On the significance of song amplitude in birds – function, mechanisms, and ontogeny

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Mathias Ritschard

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“Laute Sänger sind für Zebrafinkenweibchen offensichtlich attraktiver als leisere Artgenossen. Das haben Forscher des Max-Planck-Instituts für Ornithologie in Seewiesen bei Starnberg herausgefunden. (...) Das hätten wir den Vögelforschern allerdings auch ohne Forschungsstudie vorher sagen können. Wir nennen das schon lange den „Bushido-Effekt“. Das ist schliesslich der Grund, warum junge Männer dauernd so laut in der Gegend herumkrakeelen.“

TAZ (Die Tageszeitung), 22. Februar 2010

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General introduction

Communication is a central field in behavioural ecology. Animals rarely live in isolation, but rather share resources like living space and food or, in the case of sexual reproduction, are even dependent on other individuals to make sure that their own genes are passed on to the next generation. Communication is the process of exchanging information between individuals in the form of a signal, and this signal may, or may not, change the behaviour of the targeted receiver.

Animals make use of a number of signal modalities to code and share information. We can distinguish between several broad categories of communication signals: acoustic, optical, chemical, electrical and tactile signals. Acoustic signals are widespread in vertebrates and insects because they have some unique advantages. They can easily travel across long distances and through complex habitats and they make it possible to share a large amount of information within a short time. Acoustic signals range from simple drumming sounds produced by wolf spiders to attract females (e.g. Allard 1936; Parri *et al.* 2002) to the complex and multifunctional human speech (e.g. Paget and Rae 1978).

The study of birdsong

Without doubt, birdsong is among the most remarkable and beautiful sounds produced by nature, and it has inspired writers, musicians and researchers for centuries. Thanks to the invention of sound spectrograph technologies in the 1950s, it became possible to make birdsong visible on paper and, later, on screens, which ushered in a new field of modern birdsong research.

Birdsong has been under extensive research during the last decades and has been established as an important model in various biological disciplines. Anatomists and biophysicists are studying the anatomy and functionality of the avian sound producing system, physiologists are concerned with the endocrinology and energetics of birdsong, neuroscientists are investigating the neuronal pathways that are necessary for the production and perception of birdsong as well as for song learning, evolutionary biologists and behavioural ecologists ask questions about how birdsong is shaped by natural and sexual selection, and biogeographers and taxonomists are studying the biogeographic variation of birdsong and the relationship between birdsong and speciation.

Birdsong has two main functions: defense of territories and attraction of sexual partners (see below). While songs are usually the most obvious and complex acoustic signals that birds produce, their signal repertoire also includes a variety of simpler vocalizations with different functions, commonly referred to as calls. For instance, begging calls of juveniles signal their need for food, contact calls help to keep in touch with mates or flock members, and alarm calls warn conspecifics of predators (Marler 2004). Many bird species have repertoires of ten or more different call types, but their exact function is often little studied.

Birdsong and sexual selection

In his groundbreaking book “The descent of man”, Charles Darwin (1871) proposed that birdsong is shaped by sexual selection. This view is still hold today and has received plenty of experimental confirmation since Darwin’s time. Sexual selection is usually thought to occur in two ways: within sexes (male-male competition) and between sexes (female attraction).

There are several lines of evidence suggesting that song plays an important role in male-male competition. Experiments where territory holders were muted or were removed from their territories and partly replaced by speakers showed that muted males suffered higher intrusion rates into their territory (McDonald 1989), and that territories where the resident male had been replaced by a speaker were reoccupied much later than control territories (Krebs 1977). Moreover, playing back the song of a conspecific in the territory of a male usually stimulates the territory holder to sing back and to approach the speaker or even to physically attack it (Catchpole and Slater 2008).

Many experimenters have tested the role of birdsong in female attraction. Several studies demonstrated that females are more attracted to nest sites from where a male song was played back than to control nest sites (e.g. Eriksson and Wallin 1986; Mountjoy and Lemon 1991). Similarly, females in captivity have been shown to approach male songs (e.g. Miller 1979; Clayton 1988) played from speakers. Moreover, female songbirds sample a number of singing males before they settle with one of them (Bensch and Hasselquist 1992; Roth *et al.* 2009). Apart from its importance in attracting females, male song has also been shown to affect female reproductive behaviour and physiology. For instance, in many species, females that are exposed to male song may show copulation solicitation displays, a behaviour which signals their willingness to copulate (Searcy 1992).

Individual variation in the expression of sexually selected traits affects reproductive success (Andersson 1994). Thus, to ascertain the reliability of a sexually selected trait, the production or maintenance of the trait is expected to be costly (Grafen 1990). Birdsong is not one single trait, but encompasses multiple traits that may be sexually selected and that may each have its own costs. In their influential review on the honesty of birdsong, Gil and Gahr (2002) listed a number of song traits that may be important in sexual selection: performance-related traits like singing rate and song amplitude, repertoire size, and specific song content (e.g. dialects, the presence or absence of specific syllables, song frequency or song sharing). The costs that act on these traits may be related to time or energy budgets, developmental constraints, social aggression, predation and immunocompetence, or they may be neural (Gil and Gahr 2002).

Song learning

As with most types of behaviour, birdsong is a product of the interaction between nature and nurture, i.e. between genes and environment (Baptista 1996). In oscines, parrots and hummingbirds, vocal production learning plays an important role in song development (Catchpole and Slater 2008).

Birds that are raised in acoustic isolation develop a rudimentary song, although these "isolate" songs may be structurally similar to the songs of conspecific birds raised under normal conditions (e.g. Thorpe 1958; Nottebohm 1968). This observation led to the development of the auditory template model: songbirds are born with a crude template of their own species' song. In a memorization phase early in life, the template is matched to the songs the bird is hearing (the 'tutor' songs) and an exact template is acquired. In a subsequent motor phase, when the bird starts to produce songs, its own song output is matched to the exact template and after some training, the bird reproduces a more or less accurate copy of its tutors' songs (Catchpole and Slater 2008).

The auditory template model seems to apply for most songbird species studied to date. However, there is a huge variability between species in the timing of memorization and the social conditions that are required for song learning. Some species may learn only as juveniles, other may learn during their entire lives. Some species will incorporate songs or song elements from many different tutors, while others will learn from one tutor only; some learn only their own species' songs, others may imitate the songs of other species.

An introduction to song amplitude

Physics

Sound can be defined as mechanical vibrations that travel through a medium, usually a gas like air. In air, sound is transmitted as longitudinal waves, i.e. through local compression of molecules in the axis of propagation. These sound waves can be characterized by wavelength (the distance between two peaks of the sinusoidal pressure curve), amplitude (the difference between peak and ambient pressure) and speed of propagation. The speed depends on the medium and is ca. 343 m/sec for air. The pitch, or frequency, of a sound relates to the time interval between two pressure peaks at a fixed location and therefore depends on wavelength and speed of propagation. Frequency is measured in Hertz (Hz).

Sound amplitude is usually given in decibel (dB). Decibel is a relative measure, typically expressed in relation to the human hearing threshold level which is arbitrarily set to 0 dB. An amplitude value given relative to this threshold is indicated by adding 'SPL' (sound pressure level). Moreover, decibel is a logarithmic scale; as a rule of thumb, an increase of 6 dB corresponds to a doubling of the sound pressure. Rustling leaves have a sound pressure level of approximately 10 dB SPL, a normal conversation reaches about 60 dB SPL (323 times higher pressure than the leaves) and the noise of a jet taking off is around 120 dB SPL (1024 times higher pressure than a conversation; data taken from Bradbury and Vehrencamp 1998).

To compute the amplitude of a sine wave at a certain point in time, one can either measure the difference between maximum and minimum pressure ('peak-to-peak' measure) or between one of the extreme values (maximum or minimum) and ambient pressure ('peak' measure). To measure amplitude across a certain duration of time, a large number of measurements of deviations from ambient pressure are taken and the root mean squared (RMS) value of pressure deviations is calculated. A simple arithmetic mean of pressure deviations can not be used because positive and negative deviations would cancel each other. This RMS method has been employed throughout my thesis to assess sound pressure levels.

How to measure sound amplitude

Assessing the vocal amplitude of birds is not a simple matter. This may be one of the main reasons why amplitude has received relatively little attention in bioacoustic research. Imagine an observer attempting to measure the song amplitude of a bird singing in a nearby tree. The amplitude measurement at the position of the observer depends on several factors: (1) the distance from the singing bird, (2) the orientation of the singer (Witkin 1977; Larsen and

Dabelsteen 1990; Brumm 2002; Patricelli *et al.* 2007) and (3) environmental acoustics; sound absorption depends on vegetation, humidity and air temperature (Wiley and Richards 1982). Therefore, all these variables should be taken into account when assessing the song amplitude of a bird. This can be done by measuring the distance to the singing bird, standardizing the orientation of the bird (e.g. by only using measurements obtained while the bird faces directly towards the microphone or sound level meter), minimizing the distance to the singing bird (which reduces effects of environmental acoustics), making sure that there are no obstacles in the direct sound path between the singer and the measuring instrument, and controlling for air temperature and humidity (Brumm 2004b). In the laboratory, measuring song amplitude is usually easier since environmental conditions can be kept constant and the distance between the singer and the measuring instrument is minimal. Moreover, the orientation of the bird can be controlled for by measuring sound amplitude from above rather than from the side (Brumm 2009). More elaborate set-ups have been used, for example microphone arrays arranged in a circle around a singing bird to determine its exact orientation, but these techniques are very laborious and usually difficult to apply in the field (Patricelli *et al.* 2007).

Production

The avian sound production organ, the syrinx, is located at the position where the two bronchi meet (the tracheobronchial junction). Songbirds do not have vocal chords like mammals; instead, sound is produced by two pairs of small tissue pads (one pair per bronchus), called labia, which are located at the cranial end of each bronchus. The labia are moved in and out of the airflow by muscle activity, which causes them to vibrate (see Suthers and Zollinger 2008). The two pairs of labia can be controlled independently and sound can therefore be produced either with the right or the left side of the syrinx or with both sides at the same time (Zollinger *et al.* 2008).

Although birdsong is essentially produced by the syrinx, vocal tract resonances greatly affect the final form of the song that we can hear when listening to a singing bird (Nowicki 1987). Like humans, birds can actively alter the filtering properties of the vocal tract system (Riede *et al.* 2006; Suthers and Zollinger 2008; Riede and Suthers 2009). Vocal amplitude may therefore be regulated by at least two different mechanisms. Firstly, increased airflow speed may increase the vibration amplitude of the labia, which results in higher sound pressure levels. Airflow speed is related to the pressure in the avian air sac system, a specialized part of the respiratory system in birds that increases the efficiency of oxygen absorption in the lungs. Indeed, there is evidence that air sac pressure is related to vocal amplitude (Suthers *et al.*

2002; Goller *et al.* 2004; Goller *et al.* 2006; Plummer and Goller 2008). Secondly, altering the resonance properties of the vocal tract results in a shifting of resonance peaks, which ultimately has a strong effect on amplitude levels within a certain frequency range (Riede *et al.* 2006). Thus, birds may control vocal amplitude by manipulating airflow, by adjusting the resonance properties of the vocal tract, or both.

Interspecific variation

Song amplitude varies greatly between species. Large species tend to sing louder than small species, but body size can only account for a part of the amplitude variation between species. Most amateur ornithologists in Europe have made acquaintance with the song of the tiny winter wren *Troglodytes troglodytes* and have been wondering how such a small bird is able to produce such loud sounds. At the same time, these ornithologists may have overheard the soft songs produced by a male bullfinch *Pyrrhula pyrrhula* in the treetop directly overhead. It remains a mystery why some species sing much louder than others. Due to the difficulties involved in measuring sound pressure levels in the field, not many attempts have been made to quantify the loudness of birdsong and compare them between species. Mayfield (1966) measured song amplitude in four species of North American passerines. Brackenbury (1979) did the same in 17 European songbirds and found amplitude values between 74 dB (garden warbler *Sylvia communis*) and 100 dB (song thrush *Turdus philomelos*) at 1 m distance from the bird. Doubtlessly, there are numerous species that vocalize much louder or softer than that. The calls of cranes *Grus spec.* or screaming pihas *Lipaugus vociferans* reach amplitudes far beyond 100 dB (Gaunt 1987; Nemeth 2004), and to my knowledge, nobody has ever attempted to measure the sound pressure level of species like the aptly named South American screamers, whose calls can be heard from several kilometers away. On the other hand, the songs of birds like the Australian Gouldian finches *Erythrura gouldiae* are so soft that they are barely audible even at close range (Thorpe 1961).

Intraspecific variation

There are two types of intraspecific variation in song amplitude: (1) individual flexibility, i.e. regulation of amplitude depending on environmental or social conditions and (2) consistent amplitude differences between individuals given that environmental and social conditions are standardized.

Flexibility

Songbirds are able to adjust the loudness of their vocalizations depending on environmental or social factors. In particular, birds have been shown to sing louder if background noise levels increase, in order to compensate for masking effects of noise (Potash 1972; Cynx *et al.* 1998; Manabe *et al.* 1998; Brumm and Todt 2002; Brumm 2004b). This phenomenon, which is also found in mammals including humans, is termed the “Lombard effect” (Lombard 1911). Birds may also adjust the loudness of their songs according to the distance to the targeted receiver (Brumm and Slater 2006b) and depending on urgency or motivation, including for instance hunger state in nestlings (Leonard and Horn 2001; Boncoraglio and Saino 2008) and countersinging during territorial interactions (Brumm and Todt 2004). These examples demonstrate a level of plasticity in the control of vocal amplitude within individuals. The magnitude of amplitude adjustment varies generally between ca. 2 and 8 dB (Cynx *et al.* 1998; Leonard and Horn 2001; Brumm and Todt 2002; Brumm 2004b; Brumm and Todt 2004; Brumm and Slater 2006b).

Interindividual differences

Since vocal amplitude is flexible, we need to keep environmental and social factors constant when comparing song amplitude between individuals. Dabelsteen (1981) found a maximal interindividual difference of 5 dB in a small sample of full dawn song of blackbirds *Turdus merula*. Brumm (2009) measured an interindividual variation of 10 dB in captive nightingales *Luscinia megarhynchos*, 15 dB in wild nightingales and 15 dB in captive zebra finches *Taeniopygia guttata*. As a reminder, an increase of 6 dB equals a doubling in sound pressure. In conclusion, individuals vary extensively in the loudness of their songs, but the causes and consequences of this variation are poorly understood to date.

Outline and structure of this thesis

As outlined above, songbirds can regulate their vocal amplitude depending on environmental and social conditions. Irrespective of that, some individuals within a population appear to sing consistently louder than others. Variation in a trait can be maintained through different mechanisms. (1) Alternative forms of a trait are selectively neutral, i.e. phenotypic differences result from mutation, migration or genetic drift (Lande 1976; Lynch and Hill 1986). (2) The trait in question is under natural and/or sexual selection and alternative phenotypes are adaptive (Smith and Skúlason 1996). This may be the case when selection on the trait is frequency-dependent or disruptive, but also when selection is directional or stabilizing but costs and benefits of the trait are not the same for all individuals.

Song amplitude is unlikely to be selectively neutral; the two main functions of birdsong are territory defense and mate attraction, and to this end, the signal needs to reach a certain intensity to transmit over large distances (Brumm and Naguib 2009). However, the factors that determine how loud a bird sings are largely unknown. In my thesis, I investigated some aspects of the function and evolution of song amplitude. The thesis is arranged in five chapters; each chapter is written in the form of a research manuscript and has either been published in a scientific journal or is currently under review. The first two chapters address the question whether song amplitude has the potential to be sexually selected; in particular, I tested whether the loudness of songs is subject to female choice and whether it plays a role in male-male territorial interactions. The third and fourth chapters deal with proximate aspects of song amplitude; I investigated whether testosterone and body condition affect song amplitude and ultimately, whether song amplitude may signal the physiological state and phenotypic quality of the singer. Finally, in the last chapter I address the question whether song amplitude is affected by vocal learning, i.e. whether vocal learning may result in a default amplitude (which is subject to short-term plasticity), and I investigated whether there may be a genetic component to vocal amplitude.

Chapter 1

Female zebra finches prefer high amplitude song

M. Ritschard, K. Riebel, H. Brumm

The intensity of a signal is crucial for animal communication because a high signal-to-noise ratio improves signal reception. However, variation in amplitude may also have signalling value. In songbirds, song amplitude varies considerably between males but little is known about the causes and consequences of this variation. One of the main functions of birdsong is mate attraction, but whether and how amplitude variation (beyond reception thresholds) affects female choice at all is poorly understood, as this parameter is notoriously difficult to measure in the field. However, levels of signal amplitude at the source and at the receiver's end can be reliably controlled under laboratory conditions. Here we addressed the question whether differences in male song amplitude may influence female choice. Song preferences of female zebra finches Taeniopygia guttata were tested in an operant conditioning task involving sets of four song stimuli, in each case derived from one original song. Within a set, stimuli differed in amplitude or in level of degradation but not in song phonology or syntax. We found that females preferred songs with higher sound pressure level within the tested range of naturally observed amplitude variation regardless of the two reverberation levels. As song preferences are highly predictive of mate choice in this species, our results suggest that female zebra finches may use song amplitude as a criterion in mate choice. This raises the question what information the naturally occurring variation in amplitude between males in this and other songbird species conveys about the singer.

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Song plays an important role in mate choice in oscine birds. Studies to date have identified aspects of repertoire, geographical variation and performance as the foremost involved in choice (Searcy and Yasukawa 1996). Regarding song performance, song rate and song length have been investigated in several songbird species but song amplitude has hardly been explored at all (Gil and Gahr 2002). This is rather surprising as amplitude is known to play an important role in female choice in other taxa, for example insects and anurans (Latimer and Sippel 1987; Arak 1988; Castellano *et al.* 2000).

Recently, the causes and mechanisms of individual song amplitude adjustment in birds have received some attention. Zebra finches *Taeniopygia guttata* (Cynx *et al.* 1998), nightingales *Luscinia megarhynchos* (Brumm and Todt 2002) and Bengalese finches *Lonchura striata domestica* (Kobayasi and Okanoya 2003), have been shown to increase the sound pressure level (SPL) of their songs in response to increased levels of environmental background noise. Zebra finches also adjust the amplitude of their songs with increasing communication distance when directing song at a specific female (Brumm and Slater 2006b) and depending on whether they are in visual contact with conspecifics (Cynx and Gell 2004). Moreover, in some species, low-amplitude songs are often uttered in aggressive encounters between males or during courtship (Dabelsteen *et al.* 1998; Anderson *et al.* 2007). Thus, song amplitude appears to be a flexible trait which individual birds can adjust to maintain a given active space (see Brenowitz 1982) that is used for the signal to be effective.

Over and above such context-dependent variation within individuals, song amplitude in birds also differs considerably between individuals (Brumm and Slater 2006b; Brumm 2009). It has been hypothesized that song amplitude may play a role in sexual selection (Gil and Gahr 2002). Several studies have measured differences in song amplitude between males and reported marked interindividual variation ranging from 5 to 15 dB SPL (Heuwinkel 1978; Dabelsteen 1981; Brumm and Slater 2006b; Anderson *et al.* 2008; Brumm 2009). However, the methods for measuring song amplitude have varied between studies and some of them did not take the context of singing into account; thus, individual variation might have been overestimated because of differences in motivation of the singing males. An effect of motivation on vocal amplitude has been demonstrated in begging calls of tree swallows *Tachycineta bicolor*, and barn swallows *Hirundo rustica* (Leonard and Horn 2001; Boncoraglio and Saino 2008) and the territorial song of nightingales (Brumm and Todt 2004).

Although intraspecific differences in vocal SPL between male songbirds need further investigation, present evidence suggests that it is substantial, which makes song amplitude a possible candidate for a signal used in mate choice. If loud singing is costly, song amplitude could be used by females as an honest signal of male quality (Gil and Gahr 2002), with loud songs being preferred over soft songs. To our knowledge only one published study has looked at female preferences of absolute song amplitude (Searcy 1996). Female red-winged blackbirds *Agelaius phoeniceus* treated with oestradiol implants performed more copulation-solicitation displays in response to high-amplitude than to low-amplitude songs. Although another study found that female song sparrows *Melospiza melodia* reacted more to normal broadcast song than to two types of 'soft song' (Anderson *et al.* 2007), these results cannot be unambiguously assigned to differences in amplitude as 'soft song' is functionally different from normal broadcast song and differs in phonology as well as amplitude (Anderson *et al.* 2008).

Experimental tests of a putative female preference for song amplitude also need to conceptually separate and empirically control for the different ways in which song amplitude may affect female choice. It is conceivable that vocal amplitude has to reach a certain threshold only above which a song is relevant at all to a female as a mating signal. This threshold may coincide with, or may be higher than, the detection threshold. In addition to this, amplitude modulations within the signal (Forstmeier *et al.* 2002; Pasteau *et al.* 2009; Hoeschele *et al.* 2010) or specific loud song elements (e.g. inspiratory high notes [Leadbeater *et al.* 2005]) could affect female preference. Third, the observed natural variation between males in peak or average singing amplitude (Brumm 2009) may be meaningful to females. It is the latter we aimed to test with this experiment.

Zebra finches are one of the few songbird species in which variation in song amplitude between males has been studied (Brumm and Slater 2006b; Brumm 2009). Song plays a crucial role in zebra finch mate choice (reviewed in Riebel 2009). During courtship, males direct their songs over short distances at a particular female, so the female is able to perceive the source level of a singing male accurately. This so-called directed singing makes the zebra finch a very suitable study species to address the topic of female preferences for song amplitude.

For song preference testing, we used an operant task with song as the sole reward (Stevenson-Hinde 1973; Riebel and Slater 1998). This behavioural preference assay is based on the finding that song alone can reinforce behaviour that precedes it (Stevenson 1967; Stevenson-Hinde 1973), for example perch hopping (Riebel and Slater 1998; Leitão *et al.* 2006) or key pecking (Adret 1993; Collins 1999; Houx and ten Cate 1999; Riebel 2000; Riebel *et al.* 2002; Holveck and Riebel 2007). In zebra finches, key pecking for song reward has been shown to be a method with excellent internal and external validity: female preferences are highly repeatable within and between tests (Riebel 2000; Riebel *et al.* 2002) and females' preferences in the operant tests predict their preferences for live males (Holveck and Riebel 2007) and latency to first egg (Holveck and Riebel 2010). In our tests, females could actively choose between different song stimuli by pecking four small response keys. To test whether song amplitude would affect the reinforcing quality of song to females, each female received a unique stimulus set consisting of four different edited versions of a male song. Three of the stimuli differed in amplitude only (high, medium and low amplitude) and a fourth (at medium amplitude) was acoustically degraded, after it had been recorded over 5 m distance in a highly echoic chamber. Sound pressure levels for all stimuli were clearly above detection levels (Dooling 1982) and were representative of the naturally observed variation in song amplitude. Sound degradation has been shown to be used as a distance cue in many species (reviewed in Naguib and Wiley 2001). A potential preference for loud over soft song may be explained by (1) females preferring males that sing with a high SPL or by (2) females preferring males that are spatially close. Using moderately degraded and highly degraded song stimuli with the same amplitude may help us to distinguish between these two explanations. If females prefer louder males and use ranging, then they should prefer the highly degraded over the moderately degraded stimulus; however, if absolute SPL as such guides their preferences, the highly degraded and moderately degraded song should be equally preferred at equal amplitude.

Methods

Subjects and housing

We used wild-morph domesticated zebra finch females from an outbred breeding colony at Leiden University, The Netherlands. Zebra finches are small passerines occurring in almost all Australian mainland habitats and on the Lesser Sunda Islands. They are socially monogamous, but breed in colonies and form feeding flocks outside the breeding season (Zann 1996). Males and females are highly vocal, and constant calling can be heard in flocks, but only males also perform the more complex courtship song. Female song preferences for specific song variants are highly influenced by early song experiences (reviewed in Riebel 2003; 2009). As nothing is known about age or experience effects on female perception of song amplitude, we kept our sample of test subjects as heterogeneous as possible with respect to age, social and housing experiences, randomly selecting them from the holding stock of currently nonbreeding individuals. Females were 3–59 months old, which means that all can be considered as sexually mature (females in captivity may lay their first eggs as early as 90 days posthatching [Zann 1996]). Females also varied in a nonsystematic manner regarding in which sequence and for how long they had been housed in unisex holding cages and single or mixed-sex aviaries, but all had been housed with live tutors during the sensitive phase for song learning (Riebel 2003) and all had throughout been exposed to a variety of songs of adult males within their own or neighbouring holding cages or indoor aviary facilities.

During the preference tests, birds were kept singly in experimental cages (see below) placed in sound attenuation chambers. In their home cages as well as during the trials they had ad libitum access to a commercial tropical seed mixture enriched with GistoCal mineral and vitamin powder (Beaphar B.V., Raalte, The Netherlands), cuttlefish bone and water, supplemented with egg food and germinated seeds twice a week. Before and after the experiment, birds were housed in aviaries or in cages in groups of 2–10 females in large bird-holding rooms with a 13.5:10.5 h light:dark schedule (lights on between 0700 and 2030 hours). They could hear the songs of males kept in the same room and could see males housed in the cage rows opposite them (at 1.5–2 m distance).

Stimuli

Four categories of experimental stimuli were used: moderate-reverberation male song at low, medium and high amplitude and high-reverberation male song at medium amplitude. We used song recordings (each from a different male) to produce a set of four stimuli from each original song to ensure that the test stimuli within a set differed only in amplitude and degradation level. Each female was tested with a different stimulus set made from the song of a different male. The original songs had been high-quality recordings made in anechoic chambers from males in the laboratory populations at the Universities of St Andrews, U.K. (see Brumm and Slater 2006b for details on recording procedure) and Leiden, The Netherlands (see Holveck *et al.* 2008 for details) as well as from the population at the Max Planck Institute for Ornithology in Seewiesen, Germany (see Forstmeier *et al.* 2009 for details). Song of adult male zebra finches consists of individual-specific phrases or motifs (duration <1 s) which are repeated several times in a song bout. The first phrase is usually preceded by a few short introductory notes. To ensure that each phrase within a song bout had the same amplitude, we constructed stimulus songs from one introductory phrase followed by four identical copies of a randomly chosen song phrase, with interphrase intervals of more than 0.1 s. All songs were played back through a Braun L46 loudspeaker powered by a TEAC A-H300 amplifier in an empty concrete-lined room (7.0 × 1.7 m and 2.9 m high) and then re-recorded with a Sennheiser ME 62 omnidirectional microphone connected to a Marantz PMD 660 solid state recorder. The microphone was placed at a distance of 0.5 m for the moderately degraded stimuli and 5 m from the loudspeaker for the highly degraded stimuli, respectively. This procedure yielded two sets of stimuli for each song type that differed considerably in the amount of reverberation, which was clearly audible to human listeners. We define reverberation here as a blurring of the temporal sound structure, which is caused by the sound waves being reflected by echoic surfaces along the transmission path. Such reverberation can be quantified by the degree of equalization of SPL over time (see below and Fig. 1). Although reverberations created in a concrete-lined room may be different from what zebra finches experience in their natural habitat, we chose this procedure because all experimental birds had been raised and spent their whole lives in such rooms and were therefore familiar with the acoustic properties of highly echoic rooms. This approach seems advisable because it has been suggested that birds learn the relationship between degradation and distance by listening to conspecific songs at various distances (McGregor *et al.* 1983; Naguib 1996; 1998). By playing back and re-recording all the stimuli we made sure that moderately and highly degraded stimuli went through the same production procedure and did not differ in any parameter other than degradation level. To quantify degradation levels, we cross-correlated the original recording with the two experimental stimuli to accurately define the starting point of the first element. We then measured SPL (integration time = 12.5 ms) in the highly degraded and the moderately degraded stimulus at the position where the first element reached peak intensity in the original recording (point 1) and again 50 ms later (point 2). Relative sound pressures (mean ± SD) of point 2 in relation to point 1 were 76.9 ± 6.2% for moderately degraded and 93.6 ± 5.5% for highly degraded song (compared to 68.5 ± 2.9% for the original recording; $N = 8$ each).

To ensure that the amplitude levels used for the playbacks were within the natural range of interindividual variation in zebra finch song, playback amplitude was set according to published measurements of peak amplitudes of directed song from 41 males, which ranged from 62.1 to 79.2 dB SPL at 0.5 m above the bird (Brumm 2009). In this previous study, singing context was controlled by placing a female zebra finch 20 cm from the male. We added 2 dB to these measurements to account for the fact that the measurements were not taken from in front of the bird but from vertically above the bird's head to control for song directionality. Then the 10% quantile (68 dB re 20 mPa at 50 cm), median (73 dB) and 90% quantile (78 dB) were calculated and used to set the playback peak amplitude for the soft, medium and loud stimuli. The sound level of the playback (re 20 µPa, root mean square

[RMS] method) was measured between the two central perches inside the experimental cage, using a Rion NL-15 sound level meter (Rion Co. Ltd., Tokyo, Japan).

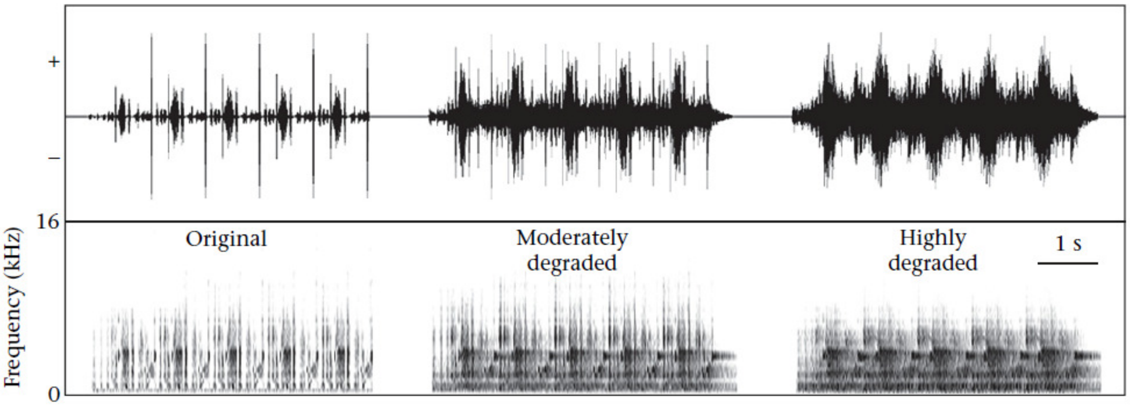

Figure 1. Oscillogram (above) and spectrogram (below) of song stimuli. *Original song used to prepare stimuli recorded in an anechoic chamber, moderately degraded stimulus song played back in a concrete-lined room, as used for bird housing, at 0.5 m distance, and highly degraded stimulus (song played back in a concrete-lined room at 5 m distance). Note that both the amplitude dynamics and the syllable structure are blurred by the reverberation.*

Preference tests

For preference testing, females were moved into an experimental cage (70 × 30 cm and 45 cm high) placed in a sound attenuation chamber (100 × 200 cm and 220 cm high). Front, sides and top of the cage were made of wire mesh and the cages were evenly illuminated by a single true light fluorescent tube (Lumilux De Luxe Daylight, 1150 lm, L 18 W/ 965, Oscam, Capelle aan der Ijssel, The Netherlands) placed along the whole length on top of the cage. Although the general set-up and protocol followed earlier published studies using this method (e.g. Riebel 2000; Riebel *et al.* 2002; Holveck and Riebel 2007) there were some modifications. All earlier studies had offered only bimodal choices. However, we aimed to test a gradient and thus had the set-up modified such that females had access to a total of four pecking keys. To this end, there were four perches evenly arranged in a horizontal line. Perched at the back end of each perch, a bird could peck one (of four) red response keys (diameter: 1 cm) fitted into the rear wall of the cage with a red light-emitting diode (LED) in the centre. Pecking a key triggered a playback of the song stimulus assigned to the key. The playback was broadcast by a Vifa MG10SD-09-08 broadband loudspeaker fixed 50 cm above the experimental cage facing downwards. A custom-built electronic device (Electronics workshop of the faculty of Natural Sciences at Leiden University) controlled the playbacks and registered the number of pecks per key.

From a total of 53 females, 34 (i.e. 64%) learned to peck the keys. We did not target specific females from the colony but focused on the females that learned either by autoshaping or after brief shaping sessions, and we aborted trials with slower learning females. The resulting 64% learning success is well within the range of 55–100% training success of earlier studies in Leiden using this set-up (e.g. Leadbeater *et al.* 2005; Holveck and Riebel 2007; Riebel *et al.* 2009; Holveck and Riebel 2010). While this seems a rather wide range, we would point out that for these earlier studies experimenter identity (and level of experience with training the birds in the task) and training intensity varied substantially. For earlier experiments where the birds had unique developmental and tutoring histories we shaped the birds that were not learning spontaneously and thus reached 98–100% training success (e.g. Riebel *et al.* 2009; Holveck and Riebel 2010). For the present study we aimed for a high throughput rather than an 'intensive training' approach. Although we might have introduced a biased sample with respect to hormonal/motivational status or exploration or neophobia scores of these females, we have found as yet no evidence that how quickly a female learns is linked to female condition or preference strength (Riebel *et al.* 2009).

Also, in line with earlier studies, females showed great interindividual variation in key-pecking activity (see e.g. Holveck and Riebel 2007; Riebel *et al.* 2009). From the 34 females that did learn to peck the keys, some showed a rather low pecking activity on some or all days. Because there were four keys, those females with low pecking activity might one day peck a particular key only on one visit and in one bout. We therefore decided to include only females that pecked on average at least 50 times per day (minimum 200 pecks over 4 days) and analyzed the effect of pecking activity on preference strength (see below). In addition, females had to have pecked all four keys before we started the actual preference test. Each test lasted 4 days so that each song stimulus could be presented at each key for 1 whole day. Of the 34 females that learned to peck, 10 were excluded because they did not comply with the criteria given above. We found no systematic effect of age or breeding experience on learning and, from the successfully tested subjects, nine females had no breeding experience and 15 females had bred at least once. The actual preference test started 1 day after the subject had pecked each of the four keys (average time to reach this criterion: 1.5 ±1.4 days). For logistic reasons, the experiment was started 1 day later for one bird and 2 days later for another bird. Also, in one case, owing to a reading error, data registration was started before a female had pecked all four keys but she did so within the first day of the test period. As exclusion of these birds did not alter the results qualitatively, we included them in the final analysis. Keys were active from 0700 to 2030 hours (lights on 0700–2030 hours with a 15 min twilight phase with the light fading in and out at the beginning and the end of each day). Every night, the allocation of the stimuli to the pecking keys was changed so that each of the four stimuli was assigned to each of the four keys for 1 day only. This was done to control for effects of a possible location preference. Stimulus allocation on day 1 was randomized.

The experimental procedures were reviewed and approved by Leiden University's animal experimentation ethical committee.

Data analysis

All statistical tests were performed with R 2.8.1 (R Development Core Team 2008) . The function *lmer* (R package *lme4*) was used to fit linear mixed-effects models with female as a random factor, test day (1–4), key (1–4) and degradation (medium or high) as fixed factors and stimulus amplitude as a covariate. Alternative models were compared with the ANOVA function, because the *lmer* function does not provide P values. The dependent variable was log transformed to reach the best fit with our models (according to the distribution of the residuals and the Akaike's information criterion). Nonsignificant interactions and nonsignificant factors were backward eliminated. Breeding experience (yes/no) did not explain a substantial amount of variation, but note that because of the large variation in housing conditions and time since last breeding, females' experience levels with male songs and male company are probably poorly reflected by this dichotomous variable.

Results

The 24 birds included in the final analyses pecked on average 764 times (range 207–3313) within the 4-day experimental period. We found that the birds' pecking rates were strongly nonrandom with regard to the four different keys ($\chi^2 = 73.123$, df = 3, $P < 0.001$), with exterior keys (1 and 4) preferred over interior keys (2 and 3; Fig. 2). In contrast, test day ($\chi^2 = 0.183$, df = 3, $P = 0.18$) and stimulus degradation ($\chi^2 = 0.047$, df = 1, $P = 0.83$) had no significant effects on the number of pecks. Females showed a clear preference for high-amplitude songs ($\chi^2 = 6.861$, df = 1, $P = 0.009$; Fig. 3).

A similar model comparing only highly degraded song and moderately degraded song at medium amplitude confirmed that degradation had no effect on female preference ($\chi^2 = 0.04$, df = 1, $P = 0.84$; Fig. 3).

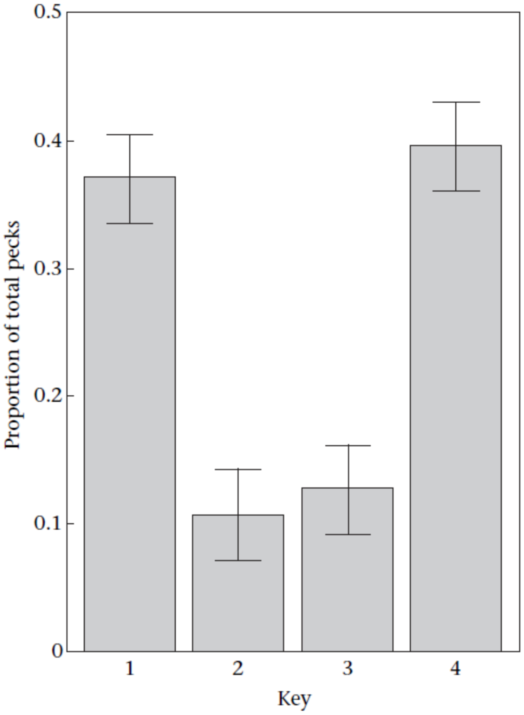

Figure 2. Spatial key preferences of female zebra finches (N = 24) in an operant conditioning test with four sensors: *mean proportion of pecks per keys 1–4 (pecks per key divided by total number of pecks). Least significant difference bars are shown.*

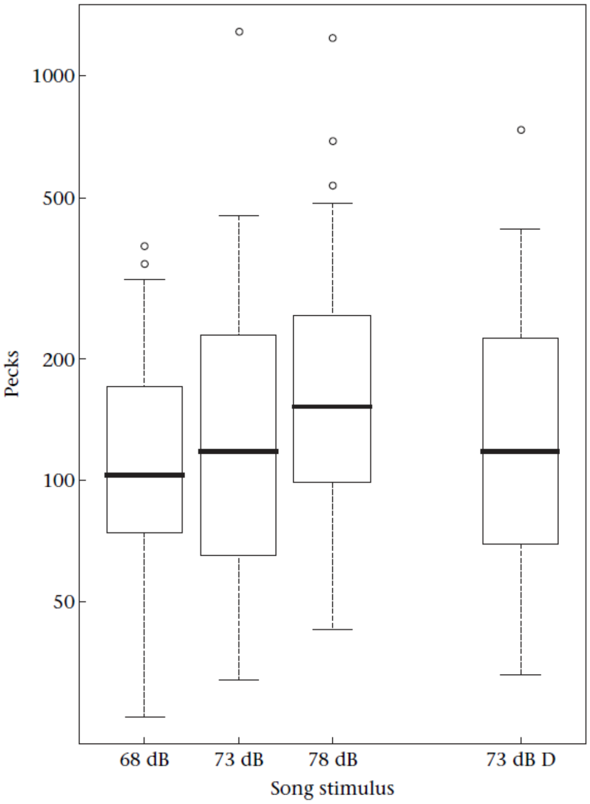

Figure 3. Preferences of female zebra finches for male song amplitude: *number of triggered playbacks (N = 24 females) of moderately degraded low-, medium- and high-amplitude songs and of highly degraded medium-amplitude song ('73 dB D'). Box plots show median, first and third quartiles, 'whiskers' (1.5 times interquartile range or maximum value) and outliers. Note logarithmic scale of Y axis.*

To test for a possible link between preference strength and motivation, we tested whether those females pecking most also had the most pronounced preferences. However, there was no significant correlation between the total number of pecks and preference strength measured as the number of pecks for the most preferred stimulus divided by the number of pecks for the least preferred stimulus (Pearson product – moment correlation: $r = -0.12$, df = 23, $P = 0.55$) or the number of pecks for the high-amplitude stimulus divided by the number of pecks for the low-amplitude stimulus ($r = -0.27$, df = 23, $P = 0.18$).

Discussion

We found that, when given a choice between three different amplitude levels within the natural range measured in a domesticated population, female zebra finches showed a preference for high-amplitude over low-amplitude songs. Such a directional preference for loud songs is well known in female anurans and insects, which have been found to be attracted to the louder of two signals (Latimer and Sippel 1987; Arak 1988; Castellano *et al.* 2000). Arak (1988), for instance, showed that female natterjack toads *Bufo calamita* preferred males that produced louder, lower frequency calls and were heavier than rejected males. Our findings in zebra finches are corroborated by the only other study on absolute amplitude preferences in birds that we know of: Searcy (1996) found that oestradiol-treated female red-winged blackbirds performed more copulation-solicitation displays in response to high-SPL than to low-SPL songs.

At a proximate level, high-intensity songs may simply be stronger stimuli, that is, they may be more efficient at stimulating the receiver's sensory apparatus, causing auditory neurons to fire at higher rates, and therefore may be more attractive to females (Castellano *et al.* 2000). At an ultimate level, vocal intensity may be positively correlated with genetic or phenotypic male quality (Castellano *et al.* 2000; Brumm 2004a; 2009). Body size may be one such quality; females are thought to benefit from choosing large mates because large males may be more successful in competing for resources than small males, although direct benefits may also be involved (Andersson 1994; Savalli and Fox 1998). Evidence of a positive correlation between vocal amplitude and body size comes from the American toad *Bufo americanus* (Gerhardt 1975) and the natterjack toad (Arak 1988), in which species larger males produced more intense calls than smaller males. Forrest (1991) found that heavy males of the cricket *Oecanthus quadripunctatus* had a higher power output. In contrast, no evidence for an intraspecific correlation between body size and median SPL of song has been found in birds (Brumm 2009), but to date only very few species have been studied in this respect. A positive relationship between body size and song amplitude appears plausible in birds because sound intensity may be partly determined by the size of the respiratory muscles (Brackenbury 1979). If song amplitude is positively correlated with male genetic quality, it needs to have a heritable component. A recent study on the heritability of zebra finch vocalizations (Forstmeier *et al.* 2009) indeed suggests some heritability of song amplitude. However, as Forstmeier *et al.* (2009) stated, their estimates of song amplitude heritability have to be treated with caution, because of methodological caveats regarding the measurement procedure.

In our experiment, female preference strength increased with amplitude and females may thus exert a directional selection on males to sing loudly. However, if singing loudly is beneficial, the fact that, in the zebra finch, song amplitude is relatively low compared to other species (Brackenbury 1979) indicates that substantial costs of loud songs may be involved or that the benefits of singing loudly are small. Studies of the metabolic costs of birdsong found only very small amounts of energy expenditure related to song production (Franz and Goller 2003; Ward *et al.* 2003; Ward *et al.* 2004). In particular, Oberweger and Goller (2001) found only a 1.16-fold increase in the rate of oxygen consumption in loud compared to soft song elements differing by 16 dB in the starling *Sturnus vulgaris*. For comparison, basal metabolic rate during flight increases 10-fold for a bird of the mass of a canary *Serinus canaria* (Butler and Bishop 2000). Possibly, singing loudly is constrained by predation costs and costs induced by sound-orienting parasites (Cade 1975), which, however, remains to be demonstrated in birds. Furthermore, social costs may also constrain the production of loud songs (Dabelsteen *et al.* 1998; Gil and Gahr 2002). In certain cases, such as territorial disputes or during courtship, it may benefit birds to produce songs with restricted transmission distance to conceal them from eavesdropping conspecifics. Dabelsteen *et al.* (1998) predicted that the amount of low-amplitude singing will increase with the density of the population, which may provide an explanation for the relatively low song SPL in the colonial-breeding zebra finch.

While we were able to show a clear preference of females for loud songs, strong degradation of the signal by reverberations did not have a significant effect on the attractiveness of the stimulus. In the context of amplitude preference, this suggests either that the birds tested did not perceive the highly degraded song as being more distant and/or that the amount of neuronal stimulation is an inflexible and linear predictor of amplitude preference. While the ability of acoustic ranging through degradation cues has been well documented in many territorial bird species (reviewed in Naguib and Wiley 2001), long-range communication in the colonially living zebra finch may have less importance. Moreover, zebra finches are inhabitants of open habitats, where the degree of sound reverberation is expected to be low (Brumm and Naguib 2009). Thus, our findings may reflect the potentially little importance of ranging in this species. However, one has to keep in mind that under natural conditions, degradation not only affects spectral and temporal patterns of a sound but also causes sound waves to scatter in a three-dimensional space, while degraded sound waves emitted by a loudspeaker are inevitably focused. A loudspeaker may thus not be suited to simulate sound degradation properly. Thus, we found no evidence that females choose loud songs because they prefer males that are close, although such an effect cannot be entirely ruled out. For female anurans and insects, it has been suggested that predation costs may select females, given all other things being equal, simply to choose the closest male (Arak 1988). However, for highly mobile animals such as birds, where females are known to sample several males over long distances (Bensch and Hasselquist 1992; Roth *et al.* 2009), this explanation seems very unlikely.

In conclusion, we found that female zebra finches showed a linear preference for loud song (within the naturally observed range of male song amplitude) when other song parameters were kept constant. Future work will have to investigate how song amplitude influences choice in relation to other parameters and whether there are correlations between song amplitude and male quality as well as fitness benefits for females resulting from mating with loud males.

Chapter 2

Song amplitude affects territorial aggression of male receivers in chaffinches

H. Brumm, M. Ritschard

The evolution of birdsong is closely related to sexual selection as birds use their songs for mate attraction and territorial defense. Recently, it has been shown that song amplitude can differ markedly between males and that females prefer louder songs. However, it is not known what constrains the production of loud songs. Here, we investigated how interindividual variation in song amplitude affects male–male territorial interactions. We simulated territorial intrusions by exposing male chaffinches Fringilla coelebs to song amplitudes consistent with the high and low end of the distribution of naturally occurring interindividual variation and found significantly stronger territorial behaviors in males exposed to high-amplitude songs. In particular, males sang less, approached the simulated rival closer, and stayed longer in the vicinity when the playback loudspeaker broadcasted louder songs. Our results demonstrate that the intensity of territorial behavior in chaffinches is modulated by the amplitude of songs to which they are exposed, suggesting that social aggression is likely to constrain song performance in this species. We conclude that the variation in song amplitude between males is, at least partly, a result of male–male competition as probably not all males are able to bear the potential costs of increased social aggression.

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Bird song is a particularly useful model in the study of sexually selected signals (ten Cate 2004; Searcy and Nowicki 2005). In the temperate zones, it is mostly the males that sing, and they mainly use their songs to attract females and defend their territories against rival males (Catchpole and Slater 2008). It has been hypothesized that the amplitude of birdsong plays a part in sexual selection (Gil and Gahr 2002). This notion is strongly supported by experimental evidence showing that female birds prefer louder songs, which has been demonstrated in red-winged blackbirds *Agelaius phoeniceus* using the copulation–solicitation assay (Searcy 1996) and in zebra finches using operant conditioning techniques (Ritschard *et al.* 2010). Moreover, male nightingales *Luscinia megarhynchos* increase the sound pressure level (SPL) of their songs when interacting with a rival male, suggesting that vocal amplitude may be important for territorial competition (Brumm and Todt 2004). In particular, it has been speculated that the song amplitude during territorial disputes may signal a singer's quality or motivation (Kroodsma 1979; Brumm and Todt 2004; Lampe *et al.* 2010).

In line with this notion, it has been found that song amplitude can vary considerably between individuals. Heuwinkel (1978) found a maximum between individual difference of 1.5 dB in a sample of 5 Eurasian reed warblers *Acrocephalus scirpaceus*. Later studies on bigger sample sizes also found bigger interindividual differences: 5 dB in wild blackbirds *Turdus merula* (Dabelsteen 1981), 10 dB in captive nightingales (Brumm 2009), 13 dB in captive Bengalese finches *Lonchura striata* (Ritschard *et al.* 2011), and 15 dB in wild nightingales and captive zebra finches *Taeniopygia guttata* (Brumm 2009). An increase of 6 dB equals a doubling in sound pressure, thus a bird has to produce a more than 5.6-fold increase in sound pressure to achieve the 15 dB difference found in nightingales and zebra finches. However, the striking song amplitude differences between individuals, as well as between species, are only poorly understood, and the question of what constrains the production of loud songs remains an open one. Most studies on the metabolic costs of song production indicate that singing in birds requires little energy per se (Oberweger and Goller 2001; Franz and Goller 2003; Ward *et al.* 2003; Ward *et al.* 2004). More specifically, rates of oxygen consumption of zebra finches were not considerably higher for loud songs than for songs produced at lower sound levels (Zollinger SA, Goller F, Brumm H, unpublished data). Thus, singing is energetically cheap in songbirds, and therefore song performance is very unlikely to be constrained by metabolic costs. Another proximate constraint of song amplitude that has been discussed in the literature is body size; the hypothesis is that bigger birds may be able to produce louder songs (Brackenbury 1979). However, a recent study (Brumm 2009) did not find any evidence in support of this hypothesis.

Gaining insight into the constraints of song amplitude production will also help to clarify the issue of whether and how song amplitude is used as a signal in male–male competition. In their review on the constraints of birdsong, Gil and Gahr (2002) suggested that higher song amplitudes probably increase aggression by other males. The mechanism underlying a possible amplitude-dependent increase of social aggression could be either that louder songs are simply detected more easily (Dooling 2004) or, for songs beyond detection thresholds, that the intensity of territorial aggression is modulated by the level of the rival's song performance (de Kort *et al.* 2009a).

In most songbirds, social aggression by territorial males can easily be elicited with playback experiments, and this technique is widely used to test hypotheses on territorial behavior (Catchpole and Slater 2008). Several playback studies have shown that individual song performance can affect male–male territorial interactions (e.g. Hultsch and Todt 1982; Illes *et al.* 2006; Schmidt *et al.* 2008; de Kort *et al.* 2009b). Song amplitude is a performance trait of particular significance because it is crucial for signal transmission (Brumm and Naguib 2009). However, only very few studies have addressed the question how song amplitude affects territorial aggression in birds. Blackbirds were found to react more weakly to an unnaturally low SPL (65 dB at 1 m distance) compared with natural song amplitude (85 dB SPL; Dabelsteen 1981). In the laboratory, blackbirds reduced their song duration when overlapped by a playback of conspecific song, and this response was augmented by an increase in playback level in the one subject tested on song amplitude differences (Todt 1981). More recently, Lampe *et al.* (2010) examined the responses of redwing males *Turdus iliacus* to variation of sound amplitude in twitter song, a particular type of song that is used in short-range male–male interactions in this species. In their experiment, Lampe *et al.* (2010) used 2 versions of twitter song differing by 6 dB and found that males responded more strongly to the loud playback. However, in these previous studies, it is difficult to disentangle the effects of source level from distance because an increase in playback amplitude could either connote that the simulated rival sang louder or that the rival was closer. Recently, Sprau *et al.* (2010) showed that the distance between the subject and the playback loudspeaker can crucially affect the responses of territorial birds. Therefore, it is advisable to use not only a song playback to simulate a territorial intrusion but also a dummy bird that allows the tested male to precisely locate the distance to the rival and thus to assess the source level of the songs. Moreover, it is also preferable to measure the natural song amplitudes prior to playback experiments to adjust the level of experimental song to the levels of natural song (Dabelsteen 1981). This is generally true for all playback experiments but of particular importance for studies aiming to test hypotheses about signal amplitude.

In this study, we addressed the question whether differences in song amplitude affect territorial behavior in chaffinches *Fringilla coelebs*. This species is not only one of the most common songbirds in Europe but also an excellent species in which to test our hypothesis because many aspects of its singing and territorial behaviors have been studied in detail (Hinde 1958; Slater 1981; Slater and Catchpole 1990; Naguib *et al.* 2000; Riebel and Slater 2000; Leitão and Riebel 2003). First, we measured the naturally occurring interindividual variation in song amplitude in the study population and then we simulated territorial intrusions using a dummy bird together with song playbacks that matched the high and low end of the distribution of the observed song amplitude variation. If the performance of high-amplitude songs is constrained by social aggression by rival males, then we expect territorial birds to show stronger responses toward males that sing loud songs and weaker responses toward males with softer songs.

Material and methods

Study species and site

Chaffinches have a small repertoire of 1–6 song types, with the majority of birds having 2 or 3 (Slater 1983). Birds that sing more than one song type typically sing short sequences of one song type and then switch to another type (Riebel and Slater 1999b; Brumm and Slater 2006a). As in most songbirds, the 2 major functions of chaffinch song are mate attraction and territory defense, and therefore variations in song characteristics are meaningful to both female and male receivers (Slater 1981; Riebel and Slater 1998; Leitão *et al.* 2006). In central Europe, male chaffinches establish their territories from mid-February until March, and egg laying does not typically begin until mid-April (Bauer *et al.* 2005). The song level measurements and playback experiments were carried out in Starnberg district, Germany. Study sites were selected based on a good visibility of the subjects and away from noise sources, such as busy roads or railway lines.

Song level measurements

We measured the SPLs of the songs from 20 male chaffinches between 19 and 23 March 2010 from 0700 to 1100 h with a CEL 573.B1 Sound Level Analyser. The Sound Level Analyzer was used in a measurement mode as a precision (class 1) real time sound level meter, which allowed us to manually store measurements in an internal memory. For an average of 12 songs per male (range 10–21 songs), we recorded the A-weighted SPL with an integration time of 125 ms (dB SPL, re. 20 lPa).

Quantifying song amplitude in the field is challenging because several factors may affect the measurements. First, birds may adjust their song amplitude in response to varying levels of background noise (termed the Lombard effect, reviewed in Brumm and Slabbekoorn 2005) and dependent on the social context, that is, the presence and distance of targeted receivers (Brumm and Todt 2004; Cynx and Gell 2004; Brumm and Slater 2006b). Second, the measuring procedure is also crucial because the measured sound pressure values vary with distance from the singing bird as well as its orientation (Brumm 2004b). Finally, the environmental acoustics of the habitat can affect sound level measurements due to varying levels of sound absorption by differences in vegetation as well as air temperature and humidity (Wiley and Richards 1982). The effect of the latter increases with the recording distance; thus, it can be minimized by keeping the recording distance to a minimum with no obstacles in the direct sound path between the measuring microphone and the singing bird. We did our measurements (and the subsequent playback experiments) during a short period of just a few early days in the breeding season, which had 2 advantages: all subjects were in the same breeding stage (i.e., territory establishment, several weeks before egg laying) and there was no foliage, which allowed good visibility of the birds and minimal sound absorption and scattering (Blumenrath and Dabelsteen 2004). Song levels were only recorded provided there were no obstacles (e.g., branches) between the singing bird and the sound level meter, and the bird was closer than 30 m (mean 17.5 m, range 10–27 m, assessed with a Leica Rangemaster 800 CRF laser range finder). Readings were only taken from an angle of incidence of about 90° in relation to the animal's longitudinal axis, and the microphone of the sound level meter pointing directly at the singing bird. For each song, 2 measurements were taken: one of the maximum SPL of the song and one immediately after the end of the song (see above). The second reading was used as a proxy for the environmental noise during the song (see below). Air temperature and humidity were measured with a Conrad WS-7138 thermo/hygrometer. To ensure that all birds were in a similar social context, we only measured individuals that had their territory within earshot of other singing chaffinches. To further control for a potential effect of the density and distance of neighboring males, we recorded the number of males singing within earshot as well as the distance from the measured bird to the nearest singing neighbor.

Playback experiment

Construction of playback stimuli

Each male was tested with the songs of a different source male. All source males were recorded in previous years more than 1300 km away from the test sites (Brumm *et al.* 2009a), which ensured that birds tested were neither familiar with the songs nor the singer. This is advantageous because chaffinches have been found to show stronger territorial responses toward strange than toward familiar songs (Slater 1981). From the recording of each source male, we took one high-quality recording of each of 2 song types. These 2 songs were then high-pass filtered at 1.4 kHz and subsequently normalized to 95%. Eight copies of one song type followed by 5 copies of the other were then copied in regular intervals into a 2-min playback file to form a song sequence with a song rate of 6.5 songs per minute. This song rate, and the switch in song types after 8 renditions, was derived from the average song performance values for chaffinches measured in a sample of over 100 individuals (Brumm *et al.* 2009a). All digital editing and construction of the playback files were done with Avisoft SASLab Pro (Raimund Specht, Berlin). Finally, the playback files were stored in the digital memory of a playback device (Foxpro Scorpion model X1-A) that allowed a remote controlled broadcast of the files. Half of the stimulus files were randomly assigned to the high-amplitude treatment, the other half to be low-amplitude treatment. The volume control of the playback device was set to values that resulted in 87 dB SPL at 1 m distance for the high-amplitude songs and 78 dB SPL at 1 m distance for the low-amplitude playback (as measured with a CEL 573.B1 Sound Level Analyser). These SPL values were consistent with the high and low end of the distribution of chaffinch song SPL measured before the playback (see below). The 2 experimental treatments were carried out alternating, that is, the first subject received one treatment, the second the other, and so on.

Procedure

Playback experiments were carried out from 24 to 31 March 2010 between 0630 and 0930 h. As with the song level measurements, we restricted the playback experiment to a brief period of a few days earlier in the breeding season to ensure that all males were in a similar breeding stage, that is, territory establishment and pair formation. Females were only observed in some of the territories on the last 3 days of the experimental period.

In total, we tested 22 males, 11 with the high-amplitude treatment and 11 with the low-amplitude treatment. At the beginning of each experiment, each bird was observed until at least 2 song posts could be mapped. Then a dummy bird (a taxidermic mount of a male chaffinch that was stuffed in a singing posture) was mounted on a tripod in 170 cm height immediately above the playback device. The tripod with the dummy and the playback device was positioned in between 2 song posts of the targeted bird to ensure that all playbacks were done within the birds' territories. (Although the song playback was fully replicated, the use of only one dummy could be regarded as pseudoreplication. However, the same taxidermic mount was used in all experiments so that any potential bias induced by the particular dummy used was the same for high- and low-amplitude playbacks.).

Previous playback studies with chaffinches found that the latency to approach the loudspeaker, the time spent within 5 m of it, the closest approach, the number of flights, and the number of calls are the most useful response variables (Slater 1981; Slater and Catchpole 1990; Leitão and Riebel 2003). We adopted these response variables, and in addition also considered the number of songs because chaffinches decrease their song output in response to playbacks inside their territories (Slater 1981).

The males' territorial behaviors were registered 2 min before, during, and after the playback. During these periods, we recorded their vocalizations with a Sennheiser Me 66 directional microphone connected to a Marantz PMD 660 solid state recorder. The recorded digital audio files had a sampling rate of 44.1 kHz and an accuracy of 16 bit. A spoken commentary reporting the other response variables was simultaneously recorded with the same equipment. The playback was started after the 2-min preplayback observation period and when the bird was not further away than 30 m from the dummy bird (measured with a Leica Rangemaster 800 CRF laser range finder). The initial distance between the subjects and the dummy at the beginning of the playback did not differ systematically between the 2 treatments (Welch 2-sample t-test, $t = 1.06$, df = 18.20, $P = 0.30$; mean distance high-amplitude group: 23.7 m [range 15–30 m] and low-amplitude group: 21.6 m [range 13–29 m]) nor did the time of day when the animals were tested ($t = 0.70$, df = 18.71, $P = 0.49$).

Data analysis

The reading of the sound level meter gives the SPL of all incoming sound energy, that is, the song and the background noise. To calculate the SPL of the song without the background noise, we subtracted the second SPL value taken immediately after each song from the SPL measurement taken during the song (for details of the logarithmic calculation procedure, see Brumm *et al.* 2009b). However, all birds were recorded in areas with low levels of ambient noise and because the measurements were done very close to the birds, the background noise had very little or virtually no effect on measured values of song (signal-to-noise ratios: 10–26 dB). In a second step, the SPL values for each song were normalized to the standard distance of 1 m according to the spherical spreading of sound. The SPL in 1 m distance is $L_{1m} = 6 \log(d) + L_d$, where d is the distance (in m) from which the SPL measurement was taken, and L_d is the SPL in distance d. Finally, the mean L_{1m} value for all the songs of each male were calculated and used for further analysis.

The validity of our method was checked by calculating a repeatability score. Therefore, we compared SPL values within 14 males that have been taken at different distances. For each individual, the mean SPL values of the closest and the furthest recording were correlated with each other. A strong correlation would show that variation within males is smaller than the variation between males, indicating consistent amplitude differences between males and high internal validity of the method.

To control for potential environmental effects on the song amplitude measurements, we conducted a general linear model with time of the day, temperature, humidity, number of singing neighbors, distance to the nearest neighbor, and background SPL as explanatory variables. Nonsignificant variables were backward eliminated.

When analyzing the data of the playback experiments, pairwise comparisons between the response variables revealed that all but 2 (number of calls uttered during the playback and the latency of approach) were correlated with other variables. Therefore, to obtain uncorrelated response measures, we performed a principal component analysis (PCA) on the correlated variables (during the playback: number of songs and flights, closest approach, time spent within 5 m; after the playback: number of songs, calls and flights and time spent within 5 m during the postplayback period). A Kaiser–Meyer–Olkin test confirmed that our correlation matrix was suitable for a PCA (sampling adequacy = 0.65). The PCA yielded 2 components with an eigenvalue higher than 1, which accounted for 70.3% of the overall variance. PC1 was mainly a measure of number of flights and songs during the playback, of closest approach and of number of songs after the playback, whereas the other variables loaded to similar extent on the 2 main components (Table 1).

Comparisons of the PCA scores, as well as comparisons of the raw response variables, were made with Welch 2-sample *t*-tests, following the recommendations of Ruxton (2006). For nonnormally distributed data, we used ranks instead of the original ratio measurements.

All statistical tests were performed with R 2.8.1 (R Development Core Team 2008) and SPSS 15.0 (SPSS Inc.). All tests were 2-tailed.

Results

We found considerable differences in the mean song amplitude between males, with a maximum between-individual difference of 9 dB (range between males: 78–87 dB, mean value of all males 82 dB; Fig. 4). Our method of measuring song amplitude in the field proved to be highly repeatable with a repeatability score of $r^2 = 0.81$. None of the environmental variables varied significantly with the measured SPLs. The only exception being air temperature ($F = 6.11$, df = 1.19, $P = 0.02$): song amplitude tended to be higher when temperature was low. However, this effect was fairly weak, with temperature explaining less than 20% of the variation in song amplitude ($r^2 = 0.19$).

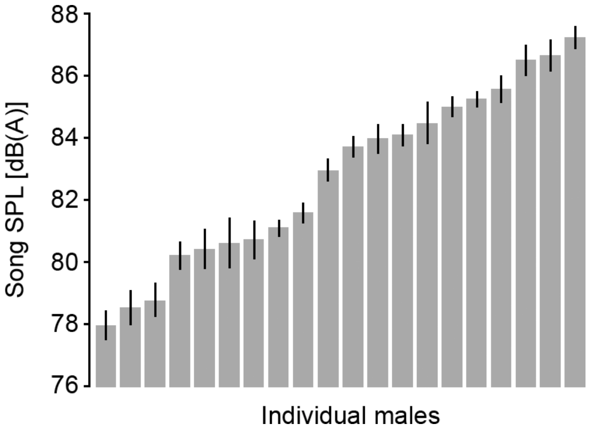

Figure 4. Song amplitudes of 20 free-ranging male chaffinches measured in their natural habitat. *Mean and standard error are given for each individual.*

Before the playback, none of the measured behavioral variables differed significantly between the subjects that received the high-amplitude playback and those that received the low-amplitude playback (number of songs [Welch 2-sample t-test: $t = 0.40$, df = 16.14, $P = 0.69$], number of calls [$t = 1$, df = 10, $P = 0.34$] and number of flights [$t = 21.17$, df = 19.37, $P = 0.26$]).

During the playback, all 22 males approached the loudspeaker, and there was no significant difference in the latency of approach during high- and low-amplitude playback ($t = 0.21$, df = 19.15, $P = 0.83$; Fig. 5). This suggests that the playback amplitude had no effect on the detectability of the simulated intruder.

PCA revealed that males responded significantly stronger in response to high-amplitude than to low-amplitude songs according to PC1 ($t = 2.79$, df = 14.59, $P = 0.01$) but not according to PC2 ($t = 1.45$, df = 11.67, $P = 0.17$). PC1 explained more than twice as much variation in the data than PC2. To further analyze the response difference between the 2 experimental groups, we investigated each response variable singly (Fig. 5): During playback, birds exposed to loud songs sang less ($t = 22.59$, df = 16.76, $P = 0.02$), spent more time within 5 m of the loudspeaker ($t = 5.62$, df = 16.20, $P < 0.01$), and approached closer ($t = 24.38$, df = 17.65, $P < 0.01$) than those exposed to low-amplitude song. The number of calls ($t = 1.68$, df = 20.00, $P < 0.11$) and the number of flights ($t = 1.85$, df = 18.19, $P = 0.08$) did not differ significantly between the treatment groups. During the postplayback period, birds also spent more time within 5 m of the loudspeaker when exposed to loud song ($t = 4.01$, df = 10, $P < 0.01$), while the number of songs ($t = 20.93$, df = 17.12, $P = 0.37$) and calls ($t = 1.01$, df = 18.15, $P = 0.32$) and the number of flights ($t = 0.30$, df = 19.38, $P = 0.77$) did not differ significantly. All significant differences between the single response variables were retained after Bonferroni-Holm correction.

Table 1. PCA including 8 response variables

	PC1	**PC2**
during the playback		
number of flights	**0.83**	-0.19
number of songs	**-0.85**	0.08
time within 5m	**0.54**	**0.75**
closest approach	**-0.82**	-0.19
after the playback		
number of flights	**0.52**	**-0.57**
number of songs	**-0.75**	0.22
number of calls	0.44	**-0.62**
time within 5m	**0.67**	**0.59**

All principal components with an eigenvalue higher than 1 are given (PC1 and PC2). Loadings of variables that made an important contribution to the PC score (<0.5) are indicated in bold.

Discussion

We found considerable differences in song amplitude between male chaffinches, with the loudest male in our sample of 20 birds singing on average 9 dB SPL higher than the male with the softest songs. Similar maxima between individual differences in song amplitude have also been reported in previous studies on free-ranging songbirds (Dabelsteen 1981; Brumm 2009).

Our playback experiment demonstrated that this 9 dB difference in song amplitude significantly affected the territorial behavior of male chaffinches: During a simulated territorial intrusion, males reacted more strongly in response to high-amplitude songs than to low-amplitude songs. In particular, males sang less and approached the dummy closer when we broadcasted high-amplitude songs, and they stayed longer in its vicinity, both during and after the playback.

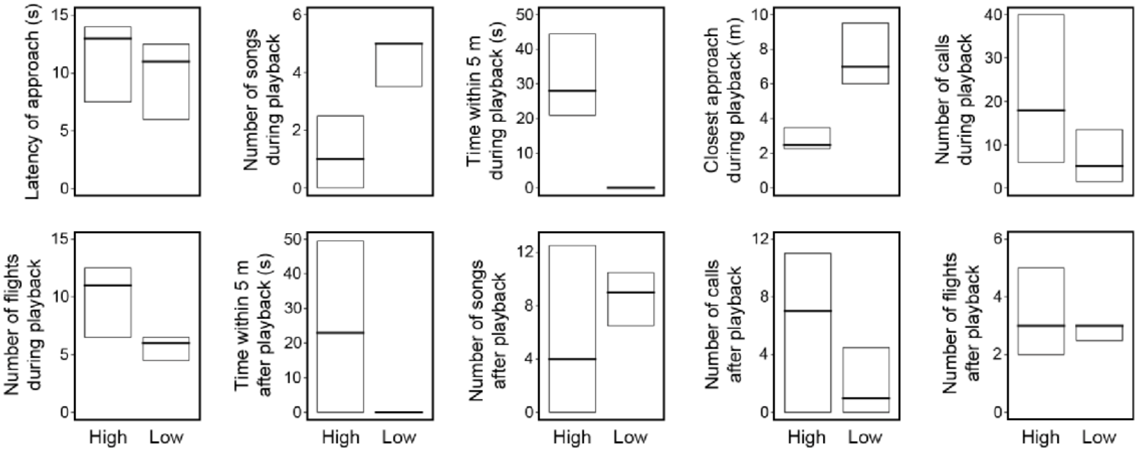

Figure 5. Responses of territorial chaffinches to playback of loud and soft songs, across 10 raw response variables. *X-axis shows the playback amplitude (high: 87 dB SPL at 1 m distance, low: 78 dB SPL at 1 m distance). Medians and interquartile ranges are shown for N = 11 males for each treatment. Statistically significant differences between the 2 playback treatments were found for the number of songs, the time within 5 m, and the minimum approach distance during the playback and the time within 5 m after the playback (see text).*

The differences in response strength cannot be explained by a limited audibility of the low-amplitude songs because there was no significant difference in the latency with which the tested birds approached the low- and high-amplitude playback. The validity of our results is further confirmed by a comparison with earlier playback studies with chaffinches (Slater 1981; Slater and Catchpole 1990; Leitão and Riebel 2003). Territorial males in these studies showed similar responses to playback in terms of number of songs during the playback, time spent within 5 m of the loudspeaker, and nearest approach as the males in our study did. Moreover, as we presented a stuffed male chaffinch together with the song stimulus and because all birds closely approached the dummy, we can exclude that territory owners simply perceived the simulated loud singers to be spatially closer. In one case, a territory owner showed close approach and antagonistic behavior toward the dummy before the playback had even started, which highlights the fact that the birds recognized the taxidermic mount as a conspecific male and potential rival.

Modulation of territorial behavior

The findings of our playback experiment indicate that chaffinches exhibit stronger territorial behaviors toward rival males singing loudly than toward rivals that produce softer songs. On a proximate level, louder songs can be interpreted as stronger stimuli, with more sound energy stimulating the listener's sensory apparatus and thus leading to an increased arousal and stronger responses. Such a proximate mechanism may be sufficient to account for the differential reaction in our 2 treatment groups. However, as territory defense is costly (Davies 1980; Huntingford and Turner 1987), it will be adaptive for territory holders to adjust the strength of response toward an intruder to the level of threat the intruder poses (de Kort *et al.* 2009a).

At least 3 mutually nonexclusive explanations may account for why rival males with loud songs are a stronger threat to territory owners: 1) loud singers are presumably highly motivated and thus more likely to escalate a territorial interaction. Several studies have demonstrated such a correlation between vocal amplitude and motivation: male nightingales increase their song amplitude when countersinging with conspecific males (Brumm and Todt 2004). Juvenile tree swallows *Tachycineta bicolor* and barn swallows *Hirundo rustica* increase the amplitude of their begging calls with increasing hunger (Leonard and Horn 2001; Boncoraglio and Saino 2008). So, if high-amplitude songs signal motivation to fight, then territory holders need to put more effort into defending their territory against a loud intruder. 2) Loud singers might be physically stronger than soft singers and thus pose a bigger threat to territory owners. However, body size, a physical attribute which may be of importance in intrasexual aggression, has been found to be unrelated to song amplitude in birds (Brumm 2009). 3) Loud intruders may be more likely to win extrapair copulations from the territorial female and thus pose a higher risk of loosing paternity to territory owners. This seems plausible as loud intruders will be detected over greater distances and as female songbirds are known to prefer loud songs (Searcy 1996; Ritschard *et al.* 2010). However, in the present case, this explanation cannot explain the modulation of territorial behavior because almost all the birds tested were not mated at the time of the experiment.

Studies on frogs indicate that a male's response to calls of different amplitude is affected by the density and distance of neighboring males (e.g. Rose and Brenowitz 1991; Burgmeister *et al.* 1999). Marshall *et al.* (2003) found that the stimulus amplitude at which male spring peepers *Pseudacris crucifer* respond aggressively is positively correlated with the amplitude of the advertisement call of its nearest neighbor. In other words, the closer the nearest calling neighbor, the higher the aggressive threshold of a male. By contrast, we found no effect of the distance to the nearest neighbor or the number of neighbors on the response strength of territorial chaffinches. During our experiments, the distance to the nearest singing neighbor varied markedly between individuals (30–190 m), but the overall number of neighbors was similar between all subjects (all birds had 1 or 2 neighbors). Although frog choruses, in which numerous males call simultaneously in big aggregations, are rather different from the more widely spaced territories of songbirds, we cannot rule out that social factors (including the perceived song amplitude of neighboring males) might affect male territorial responses in birds, too. Future studies that specifically address this issue will be very useful to further elucidate the flexibility and modulation of territorial behaviors in birds.

Consequences of increased territorial aggression

Variation in the defense behavior of a territory holder has consequences for an intruder or a neighboring male. The stronger territorial reactions in response to loud songs demonstrated in this study suggest that the performance of high-amplitude songs is likely to be constrained by the territorial aggression of resident males. This, in connection with previous studies on vocal amplitude in birds, indicates that song amplitude is individually regulated within the boundaries set by the requirements for successful signal transmission (Brumm and Naguib 2009) on the one side, and on the other side by constraints, such as social aggression (this study), eavesdropping (Dabelsteen *et al.* 1998), and predation (Krams 2001).

The strength of the social aggression experienced by a singing male will depend on the quality and/or motivation of the other males in the population (de Kort *et al.* 2009a). As it is unlikely that all males in a population would be able to bear the potential costs of increased social aggression, we conclude that the striking variation in song amplitude between individuals is, at least partly, a result of male–male competition. Because only high-quality males can counter the increased levels of aggression, high-amplitude songs would thus reliably reflect male quality. This idea is corroborated by earlier playback studies on other aspects of song performance, such as song overlapping (Naguib 1999) or song consistency (de Kort *et al.* 2009b). As in these previous studies, we found that higher levels of simulated song performance elicited stronger aggressive responses from territorial birds.

It remains to be shown, however, whether increased territorial aggression is translated into fitness-relevant costs for the recipient, for instance, through significant energy expenditure or physical injury. If this is true, then sexual selection would exert opposing selection pressures on song amplitude because females prefer louder songs (Searcy 1996; Ritschard *et al.* 2010) but social aggression by other males will limit the production of loud songs.

Interestingly, similar converse intra- and intersexual selection of male acoustic signals has been suggested for anurans and insects (Gerhardt and Huber 2002). All other call parameters being equal, female anurans and insects are attracted to the louder of 2 male advertisement signals. When encountering a rival, male insects and anurans may switch from advertisement signals to aggressive signals with a distinctly different structure. In frogs, such switches to aggressive signaling depend on the amplitude of the rival's call, that is, calls elicit aggressive responses only above a certain amplitude threshold (e.g. Brzoska 1982; Arak 1983; Brenowitz 1989; Marshall *et al.* 2003). This means that loud (or spatially close) male frogs experience increased levels of aggression from other males. Thus, the overall, comparative picture—including our results from a songbird—suggests a general pattern of male–male competition mediated by acoustic signals, in which the production of high-intensity signals is constrained by social aggression from rival males.

Conclusions

We have found that 1) song amplitude differed markedly between individuals and 2) the intensity of territorial behavior in chaffinches is modulated by the amplitude of songs to which they are exposed, suggesting that song amplitude may bear information about the singer's quality or motivation and that song amplitude may be socially constrained. The latter supports the hypothesis of Gil and Gahr (2002) that social aggression may limit the expression of song amplitude in birds, thus emphasizing the potential of vocal amplitude for sexual selection.

Chapter 3

Enhanced testosterone levels affect singing motivation but not song structure and amplitude in Bengalese finches

M. Ritschard, S. Laucht, J. Dale, H. Brumm

Song is a fundamental component of territory defense and mate attraction in birds, and androgens (like testosterone) are known to play a key role in controlling it. However, little is known about how differences in testosterone levels between males translate into inter-individual song variation. Indeed, testosterone could affect both the motivation to sing and the structure of song itself. Here, we tested whether experimentally elevated testosterone levels in adult Bengalese Finches Lonchura striata var. domestica, an oscine bird species, have an activational effect on 1) song performance, and 2) song structure. Our results show that testosterone treated males, in contrast to sham-control males, sang more when confronted with a female. Other performance-related traits, however, such as latency to sing and song amplitude, were not affected. Testosterone treated males also showed no differences in our two measures of song structure: fundamental element frequency and mean song frequency. Because song structure is known to be organizationally affected by testosterone, our results, synthesized together with findings from the current literature, suggest that in oscine birds, song contains multiple messages about the signaler's hormonal status. First, song performance may reflect current hormonal condition, and second, song structure may reflect the past hormonal state.

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The sexual behaviour of many vertebrates is strongly influenced by testosterone, a steroid hormone produced in the gonads (Adkins-Regan 2005). In oscine birds, song is one of the fundamental components of mate attraction and territory defense and there are many lines of evidence that suggest that singing is controlled by testosterone. For example, in the great tit *Parus major* and willow tit *Parus montanus* plasma testosterone levels co-vary with singing activity over the breeding season (Rost 1990; 1992). In zebra finches *Taeniopygia guttata* and canaries *Serinus canaria*, castration of males decreases song rates, but normal singing activity is re-established after the castrated birds have been injected with testosterone (Pröve 1974; Arnold 1975; Heid *et al.* 1985). In a number of species, such as white-crowned sparrows *Zonotrichia leucophrys* and canaries, females supplied with exogenous testosterone develop a male-like song and singing behaviour (Shoemaker 1939; Mulligan and Erickson 1968; Kern and King 1972), even though in those species females normally do not sing. Finally, neurobiological studies demonstrated that songbirds have androgen receptors in their specialized song control nuclei in the forebrain (Gahr and Metzdorf 1997; Ball *et al.* 2003) as well as in the *ventralis* muscle of the adult avian syrinx (Veney and Wade 2004).

Even though the overall relationship between androgens and singing activity is well-documented, there is actually only a limited number of studies that have looked at how differences in testosterone levels between males translate into inter-individual song variation (Gil and Gahr 2002). Two fundamentally different properties of song can be used to describe inter-individual song variation: song performance and song structure. First, song performance is the quantity and amplitude of song as well as the overall temporal pattern of song utterance. Second, song structure includes spectral and temporal properties of the sound waves that are emitted during singing. Song performance and song structure can be measured independently of each other (Gil and Gahr 2002).

Most studies that have looked at how differences in testosterone levels between males translate into inter-individual song variation have considered song performance only. The majority of these reported increased call or song rates in testosterone-treated oscines (Silverin 1980; Nowicki and Ball 1989; Ketterson *et al.* 1992; McDonald *et al.* 2001; Cynx *et al.* 2005; Boseret *et al.* 2006; Kurvers *et al.* 2008; Strand *et al.* 2008), as well as in a suboscine (Day *et al.* 2006) and a non-oscine species (Mougeot *et al.* 2005). Another study employing experimental manipulation of testosterone level did not find an effect on singing rates (Kunc *et al.* 2006) and several investigations relating natural testosterone levels to inter-individual differences in song rate also failed to find such a relationship (Saino and Møller 1995; Galeotti *et al.* 1997; Saino *et al.* 1997). In some cases, this may be explained by seasonal variation in the level of the estrogen-synthesizing enzyme aromatase (Fusani 2000), which plays a crucial role in linking testosterone and behaviour (Gil and Gahr 2002; Ball *et al.* 2003). Song bout length, another performance-related trait was not found to be positively related with experimentally increased androgen levels (Kunc *et al.* 2006; see also Voigt and Leitner 2010). At the level of song element types, Galeotti *et al.* (1997) found that the length of the distinctive 'rattle' syllable in the song of the barn swallow *Hirundo rustica* as well as the number of pulses correlates with natural plasma testosterone levels.

One song performance parameter, amplitude, has been ignored among the several studies that have investigated the effect of testosterone on song performance. One reason for this may be that song amplitude can only be measured reliably in a highly controlled set-up. However, amplitude is important because 1) it determines the active space of the signal, 2) female birds prefer loud songs (Searcy 1996; Ritschard *et al.* 2010) and 3) song amplitude is controlled by air sac pressure (Plummer and Goller 2008), and it has been shown that certain muscles and other tissues that may affect the gas pressure in the syrinx are sensitive to androgens (Veney and Wade 2004).

In contrast to song performance traits, there are very few studies that have addressed possible effects of sex steroids on song structure. From a proximate perspective such an effect is expected simply because of the high androgen sensitivity of the song control system (see Ball *et al.* 2003 for review). From an ultimate perspective such an effect is expected because birdsong is known to play a key role in male–male competition and mate choice (Catchpole and Slater 2008). As sex steroids strongly affect aggression and courtship behaviour (Adkins-Regan 2005; Fusani 2008b), high testosterone levels may optimize song parameters for efficient sound transmission and mate attraction. Nevertheless, the current evidence for a link between testosterone and song structure is inconsistent. Repertoire size (Weatherhead *et al.* 1993), rate of song type switching (Weatherhead *et al.* 1993) and song versatility (repertoire size multiplied with the number of song type switches; Kunc *et al.* 2006) were not affected by administration of exogenous testosterone. However, Cynx *et al.* (2005) demonstrated that experimentally elevated testosterone levels in adult zebra finches decreased their fundamental song frequency. Remarkably this effect was only observed in the long term as treatment birds decreased their song pitch after five weeks post testosterone implantation. Moreover, the effect was still present one year later, indicating long term effects of sex steroids during adulthood (see Arnold and Breedlove 1985 for a discussion of organizational vs. activational androgen effects). To date, no studies have demonstrated an activational effect of androgens on song structure.

In the present study we test for activational effects of testosterone implants (i.e. an increase of testosterone above baseline levels) on a thorough suite of both performance-related and structure-related song parameters, some of which have never (or rarely) been studied in the context of androgen sensitivity.

Our study species, the Bengalese finch *Lonchura striata* var. *domestica*, belongs, as the zebra finch, to the Estrildidae family. Like many other members of that family, Bengalese finches display two types of song: directed song and undirected song (Sossinka and Böhner 1980; Ikeda *et al.* 1994). Both types of song are structurally similar, but directed song is addressed at a female during courtship while undirected song is sung in contexts not clearly associated with a targeted receiver. Although it has been shown that testosterone levels in the Bengalese finch are significantly increased during the nest construction phase (Seiler *et al.* 1988; Seiler and Güttinger 1988), overall levels and fluctuations of plasma testosterone are low compared to songbirds of temperate latitudes, which is a pattern commonly observed in tropical species (Goymann *et al.* 2004; Hau *et al.* 2008). As in the zebra finch (Pröve 1974), the occurrence of directed song in the Bengalese finch is controlled by testosterone, while undirected song is largely independent of androgen levels (Ikeda *et al.* 1994). To test for activational effects of androgens, we measured performance- related song parameters and song structure of directed song in birds implanted with testosterone pellets and the control birds with sham pellets and compared their singing behaviour before and after implantation.

Methods

Subjects and housing

The Bengalese finch is a domestic strain of the white-rumped munia *L. striata*, an estrildide finch which is distributed throughout tropical Asia (Restall 1996). White-rumped munias are gregarious birds often found in groups of several dozen individuals (Restall 1996). The domesticated form is highly gregarious, reaches sexual maturity at about 3 months of age, and may breed all year round (Eisner 1960). Bengalese finches have relatively simple, stereotyped songs, similar to those of zebra finches. Songs consist of individual-specific phrases or element sequences repeated several times in a song bout (Okanoya and Yamaguchi 1997; Woolley and Rubel 1997). A song element is a single note, visible as a continuous tracing in a spectrogram. A phrase is a stereotyped sequence of elements, whereas a song bout is a sequence of uninterrupted song (usually containing several phrases) which is separated by other song bouts by a clear break of at least a few seconds. While birdsong often has a dual function of male–male competition and mate attraction, Bengalese finch song is never used in aggressive contexts but primarily functions in female attraction and during courtship (Honda and Okanoya 1999).

The tested birds were obtained from local pet suppliers and kept for several months in a large aviary at the Max Planck Institute for Ornithology in Seewiesen to make sure that they had all reached sexual maturity. The experiments were conducted between June 2nd and June 21st. Two weeks before the experiment started, the males were moved to single cages (61 × 40 cm and 50 cm high) set up in a large outdoor aviary with three wooden walls, wooden ceiling and one wall made of wire mesh. Since Bengalese finches are highly social, all birds were kept in constant visual and acoustic contact with other males to allow some degree of social interactions. They were exposed to the outside light/dark cycle (ca. 16 h of full daylight during the time of the experiments) and temperature fluctuations. Cages were equipped with three perches and a cuttlefish bone, and birds had ad libitum access to a commercial tropical seed mixture and water. Their diet was supplemented with fresh lettuce once a week. The test birds could hear the songs of the other males kept in the same room and they could also see some of the other birds. No females were present in the room.

Testosterone implantation and hormone assay

A total of 34 birds were randomly assigned to the experimental groups, but we made sure that the same number of different colour morphs was included in the treatment and control group. Testosterone birds were implanted with testosterone pellets (1.5 mg testosterone, 60 day release, Innovative Research of America, Sarasota, Florida) subcutaneously through a small dorsal incision of the skin that was then closed with VetGlu tissue adhesive (Heiland Vet GmBH, Germany). For an assessment of release rates of pellets and a comparison with silastic tubes see Fusani (2008a). The control birds were implanted with sham pellets consisting of pure binding material (Innovative Research of America, Sarasota, Florida). Blood samples were taken twice: 7 days before implantation and again 7 days after implantation. 70–150 µl of blood was collected with heparinized micropipettes following venipuncture of the alar vein. The samples were centrifuged and the plasma separated and stored frozen until further analysis. For each individual, the pre-implantation and the post-implantation blood samples were taken at the same time of day to control for circadian fluctuations of plasma testosterone levels (Schanbacher *et al.* 1974; Balthazart 1976; Balthazart *et al.* 1981; Bachmann *et al.* 1987). Blood sampling took place between 1400 and 1630 h.

Plasma testosterone concentration was determined by direct radioimmunoassay (RIA) employing testosterone antiserum T3-125 (Esoterix Endocrinology, Calabasas, CA, USA), following the protocols described in Goymann *et al.* (2002) and Goymann *et al.* (2006). Cross reactivities of this antiserum are testosterone (100%), 5a-dihydrotestosterone (44%), d-1-testosterone (41%), d-1-dihydrotestosterone (18%), 5a-androstan-3b, 17b-diol (3%), 4-androsten 3b,17b-diol (2.5%), d-4-androstenedione (2%), 5bandrostan- 3b, 17b-diol (1.5%), estradiol (0.5%), and less than 0.2% with 23 other steroids tested. Plasma samples were equilibrated with 1500 dpm of tritiated testosterone (Perkin Elmer, Wellesley, MA, USA) to calculate recoveries. Mean ± SD extraction efficiency for plasma testosterone was 92 ± 3%. Standard curves and sample concentrations were measured in duplicates and calculated with Immunofit 3.0 (Beckman Inc. Fullerton, CA), using a four parameter logistic curve fit. The lower detection limit of the standard curves was determined as the first value outside the 95% confidence intervals for the zero standard (B_{max}) and was 0.36 pg/tube. Samples were analyzed in two assays with an intra-assay coefficient of variation of 1.7 and 6.2% (determined from standard testosterone). The inter-assay coefficient of variation was 11.5%, the intra-extraction coefficient of variation of extracted plasma pools was 4.4 and 7.6%, and the inter-extraction coefficient of variation was 21.2%. Since the testosterone antibody used shows significant cross-reactions with 5a-dihydrotestosterone (44%), our measurement may include a fraction of 5a-DHT.

The experimental procedures were reviewed and approved by the administration of Upper Bavaria.

Song recording and song analysis

For each male, song was recorded 1–3 days after the first blood sampling (i.e. 4–6 days before implantation of the testosterone/control pellet) and again 1–3 days after the second blood sampling (i.e. 8– 10 days after implantation).

For the song recordings, each bird was moved singly to the experimental cage, a small wire mesh cage (54 × 28 cm and 41 cm high) equipped with a single perch. The cage was placed in the center of an empty room (4.60 × 3.40 m and 2.45 m high) that was partly lined with anechoic foam to reduce reverberations. The back and side walls of the cage were foam-padded while the top and the front were open. A Sennheiser ME 62 omnidirectional microphone connected to a Marantz PMD 660 solid state recorder was installed 80 cm vertically above the center of the perch. This set-up allowed us to reliably measure sound amplitude independent of the singing direction of the subject (Brumm 2009; Brumm *et al.*

2009c). A second cage with two females was placed in one meter distance from the uncovered front of the male cage to stimulate the test bird to sing. The females had access to ad libitum food and water. The sound recording was started as soon as the male was released into the experimental cage and lasted for 1 h. Simultaneously, the males' behaviours were recorded with a video camera (JVC Everio GZ-MG77E). This allowed us to later distinguish songs uttered from the perch from songs uttered from the floor of the cage. After 1 h, the male was moved back to its home cage.

Of the 34 males tested, one died between the first and the second session due to unknown causes. 12 (seven treatment birds and five control birds) sang during both recording sessions. Five males sang in only one of the two sessions, 16 birds did not sing at all. In our experience, the high rate of non-singers is typical for Bengal finches and related species. Zebra finches, for instance, show very similar singing rates with comparable testing set-ups when a female is kept in 1 m distance (Brumm and Slater 2006b).

Sound analysis was performed both on the level of song bouts and on the level of single song elements. For the latter, we analyzed 3–8 different song element types per bird. We chose song elements depending on whether several good quality renditions of the same element were available. Only those songs which were uttered while the male was sitting on the perch were used in the analyses, as some song parameters, in particular song amplitude, can vary with the distance of the singing bird to the microphone. All recordings were high-pass filtered at 350 kHz to remove low-frequency background noise. The following song parameters were measured using Avisoft-SASLab Pro (R. Specht, Berlin, Germany): latency to sing (the time it took from the start of the experiment until the bird uttered the first song), time spent singing, element rate (number of elements per second), peak amplitude (the amplitude of the loudest element in the song, averaged over 1–10 renditions; root mean square (RMS) values, averaging time 125 ms) and mean amplitude across the whole song bout (RMS method, averaged over 1–10 song bouts). Song element parameters included fundamental frequency (only measured in harmonic elements), mean frequency and amplitude (RMS method with averaging time 125 ms), all averaged over 3–10 renditions. Recording settings were kept constant between birds, which allowed us to compare the sound levels of songs from different recordings. To calibrate the sound amplitude measurements, we played a calibration tone (f_0 = 2 kHz) with constant amplitude from inside the experimental cage (with a FOXPRO Scorpion X1A digital player, FOXPRO Inc, Lewistown, USA), which was recorded with the same recorder and settings as the song recordings. We then replaced the microphone with a CEL 314 precision sound level meter (integration time 125 ms) to measure the sound level at the location of the microphone. Mean of peak amplitude of the Bengalese finch songs during the pre-implantation session was 52.9 dB SPL re 20 μPa at 1 m (range: 44.1–61.7 dB), mean of mean amplitude was 49.3 dB (range: 43.1–55.9 dB).

Data analysis

All statistical tests were performed with R 2.8.1 (R Development Core Team 2008). The function *lmer* (R package *lme4*) was used to fit generalized linear mixed-effects models (GLMM). We used a Wald χ^2 test (see Bolker *et al.* 2008) to investigate whether there was a significant interaction between the fixed factor "timing" (before/after the treatment) and the "group" (control/treatment group), i.e. whether the reaction of the individuals depended on the treatment. The variable "latency to sing" had a complex distribution because birds that did not sing during one session were assigned a maximum value. We were not able to fit a GLMM to the original data and therefore used a rank transformation following the procedures outlined by Conover and Iman (1982).

Birds that did not sing during at least one of the two recording sessions (i.e. 16 out of 33) were excluded from the analyses. One bird from the treatment group did not show an elevated testosterone level but a decrease by 30% (from 994 to 695 pg/ml), while all other treatment birds had a more than two-fold increase in testosterone levels. Therefore, we assumed that the implant probably fell off and the individual was removed from the analysis.

We accounted for the repeated sampling of the same individuals (one sampling before the treatment and one sampling after the treatment) by using individual subject as a random factor. For the analysis of the song element parameters (fundamental frequency, amplitude, mean frequency), we used a random factor "element type" that was nested in the individual subjects. Body weight was always included in our models as a fixed factor because the control birds tended to be heavier than treatment birds before implantation, but this difference was not significant (Welch Two Sample *t*-test: $t = -2.09$, df = 7.85, $P = 0.07$).

Results

Before implantation, the two experimental groups neither differed statistically in their testosterone levels ($t = -0.60$, df = 13.90, $P = 0.57$) nor in any of the measured song parameters: total time singing ($t = -1.20$, df = 8.67, $P = 0.26$), latency to sing ($t = -0.19$, df =

14.00, P = 0.85), peak amplitude (t = −1.60, df = 8.41, P = 0.15) and mean amplitude (t = −0.87, df = 7.83, P = 0.41). Similarly, baseline testosterone level was not correlated with body weight (Spearman's rank correlation, r^2 = 0.01, t = 0.44, df = 14, P = 0.66), nor were any of the song parameters ($0.22 \geq r^2 \geq 0.003$, $1.66 \geq t \geq -0.20$, $14 \geq$ df ≥ 10, $0.84 \geq P \geq 0.13$). Likewise, none of the song parameters varied with individual testosterone levels before implantation ($0.16 \geq r^2 \geq 0.01$, $1.38 \geq t \geq -0.37$, $14 \geq$ df ≥ 10, $0.72 \geq P \geq 0.20$).

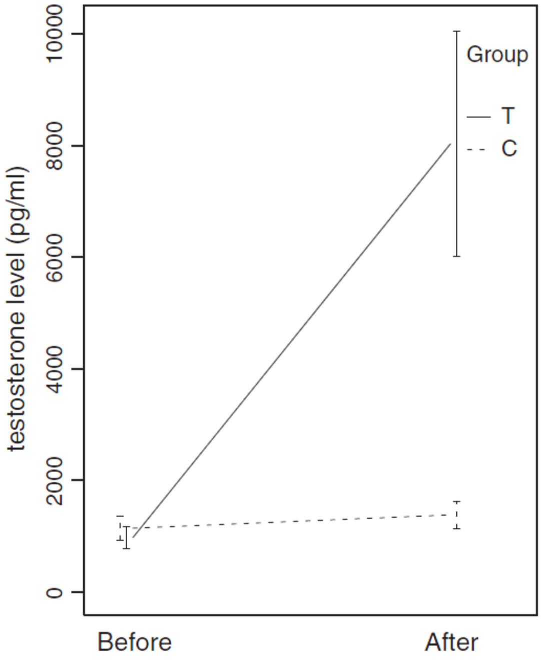

Fig. 6. Interaction plot of plasma testosterone levels before and after pellet implantation in male Bengalese finches. *Means ± SE of testosterone levels are given for treatment birds (solid line; N = 8) and the control birds (dashed line; N = 8). In contrast to the control birds, treatment birds show significantly elevated testosterone levels after implantation (P < 0.001).*

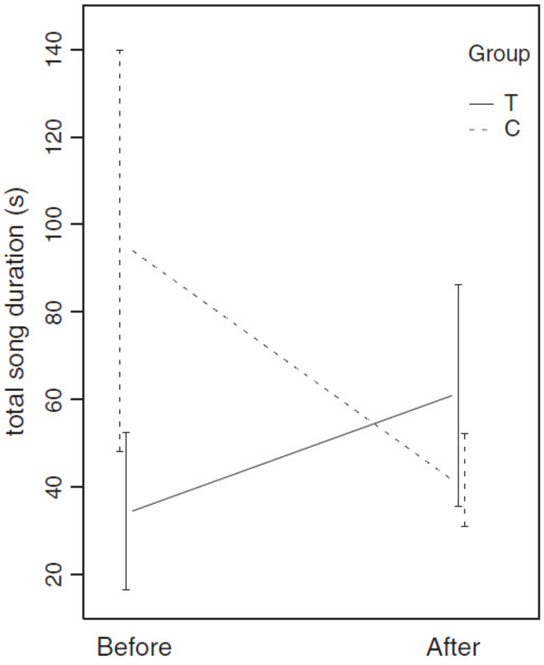

Fig. 7. Effect of testosterone on the total duration of courtship song in Bengalese finches. *Interaction plot of the song duration before and after pellet implantation. Means ± SE of total song duration are given for treatment birds (solid line; N = 8) and the control birds (dashed line; N = 8). In comparison with the control birds, testosterone-implanted birds show a significant increase in total song duration (P < 0.001).*

After implantation, the treatment birds had considerably increased plasma testosterone levels compared to the control birds (χ^2 = 9812.1, df = 1, P < 0.001, Fig. 6), which indicates that our hormone treatment was successful. Body weight was not affected by the treatment (χ^2 = 0.35, df = 1, P = 0.55), with both treatment group and control group showing a slight, but statistically insignificant, increase in weight.

Song performance

Our testosterone treatment did not affect the overall likelihood to sing (χ^2 = 0.09, df = 1, P = 0.76); in the treatment group, a total of seven birds sang during the first session and eight birds during the second session, while in the control group, a total of six birds sang in the first session and seven birds (whereof two did not sing before) in the second session. In contrast, the treatment had a strong effect on the total singing duration (χ^2 = 206.63, df = 1, $P < 0.001$) with testosterone birds spending more time singing and the control birds decreasing their total singing duration (Fig. 7). The decrease within the control birds suggests that the baseline singing motivation was lower in the second recording session compared to the first session. However, while the two slopes differed significantly, both the increase within the testosterone birds (t = −0.76, df = 12.32, P = 0.46) and the decrease within the control birds (t = 1.10, df = 9.14, P = 0.30) were statistically not significant. The testosterone-related difference in performance time was mainly due to the treatment birds producing more song bouts, but they also tended to increase their average song bout length compared to the control birds (χ^2 = 2.77, df = 1, P = 0.10). Latency to sing (χ^2 = 0.44, df = 1, P = 0.51) and element rate were not significantly affected by the testosterone treatment (χ^2 = 2.21, df=1, P = 0.14), nor were mean song amplitude (χ^2 = 0.30, df = 1, P = 0.58), peak song amplitude (χ^2 = 0.15, df = 1, P = 0.70) and element amplitude (χ^2 = 1.26, df = 1, P = 0.26).

Song structure

We found no effect of the testosterone treatment on our two structural song measures, fundamental element frequency (χ^2 = 0.30, df = 1, P = 0.58) and mean element frequency (χ^2 = 0.97, df = 1, P = 0.32).

Discussion

We found that within 10 days after administration, testosterone implants increased the total time that male Bengalese finches spent singing. In contrast, no evidence for activational effects of testosterone on latency to sing and on song amplitude could be observed. Thus, we conclude that in Bengalese finches, high plasma testosterone levels augment the motivation to sing. A similar effect has been described in a number of avian species, including the pied flycatcher *Ficedula hypoleuca* (Silverin 1980), song sparrow *Melospiza melodia* (Nowicki and Ball 1989), dark-eyed junco *Junco hyemalis* (Ketterson *et al.* 1992), rufous whistler *Pachycephala rufiventris* (McDonald *et al.* 2001), red grouse *Lagopus logopus scoticus* (Mougeot *et al.* 2005), zebra finch (Cynx *et al.* 2005), canary (Boseret *et al.* 2006), golden-collared manakin *Manacus vitellinus* (Day *et al.* 2006), blue tit *Cyanistes caeruleus* (Kurvers *et al.* 2008) and the house finch *Carpodacus mexicanus* (Strand *et al.* 2008).

As singing functions in territory defense and mate attraction (Catchpole and Slater 2008), a high motivation to sing is likely to be required during the periods when competition for resources and access to mates is high. Such periods may vary between species depending on their ecological requirements and life-history traits (see Hau *et al.* 2008 for review). Peak androgen levels are correlated, among other factors, with, seasonality (Rost 1990; 1992; Wikelski *et al.* 2003; Goymann *et al.* 2004), mating system (Wingfield *et al.* 1987; Goymann *et al.* 2004) and sociality (Reyer *et al.* 1986; Wingfield *et al.* 1991). In the Bengalese finch, testosterone levels are highest during the nest building stage, when males compete for access to females (Seiler and Güttinger 1988). In combination, all these pieces of evidence strongly suggest that a direct physiological link exists between circulating testosterone levels and singing motivation.

Contrary to song output, song amplitude did not differ between testosterone birds and the control birds in our study. This indicates that song amplitude is not affected by variation in circulating testosterone levels, at least in the Bengalese Finch. It is possible that an effect of testosterone on song amplitude will be found in more territorial species that use their songs for long-range communication, because loud songs will be advantageous both in territorial defense and in passive female attraction, however we presume that modulation of song amplitude will generally tend to be under more direct (i.e. neuronal) control mechanisms (see later discussions).

The challenge hypothesis predicts short term increases of testosterone levels during aggressive encounters (Wingfield *et al.* 1990a), which is particularly interesting with respect to the potential relationship between song amplitude, testosterone and territorial defense. In nightingales, territorial males have been found to increase their song amplitude during simulated challenges by rival males (Brumm and Todt 2004). However, all nightingales increased their vocal effort within one minute after hearing a rival male (some individuals even within a few seconds) indicating that the vocal regulation of song amplitude can be very fast. These brief reaction times suggest that testosterone may not be the immediate mediator of the increase in song amplitude because the secretion of testosterone is expected to take at least several minutes after stimulation (Wingfield 1985). Therefore short term changes in song amplitude are more likely to be mediated by other, i.e. neuronal, mechanisms.

In contrast to the effects on song performance, our testosterone treatment did not affect our two measures of song structure, fundamental frequency and mean frequency of song elements. This is in line with another study (Cynx *et al.* 2005) that found organizational, but no activational effects of androgens on fundamental frequency of zebra finch song elements: the fundamental frequency of testosterone-treated males did not change within three weeks after implantation, but it was significantly lower five weeks after implantation and remained low for at least one year. However, Cynx *et al.* (2005) did not assess plasma testosterone levels, so it is unclear how effective the treatment was and for how long testosterone levels remained elevated.

Although in our study administration of additional testosterone did not alter song structure on the short term, this does not exclude that testosterone below the observed baseline levels may have an impact on song structure. It might be that a comparably low testosterone level has activational effects on song structure and/or amplitude, but these song features may remain stable beyond a certain threshold of androgen concentration. This hypothesis can be tested with experiments using androgen receptor blockers or employing methods to reduce the amount of circulating testosterone (Adkins-Regan 2005).

Taken together, our study and the study of Cynx *et al.* (2005) suggest that immediate, activational effects of enhanced plasma testosterone affect singing motivation, while long term effects may alter song structure. It is possible that some changes in song structure involve complex physiological processes altering neural and muscular actions and/or the structure of the syrinx. As a consequence, singing rate and perhaps song bout length, may reflect current hormonal state, while song structure may reflect past hormonal state, both during early development (Grisham and Arnold 1995; Veney and Wade 2005) and during adulthood (Cynx *et al.* 2005). However, this hypothesis regarding the hormonal basis of singing motivation and song structure needs to be substantiated by further experimental data. While there is a rather large body of evidence for a strong link between current androgen levels and overall courtship motivation in birds, activational and organizational effects of testosterone on song structure are still neglected. Therefore, future studies of testosterone-sensitivity of birdsong should assess both short term and long term effects. Moreover, such studies may focus on song parameters which have been found to be under positive female preference, as testosterone during early development as well as during adulthood may have long-time effects and perhaps also short-time effects on song quality. High testosterone levels have been shown to be costly to maintain (Wingfield *et al.* 2001) and good singers may thus signal their ability to bear these costs. Song parameters which may be tested for testosterone-sensitivity include repertoire size (Mountjoy and Lemon 1996; Searcy and Yasukawa 1996) and specific song elements (‘sexy syllables’) like the broadband trills in canaries (Vallet and Kreutzer 1995), which both have been shown to be important for female choice.

Chapter 4

Zebra finch song signals male body condition

M. Ritschard, H. Brumm

The honesty of sexually selected traits can be maintained if the expression of such a trait is sensitive to the signaler's phenotypic condition. Male birdsong is a secondary sexual trait which is under female choice and it has been suggested that (1) body condition is reflected in inter-individual song variation and (2) females will prefer songs that indicate the signaler is in good condition. To test these ideas, we manipulated the body condition of adult male zebra finches Taeniopygia guttata and assessed the impact of the treatment on song parameters that may be important in mate choice in this species. We found no effect on syllable repertoire, proportion of sound versus silence within a song and mean song frequency. In contrast, treatment birds showed a reduced song rate, an increased latency to sing, and a lowered song amplitude and fundamental frequency. Several song parameters were thus affected in a converse direction to that of female preferences. Our study demonstrates that zebra finch song reflects male body condition and that songs of high condition males contain traits that are more attractive to females. This adds strong support to the general assumption that female song preferences evolved because song reflects male quality.

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Sexual selection theory assumes that sexually selected traits reflect the quality of the signaler. The honesty of a secondary sexual trait can be maintained if (1) it is costly to produce or maintain and (2) the expression of the trait is sensitive to the males' phenotypic condition (Nur and Hasson 1984; Andersson 1986; Pomiankowski 1987; Andersson 1994; Johnstone 1995; Rowe and Houle 1996; Cotton *et al.* 2004). Such traits are thought to play a role both in male-male competition and in female choice (Andersson 1994). Indeed, it has been shown in a number of taxonomic groups that the expression of morphological and behavioral traits that are subject to sexual selection varies with male body condition (e.g. insects [Emlen 1994; David *et al.* 1998; Holzer *et al.* 2003], spiders [Kotiaho 2000], fish [Candolin 1999], amphibians [Green 1991], and birds [von Schantz *et al.* 1997; Birkhead *et al.* 1998; McGraw *et al.* 2002]).

Birdsong is a secondary sexual trait which is thought to be important in male-male competition and to be under intense female choice (Searcy and Andersson 1986; Searcy and Yasukawa 1996; Catchpole and Slater 2008). Similar to other sexually selected traits, individual song variation is likely to signal the singers' physical condition (Gil and Gahr 2002), but to date only few studies have addressed this issue. However, such studies on the condition dependence of birdsong are important, since body condition affects survival and reproductive success (Iwasa *et al.* 1991; Roff 1992; Stearns 1992), and because song plays a crucial role in sexual selection in birds. There is some correlative evidence that song may be an important link between body condition and reproductive success, assuming that females assess the quality of a male through its song. For instance, male pied flycatchers *Ficedula hypoleuca* which were provided with supplemental food or were in better condition sang more, had more complex songs, and they also paired earlier and were more likely to find a mate (Alatalo *et al.* 1990; Lampe and Espmark 2003). Dunnocks *Prunella modularis* supplemented with additional food sang with a higher rate than control males and shifted their mating system from polyandry towards monogamy and polygynandry, which resulted in higher mating success (Davies and Lundberg 1984). Moreover, several studies have looked at the relationship between song variation and body condition (Searcy 1979; Davies and Lundberg 1984; Gottlander 1987; Reid 1987; Strain and Mumme 1988; Alatalo *et al.* 1990; Houtman 1992; Lampe and Espmark 1994; Galeotti *et al.* 1997; Mager III *et al.* 2007; Brumm 2009; Grava *et al.* 2009); however, the conclusions that can be drawn from these previous investigations are limited because the data are purely correlative or, if food availability was manipulated, body condition was not assessed. A causal relationship between body condition and song can therefore not be inferred from these studies.

Although sexual selection undisputedly plays a key role in the evolution of birdsong (Searcy and Andersson 1986; Gil and Gahr 2002; Catchpole and Slater 2008), it is difficult, if not impossible, to experimentally demonstrate a direct link between male song and either male or female fitness, because in natural situations, song can not be disentangled from other attributes of an individual (Searcy and Andersson 1986). Therefore, fitness consequences of song variation are usually studied indirectly by looking at female song preferences or the outcome of male-male singing interactions. However, the crucial issue is whether differences in song features indeed signal differences in fitness-related traits.

To test whether body condition is reflected in song and whether such condition-dependent song variation is likely to be used by females to assess male quality, we manipulated the body condition of male zebra finches *Taeniopygia guttata*. We determined the effects of our treatment on a number of song parameters that have been shown to affect mate choice in the zebra finch. In this species, song is closely linked to mate choice and female song preferences are well studied (Zann 1996; Riebel 2009). Song rate, song amplitude, syllable repertoire, and proportion of sound versus silence within a song have been shown to be particularly important in mate choice (Collins *et al.* 1994; Riebel *et al.* 2009; Ritschard *et al.* 2010). To understand why females pay attention to these song traits, we measured the effects of male body condition on these song parameters, and also analyzed whether our treatment affected additional parameters like song frequency and the latency with which males start to sing. Previous studies in zebra finches have looked at the effects of early nutrition on song development (Spencer *et al.* 2003; Holveck *et al.* 2008; Zann and Cash 2008; Brumm *et al.* 2009c; Bolund *et al.* 2010); however, in this study we manipulated body condition in adult birds to investigate the effects of current condition.

The assessment of body condition in birds remains a subject of debate, and commonly used indices of condition, such as mass/length residuals, are controversial (Brown 1996; Green 2001; Cotton *et al.* 2004). To circumvent some of the challenges related to the determination of body condition, we chose an experimental design that allowed us to directly investigate effects of condition within individuals rather than between individuals. To this end, we used food restriction as a means to manipulate body condition in our treatment group, while a control group was kept on an unrestricted diet. Nutritional status is generally closely related to phenotypic condition, and reduced nutrient levels in birds result in loss of body mass (Birkhead *et al.* 1998), poorer reproductive performance (Jones and Ward 1976; Ankney and MacInnes 1978; Lemon 1991), and they also affect immune capacity (Alonso-Alvarez and Tella 2001). If zebra finch song is indeed a signal of male current condition, then we expect that the treatment birds would show changes in the song parameters that have been found to be important in female choice (see above), in such a way that the songs of males in reduced condition would be less attractive.

Methods

Subjects and housing before the experiment

We used 30 wild-morph domesticated male zebra finches from our colony at the Max Planck Institute for Ornithology in Seewiesen, Germany. Zebra finches are socially monogamous and breed in colonies. They occur in most Australian mainland habitats and on the Lesser Sunda Islands (Zann 1996). Males have two types of song, which are structurally very similar: directed song, which is a courtship display addressed to a nearby female, and undirected song, which is uttered in situations where the male is alone (Sossinka and Böhner 1980). The song is usually preceded by a few short introductory notes and consists of individual-specific phrases or motifs (duration < 1 s) which are repeated several times in a song bout.

Before the start of the experiment, the males were housed together with females in a large aviary (3.0 m × 2.0.m and 2.0 m high) with a 13:11 h light:dark schedule (lights on between 0500 and 1800 hours). The aviary was equipped with a number of perches and tree branches, the floor was covered with wood litter. The birds had ad libitum access to a commercial tropical seed mixture, water, and cuttlefish bone, supplemented with fresh lettuce once a week. No nesting aids or nesting materials were provided and birds did not breed, but approximately half of the males had previous breeding experience.

Food manipulation experiment

After the first recording session (see below), the birds were weighed and then divided into two equal-weight groups, a treatment group and a control group, each containing 15 individuals. Each group was further split up into 3 subgroups containing 5 birds each, and each subgroup were was then moved to a small cage (124 cm × 40 cm and 40 cm high) and kept on the same light:dark schedule as before. We grouped lighter birds and heavier birds together to reduce potential dominance skews within subgroups. Treatment birds then received ad libitum access to water but a restricted diet, initially consisting of 3.6 g / day / bird of a commercial tropical seed mixture per bird, blended with 3 times the same volume of husk to increase feeding time (see Lemon 1991). The aim of the increased feeding times was to equalize food apportionment between the individuals within each cage. For the same reason, the food was split up between two cups. Control birds received the normal food regime including ad libitum access to a commercial tropical seed mixture supplemented with fresh lettuce. A similar food restriction regime in zebra finches has been successfully employed in earlier studies (Lemon 1991; Buchanan *et al.* 2004; Brumm *et al.* 2009c).

Body weight was measured at least once per week. As long as no decline in body weight was observed, food was step-wise restricted further, but not below 1.5 g /day / bird. As soon as a bird from the treatment group decreased in weight (≥ 1g, which happened 9 – 15 weeks after the onset of the treatment), its song was re-recorded and the bird was then set back to an unrestricted diet. Control birds were re-recorded 10 – 13 weeks after the onset of the treatment.

Treatment birds lost 9.5 ± 2.7 % (SE) of their body weight. None of the birds weighed less than 11.8 g during the food restriction treatment, which is well within the usual range for zebra finches (Zann 1996). All birds quickly re-attained their original weight after the experiment when they were put back to an unrestricted food regime and no adverse effects on their health could be observed.

Song recording and song analysis

The song of all subjects was recorded twice, once before the start of the food restriction experiment and once after a period of food restriction, when treatment birds showed a significant reduction in body weight. For the song recordings, each male was placed in a cage (54 cm × 27 cm and 39 cm high) together with a randomly chosen female in an anechoic chamber. 6 different females were used in total. Each cage was equipped with a single perch in the center and wood litter on the floor, and food and water was provided. A Behringer C2 condenser microphone was placed directly above the perch. This set-up allowed us to control for variation in song amplitude arising from changes in singing direction (Brumm 2009). Song activity was continuously recorded using the Sound Analysis Pro (SAP) software (Tchernichovski *et al.* 2004) on a PC equipped with a SoundMax HD Audio sound card. After 1–4 days, when we had obtained several good quality song recordings, the male was weighed and moved back to its home cage. During the first recording session, all the birds were at least 20 weeks old and thus sexually mature (Zann 1996). During the second recording session, the food for treatment birds was limited (see above). When birds were temporarily removed from their home cages for sound recording, we replaced them with individuals that were not part of the experiment to keep the bird density in the cages constant (5 birds per cage).

In most cases, it was easy to determine whether the birds were sitting on the perch or on the floor just by listening to the recording, because background noises differed. For analyses, we chose only those recordings were the song was uttered from the perch (uncertain cases were excluded) to ensure that the distance between the singing bird and the microphone was always the same (as recorded song amplitude varies with the distance of the singing bird to the microphone). All recordings were high-pass filtered at 400 kHz to remove low-frequency background noise. We analyzed song rate, latency to sing, song amplitude, syllable repertoire, the proportion of sound versus silence within a song, mean song frequency and fundamental song frequency. As a measure of song rate, we counted the number of song bouts uttered during the first four hours of daylight on the first day after the male had been moved to the recording chamber. Similarly, song latency was the delay between when the lights were switched on in the morning and the onset of the first song (on the first morning after the birds were moved to the recording box). One bird did not sing during the whole first day of the recording, and for this individual we assigned a song latency equal to one day length (13 hours). Excluding this bird from the analyses does not change the direction of the results. We measured two aspects of song amplitude (Brumm and Todt 2002; Brumm 2009): peak amplitude (the amplitude of the loudest element in the individual-specific phrase, averaged over 3 – 5 renditions; root mean square (RMS) values with 125 ms averaging time) and mean amplitude across the whole phrase (RMS method, averaged over 3-5 phrases). As a measure of syllable repertoire we counted the number of different syllables across a total of 10 seconds of song (which corresponds to ca. 10 phrase repetitions). The proportion of sound versus silence within a song was determined by using a gate function with a threshold of 5% (i.e. song fractions which exceeded 5% of the maximum amplitude were categorized as sound, while softer fractions were classified as silence; delay = 5 ms, time constant = 1 ms; averaged over 5 song phrases). Mean song frequency was measured across a whole phrase. To measure fundamental frequency, we chose one song element with a clear harmonic structure and a constant frequency per bird. To increase the accuracy of measurement, we determined the frequency of a high harmonic (4th to 9th) and divided it by the number of harmonics to obtain the fundamental frequency. The frequency measures were also averaged over several (3-5) phrase renditions.

All song analyses were performed in Avisoft-SASLab Pro (R. Specht, Berlin, Germany). To calibrate the sound amplitude measurements, we played a tone with a fundamental frequency of 1.4 kHz in a fixed location inside the recording chamber and recorded it using the same recording setting as for the song recordings. Its recorded amplitude was highly constant, as repeated calibrations exhibited a variation of less than 2 dB in most cases. Since we are only interested in treatment-related changes of song amplitude, we did not determine absolute amplitude values, but song amplitude was expressed relative to the amplitude of the calibration tone.

Data analysis

One bird from the treatment group died for unknown reasons before the food restriction treatment had an effect on body weight. On the contrary, it even showed a slight increase in body weight compared to before the start of the treatment. This left us with a sample size of 29 birds (14 treatment birds, 15 control birds). In the second recording session, two treatment birds sang only from the floor of the cage but uttered no songs from the perch within 4 days. Since the position of the bird relative to the microphone affects song amplitude, we did not measure amplitude in these birds. Moreover, one treatment bird had no harmonic song element with constant frequency in its repertoire and we did therefore not measure fundamental frequency in this bird.

Since we were only interested in the treatment effect, we eliminated any inter-individual pre-treatment variation by using the measurements that we took before the treatment as a baseline and setting them to zero. The post-treatment measurements were then expressed as the difference between post-treatment and pre-treatment measurements.

All statistical tests were performed with R 2.10.1 (R Development Core Team 2009). For body weight, number of song bouts and latency to sing, the function lmer (R package *lme4*) was used to fit generalized linear mixed-effects models (GLMMs) with our song measures as the dependent variable and individual subject as a random factor to account for repeated sampling of the same individuals (before and after the treatment). Female subject (i.e. the partner of the male in the sound box) was also included as a random factor, because a male’s singing behavior may be affected by the behavior of the female (Collins 1994; Rutstein *et al.* 2007). A Wald χ^2 test was used to test whether there was a significant interaction between the fixed factors “experimental stage” (before or after the treatment) and the experimental group (control / treatment).

Results

Before the start of the food treatment, treatment birds and control birds did not differ significantly in body weight (Welch Two Sample *t*-test: $t = 0.47$, df = 26.06, $P = 0.64$) or behavioral traits (number of song bouts: *t* = 1.03, df = 21.25, *P* = 0.32; latency to sing: *t* = -1.63, df = 17.86, *P* = 0.26).

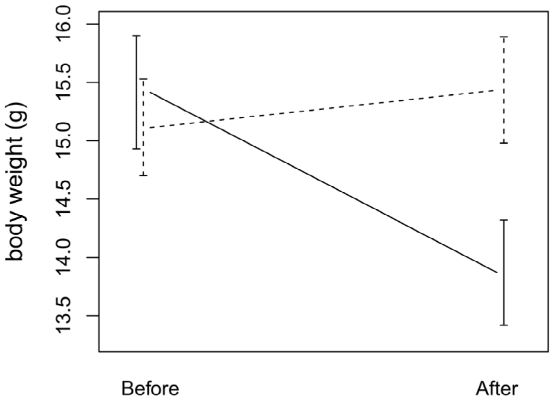

Figure 8. Interaction plot of body weight. *Means ± SE are given for treatment birds (solid line) and control birds (dashed line). The change in body weight differed significantly between treatment birds and control birds (p < 0.01).*

In contrast, we found a highly significant interaction between the experimental stage (before / after the treatment) and the treatment (food restricted / unlimited food) on body weight (χ^2 = 12.86, df = 1, *P* < 0.01; Fig. 8), number of song bouts (χ^2 = 73.50, df = 1, *P* < 0.01), latency to sing (χ^2 = 1098.1, df = 1, *P* < 0.01), mean song amplitude (χ^2 = 11.99, df = 1, *P* < 0.01), peak amplitude (χ^2 = 8.57, df = 1, *P* < 0.01) and fundamental frequency (χ^2 = 7.30, df = 1, *P* < 0.01), i.e. treatment birds not only showed a reduction in weight but also a reduced singing activity, mean and peak song amplitude and fundamental frequency, and an increased latency to sing (Fig. 9). Control birds increased the number of song bouts significantly (*t* = -2.33, df = 21.64, *P* = 0.03) while treatment birds reduced it (*t* = 2.65, df = 15.08, *P* = 0.02). Mean song frequency also tended to be reduced in treatment birds, but this was not significant (χ^2 = 3.31, df = 1, *P* = 0.07). Syllable repertoire (χ^2 = 1.44, df = 1, *P* = 0.23) and the proportion of sound versus silence (χ^2 = 1.62, df = 1, *P* = 0.20) were not significantly affected by the treatment (Fig. 9).

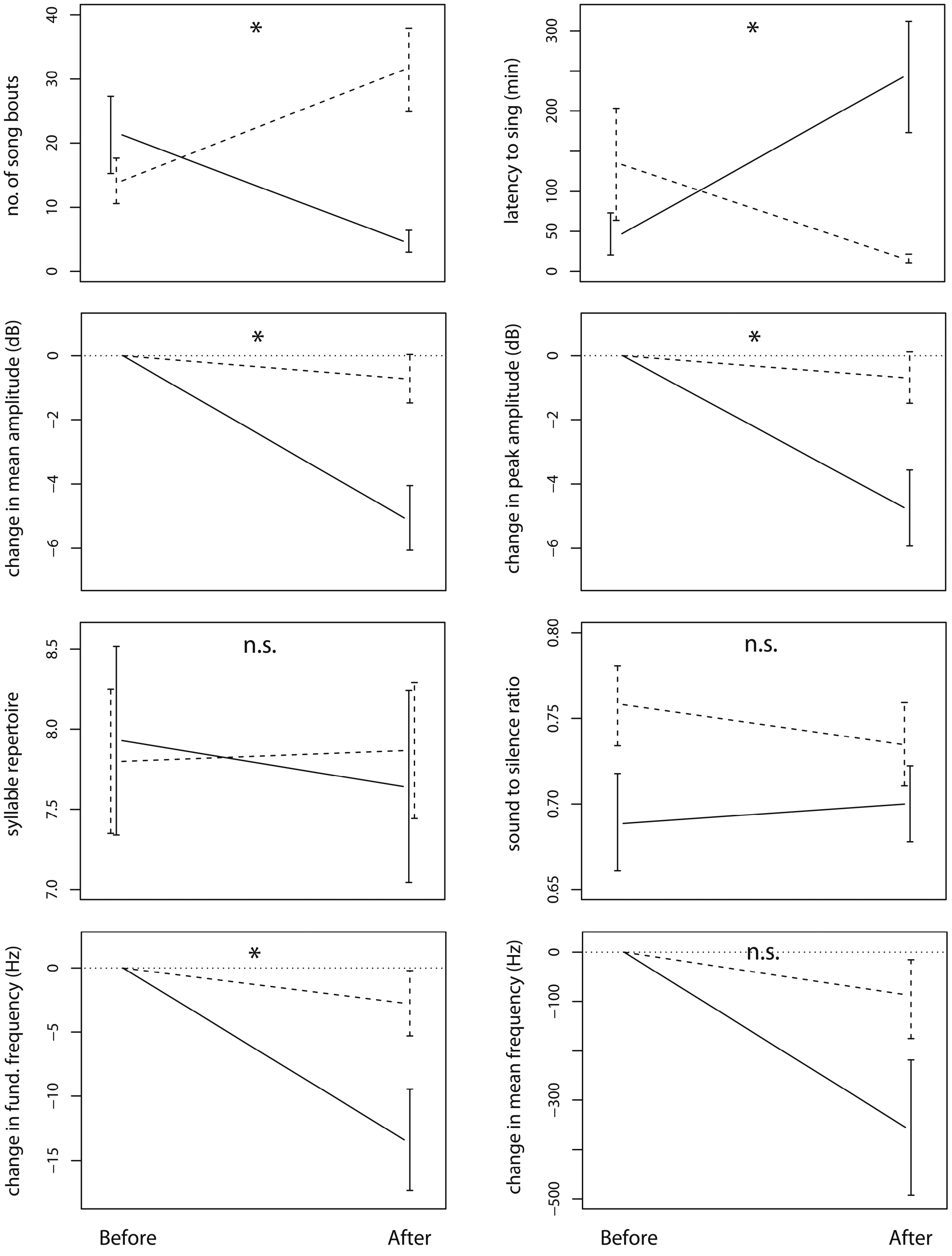

Figure 9. Interaction plots of number of song bouts, latency to sing, mean and peak amplitude, syllable repertoire, sound to silence ratio, fundamental frequency and mean frequency. *Means ± SE are given for treatment birds (solid line) and control birds (dashed line). Stars indicate that the interaction between the experimental stage (before / after the treatment) and the treatment is significant (see results).*

Discussion

We found that a variety of song traits in the zebra finch were sensitive to changes in body condition. In particular, birds with experimentally lowered condition, as reflected by body weight, showed a strongly reduced song rate, a reduction in song amplitude, and took longer to commence singing in the morning. The increase in latency may be a side effect of the reduced song rate, since fewer bouts per time unit results in prolonged silence intervals. In accordance with this, treatment birds showed a 4.5-fold decrease in song rate and a 5-fold increase in song latency. Song amplitude in the treatment birds was reduced on average by ca. 4 dB compared to control birds, which equals approximately a 60 % reduction in sound energy and a 40 % reduction in transmission distance. Males in lower condition are therefore less likely to attract distant females. On the other hand, courtship in zebra finches usually takes place over short distances of just a few centimeters (Zann 1996; Brumm and Slater 2006b), but even then the reduced amplitude of low-condition males is likely to have fitness consequences because females prefer high-amplitude songs (Ritschard *et al.* 2010).

Birds in lower body condition also showed a decrease in fundamental song frequency by ca. 13 Hz. Although this decrease is statistically significant, 13 Hz is not much considering that the absolute frequency values ranged between 447 and 1298 Hz (mean: 696 Hz) before the experiment, and may even be beyond discrimination levels (Dooling 1982). While decreased song rate may be a means to reduce energy expenditure, the frequency changes we observe may have no functional value but may rather be a physiological side effect of singing at lower amplitude (Nelson 2000; Brumm and Naguib 2009; Osmanski and Dooling 2009; Nemeth and Brumm 2010).

As indicated in the methods section, the birds were kept in mixed-sex groups in large aviaries during the first recording session and in small unisex groups during the second recording session. However, this was the same for both experimental groups, thus the effects of the treatment on song can be explained by the lowered body condition and may also be affected by an interaction between body condition and change in housing condition. Differences in housing conditions may explain the finding that control birds increased their song rates from the first to the second recording session, for separation from females for several weeks may have increased the males' singing motivation. In the treatment birds, however, this effect was masked by the strong decrease of singing motivation induced by the lowered body condition.

In the zebra finch, female attraction is the main function of song, and female song preferences are well studied. Song rate, song amplitude, syllable repertoire size and the proportion of sound versus silence within songs are positively associated with female preference (Collins *et al.* 1994; Riebel 2009; Ritschard *et al.* 2010). We found that two of these parameters, song rate and song amplitude, are condition-dependent. Song rate and song amplitude therefore convey information about male body condition, which suggests a direct link between female choice and male quality. In contrast, our experiment did not have an effect on the proportion of sound versus silence within a song. Interestingly, the consistency of this parameter can be affected by early nutrition (Holveck *et al.* 2008). Early nutrition is key in the 'nutritional stress hypothesis' (Nowicki *et al.* 1998; Nowicki *et al.* 2002; Spencer and MacDougall-Shackelton 2011), which proposes that learned song features are an indication of male quality because the development of brain structures responsible for song learning and song production is very sensitive to environmental conditions during the period of fastest development. In contrast, song amplitude and song rate are not affected by early nutritional stress in zebra finches (Holveck *et al.* 2008; Brumm *et al.* 2009c). However, our results indicate that these parameters may signal current condition. Thus, taken together with these previous studies, our results show that female zebra finches could use different song parameters to assess both past and current condition of a potential mate.

Two categories of costs imposed by singing loudly have been discussed in the literature, direct (energetic) expenses and indirect costs (e.g. increased predation risk or social aggression). Experimental studies measuring energy expenditure in singing birds showed that high song amplitudes do not incur considerable metabolic costs (Oberweger and Goller 2001; Zollinger *et al.* under review). In contrast, the production of loud songs is most likely constrained by social aggression (Brumm and Ritschard 2011). Such social costs may also account for the observed changes in song amplitude in the current study. Males in lower condition may have decreased their vocal amplitude to reduce competition with other males. Housing our birds in all-male groups in cages might even have increased dominance interactions among males and thus perhaps increased the effect of body condition on song amplitude.

Our experimental data shows that zebra finch song parameters that may be important in mate choice are condition-dependent. Previous studies have indicated that food restriction can affect singing activity (Rashotte *et al.* 2001), and there is correlative evidence suggesting that song rates may be related to body condition (Lampe and Espmark 1994; but see Galeotti *et al.* 1997) or deposition of clavicular fat (Houtman 1992). An experimental study by Dreiss (2008) showed that challenging the immune system of barn swallows *Hirundo rustica* results in decreased strophe duration and decreased rattle duration. Moreover, supplemental feeding has resulted in increased song rates in a number of passerines (Searcy 1979; Davies and Lundberg 1984; Gottlander 1987; Reid 1987; Strain and Mumme 1988; Alatalo *et al.* 1990; Grava *et al.* 2009; Barnett and Briskie 2011). However, the link between supplemental feeding and body condition is speculative, for increased singing rates following supplemental feeding in free-ranging birds may simply be a consequence of time budget adjustments: if extra food is made available, birds need to invest less time in foraging activities and may spend more time for territory defense and advertisement. We cannot entirely rule out an effect of time restriction on singing activity for our experiment, however such an explanation seems unlikely because food deprived zebra finches show an overall decrease in daytime activity, i.e. they spend much of the day resting (Rashotte *et al.* 2001).

Until recently, the function and evolutionary consequences of song amplitude have received little attention in this and other species (Gil and Gahr 2002; Brumm 2004a). Nevertheless, the amplitude of birdsong has been found to play an important role in social interactions (Brumm and Todt 2004; Brumm and Slater 2006b) and sexual selection (Dabelsteen 1981; Searcy 1996; Lampe *et al.* 2010; Ritschard *et al.* 2010; Brumm and Ritschard 2011). The only study which has previously looked at song amplitude and body condition in a bird found no relationship between the two (Brumm 2009). However, Brumm (2009) did not experimentally manipulate condition but used a correlative approach instead.

To conclude, our study demonstrates that body condition affects several attributes of zebra finch song: song rate and latency to sing, song amplitude, and fundamental frequency. Previous studies have shown that some of these song parameters are under positive intersexual selection, i.e. females may use these song parameters to assess the current condition of a singing male. Our results add strong support to the general assumption that song signals male quality, and therefore expand our understanding of the function and evolution of female song preferences and, ultimately, of birdsong in general.

Chapter 5

Effects of vocal learning, phonetics and inheritance on song amplitude in zebra finches

M. Ritschard, H. Brumm

Birdsong is an important model in the study of evolutionary processes. Vocal amplitude, a song trait that has received little attention to date, varies considerably between individuals, and this variation is important in both female choice and male-male competition. To understand the function of a trait, it is often insightful to look at its origin and ontogeny. Like human speech, birdsong is a learned behaviour, and song amplitude may be adopted from the tutor during vocal ontogeny. However, vocal amplitude may also be bound to song phonetics due to production constraints. We addressed these ideas with song learning experiments in the zebra finch Taeniopygia guttata, a widely used species in vocal learning studies. The songs of young were compared to the songs of their genetic fathers and to those of their tutors. We found that the amplitude of tutee song elements was strongly related to the tutor element amplitude, indicating that song amplitude is adopted during song learning. Mean element amplitude of the genetic father was not related to tutee element amplitude. Along this line, our heritability estimates of song amplitude were not significant (but statistical power was low). Moreover, element amplitude varied with structural element properties (duration and pitch), suggesting that the relationship between tutor and tutee element amplitude may be partly accounted for by production constraints. Our findings indicate that the ontogenetic development of adult song amplitude is based on an interplay between vocal production learning and physiological constraints.

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The two main functions of birdsong are territory defense and mate attraction (Catchpole and Slater 2008). Since the second half of the last century, birdsong has become an important model in the study of evolutionary processes, in particular regarding speciation (Grant and Grant 1996; Slabbekoorn and Smith 2002; Lachlan and Servedio 2004; Podos and Warren 2007; Brumm *et al.* 2010), environmental adaptation (Wiley and Richards 1978; 1982; Wiley 1991; Brumm and Naguib 2009), and sexual selection (Searcy and Andersson 1986; Catchpole 1987; Searcy and Yasukawa 1996; Catchpole 2000; Gil and Gahr 2002). As to sexual selection, it has been shown that not only song structure but also song performance may be important (Forstmeier *et al.* 2002; Riebel 2009; Rivera-Gutierrez *et al.* 2010). Song amplitude is a performance-related trait that varies considerably between species (Brackenbury 1979), within species (Brumm 2009; Ritschard *et al.* 2010), and within individuals (Cynx *et al.* 1998; Brumm and Todt 2004; Cynx and Gell 2004; Brumm and Slater 2006b). The sound pressure of male songs is subject to female preference (Searcy 1996; Ritschard *et al.* 2010) and affects male-male territorial interactions (Dabelsteen 1981; Lampe *et al.* 2010; Brumm and Ritschard 2011) and may therefore play an important role in sexual selection.

To understand the function of a trait, it is usually very insightful to look at its provenance and ontogeny because physiological mechanisms or ontogenetic pathways may constrain the ability of a trait to respond to selection (Gould and Lewontin 1979; Ryan and Brenowitz 1985). Brumm and Hultsch (2001) tracked the ontogenetic trajectory of song amplitude in juvenile nightingales *Luscinia megarhynchos* and discovered that, embedded in an age-dependent increase in overall song amplitude, sound intensity is related to vocal production learning. During plastic singing, song episodes containing many imitations of acquired model songs are produced with higher amplitude than episodes with only few or no imitations. However, it is unclear whether the target of the trajectory is also determined by vocal imitation, i.e. whether the amplitude of adult full song is affected by song learning. Alternatively or in addition to vocal imitation learning, song amplitude may be genetically inherited from the parents or it may be related to the phonetic characteristics of the song due to production constraints. The latter refers to the phenomenon that certain birdsong elements are more difficult to produce than others and may therefore not be produced at high amplitude (Suthers and Zollinger 2004). Hence, adult song amplitude might be affected by the phonetic structure of the acquired song rather than the amplitude of the tutor song per se.

While we are often tempted to classify behaviours as either learnt or innate, the nature/nurture debate which started more than half a century ago (Hebb 1953; Lehrman 1953) made it clear that, in most cases, it is not reasonable to cling to such a rigid dichotomy.

Although there is ample evidence that songbirds learn their songs (Hultsch and Todt 2004), there is also a genetic basis to birdsong. This becomes evident when birds are raised in acoustic isolation. The songs such birds develop are often very rudimentary, but usually their structure resembles that of conspecifics that copied their songs from tutors (Thorpe 1958; Nottebohm 1968; Price 1979; Marler and Sherman 1985).

Vocal production learning has been shown to occur in all oscine birds studied to date, as well as in some non-passerines, including parrots and hummingbirds (Todt 1975; Kroodsma and Baylis 1982; Baptista and Schuchmann 1990; Catchpole and Slater 2008). While the reasons for the evolution of song learning remain obscure (Catchpole and Slater 2008), the patterns of song learning and the neurophysiological processes involved have been relatively well investigated (Zeigler and Marler 2008; Mooney 2009b; a). There are large interspecific differences in when birds learn, what they learn and from whom they learn (Catchpole and Slater 2008). Some species acquire new vocal patterns only as juveniles, others may learn new songs during their whole life. Birds may learn from their fathers or from unrelated males, and they may only copy songs from their own species or they may also imitate the songs of other species. Previous studies of vocal learning in birds have focused on song structure, while performance-related parameters such as song rate or song amplitude have been largely ignored (Hultsch and Todt 2004).

In contrast to song learning, little is known about the genetic basis of birdsong. Recently, Forstmeier *et al.* (2009) have estimated heritabilities of vocal traits in the zebra finch *Taeniopygia guttata*. They found significant heritabilities of morphology-related voice characteristics, for instance timbre and mean frequency, but very low heritabilities of song features that are often thought to be sexually selected, such as song bout duration or repertoire size.

In the current study, we investigated the importance of learning, song phonetics and inheritance for the determination of song amplitude in adult zebra finches. In particular, we tested whether song amplitude is affected by vocal learning and whether the flexibility of amplitude may be constrained by element structure. Moreover, we estimated the heritability of song amplitude using cross-fostered birds. Zebra finches are easy to keep in the laboratory and their song development has been extensively studied. Males of this species sing one short (duration < 1 s), individual-specific and very stereotyped song type consisting of ca. 3–13 different element types (Slater *et al.* 1988) that is normally repeated several times within a song bout. Juveniles learn their songs from their fathers or from neighboring males (Clayton 1987; Slater *et al.* 1988; Williams 1990; Zann 1990; Mann and Slater 1995) when they are between 35 and 65 days old (Eales 1987). Usually, they do not copy complete songs, but chunks of song elements (Immelmann 1969; ten Cate and Slater 1991; Williams and Staples 1992), and they may learn from more than one male (Williams 1990; ten Cate and Slater 1991; Mann and Slater 1995).

Methods

Subjects, housing, and breeding

We used wild-morph domesticated zebra finches from our colony at the Max Planck Institute for Ornithology in Seewiesen, Germany. Zebra finches naturally occur in most Australian mainland habitats and on the Lesser Sunda Islands. They are socially monogamous and breed in colonies. Males sing both during courtship ('directed song') and when they are alone ('undirected song' [Sossinka and Böhner 1980]).

Before the start of the breeding experiment, the birds were kept in small cages (124 cm × 40 cm and 40 cm high) in unisex groups of 4 to 6 individuals. For breeding, 22 males were moved into a large aviary (3.0 m × 2.0 m and 2.0 m high) together with 22 randomly chosen females. All individuals were at least 6 months old. The aviary was divided into 3 equally large compartments which were interconnected by small openings. It was equipped with a number of perches and tree branches and with cuttlefish bone, the floor was covered with wood litter. The birds had ad libitum access to a commercial tropical seed mixture and to water; the food was supplemented with fresh lettuce once a week.

We fixed 24 nesting aids to the internal walls of the aviary and provided unlimited access to nesting material. The birds instantly started to pair up and build nests, the first eggs were laid 4 days after the birds were moved into the aviary. We performed a full cross-fostering between pairs of clutches in the period just before hatching of the first egg until 1 day after hatching of the first egg. This was done to be able to disentangle genetic and environmental effects on song. Some clutches could not be cross-fostered due to a lack of a suitable foster nest, which reduced the sample size for the heritability analysis (see below).

The birds were let to interbreed freely for 40 days. After that, we started to remove empty nests and nesting opportunities to prevent further breeding attempts. In total, 18 juvenile males from 9 different genetic fathers survived to adulthood (90 days post hatching). Both parental and F1 generations were kept together in the aviary until all recordings were completed (see below).

Song recording

The songs of the 22 parental and the 18 F1 males were recorded when the latter were between 19 and 24 weeks old, i.e. after they have reached sexual maturity. Under normal conditions, zebra finches learn their songs when they are between 35 and 65 days old (Eales 1987) and song development is completed at around 12 weeks of age (Slater *et al.* 1988).

For song recording, each male was placed in a cage (54 cm × 27 cm and 39 cm high) together with a female (which did not come from the experimental aviary) in an anechoic chamber for 24 hours. Each cage was equipped with a single perch in the center and wood litter on the floor, and food and water was provided. Song activity was continuously recorded using the Sound Analysis Pro (SAP) software (Tchernichovski *et al.* 2004) on a PC equipped with a SoundMax HD Audio sound card. A Behringer C2 condenser microphone was placed directly above the perch, ca. 25 cm from the bird's head. This set-up allowed us to control for variation in song amplitude due to changes in singing direction (Brumm 2009). In most cases, it was easy to determine whether the birds were sitting on the perch or on the floor just by listening to the recording, because background noises differed. For analyses, we chose only those recordings where the song was uttered from the perch (uncertain cases were excluded) so that the distance between the singing bird and the microphone was always the same. This was done because the recorded song amplitude varies with the distance of the singing bird to the microphone.

Zebra finches, like other songbirds, vary their song amplitude dependent on the level of background noise (Cynx *et al.* 1998) or the social context (Cynx and Gell 2004; Brumm and Slater 2006b). Our experimental set up with a pair of birds in a sound-shielded chamber allowed us to keep these environmental variables constant across as well as within all males recorded. To calibrate the sound amplitude measurements, we played a tone with a fundamental frequency of 1.4 kHz in a fixed location inside the recording chamber and recorded it using the same recording setting as for the song recordings. Its recorded amplitude was highly constant, as repeated calibrations exhibited a variation of less than 2 dB in most cases. To obtain absolute amplitude values, we replaced the standard microphone with a calibration microphone (Earthworks M23 in combination with a sound level calibrator ND9) to determine the loudness of the calibration tone.

Song analyses

In a first step, we split up the songs of all 18 F1 males into elements. An element corresponds to a single note, visible as a continuous tracing in a spectrogram. In a second step, two observers assigned elements or element chunks from the songs of the F1 males to the 22 tutor songs by visual and acoustical comparison. Accordance between the two observers was 99%, which proves that our method of assigning elements was appropriate. For the subsequent analyses, we only used song elements of F1 males for which we had been able to assign one or several tutors.

To calculate heritability, we measured two aspects of song amplitude of F1 males and fathers, peak and mean amplitude. Peak amplitude was measured as the amplitude of the loudest element in the individual-specific phrase, averaged over 3–5 renditions (root mean square (RMS) values with 125 ms averaging time), and mean amplitude was the average amplitude across the whole phrase, averaged over 3–5 phrases (RMS method, 125 ms averaging time).

To investigate whether song amplitude was affected by the amplitude of the tutor song, we compared the amplitudes of tutor-tutee element pairs, i.e. element types that were shared between a tutor and a tutee. Element amplitude was averaged over 3–5 renditions of the same element (RMS method, 10 ms averaging time). We did not only test whether, but also how accurately song amplitude was copied from tutors and whether element amplitude was related to structural attributes of the element. In order to do this, we analyzed tutor element amplitude in relation to three characteristics of element structure: element length, Wiener entropy, and pitch. Wiener entropy is a measure of the pureness of a sound, with low values corresponding to pure, harmonic sounds and high values corresponding to noisy sounds. Pitch is a measure of the period of oscillation and corresponds to the fundamental frequency of a pure sound or to an estimate of the fundamental frequency of a complex sound.

Song amplitude, element amplitude and element duration were measured in Avisoft-SASLab Pro (R. Specht, Berlin, Germany; measured with FFT length = 512, samle rate = 44.1 kHz), Wiener entropy and pitch were determined using Sound Analysis Pro 1.02 (Tchernichovski *et al.* 2004). Prior to all measurements, the audio recordings were high-pass filtered at 400 kHz to remove low-frequency background noise.

Paternity analysis

Blood was sampled from all juveniles and from all adult (male and female) zebra finches in the aviary (510 µl from the brachial vein). Samples were genotyped using 18 highly polymorphic microsatellite markers: Tgu1, Tgu3, Tgu4, Tgu5, Tgu6, Tgu7, Tgu8, Tgu9, Tgu10, Tgu12 (Forstmeier *et al.* 2007), and 21, 25(5), 26, 31(11), 32, 33, 34 and 36 (W. Forstmeier, unpublished). Parentage was assigned by exclusion, as all potential parents were known.

Data analysis

Heritability analyses were performed in PASW 18.0.0 (formerly SPSS). To calculate the heritability of song amplitude we employed parent-offspring regressions (POR) using mean offspring traits. We followed the standard procedures for parent-offspring regression as outlined by Falconer and Mackay (1996) and Lynach and Walsh (1998). 14 F1 males from 9 fathers were included in the analysis. Only cross-fostered offspring were taken into account. Families were weighted according to family size, following Kempthorne and Tandon (1953). The solution was found iteratively, because family weights depend on heritability. We used an ANOVA to calculate repeatability among brothers (fullsib analysis). Since we performed a single parent-offspring regression, we multiplied the slope and standard error of the regression by 2 to obtain h^2.

Analyses to test for the effects of the tutor and the genetic father on tutee element amplitude were performed in R 2.10.1 (R Development Core Team 2009). The function *lmer* (R package *lme4*) was used to fit a linear mixed-effects model (GLMM). Subject (tutee, N = 14), foster nest (N = 8) and song element (N = 52) were entered in the model as random factors to account for repeated sampling of the same individual, the same nest (common rearing environment of some tutees) and the same song element (some tutor elements were copied by several tutees). We used a model comparison approach (Wald χ^2 test) to assess whether omitting a factor from the model caused a significant change of the model fit.

To test for the effects of our measures of element characteristics on element amplitude, we fitted a similar GLMM. Amplitude was our response variable, subject (tutee) was entered in the model as random factor to account for repeated sampling of the same individual.

To test for the effects of the tutor on tutee element amplitude, 4 tutees had to be excluded because none of their song elements could be unequivocally assigned to a tutor, which left us with a sample size of N = 14 tutees. In our analysis on the effects of element structure on element amplitude, we included all tutor elements which had been copied by at least one tutee ($N = 51$).

Results

Mean amplitude of entire songs was 72.5 dB SPL in the tutor males (range: 67.7–78.2 dB; measured in the anechoic sound box 25 cm above the bird's head; $N = 22$ males) and 75.0 dB SPL in the F1 generation (68.2–80.3 dB; $N = 18$ males). Peak song amplitude was 74.7 dB SPL (68.3–79.4 dB) in tutors and 77.1 dB SPL (70.5–82.3 dB) in F1 males. Both mean song amplitude (Welch Two Sample *t*-test: $t = -2.51$, df = 36.42, $P = 0.02$) and peak song amplitude ($t = -2.48$, df = 35.50, $P = 0.02$) were significantly higher in tutees than in tutors.

Is song amplitude copied from the tutor?

Tutor element amplitude had a very strong effect on the element amplitude of the tutee ($\chi^2 = 44.51$, df = 1, $P < 0.001$, Fig. 10). On the other hand, mean element amplitude of the genetic father did not significantly affect tutee element amplitude ($\chi^2 = 2.01$, df = 1, $P = 0.16$).

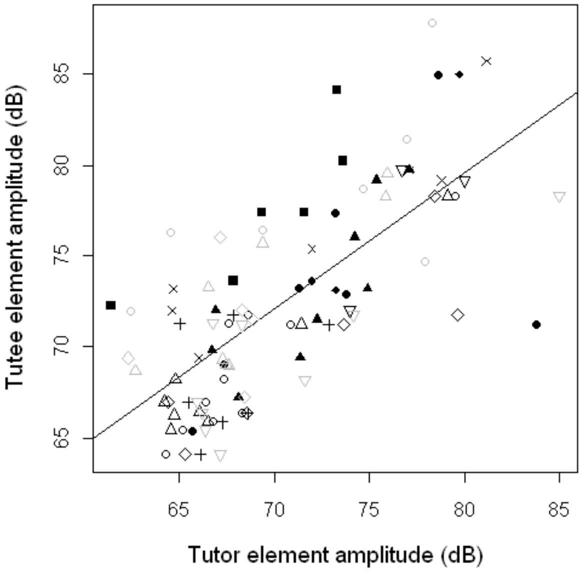

Figure 10. Element amplitude of tutees regressed against element amplitude of tutors. *Each dot represents a tutee element that could be assigned to a specific tutor. Each tutee is represented by a different symbol. Tutee element amplitude is highly dependent on tutor element amplitude (p < 0.001).*

In a next step of our analyses, we investigated how accurately element amplitudes were copied by calculating the amplitude difference for all tutor-tutee element pairs. Then, we calculated the amplitude differences of all possible combinations of element pairs for each of the 22 tutors. If tutees adopt the mean amplitude of the tutors, but not the amplitude patterning (i.e. relative amplitude differences between elements), we would expect the two frequency distributions of amplitude difference between tutor-tutee element pairs and amplitude difference between elements within a tutor to be similar. However, this was not the case: the median amplitude difference for tutor-tutee element pairs was 2.6 dB, whereas the median difference for within tutor element pairs was 5.6 dB (Wilcoxon Rank Sum Test: $W = 53104$, $N_1 = 804$, $N_2 = 95$, $P < 0.001$; Fig. 11). The maximum element difference within tutor songs was more than twice as big as between tutor-tutee element pairs (within tutors: 28.1 dB; between tutors and tutees: 12.5 dB). This indicates that tutees adopt the amplitude of each element from the tutor, so that the amplitude modulation pattern of tutees and their tutors is very similar (Fig. 12).

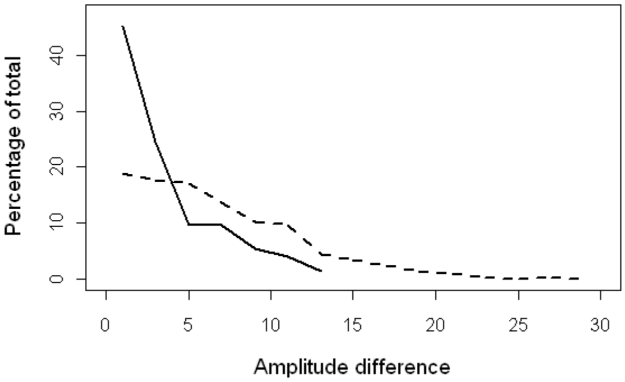

Figure 11. Frequency distribution of amplitude differences of tutor-tutee element pairs (solid line) and amplitude differences between elements within tutor songs (dotted line). *Amplitude differences of tutor-tutee element pairs are considerably smaller than amplitude differences within a song.*

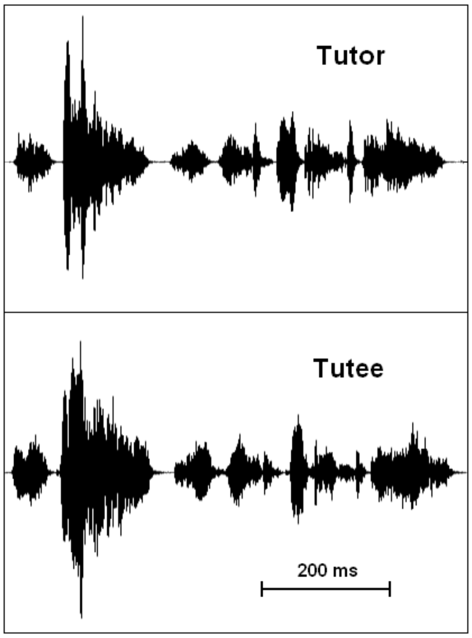

Figure 12. Oscillograms of songs from one exemplary tutor-tutee pair illustrating the similarity in amplitude patterns between the song imitation (tutee) and the model song (tutor). *The tutee song is a full match, i.e. the young bird copied the entire song phrase of the tutor.*

Does element structure affect amplitude?

Element duration had a strong effect on element amplitude in the tutor birds (χ^2 = 17.31, df = 1, *P* < 0.001), long elements being louder than short elements (Fig. 13). We also found a significant effect of pitch on element amplitude (χ^2 = 5.00, df = 1, *P* = 0.02). Visual inspection of the regression graph between pitch and amplitude (Fig. 14) suggests that this effect was due to elements with a pitch below 1200 Hz. Thirty-three out of 51 elements had a pitch between 473 and 1138 Hz, while the rest had a pitch higher than 1700 Hz. When analyzing the elements with a pitch below 1200 Hz separately, we found that the effect of pitch on amplitude became very strong (χ^2 = 20.26, df = 1, *P* < 0.001; Fig. 14), which suggests a trade-off between pitch and amplitude for elements with a pitch below ca. 1200 Hz. Wiener entropy had no significant effect on element amplitude (χ^2 = 0.85, df = 1, *P* = 0.36).

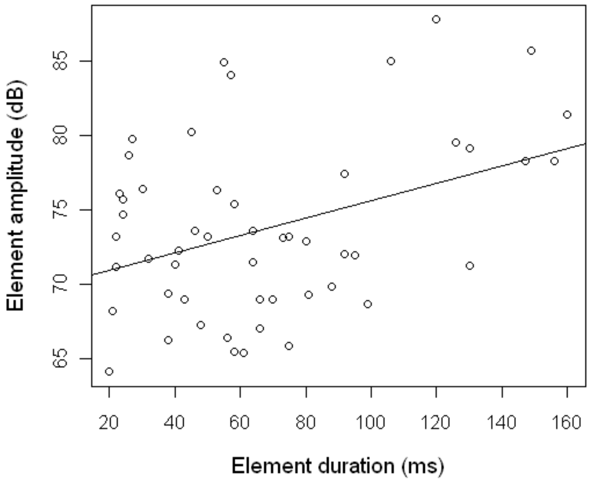

Figure 13. Regression of element amplitude against element duration. *Long elements are significantly louder than short elements (p < 0.001).*

Heritability of song amplitude

The parent-offspring regression revealed no significant heritability of mean (h^2 = 0.980 ± 0.884 SE, *P* = 0.30) nor peak song amplitude (h^2 = 0.602 ± 0.849, *P* = 0.50).

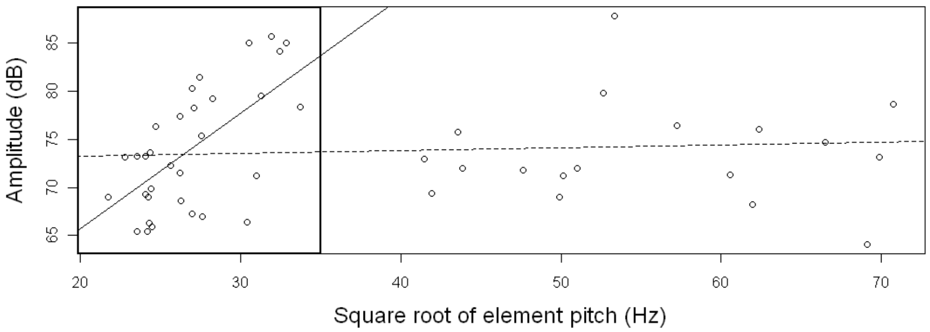

Figure 14. Regression of element amplitude against element pitch*. Dotted regression line is calculated across all elements, solid regression line is calculated across elements with a pitch below 1200 Hz (framed with black square). High-pitched elements are louder than low-pitched elements, particularly for elements with a pitch below 1200 Hz ($p < 0.001$).*

Discussion

We found a strong relation between the amplitude of tutor and tutee song elements in zebra finches. Young zebra finches have been shown to integrate chunks of elements of one or several tutors into their own songs (Immelmann 1969; Zann 1990; Williams and Staples 1992). Birds do not only copy the spectrographic properties of song elements but also the temporal structuring of the song, regarding both the element sequence within songs (Hultsch 1991) and the sequence in which alternative song types are sung (Riebel and Slater 1999a). Moreover, there is some evidence that songbirds may even learn the situations (time of the day) in which song should be produced (Kroodsma 1988; Spector *et al.* 1989). Our results demonstrate that, analogous to song structure, song amplitude is also adopted from the tutor. Moreover, our results show that tutors and tutees do not simply share a similar mean song amplitude, but that tutees copy the amplitude of each single element, i.e. they take over the amplitude pattern of the tutor's song. However, we also found that song amplitude of most element types was likely to be constrained by song structure, for longer elements or high-pitched elements were produced at higher amplitudes. The effect of pitch was particularly strong in elements with a fundamental frequency below 1.2 kHz suggesting that, in the low-frequency range, there is a trade-off between singing with low pitch and singing loud. Vocal tract filtering does not only play an important role in human speech but also in avian vocal communication (Nowicki 1987; Williams 2001; Riede *et al.* 2006; Riede and Suthers 2009). While evidently the syrinx of even small birds can produce sounds way below 1 kHz, the vocal tract system acts as a high-pass filter. In the present case, the lowest resonant frequency of the vocal tract is likely to be at around 1.2 kHz and consequently, lower frequencies can only be produced at relatively low amplitudes.

Similar to our results, Nelson (2000) found a positive correlation between vocal amplitude and frequency parameters in the calls of eastern towhees *Pipilo erythrophthalmus*. It is unlikely, however, that the association between vocal amplitude and frequency in towhee calls can be accounted for by the same production constraints we are proposing for zebra finch vocalizations. The lowest frequency of the towhee calls was around 2 kHz, while our data suggest a trade-off between amplitude and frequency in the zebra finch (which is even smaller in size than towhees) only for frequencies below 1.2 kHz.

Production constraints may also explain the positive correlation between element length and amplitude found in our birds. Song amplitude is related to air sac pressure and airflow speed (Suthers *et al.* 2002; Goller *et al.* 2004; Goller *et al.* 2006; Plummer and Goller 2008), and birds may not be able to reach a high airflow speed during very short notes. In contrast to our results, Nelson (2000) found a negative correlation between call duration and amplitude in towhees, i.e. short calls were produced at higher amplitudes than long calls. However, the analyzed towhee calls varied mostly between 200 and 300 ms and were thus considerably longer than zebra finch song elements, which ranged between 20 and 160 ms. This difference in element duration may account for the opposite associations with vocal sound pressure levels in the two species, although the particular mechanisms involved remain unknown. Interestingly, the positive correlation between amplitude and element length in zebra finch song is similar to the regulation of vocal parameters in primates: when vocalizing in noise, New World monkeys increase both the duration and the amplitude of their calls, so that longer calls are produced at higher amplitudes. This applies for brief calls below 100 ms (Brumm *et al.* 2004) as well as long calls above 300 ms (Egnor and Hauser 2006).

The relationship between the amplitude and spectro-temporal characteristics of song elements reported in this study suggests that phonetic properties may indeed restrict the amplitude range within which an element can be produced by zebra finches. This effect may at least partly explain the similarity of tutor and tutee song amplitude that we found. It does not signify, however, that there is no room for learning. Studies on zebra finch song adjustment in fluctuating environments indicate that song amplitude within individuals is flexible at least to a certain extent (Cynx *et al.* 1998; Cynx and Gell 2004; Brumm and Slater 2006b). The effects of vocal learning and production constraints on song amplitude can be disentangled with experiments in which juvenile zebra finches are tutored with songs that have manipulated amplitude envelopes.

We found no evidence for a genetic basis of song amplitude in our birds. The yielded heritability estimates for mean song amplitude and peak song amplitude had large standard errors and were therefore far from significant. In their comprehensive study on the heritability of zebra finch vocalizations, Forstmeier *et al.* (2009) found a weak but significant heritability of song amplitude. Our sample size was probably too low to reliably detect such a weak heritability. However, it is important to note that the amplitude measurements by Forstmeier *et al.* (2009) were not conducted in a controlled set-up. In their study, the distance of the birds to the microphone and the birds' singing orientation in relation to the microphone varied so that song amplitude could have been confounded by differences between males in the position they assumed in the recording cage as well as differences in body orientation while singing.

In conclusion, while more data is needed for a thorough understanding of the genetic basis of avian song amplitude, the current evidence suggests that the heritability of song amplitude is rather low. Song amplitude has been suspected to be under sexual selection, as it affects both female preference (Searcy 1996; Ritschard *et al.* 2010) and male-male competition (Dabelsteen 1981; Lampe *et al.* 2010; Brumm and Ritschard 2011). At first glance, a low heritability may therefore indicate that song amplitude may hardly respond to selection at all. However, this is not necessarily true. Heritability is the additive genetic variance divided by the total phenotypic variance ($h^2 = V_A/V_P$); the latter includes additive and environmental effects. Despite a high additive genetic variance, an excess of residual variance may therefore result in low heritabilities (Houle 1992). Interestingly, while it is commonly assumed that birdsong has evolved as a result of sexual selection (Catchpole and Slater 2008), heritabilities of structural song traits are generally low (Forstmeier *et al.* 2009). Comprehensive studies on the evolutionary responsiveness of song traits would greatly improve our understanding of the function and evolution of birdsong, but adequate data is missing to date.

Rather intriguingly, we found that F1 males sang ca. 2.5 dB louder than parental birds. This finding may be attributed to differences in sexual motivation, as motivation has been shown to affect the amplitude of acoustic signals (Leonard and Horn 2001; Brumm and Todt 2004; Boncoraglio and Saino 2008). However, F1 birds did not sing with a significantly higher rate (Wilcoxon rank sum test: $W = 176.5$, $P = 0.11$), which would be expected if their singing motivation was higher. In fact, parental males tended to show a higher song rate than F1 birds. Alternatively, F1 could be in better condition than adult birds, as body condition has also been shown to affect song amplitude (Ritschard and Brumm under review). To test this idea, we regressed body mass against tarsus length and used the residuals as an index of condition (Jakob *et al.* 1996). Adult and F1 differed significantly in condition index (t = 2.32, df = 31.73, P = 0.03), but contrary to our expectation, adult birds had a higher condition index than F1 birds. A third explanation for the age-related difference in song amplitude may be that young birds learn preferentially from loud tutors; however, this scenario could also not be affirmed by our data (data not provided). At the current stage, we are therefore unable to provide a satisfactory explanation for the difference in song amplitude that we found between parental birds and their offspring, but it may open interesting perspectives for future research on the function and evolution of song amplitude.

General discussion

In this thesis, I set out to answer some fundamental questions about song amplitude in birds. Could song amplitude be sexually selected? Which proximate mechanisms constrain the production of high-amplitude songs? Is song amplitude affected by vocal learning during early vocal ontogeny? I was able to show that female zebra finches prefer high-amplitude over low-amplitude songs (chapter 1), and that male chaffinches behave more aggressive towards territory intruders singing loud than territory intruders singing with low amplitude (chapter 2), which indicates that song amplitude may be sexually selected. Moreover, I found that body condition, but not testosterone level, affects song amplitude (chapters 3 and 4). Finally, I could show that song amplitude is affected by vocal learning: young zebra finches adopt the song amplitude from their tutors (chapter 5). In the following sections, I will discuss these findings in more detail.

Song amplitude and sexual selection

Sexual selection is usually partitioned into two distinct categories: intrasexual selection and intersexual selection. Intrasexual selection results from the competition within one sex for access to resources like nesting sites, territories or mates. It favours traits that increase an individual's competitive ability and therefore signal physical strength. On the other hand, intersexual selection relates to the efforts of one sex (usually the male) to attract members of the opposite sex and mate with them. Intersexual selection favours traits that signal a high phenotypic or genetic quality and therefore promise high benefits (direct or indirect) for the choosy sex. Alternatively, intersexual selection may also favour traits that are not related to quality but that exploit a pre-existing perceptual preference of the choosy sex (Ryan 1998).

I performed experiments to test whether song amplitude plays a role in intrasexual and/or intersexual selection. To look at the function of song amplitude in intrasexual selection, I carried out a playback experiment in the field where male chaffinches were exposed to high-amplitude and low-amplitude song (within the natural variation of song amplitude) of conspecific males (chapter 2). Territorial males approached the loudspeaker and dummy male closer and spent more time in their vicinity (within 5 m) in reaction to a simulated intruder singing high-amplitude songs than to an intruder singing low-amplitude songs. At the same time, territorial males sang less than in reaction to the low-amplitude playback.

The fact that I found differences in the behaviour of territorial males towards simulated high-amplitude as opposed to low-amplitude intruders indicates that the sound pressure level of territorial song indeed affects male-male competition. The reaction towards loud intruders appeared to be more aggressive, as territory holders approached closer and spent more time in the proximity of the simulated intruder. From a proximate point of view, a high-intensity song may be more efficient at stimulating the receiver's sensory apparatus. A higher neuronal activity may trigger a stronger behavioural response. Ultimately, it is conceivable that a loud intruder signals a high resource holding potential (a measure of the "absolute fighting ability of the individual" [Parker 1974]), for example may be in better physical condition (see chapter 4), and therefore represents a bigger threat to the territorial male.

The costs of physical fighting are usually high in terms of energy expenditure, risk of injury and predation (Riechert 1988). Therefore, theoretical models and empirical evidence suggest that animals should choose their tactics during conflicts based on a conventional escalation sequence from low-cost to progressively more costly tactics (Parker and Rubenstein 1981; Enquist *et al.* 1990; Keeley and Grant 1993; Hack 1997). Low-cost tactics are used more often than high-cost tactics, which suggests that they may function as reliable signals to assess fighting abilities (Keeley and Grant 1993). Even if I could not observe any physical attacks during the playback experiment, social aggression may put a cost on loud singing because only high-quality individuals should risk escalating a conflict.

To test the potential role of song amplitude in intersexual selection, I performed an experiment on female song preferences, employing operant conditioning techniques (chapter 1). Song preferences in operant conditioning contexts predict preferences for live males (Holveck and Riebel 2007). My experiment revealed that females prefer high-amplitude over low-amplitude songs. Again, differences in neuronal activity due to a more intense stimulation of the female's sensory apparatus may lead to such a differential behavioural response towards the two stimuli. In ultimate terms, females may get benefits from choosing loud males if sound pressure levels of songs correlate with male quality.

Considering the potential role of song amplitude in sexual selection, an important question to ask is to which extent potential sexual partners and conspecific competitors are able to estimate the song amplitude of a singer under natural conditions. From a physiological point of view, the intensity discrimination abilities of birds appear to be slightly inferior to human discrimination abilities. On average, an intensity difference between two successive sounds must be 2–3 dB for a bird (1 dB for humans) to detect the difference, depending on species, sound frequency and absolute amplitude level (Hienz *et al.* 1980; Dooling 1982). However, considering the large interindividual variation in song amplitude (Brumm 2009), avian intensity discrimination abilities are adequate to detect differences in sound pressure level between males. My experiment on the function of song amplitude in territorial interactions (chapter 2) showed that chaffinches have interindividual differences in song amplitude of up to 9 dB, and that they can perceive these differences.

As zebra finch males direct their songs over very short distances at females (Sossinka and Böhner 1980; Brumm and Slater 2006b), it is conceivable that females can accurately estimate the source amplitude level of a singing male. However, if birds communicate over larger distances, sound is attenuated and degraded depending on environmental factors like vegetation, air temperature and humidity (Wiley and Richards 1982; Brumm and Naguib 2009), which makes it difficult to estimate source amplitude levels. Nevertheless, birds may be able to estimate amplitude levels over considerable distances since there is evidence that they use sound amplitude to estimate the distance to a sound source (Nelson 2000; Naguib and Wiley 2001). Moreover, it should be kept in mind that females actively approach singing males when sampling potential mates (Bensch and Hasselquist 1992; Roth *et al.* 2009) and that male-male territorial interactions usually take place over short distances.

To conclude, I was able to demonstrate experimentally that song amplitude plays a role in both male-male competition and female choice and therefore may be subject to sexual selection. However, as I manipulated only amplitude, it is not possible to tell how important song amplitude is in relation to other song parameters like song rate or repertoire size. Future studies on sexual selection of birdsong may try to include manipulations of several song parameters to get estimates of their relative importance, rather than addressing each parameter in isolation.

A further aspect that requires to be addressed is the signaling value of song amplitude. Most models of sexual selection, including the ‘good genes’ hypothesis (Hamilton and Zuk 1982) and Zahavi’s ‘handicap’ principle (Zahavi 1975), assume a correlation between a sexually selected trait and the genetic and/or phenotypic quality of its carrier. Does song amplitude honestly signal male quality? The following paragraph will address proximate mechanisms and the potential signaling value of song amplitude.

Proximate mechanisms and signaling value of song amplitude: the role of body condition and testosterone

If physical or physiological factors cause the differences in song amplitude that can be observed between individuals, song amplitude could signal quality to potential mates or physical strength to competitors. A relationship between vocal amplitude and body weight or body size has been described in toads (Gerhardt 1975; Arak 1988; Marquez *et al.* 2006) and crickets (Forrest 1991). In birds, such a relationship between body size and vocal amplitude has not been found (Brumm 2009).

Studies looking at the relationship between body size and vocal amplitude are necessarily bound to a correlative approach. In contrast, body condition can be changed, which allowed me to experimentally manipulate body condition of zebra fiches and to measure the effect of the treatment on song amplitude (chapter 4). Unlike control birds, those individuals which lost weight showed a considerable decrease in song amplitude. This finding is especially interesting in the context of my experiment on female song preference (chapter 1), where I showed that female zebra finches prefer loud songs. If song amplitude signals male body condition, a female preference for loud singers is likely to be adaptive.

In addition to condition-dependent effects, song amplitude might also be directly affected by the endocrinological control of birdsong. Hormones affect many aspects of a bird's physiology, morphology and behaviour. Testosterone is a sex steroid that regulates the development of some morphological characters that are important in sexual selection, and can explain interindividual differences in sexual and reproductive behaviour (Adkins-Regan 2005). For instance, in birds testosterone affects parental investment (Silverin 1980; Oring *et al.* 1989) and aggressive behaviour (Balthazart 1983; Wingfield *et al.* 1990b; Adkins-Regan 2005). Testosterone levels may relate to fitness components such as survival and reproduction and there is evidence that it is also linked to mating success (Borgia and Wingfield 1991; Alatalo *et al.* 1996). Moreover, testosterone affects vocal behaviour in songbirds, in particular singing rate (Silverin 1980; Cynx *et al.* 2005; Boseret *et al.* 2006; Kurvers *et al.* 2008): individuals with high testosterone levels sing more than individuals with low testosterone levels. It is therefore conceivable that testosterone may also affect song amplitude, especially because songbirds have androgen receptors in their specialized song control nuclei in the forebrain (Gahr and Metzdorf 1997; Ball *et al.* 2003) as well as in muscles that control the syrinx (Veney and Wade 2004). However, I could not find any evidence that enhanced testosterone levels lead to an increase in song amplitude (chapter 3). This indicates that vocal amplitude does not convey information about testosterone levels of the singer to competitors or potential mates. It may be informative to perform the same experiment in a territorial species, since Bengalese finch song functions primarily in mate attraction and courtship.

To conclude, I found that body condition, but not testosterone levels, may be reflected by song amplitude. Song amplitude may therefore serve as an honest signal of physical condition.

The role of vocal learning

Although I was able to show that a decrease in body weight leads to a reduction of song amplitude by ca. 4 dB (chapter 4), body condition is likely to account only for a part of the interindividual variation in song amplitude, which is about 15 dB for zebra finches (Brumm 2009). All oscine birds studied to date learn their songs. Analogous to spectro-temporal song characteristics, song amplitude may be affected by vocal learning.

The goal of my song learning experiment was to investigate whether the amplitude of songs is sensitive to vocal learning and whether genetic factors may play a role. Birdsong is a classical example of a behaviour that is shaped by both the environment and by genes (Hultsch and Todt 2008). I found that tutees adopt the amplitude of song elements from their tutors. This suggests that actual amplitude learning is involved and/or that element structure and amplitude are bound to each other due to production constraints. If such production constraints exist, one would expect to find correlations between the structure and the amplitude of elements. Indeed, longer elements and elements with a higher pitch were louder. The relationship between pitch and amplitude was very strong for elements with a pitch below ca. 1.2 kHz, suggesting that the lowest resonant frequency of the vocal tract is likely to be at around 1.2 kHz in zebra finches. While I could demonstrate that tutees adopt the song amplitude from their tutors, the mechanisms behind remain unclear because my experimental design did not allow to disentangle the relative importance of production constraints and learning. Both are likely to be involved.

My heritability estimates of song amplitude were not significant because confident intervals were large. The sample size was therefore too low to determine to what extent song amplitude is heritable. Nevertheless, heritability tended to be positive. In a comprehensive study on the heritability of zebra finch vocalizations, Forstmeier *et al.* (2009) found a weak but significant heritability of song amplitude. However, there are methodological problems with their procedure for measuring amplitude involved (see discussion of chapter 5).

Moreover, Forstmeier *et al.* (2009) did not control for song learning, i.e. some juveniles might have learnt the songs of their genetic fathers, which would result in an overestimation of heritability.

To conclude, song learning and/or production constraints appear to have a strong effect on the sound pressure level of fully developed zebra finch song. Vocal production learning may guide the ontogenetic trajectory of vocal amplitude towards a default level which is then modified in the short-term by the current condition of the adult male. In addition, current evidence suggests that there may also be a genetic, possibly morphology-related, component of song amplitude.

Conclusions and outlook

I found that song amplitude may be subject to sexual selection, is sensitive to body condition and is affected by vocal learning. These findings highlight the importance of signal intensity in acoustic communication in birds.

However, many questions remain unanswered. An important issue is the costs which limit song amplitude and, ultimately, determine whether song amplitude is an honest signal of physical or genetic quality. It is often assumed that energetic costs constrain song amplitude, but recent studies indicate that this is not the case (Oberweger and Goller 2001; Zollinger *et al.* under review). Alternatively to direct metabolic costs there may be indirect costs as well: singing loud may increase predation risk or might even affect parasite levels, since some parasites use acoustic orientation to find their hosts (Cade 1975; Sakaguchi and Gray 2011). I could show for the first time that loud singers may suffer higher levels of territorial aggression (chapter 2), suggesting that social aggression is a major indirect cost of song amplitude.

Moreover, further research on the signaling value of song amplitude is required; my findings suggest that body condition is important (chapter 4), but other aspects of male quality may be involved, too. Furthermore, tutoring experiments employing tutor songs with artificially manipulated amplitude envelopes would help to assess the relative importance of production constraints and of actual learning in determining the vocal amplitude of tutees. Finally, the fourth, and perhaps most challenging, field of future research that I would like to mention is the relation between song amplitude and fitness. My experiments suggest that song amplitude may be sexually selected, since females prefer loud males and loud intruders appear to represent a bigger threat for territory owners. If song amplitude is an honest signal, high-amplitude singers are expected to be of higher phenotypic or genetic quality in comparison to low-amplitude singers, which should ultimately be expressed in higher fitness returns.

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Summary

Birdsong is an important model in the study of animal behaviour, environmental adaptation and sexual selection. Its main functions are territory defense and attraction of sexual partners. Birdsong is a multidimensional trait. Each of its dimensions may be subject to different selection pressures, may be constrained by different costs and may signal different qualities. For example, a high song rate may be important to defend a territory, may be constrained by time budgets, and may signal a high foraging efficiency to females. A large song repertoire may be important to attract mates, be subject to developmental constraints and may signal early nutrition.

One song trait that so far has received little attention is amplitude, i.e. the sound pressure level of a song. It has been shown that there is a considerable variation in song amplitude between males, but the causes and consequences of this variation are poorly studied. In my dissertation, I performed several experiments in order to shed some light on the function and evolution of song amplitude in birds.

In a first step, I asked whether song amplitude is subject to sexual selection through female choice and male-male competition. To this end, I tested song preferences of female zebra finches and I simulated high-amplitude and low-amplitude intruders in territories of male chaffinches. My results demonstrated that female zebra finches have a preference for loud songs over soft songs if all other song parameters are kept constant, and that male chaffinches react considerably stronger to loud territory intruders than to intruders singing with low amplitude. This suggests that song amplitude is likely to be sexually selected. Moreover, the strong territorial response of chaffinches towards loud intruders provides the first experimental evidence that song amplitude may be constrained by social aggression. But why should females prefer loud males and why should loud males elicit stronger territorial aggression?

To answer these questions, I investigated whether song amplitude may have a signalling value. In particular, I tested whether song amplitude may reflect testosterone level or body condition, and thus signal motivation or male quality. To this end, I manipulated testosterone levels of adult male Bengalese finches and the nutritional status of adult male zebra finches. I found that elevated testosterone levels did not affect song amplitude in Bengalese finches, but that zebra finches with artificially decreased body weight sang significantly less loud than control birds. This demonstrates for the first time that song amplitude may signal the current

condition of the singer and suggests that females may make use of this information when choosing a mate.

Finally, I studied the effects of vocal learning on song amplitude. I found that young zebra finches adopted the amplitude of song elements from their tutors, which suggests that amplitude is either learned, bound to spectro-temporal element properties due to production constraints, or both. My findings indicate that vocal production learning may guide the ontogenetic trajectory of vocal amplitude towards a default level which is then modified in the short-term by the current condition of the adult male

My findings contribute to a deeper understanding of the causes and consequences of song amplitude in birds and highlight the importance of signal intensity in acoustic communication. Moreover, they emphasize that behaviour should be viewed as a complex interplay between mechanistic, functional and ontogenetic aspects.

Zusammenfassung

Vogelgesang ist ein wichtiges Modell in der zoologischen Forschung, insbesondere im Zusammenhang mit Fragen zu Verhalten, Adaptation und sexueller Selektion. Die beiden Hauptfunktionen von Vogelgesang sind die Verteidigung von Territorien und das Anlocken von Partnern. Vogelgesang ist ein multidimensionales Merkmal. Das bedeutet, jede Dimension kann eigenen Selektionskräften unterliegen, kann eigene Kosten haben und eigene Qualitäten signalisieren. Eine hohe Gesangsrate könnte beispielsweise vorteilhaft in der Territorialverteidigung sein, könnte durch das Zeitbudget beschränkt sein und könnte eine hohe Effizienz bei der Nahrungssuche signalisieren. Ein grosses Gesangsrepertoire könnte attraktiv für Partner sein, könnte Entwicklungsbeschränkungen unterliegen und könnte die Ernährungsbedingungen während der Ontogenese widerspiegeln.

Ein Gesangsmerkmal dem bisher wenig Aufmerksamkeit geschenkt wurde ist die Amplitude, also der Schalldruckpegel des Gesangs. Es ist bekannt, dass sich Individuen in der Gesangsamplitude beträchtlich unterscheiden, aber die proximaten und ultimaten Gründe dieser Variation sind kaum bekannt. In meiner Dissertation habe ich mehrere Experimente durchgeführt, die zur Klärung dieses Phänomens beitragen.

In einem ersten Schritt habe ich untersucht, ob die Gesangsamplitude sexuell selektiert sein könnte. Dazu habe ich einerseits die Gesangspräferenzen von weiblichen Zebrafinken untersucht, und andererseits die Reaktion von territorialen Buchfinkenmännchen auf laut und leise singende konspezifische Rivalen gemessen. Ich konnte zeigen, dass die Zebrafinkenweibchen lauten Gesang bevorzugten, wenn alle anderen Gesangsparameter konstant gehalten werden, und dass die Buchfinken viel stärker auf Rivalen mit lautem als mit leisem Gesang reagierten. Das lässt darauf schliessen, dass die Gesangsamplitude ein guter Kandidat für ein sexuell selektiertes Signal ist. Die starke Reaktion der Buchfinkenmännchen auf die lauten Eindringlinge stellt darüber hinaus den ersten experimentellen Beleg dar, dass die Gesangslautstärke durch soziale Aggressionen beschränkt werden könnte. Warum sollten Weibchen aber laute Männchen bevorzugen, und warum sollte lauterer Gesang eine stärkere Reaktion von Konkurrenten hervorrufen?

Um diese Fragen zu beantworten, habe ich die proximaten Mechanismen der Gesangslautstärke untersucht. Insbesondere habe ich getestet, ob die Gesangsamplitude den Testosteronspiegel und/oder die Körperkondition der Sänger widerspiegeln. Dazu habe ich den Testosteronspiegel von Japanischen Mövchen und den Ernährungsstatus von

Zebrafinken experimentell manipuliert. Meine Resultate zeigen, dass erhöhte Testosteronwerte keinen Einfluss auf die Gesangslautstärke hatten. Im Gegensatz dazu sangen Zebrafinken mit reduziertem Körpergewicht leiser als unmanipulierte Artgenossen. Damit konnte ich zum ersten mal zeigen, dass die Gesangslautstärke die aktuelle Körperkondition des Sängers widerspiegelt und dass Weibchen diese Informationen für die Partnerwahl nutzen könnten.

Schliesslich habe ich auch den Einfluss des Gesangslernens auf die Amplitude untersucht. Ich konnte zeigen, dass junge Zebrafinken die Amplitude ihrer Gesangselemente vom jeweilegen Tutor übernahmen. Das bedeutet, dass die Amplitude gelernt ist oder dass Elementstruktur und Elementlautstärke durch Produktionsmechanismen gekoppelt sind, oder beides. In jedem Fall lassen meine Resultate darauf schliessen, dass das die Amplitude durch die Mechanismen des Gesangslernens auf ein Standardlevel gebracht wird, welches dann kurzfristigen Schwankungen der Körperkondition unterliegt.

Die Befunde meiner Experimente tragen zu einem tieferen Verständinis der Mechanismen und Funktionen der Gesangsamplitude bei Vögeln bei und zeigen die Bedeutung der Signalintensität in der akustischen Kommunikation auf. Darüber hinaus unterstreichen sie die Auffassung, dass Verhalten als komplexes Zusammenspiel von mechanistischen, funktionellen und ontogenetischen Faktoren interpretiert werden sollte.

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Author contributions

Chapter 1

I collected all the data, analyzed it and wrote the first draft of the paper. My co-authors contributed with concept, study design and some practical work.

Chapter 2

I analyzed the data and wrote several paragraphs of the paper. The experiment was performed by Henrik Brumm jointly with me. Henrik Brumm designed the study and wrote the rest of the manuscript.

Chapter 3

I collected and analyzed the song data, and wrote the first draft of the paper. Silke Laucht implanted the testosterone pellets and took blood samples. The other co-authors contributed with concept and design.

Chapter 4

I collected all the data, analyzed it and wrote a first draft of the manuscript. I designed the experiment together with Henrik Brumm, who conceived the study.

Chapter 5

I designed the experiment, collected and analyzed all the data, and wrote a first draft of the manuscript. Henrik Brumm conceived the study and contributed with conceptual as well as practical work.

General introduction & discussion

Entirely my own work

Addresses of co-authors

Henrik Brumm
Communication and Social Behaviour Group
Max Planck Institute for Ornithology
Seewiesen, Germany
brumm@orn.mpg.de

James Dale
Institute of Natural Sciences
Massey University
Auckland, New Zealand
j.dale@massey.ac.nz

Silke Laucht
Department of Behavioural Ecology and Evolutionary Genetics
Max Planck Institute for Ornithology
Seewiesen, Germany
laucht@orn.mpg.de

Katharina Riebel
Institute Biology Leiden
Leiden University
Leiden, The Netherlands
k.riebel@biology.leidenuniv.nl

Curriculum vitae

Name: Mathias Ritschard

Date of birth: 6th March 1980

Place of birth: Meiringen, Switzerland

Education:

1996 – 2000	Matura at the Gymnasium Neufeld in Bern, Switzerland
2000 – 2005	Study of Biology at the University of Bern, Switzerland
2008 – 2011	Doctoral studies at the Max Planck Institute for Ornithology, Seewiesen, Germany

List of publications

Peer-reviewed publications

Ritschard M, van Oers K, Naguib M, Brumm H (2012). Song amplitude of rival males modulates the territorial behaviour of great tits during the fertile period of their mates. *Ethology* 118, 197-202.

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Declaration

Ich erkläre hiermit, dass ich die vorliegende Arbeit ohne unzulässige Hilfe Dritter und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Die aus anderen Quellen direkt oder indirekt übernommenen Daten und Konzepte sind unter Angabe der Quelle gekennzeichnet. Weitere Personen, insbesondere Promotionsberater, waren an der inhaltlichen materiellen Erstellung dieser Arbeit nicht beteiligt. Die Arbeit wurde weder im In- noch im Ausland in gleicher oder ähnlicher Form einer Prüfungsbehörde vorgelegt.

Mathias Ritschard