins ont été élevés par des parents adoptifs dans des tailles de nichée respectant l’échelle naturelle spécifique à l’espèce, à savoir soit dans des petites nichées de 2-3 oisillons ou des grandes nichées de 5-6 oisillons. Après avoir atteint leur indépendance nutritionnelle (à l’âge d’un mois), les oiseaux ont été placés dans des groupes de quatre individus (deux mâles, un de chaque taille de nichée, et deux femelles, une de chaque taille de nichée) avec un mâle adulte et sa partenaire. Ce mâle qui leur était génétiquement et socialement non familier a joué le rôle de “tuteur du chant” pendant la phase d’acquisition du chant (entre les âges d’un à deux mois).

Le Chapitre 3 montre que les conditions développementales déterminent, au moins partiellement, le taux métabolique des diamant mandarins : à l’âge d’un an (la maturité sexuelle est atteinte à trois mois), les oiseaux élevés dans les grandes nichées avaient un taux métabolique plus élevé, c’est-à-dire des besoins en énergie plus importants, que les oiseaux élevés dans les petites nichées. De plus, les femelles ont été plus affectées que les mâles. Il a précédemment été montré que des diamants mandarins issus de grandes nichées vivaient moins longtemps, et que cet effet était également plus fort chez les femelles que chez les mâles. Ces résultats combinés suggèrent que l’efficacité métabolique pourrait jouer un rôle de médiateur entre les conditions développementales et leurs conséquences à long terme pour la survie.

Le Chapitre 4 explore si les conditions développementales affectent l’apprentissage du chant chez les mâles et certaines caractéristiques de leur chant. J’ai enregistré les mâles issus des petites et grandes nichées après qu’ils aient atteint leur maturité sexuelle (à trois mois) et analysé plusieurs caractéristiques acoustiques (mesurant la complexité, la performance et la stéréotypie du chant) ainsi que le nombre d’éléments et de transitions entre éléments appris à partir du chant du tuteur à l’aide d’un programme d’analyse acoustique sur ordinateur. J’ai observé que les mâles élevés dans des grandes nichées ont appris moins de transitions entre éléments (en comparaison avec le chant de leur tuteur) que ceux élevés dans des petites nichées. De plus, les mâles provenant de grandes nichées ont chanté d'une façon moins stéréotypée que ceux de petites nichées. Ces résultats viennent donc renforcer l'hypothèse du stress développemental puisque les conditions développementales ont affecté la précision avec laquelle les mâles ont appris la structure syntactique du chant et ont conduit à une "condition-dépendance" de la stéréotypie du chant.

Le Chapitre 5 examine si les conditions développementales (taille des nichées) entraînent une variation des préférences sexuelles et des décisions reproductives des femelles. Par conditionnement opérant, une méthode maintenant bien établie pour tester les préférences des femelles (Chapitre 2), j'ai testé si les femelles issues de petites ou grandes nichées avaient une préférence pour le chant de mâles issus soit de petites soit de grandes nichées (différentes de celles dont étaient issues les femelles). J'ai trouvé que les préférences des femelles dépendaient de leur condition : les femelles provenant de petits nichées (c'est-à-dire des femelles de condition élevée) ont préféré les mâles de petites nichées à ceux de grandes nichées, alors que les femelles de grandes nichées (c'est-à-dire de faible condition) ont préféré les mâles de grandes nichées à ceux de petites nichées. Ces préférences sexuelles se sont confirmées lors de la reproduction : les femelles expérimentalement appariées à des mâles provenant d'une taille de nichées identique à la leur se sont reproduit plus rapidement que les femelles appariées à des mâles provenant d'une taille de nichées différente de la leur. Ce résultat suggère que les mâles provenant d'une taille de nichée identique à celle des femelles ont été acceptés plus rapidement comme partenaire reproducteur par les femelles. Il est important de préciser que les femelles provenant de grandes nichées avaient les même capacités d'évaluation de la condition des mâles que les femelles de petites nichées. En effet, toutes les femelles ont pondu des oeufs plus lourds, c'est-à-dire ont plus investi dans la reproduction, quand elles étaient appariées avec un mâle provenant d'une petite nichée. Ainsi en dépit de leurs préférences allant dans des directions opposées, toutes les femelles étaient en fait d'accord sur la qualité phénotypique des mâles avec lesquels elles étaient appariées. Ces résultats prouvent que les conditions développementales entraînent une variation de la condition entre individus qui se traduit par une importante plasticité phénotypique, notamment dans la direction des préférences sexuelles des femelles.

Est-ce que les études présentées dans cette thèse ont réussi à améliorer notre compréhension des causes et conséquences de la variation des traits sexuels secondaires entre les mâles et des préférences sexuelles et décisions reproductives entre les femelles ? Le Chapitre 5 montre que les conditions développementales sont une source importante de variation dans les préférences sexuelles et décisions reproductives des femelles. Les femelles de faible condition ont montré des préférences opposées à celles des femelles de condition élevée. Cela suggère un changement dans la façon dont les femelles de faible condition ont traité l'information sensorielle mais cela n'implique en aucun cas que leurs capacités cognitives aient été affectées par les conditions développementales défavorables. Il est intéressant de noter que les effets sont allés jusqu'à entraîner une préférence active pour les mâles de faible condition. Ceci peut paraître contre-intuitif de prime abord étant donné que la théorie de la sélection sexuelle prédit que les femelles devraient toujours préférer les mâles de qualité supérieure quand elles en ont le choix. Cependant des models théoriques récents suggèrent qu'une préférence pour des individus de faible qualité pourrait être une stratégie adaptative ayant évolué suite à certaines pressions de sélection. Lorsque la compétition pour acquérir un partenaire est élevée ou lorsque un choix mutuel existe entre les individus des deux sexes, les femelles de faible qualité encourent un risque important d'être fortement concurrencer par des femelles de qualité supérieure ou d'être évitées ou abandonnées (divorce) par les mâles en faveur des femelles de qualité élevée. Par conséquent en choisissant immédiatement un partenaire de qualité inférieure, les femelles de faible qualité pourraient réduire ces risques et économiser du temps et de l'énergie (qui apparemment leur est très précieux étant donné les effets des conditions développementales sur leur métabolisme (Chapitre 3) et sur leurs perspectives de survie). Enfin, une préférence active pour des mâles de qualité inférieure pourrait permettre aux femelles de faible qualité d'optimiser leur succès reproducteur (par exemple en se reproduisant plus rapidement comme le montre le Chapitre 5).

Le chant des mâles semble jouer un rôle majeur en tant que médiateur de la variation observée dans les préférences des femelles. En effet, la différence de préférence entre les femelles de petites et grandes nichées a eu lieu alors que les femelles avaient uniquement accès au chant, et leurs décisions reproductives sont venues corroborer cette variation de préférences pour le chant (Chapitre 5). De plus, les préférences pour un chant particulier se sont révélées être indépendantes du contexte expérimental dans lequel les chants étaient présentés (différents dispositifs de tests de choix) et se sont traduites par une préférence similaire pour le chanteur (Chapitre 2). Ces résultats combinés suggèrent que le chant contient une information suffisante sur le chanteur pour le choix d'un partenaire par les femelles. A l'appui de cette conclusion, j'ai apporté la preuve que certaines caractéristiques du chant reflétaient la morphologie des mâles (Chapitre 2) et/ou leur histoire développementale passée (Chapitre 4). Le chant appris par les mâles peut donc signaler leur condition à long terme et donner aux femelles une information fiable qui reflète comment les mâles se portent après avoir connu un stress développemental.

En conclusion, cette thèse indique que l'apprentissage du chant chez les mâles et les préférences apprises chez les femelles dépendent l'un comme l'autre de la variation des facteurs écologiques et sociaux précoces. Les préférences sexuelles et certains aspects de la production du chant reflètent durablement les conditions développementales des individus. Par conséquent, une approche prenant en compte à la fois les expériences passées et les traits d'histoire de vie "condition-dépendants" pourrait s'avérer extrêmement fructueuse pour améliorer notre compréhension de la sélection sexuelle et de la dynamique évolutive entre les préférences et les traits sexuellement sélectionnés et transmis culturellement.

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Curriculum vitae

I was born 27 April 1978 in Villeneuve-Saint-Georges, France. I entered the Pierre et Marie Curie national junior secondary school in Héricourt in 1989. In 1996 I passed the French scientific Baccalaureate at the Louis Aragon national secondary school in Héricourt. The same year I opted to study Biology at the University of Besançon where I got interested in studying Behavioural Biology and Ecology after a course in this field in 1999/2000. In 2000, I carried out my first behavioural biology research project which aimed at establishing the social structure and reproductive capacities in a recently restructured group of Guinea baboon, *Papio papio*, in the zoo of the History Natural Museum of Besançon. For this undergraduate project, I was supervised by Prof. Gérard Brand from the Laboratory of Neurosciences at the University of Besançon and Jean-Yves Robert, Assistant Curator at the History Natural Museum. From 2001 onwards, I investigated sexual selection and mating systems in agile frogs, *Rana dalmatina*, supervised by Prof. Thierry Lodé from the Laboratory of Animal Ecology at the University of Angers. During the course of this research project I was awarded two diplomas: In June 2001 I passed my ‘Diplôme d’Etudes Approfondies’ (equivalent of MSc Degree) in Behavioural Biology at the University of Paris XIII and in December 2002 the ‘Diplôme d’Etudes Supérieures Universitaires’ (lit. University High Education Diploma) in Biology at the University of Metz. In winter and spring of 2003, while looking for a PhD position, I worked as a substitute teacher in several Junior High Schools in Angers and its surroundings.

In September 2003 I took up the PhD project presented in this thesis investigating developmental influences on female preferences for a sexually selected and culturally transmitted trait, the song, in zebra finches, *Taeniopygia guttata*, at the Behavioural Biology group at Leiden University under the supervision of Dr. Katharina Riebel and Prof. Carel ten Cate. This work was supported by the Research Council for Earth and Life Sciences (ALW) with financial aid from the Netherlands Organization for Scientific Research (NWO).

In October 2007, I was awarded a French university post-doctoral fellowship to study whether egg color is a sexually selected signal influencing paternal investment in blue tits, *Cyanistes caeruleus*. I took up this position in January 2008 and I am working since as a post-doctoral research fellow at the Centre of Functional and Evolutionary Ecology (CEFE, CNRS) at the University of Montpellier in collaboration with Dr. Arnaud Grégoire and Dr. Claire Doutrelant (CNRS).

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