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Female calling? Song responses to conspecific call playbacks in nightingales, *Luscinia megarhynchos*

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A crucial issue for understanding the evolution, functions and mechanisms of complex communicative signals such as birdsong is to disentangle signal structures that serve to convey information in different contexts. The two main singing contexts of European male songbirds are the attraction of a female and the defence of a territory from other males. The method of choice to experimentally investigate the use of song in male–male interactions is to conduct playback studies, in which song is played to a resident male. Responses to the presence of a female, however, are hardly testable this way, since females of most European bird species do not sing. However, females do call in several situations. In a playback experiment, we investigated whether free-ranging male nightingales change their nocturnal singing in response to playbacks consisting of calls produced by either female or male conspecifics. In both cases, nightingales changed their singing style as compared to their singing before playback, and these changes were different in response to male and female calls. Males sang fewer whistle songs after male call playbacks and started to produce 'initial whistles' earlier when hearing female calls. Male call playbacks also led to an increase in song duration. An analysis of the call characteristics of both sexes used in the playbacks uncovered acoustic parameters that may account for the differentiated response. We conclude that male nightingales distinguish between female and male calls, and discuss the song characteristics that might be used to specifically address either males or females. Call playbacks proved to be useful for disentangling song characteristics that serve the multiple functions of birdsong.

Birdsong is a sexually selected communicative behaviour that serves mainly two functions: the attraction of a mate and the establishment and defence of a territory (Catchpole & Slater, 2008). Some species have two or more distinct singing styles that they use in these different contexts (e.g. Catchpole, Leisler, & Dittami, 1986; Sossinka & Böhner, 1980), but often, only gradual changes or different acoustic features within the same song serve to address different audiences. Song repertoire size, repertoire composition and structural song measures often show intra- and interindividual variability and thus allow differentiated information encoding within singing (Gil & Gahr, 2002). Given this, it is a challenge to understand the relationships among song features, encoded information and potential receivers. Investigating 'audience effects', i.e. effects of the presence of males and/or females on a singer, might offer valuable clues to find song characteristics that are modulated towards specific receivers.

Acoustic playbacks of song are a powerful tool for studying birdsong functions as they mimic a singing conspecific in or close to the territory of a resident male (Kroodsma, Byers, Goodale, Johnson, & Liu, 2001). Playback experiments have been used to address a wide array of questions concerning mechanisms and functions of song: the effects of song on territory establishment (e.g. Krebs, 1977), functions of specific song exchange patterns such as song type matching in territorial disputes (e.g. Burt, Campbell, & Beecher, 2001), endocrinological foundations of song features and aggression in territorial intrusions (e.g. Apfelbeck, Kiefer, Mortega, Goymann, & Kipper, 2012) and neuronal responses to song (e.g. Prather, Peters, Nowicki, & Mooney, 2008) have all been investigated with playback techniques. Successful as these approaches were, the examples also hint to the limitations of song playback studies: an acoustic playback can by definition only simulate the presence of a singing male, as opposed to the presence of a male in general. In this way, it is impossible to disentangle the effects of the presence of an audience (a male) from the effects of a specific song

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played. Also, since females of many species do not sing (Garamszegi, Pavlova, Eens, & Møller, 2007), playback experiments only allow for the investigation of the role of song in male–male interactions. Presenting a stuffed decoy (a model) of a male or female may also prove problematic because many bird species are monomorphic (i.e. males and females look alike, Owens & Hartley, 1998). This methodological problem contributes considerably to the bias of birdsong research towards investigating male singing strategies and neglecting potential effects of listening females on song behaviour (Riebel, 2003; Riebel, Hall, & Langmore, 2005).

Anecdotal observations led us to an alternative approach for testing audience effects on singing, including a female audience: having recorded the nocturnal singing of common nightingales over many years, we occasionally observed that a male singing at night was joined by a second nightingale that was not singing, but was calling (see Appendix Fig. A1 and the Supplementary Sound File for an example of such a recording). This caller appeared to have a conspicuous effect on the singing style of the male. Thus we decided to use calls to simulate a conspecific bystander during nocturnal singing. Different from singing, calls have not gained much attention in songbird vocalization research, although it is known that, in most species, males as well as females possess a call repertoire (Marler, 2004). Additionally, it has been shown that males and females differ in their call repertoires in some species other than songbirds (e.g. Aubin, Mathevon, Staszweski, & Boulinier, 2007; Dentressangle, Aubin, & Mathevon, 2012) as well as some songbird species (e.g. Freeberg & Branch, 2013). Such findings suggest that calls might be very well suited to investigate differentiated song responses used in inter- or intrasexual communication. We therefore decided to test the use of calls to study song responses in a nocturnal playback paradigm with common nightingales. The nightingale is a common songbird of the southwestern Palearctic. After wintering in Africa, males establish territories in spring, mostly by singing. Compared with the majority of European songbird species that sing only during the day, nightingales also sing long song bouts at night (e.g. Amrhein, Kunc, Schmidt, & Naguib, 2007; Kipper, Mundry, Hultsch, & Todt, 2004), and females prospect male territories at night (Roth, Sprau, Schmidt, Naguib, & Amrhein, 2009; personal observations). The nocturnal activity and the monomorphic appearance of the two sexes (Glutz von Blotzheim, 1989) make it reasonable to expect that the sexes are vocally distinguishable by their calls. Numerous experiments have shown that male nightingales readily interact in nocturnal song playbacks; thus vocal playbacks 'work well' in the species (see Naguib, Kunc, Sprau, Roth, & Amrhein, 2011 for examples). When playbacks are conducted during the day, males usually respond with both changes in song and/or other vocal output, as well as approaches to the sound source (e.g. Bartsch, Weiss, & Kipper, 2012; Sprau, Roth, Schmidt, Amrhein, & Naguib, 2010). At night, however, males avoid moving, and thus nocturnal playbacks allow the exclusive study of song in response to a conspecific.

Both male and female nightingales possess call repertoires that are used in many different situations during courtship and breeding, as evidenced by qualitative studies (description in Horstkotte, 1965). We therefore played strings of either male or female calls to nocturnally singing nightingales to investigate whether such calls would affect the singing behaviour. We hypothesized that males would respond to any call playback by changing song characteristics. Under the assumption that calls of females and males differ (although male and female calls are not distinguishable to the human ear), we expected the 'song response' to differ as well. These experiments were paralleled by an acoustic analysis of the playback calls to identify parameters that differ between the calls of males and females.

METHODS

Subjects and Study Population

The playbacks were conducted with 20 solo-singing male nightingales in Berlin and Brandenburg, Germany (for details on study populations and sites see Bartsch, Wenchel, Kaiser, & Kipper, 2014; Kiefer, Scharff, & Kipper, 2011; Kipper, Mundry, Sommer, Hultsch, & Todt, 2006). Males were tested at least 3 days after arrival while still engaged in nocturnal singing early in the breeding season (22 April–10 May 2009). Since males usually cease nocturnal singing after pair formation, these males were probably unmated or in an early phase of pair formation (Amrhein, Korner, & Naguib, 2002).

Stimuli and Playback Design

Given the low amplitude and rare occurrence of nightingale calls, recording calls in the field of sufficient quality for acoustic analyses and playback experiments proved very difficult. Instead, calls recorded from birds kept in captivity were used for the playbacks. These were high-quality recordings of nocturnal calls of adult nightingales kept in the laboratory (and therefore unfamiliar to the birds that would hear the playbacks). The birds were hand-reared under controlled conditions for experiments on male song learning and female song preference learning, with permission of the responsible authorities ('Landesamt für Gesundheit und Soziales', Berlin, Reg. 0128/07). Husbandry details are reported elsewhere (e.g. Kiefer, Scharff, Hultsch, & Kipper, 2014; Weiss, Kiefer, & Kipper, 2012). Call sequences of these birds were recorded in spring 2008 in sound-attenuated chambers in which individual birds were housed in their home cages overnight. The calls were thus most likely to be contact calls. In the wild, similar-sounding calls are audible throughout the breeding season: during territory establishment, courtship, nest approaches, and feeding of nestlings and fledglings (see Appendix Fig. A1 and the Supplementary Sound File for an example of calls recorded in nature). Playback preparation and analysis were done with Avisoft-SASLab Pro software (4.23e, 4.38, 4.40; R. Specht, Berlin, Germany). For the playbacks, we randomly selected 1 min segments containing at least 33 calls, blind to any specific acoustic feature of the calls. A total of 20 call playbacks were prepared this way: 10 with female and 10 with male calls. Since only six males were available for call recordings, we used two different recordings from four of these males. Call series were selected and cut after visual inspection of spectrograms, background noise from the air ventilation system in the sound-attenuated chambers was filtered (high-pass 0.4 kHz; filter type Butterworth, order 8) and call series were normalized to 75% volume. Playbacks lasted about 1 min (mean ± SD = 62.4 ± 3.7 s; no difference between female and male playbacks: Mann–Whitney U test: $W = 30.5$, $N_1 = N_2 = 10$, $P = 0.15$).

Call playbacks were played via a .wav player (MP X10i ODYS) connected to a custom-built loudspeaker (DKA Heidelberg) that closely resembled the acoustic sound radiation properties of a small songbird (Larsen & Dabelsteen, 1997). This speaker was mounted about 10 m away from the singing bird, and the amplitude was adjusted to 75 dB at a distance of 1 m to the loudspeaker measured with a precision sound level meter (CEL 314, time constant 125 ms; this corresponds to natural amplitudes measured in calling birds in both the laboratory and the field, Kipper, Kiefer, & Weiss, n.d.). The song of the target male was recorded before, during and after the playback using a solid state recorder (PMD 660, Marantz). Four song features that had been suggested to be of importance in male–male interactions and for female attraction were analysed.