Threat signaling in female song—evidence from playbacks in a sex-role reversed bird species

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Prominent research areas such as animal communication and sexual selection use birdsong as a model system. Most studies on these subjects are conducted on species with typical sex roles with male-biased song production. Accordingly, the functions of birdsong, mate attraction, and territorial defense have hardly been studied in females. We investigated the territorial function of female song in the sex-role reversed African black coucal (*Centropus grillii*) to test whether females in such species demonstrate the same principles as male birds in species with typical sex roles. When territorially challenged, female black coucals changed their vocalizations in comparison to when they were singing spontaneously: They altered the composition of songs, lowered the pitch, and increased the duration of song elements. When challenged, larger females vocalized at lower pitch than smaller ones suggesting that pitch might be a reliable indicator of competitive abilities. To study whether females pay attention to such variation, we exposed them to playback experiments in which songs varied 1) in the composition and 2) in pitch and duration of song elements. Females did not respond differently to stimuli that varied in the composition. However, they reacted more cautiously to low-pitched and long stimuli compared with unchanged stimuli. This suggests that females were intimidated by the songs with low-pitched and long elements and that those songs signaled a higher level of threat. Thus, female black coucals paid attention to song parameters that reliably indicated competitive abilities. This confirms the general role of intrasexual selection in vocal communication of birds. *Key words:* aggressive signals, female birdsong, sex-role reversal, signal evolution, signal reliability, vocal communication. *[Behav Ecol 21:1147–1155 (2010)]*

Birdsong has been studied mainly on species that breed in the temperate zone, and in most of them, only male birds sing. However, the majority of bird species breed in the tropics (Stutchbury and Morton 2001), and females of many of the tropical bird species sing as well (Slater and Mann 2004). The neglected issue of female song production started to attract more attention recently (e.g., Cooney and Cockburn 1995; Langmore 2000; Riebel 2003; Riebel et al. 2005; Brunton and Li 2006; Price et al. 2008; Geberzahn et al. 2009; Illes and Yunes-Jimenez 2009; Price 2009; Price et al. 2009), and a detailed study of the structure and significance of female song will be necessary to test whether theories about the role of song derived from studying male song also hold for female song.

In male birds, song serves to attract mates and to deter territorial rivals (Catchpole and Slater 2008). The song of passerine birds provides the best-studied case, but vocalizations of many nonpasserine species serve the same functions and can be considered as song as well (cf. ten Cate et al. 2002; Searcy and Nowicki 2005). This dual function has been studied almost exclusively in species with "typical" sex roles. In such species, females usually make a larger parental investment and are therefore the limiting sex and thus expected to be choosy. In contrast, male birds are more competitive, and most have evolved elaborate secondary sexual signals, such as song (Andersson 1994). Thus, sexual selection affects signal parameters through female mate choice and competition between rival males (Berglund et al. 1996).

Sexual selection theory suggests that it is the competing sex that should use signals accordingly, and it follows that for sex-role reversed species, it should be the song of females that serve the same functions as male song does in nonreversed species.

Vocal territorial interactions have been studied extensively in male birds—they allow them to resolve territorial conflicts without necessarily engaging in costly physical fights (Todt and Naguib 2000; ten Cate et al. 2002). During such interactions, a male needs to assess the fighting ability of a competitor to reach the appropriate decision. Among other factors, this is determined by the physical quality of the competitor. However, a male also needs to pay attention to a competitor's motivation to fight which may crucially influence the outcome of a conflict. For a territorial signal to be evolutionarily stable, it should be reliable, and a fundamental question is how such signal honesty is maintained (Gil and Gahr 2002). Honesty might, for instance, be achieved by size-, mass-, or strength-dependent vocalizations, with large or heavy birds being the only ones able to produce low-frequency sounds (e.g., Barbraud et al. 2000; Miyazaki and Waas 2003), sustained vocalizations (e.g., Ballintijn and ten Cate 1997), or particular types of vocal elements (e.g., Ballintijn and ten Cate 1999). The response of male receivers to such signals usually indicates that they perceive them as coming from stronger competitors. Here, we examine whether females in a sex-role reversed species, the African black coucal (*Centropus grillii*), show a similar response.

In African black coucals, sex roles are reversed. They are classically polyandrous, that is, females mate simultaneously with up to 4 males, whereas males form bonds with only 1 female during a given breeding season. Females are larger than males, aggressively defend large territories that contain smaller and nonoverlapping subterritories of their males, and
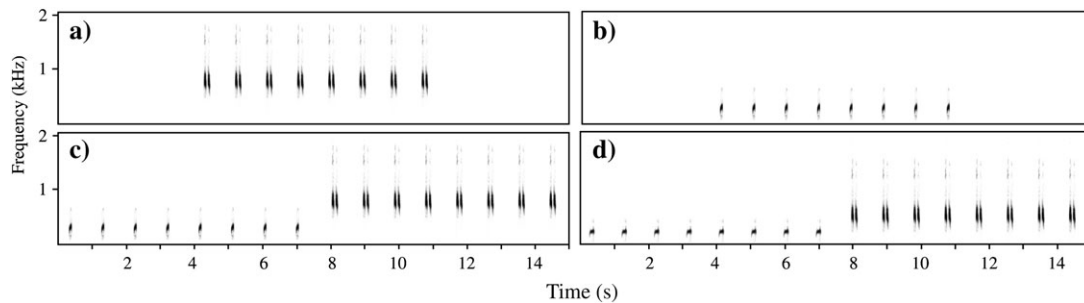
Figure 1
Spectrograms of test stimulus songs. (a and b) Composition playbacks consisted of songs containing either k'tucs syllables only (a) or of songs containing whoots only (b). (c and d) Pitch/duration playbacks consisted of songs with original pitch and duration of elements (c) or of songs with altered pitch and duration (d).

use vocalizations to defend these territories. In contrast, male birds rarely vocalize, and each male builds his own nest and incubates and cares for his social offspring in general without any help from the female (Vernon 1971; Goymann et al. 2004, 2005; Muck et al. 2009). The song of black coucals typically consists of 2 parts: the whoot part and the k'tuc part (Figure 1, see also Goymann et al. 2004 and Geberzahn et al. 2009); however, it can also be composed of either part without the other. The whoot part consists of low-pitched elements, the whoots, and the k'tuc part consists of syllables, the k'tuc syllables containing 2 elements typically.

Geberzahn et al. (2009) showed recently that singing female black coucals altered certain song features when being challenged by a playback simulating a territorial intrusion: Females changed the composition of their vocalizations (increasing the proportion of whoots), and they lowered the pitch and enhanced the duration of whoots and lowered the pitch of k'tuc syllables. When vocally responding to the territorial challenge, larger females produced k'tucs at lower pitch than smaller females. These findings suggest that vocal interactions in this species are important for mutual assessment of competitive abilities in the females (Geberzahn et al. 2009). Therefore, black coucals make an excellent model to study territorial vocal signaling in female birds. Studies on the vocal interactions of female birds will allow answering the question whether the same underlying principles and mechanisms guaranteeing reliability of signals operate in females as in males.

Although female black coucals are thus known to respond vocally to an intrusion, the current study examines whether the responses vary in relation to the vocal stimulus. Thus, we investigate the signal value of context-dependent variation in the song of female black coucals. Based on our previous study, we hypothesized that 1) a higher proportion of whoots represents a higher level of threat than a lower proportion and 2) songs at lower pitch and with longer elements represent a higher level of threat than songs at higher pitch and with shorter elements. We conducted playback experiments in which we confronted subjects with song stimuli that varied accordingly. As response measures, we used approach to the loudspeaker, agility, and number of vocalizations. Our predictions were the following: First, females should respond differently to songs with a higher proportion of whoots versus songs with a lower proportion of whoots. Second, females should respond differently to songs at lower pitch and with longer elements versus songs at higher pitch and with shorter elements. Previous playback studies on male birds investigating responses to signals conveying different levels of threat have revealed that the direction of the differences in the behavioral response varied between species (cf. de Kort et al. 2009; Searcy

and Beecher 2009): In some species, territorial males showed a stronger behavioral response to stimuli with a greater threat potential (e.g., Nelson and Croner 1991; Slabbekoorn and ten Cate 1997; Otter et al. 2002; Illes et al. 2006); in other species, male birds showed a weaker behavioral response to more threatening stimuli (e.g., Järvi et al. 1980; Hall et al. 2006; Cramer and Price 2007), whereby a strong response is one in which the territory owner approaches the stimulus closely and displays vigorously. de Kort et al. (2009) and Collins (2004) provided the "peaked curve hypothesis" that allows to reconcile this apparent discrepancy: They assume a peaked curve relationship between response intensity and signal intensity (see Figure 1 in de Kort et al. 2009): "If the experimental stimuli range from low to median intensity, representing the upward slope of the peaked curve one expects a stronger response to the higher intensity signal. If the stimuli range from average to high intensity, representing the downward slope, then the strongest response should be elicited by the lower intensity signal" (de Kort et al. 2009). For our study species, we did not know whether the signal value of our tested stimuli ranged from low to median or from medium to high intensity. Assuming such a peaked curve relationship for coucals, we therefore did not predict in which direction female black coucals would change their response.

METHODS

Study site and subjects

We studied African black coucals (family: Cuculidae) in the Usangu wetland (lat 8°41′S, long 34°5′E) in south-western Tanzania. Black coucals are short-distance migrants and establish breeding territories during the rainy season from approximately January to June. As the habitat suitable for breeding changes on a year-to-year basis, individual birds are not philopatric. In total, we exposed 22 females to playbacks, including 10 that were caught using mist nets and color ringed. In addition, 6 of these birds were equipped with a Holohil BD-2G radio transmitter (<2 g; Holohil Systems Ltd, Ontario, Canada) using a Rappole harness (Rappole and Tipton 1991) made out of soft polyethylene microtubing (0.9-mm diameter). Females weigh on average 165.5 g (Goymann et al. 2004); thus, the weight of the radio transmitter is less than 1.2% of their body mass. In fact, earlier studies showed that females carrying radio transmitters readily reproduced (Goymann et al. 2004; Goymann et al. 2005). Thus, it seems very unlikely that carrying a radio transmitter affects a female's behavior. Further details on the study site and study subjects can be found in Goymann and Wingfield (2004) and Goymann et al. (2004, 2005).
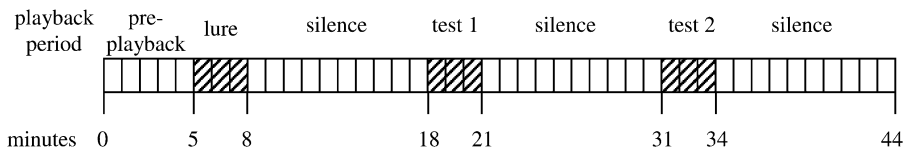
Figure 2
Playback scheme. Each experiment lasted for 44 min: 5 min preplayback period of silence and 3 subsequent 3-min playback periods each followed by 10 min of silence. The lure playback served to lure the female into the playback area. After the lure playback, we broadcast the test stimuli, with half of the subjects receiving one type of test stimulus first and the other half of the subjects receiving the other type of test stimulus first.

Experimental design and procedure of playback experiments

We conducted 2 separate experiments: one in which we tested subjects' reactions to different proportions of whoots (composition playbacks, in which stimuli varied in the composition of songs) and the other in which we tested their reaction to longer and lower pitched elements (pitch/duration playbacks, in which stimuli varied in the pitch and duration of elements). Altogether, 27 playback experiments were carried out successfully with 22 different subjects. Thus, 5 subjects were tested in both playback series. In these 5 subjects, at least 15 days elapsed between the 2 playbacks (except for 1 subject where for logistical reasons, we had to conduct the second playback 4 days after the first). Three of these subjects received a pitch/duration playback first and 2 received the composition playback first. We retested these birds as no further subjects of which we knew the territories were available. Altogether, we carried out 13 composition playbacks and 14 pitch/duration playbacks in the time between 19 March and 16 May 2006 and between 0700 and 1200 h. We placed a Blaupunkt 100W CB 4500 loudspeaker with a custom built-in Kemo MO34 amplifier connected to a Marantz PMD 670 Solid state recorder within the territory of a focal female. Songs were broadcast at an amplitude of ca. 85 dB at 1 m (CEL-231 Sound Level Meter). The playback equipment was placed in the center of a subject's territory. Experiments were aborted and considered unsuccessful if either the subject did not respond or a territorial neighbor responded to the playback (4 pitch/duration playbacks and 6 composition playbacks had to be aborted). Each experiment followed the same scheme and lasted for 44 min in total: 5 min preplayback period of silence and 3 subsequent periods each lasting 13 min consisting of 3 min playback followed by 10 min of silence each (Figure 2). During the first playback period, we broadcast a lure playback to lure the female into the playback area. During the second and third playback periods, we broadcast the 2 test stimuli, with half of the subjects receiving 1 type of test stimulus first and the other half of the subjects receiving the other type of test stimulus first.

Procedure, observations, and song recording during playback

We used 3 bamboo poles (height: 2 m) as a reference when estimating the distance of a subject to the loudspeaker. They were placed at 5, 20, and 40 m distance to the loudspeaker. Observations of the subject's behavior were made by 2 observers; 1 being positioned on the top of a ladder for better vision from a distance of approximately 50–60 m. Observations were recorded by 1 observer with FIT system software (Held and Manser 2005) on a Palm IIIx handheld computer. The second observer helped keeping track of the subjects during the

course of the experiment. As response parameters, we collected the following data: 1) number of whoots, 2) number of k'tucs, 3) number of flights per minute, 4) seconds in air per minute, 5) seconds spent within 5 m of the loudspeaker per min, 6) seconds spent within 20 m of the loudspeaker per min, 7) seconds spent within 40 m of the loudspeaker per min, 8) latency to first vocal response, and 9) latency to first approach into the direction of the speaker regardless of how close the female approached. To compare the response measures between periods of different lengths (5-min preplayback vs. 3-min test playback), we calculated all measures except latencies as number or seconds per minute. All response parameters were assessed during the preplayback (as baseline measure) and the playback periods, expect for latencies to sing. Here, we took the absolute number of seconds it took the subject to sing during the playback or in the 10 min thereafter. To assess the number of songs and the latency to singing responses, continuous recordings were made using Sennheiser ME66/K6 microphones connected to a Marantz PMD 670 Solid state recorder. These recordings were part of another study comparing singing in response to a playback and spontaneous singing to reveal context-specific variation in the song of the females (Geberzahn et al. 2009). All experiments were conducted according to Association for the Study of Animal Behavior guidelines on animal experimentation and were approved by the Tanzania Commission of Science and Technology (COSTECH) and the Tanzania Wildlife Research Institute (TAWIRI) and comply with the current laws of Tanzania.

Stimulus songs

Songs used to generate playback stimuli were recorded from 16 spontaneous singing individually ringed females during the breeding seasons in 2005 (2 February to 17 May). Recordings were made using a Sennheiser ME66/K6 microphone connected to a Marantz PMD 670 Solid state recorder (sample rate: 44.1 kHz). Spontaneous singing was defined as singing without any indication of interactions with conspecifics or predators. Given a very low recapture rate of ringed birds in our study population (<1%; Goymann W, unpublished data), it is very unlikely that the stimuli used for the playbacks (recorded in 2005) were from females known to the subjects tested in 2006. In order to simulate a territorial intrusion of only 1 rival, stimuli for a given playback experiment were all taken from recordings of 1 stimulus female (thus, lure, test 1, and test 2 were recorded from the same stimulus subject). To avoid pseudoreplication within a give playback series, any given stimulus was broadcast to 1 subject only (across the different playback series, stimuli of the same stimulus female were

broadcast to 2 subjects at most). If not stated otherwise, stimuli processing was done using Avisoft SASLAB Pro (R. Specht, Berlin, Germany).

Lure playbacks

The 3 min lure playback contained sequences of songs of a given female as they were recorded, thus with natural variation in all parameters retained (except that peak amplitude was normalized to 75%). As a consequence of our design, each subject got a different lure playback, and to rule out the possibility that differences in the lure playbacks might have affected responses to the test stimuli, we measured 7 acoustic parameters of the lure playbacks: number of whoots, number of k'tucs, proportion of whoots, mean peak frequency of whoots, mean peak frequency of k'tucs, duration of whoots, and duration of k'tucs. Avisoft SASLAB Pro was used to count the number of elements using the spectrographic view and to measure frequency and duration parameters using the automatic parameter measurement function (Fast Fourier Transform length = 1024, frame = 100%, window = ham, overlap = 99). As peak frequency, we here define the mean of the peak frequency at the start, end of element, and at the maximum amplitude of the element. We conducted a principal component analyses on these 7 parameters and included the resulting first component into further analysis comparing response measures during the different periods (see below).

Composition playbacks—variation in the composition of songs

Composition playbacks consisted of songs containing either k'tucs syllables only (0% whoots; Figure 1a) or of songs containing whoots only (100% whoots; Figure 1b). This allowed us to test the hypothesis that a higher proportion of whoots (100% whoots) indicates a higher level of threat than a lower one (0% whoots). To generate these playbacks, we selected 1 whoot element and 1 k'tuc syllable from each lure playback. Whoot elements were band pass filtered (0.1–2 kHz), and k'tuc syllables were high pass filtered (0.5 kHz). We generated stimulus songs that contained either 8 whoots or 8 k'tuc syllables, each of which was repeated at a constant rate within a song (see Figure 1a,b). Such a song was repeated 11 times at a constant rate to make up a total duration of 3 min.

Pitch/duration playbacks—variation in pitch and element duration

Pitch/duration playbacks consisted of songs with 1) original pitch and duration of elements ("normal"; Figure 1c) or with 2) altered pitch and duration ("low & long"; Figure 1d). This allowed us to test the hypothesis that songs at lower pitch and with longer elements indicate a higher level of threat. To generate these playbacks, we used whoot elements and k'tuc syllables as they had been prepared for the test stimuli of the composition playbacks. We used the constant stretch function in Cool Edit software (version 96, Syntrillum) to change the duration of whoot elements (time stretch) and the pitch in whoot elements and k'tuc syllables (pitch stretch). For both kinds of transformation, we used the high precision setting and a 150 stretching ratio. This procedure provided stimuli with parameters within the natural range of songs that females give in response to territorial challenges (see "reactive song" in Geberzahn et al. 2009). We generated songs that were composed of 8 whoot elements followed by 8 k'tuc syllables (either original or transformed; see Figure 1c,d). Within a song, whoots and k'tuc syllables were repeated at a constant rate. A song was repeated 6 times at a constant rate to make up a total duration of 3 min.

Table 1

Test statistics of GLMMs for differences in response variables during the periods: preplayback, test 1, and test 2

Playback	Response variable	F	P	Adjusted P
Composition	No. of whoots	$F_{2,19} = 8.95$	0.002	0.007
	No. of k'tucs	$F_{2,19} = 2.52$	0.11	0.11
	No. of flights	$F_{2,21} = 5.47$	0.01	0.04
	Time in air	$F_{2,21} = 3.41$	0.05	0.10
	Time within 5 m	$F_{2,21} = 25.06$	<0.001	<0.001
	Time within 20 m	$F_{2,21} = 13.57$	<0.001	0.001
	Time within 40 m	$F_{2,21} = 20.96$	<0.001	<0.001
Pitch/duration	No. of whoots	$F_{2,24} = 8.81$	0.001	0.006
	No. of k'tucs	$F_{2,24} = 3.04$	0.07	0.07
	No. of flights	$F_{2,23} = 7.26$	0.004	0.01
	Time in air	$F_{2,23} = 5.33$	0.01	0.03
	Time within 5 m	$F_{2,23} = 9.44$	0.001	0.005
	Time within 20 m	$F_{2,23} = 18.16$	<0.001	<0.001
	Time within 40 m	$F_{2,23} = 12.27$	<0.001	0.0012

P values adjusted using sequential Bonferroni procedure.

Statistical analysis

When quantifying the responses to the different stimuli, we followed a many rather than a multivariate-measure approach (sensu McGregor 1991), as different aspects of the response behavior might reflect different motivations underlying the response (e.g., aggression vs. fear, cf. de Kort et al. 2009). Furthermore, the criterion of normality which has to be fulfilled to conduct a multivariate-measure approach was not met by most of our response measures. To conduct principal component analyses on 7 acoustic parameters of the lure playbacks, we used SPSS 15.0. To test for differences in response measures between different playback periods, we used GLMMs (general linear mixed-effect models) implemented in R2.10.1 (http://www.r-project.org/) and the add-on package nlme (Pinheiro et al. 2005). To test for global differences in response measures between preplayback, test 1, and test 2, we first fitted full models where the dependent variable was the relevant response measure (# whoots; # k'tucs; # flights; time in air; time spent within 5, 20, and 40 m; latency to sing; and latency to approach); the predictors were the order of test stimulus presentation (TSP) and the first principle component of acoustic parameters of the lure playback (PC1AC). Subject identity was a random factor. TSP and PC1AC were not significant in all cases (adjusted $P > 0.3$) and were therefore removed from the models. To correct for multiple testing, we adjusted P values of this global tests using the sequential Bonferroni procedure. Furthermore, for those GLMMs that revealed a significant difference we used post hoc Tukeys contrasts for multiple comparisons (multcomp package) which provided P values adjusted by the single-step method. To test for differences in latencies to approach and latencies to sing between the playback period test 1 and test 2 (where no sufficient data were available for the preplayback period) we used one sample t-tests.

RESULTS

Responses to composition playbacks

Females generally responded to the playback: There was a significant difference in 5 of 7 response measures between the preplayback period and the playback of the 2 test stimuli (Table 1). Values for these 5 measures were lower during the preplayback period than during the playback of the 2 test
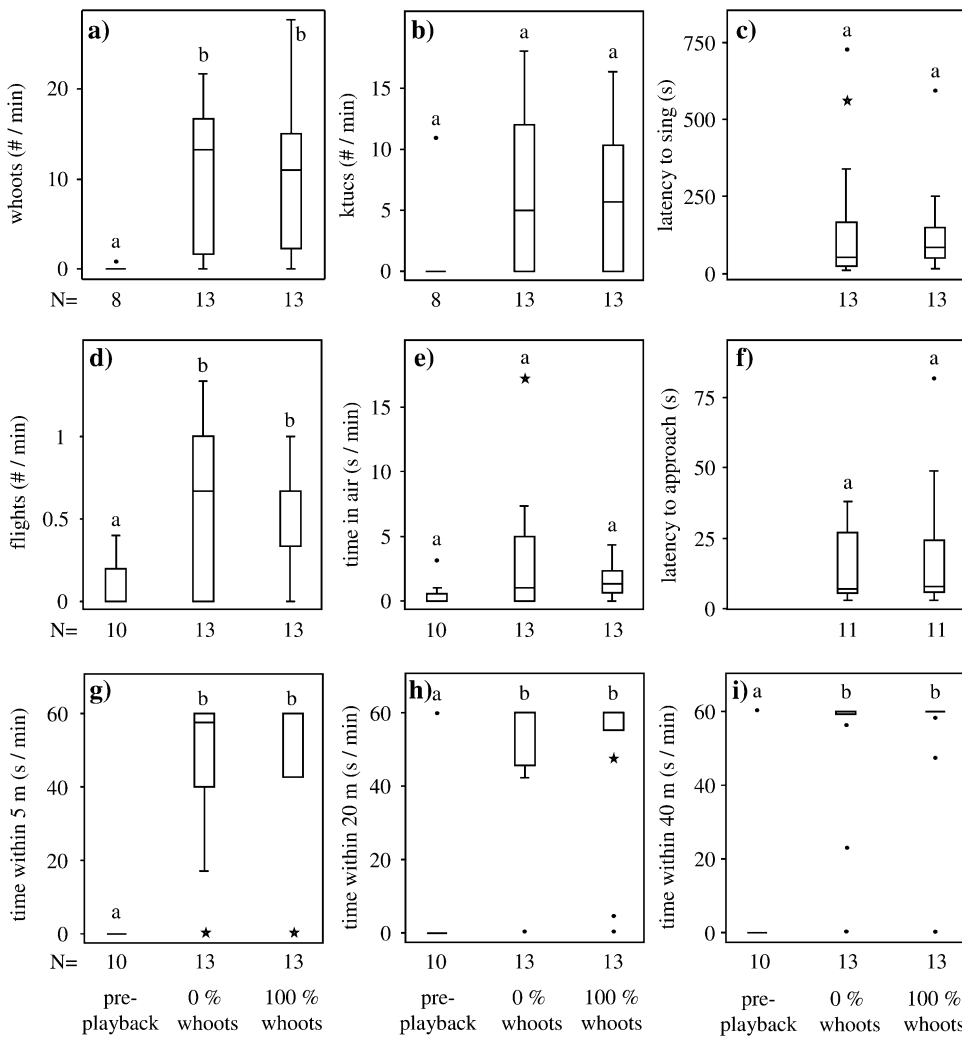
Figure 3
Response measures of 13 females for the preplayback period and the test stimuli that varied in the proportion of whoots (composition playbacks): (a) number of whoots per min, (b) number of k'tucs per minute, (c) latency to sing, (d) number of flights per minute, (e) seconds in air per minute, (f) latency to approach, (g) time spent within 5 m of the loudspeaker, (h) time spent within 20 m of the loudspeaker, (i) time spent within 40 m of the loudspeaker. Boxes indicate the interquartile range (IQR), black horizontal bars indicate medians, asterisks represent values within 1.5 times the IQR from the end of the box, and circles represent values within 3 times the IQR from the end of the box. Note that during the preplayback period, only a few females approached or sang; therefore, latencies are not indicated. Different lower case letters indicate significant differences (N is indicated below box plots, it may deviate from 13 because of missing values due to females not moving or being out of sight; for tests statistics, see Tables 1–3).

stimuli (Figure 3 and Table 2). Neither the order in which test stimuli were presented nor the first principal component of the lure playbacks had a significant effect; these factors were therefore removed from the final models. The crucial test of our hypothesis was the comparison of responses between different test stimuli: For those response measures where the global test indicated a significant difference, females did not respond differently to the 2 types of test stimuli that varied in the proportion of whoots (Figure 3 and Table 3).

Responses to pitch/duration playbacks

Females generally responded to the playback: There was a significant difference in 6 of 7 response measures between the preplayback period and the playback of the 2 test stimuli and a trend for the remaining response measure (Table 1). Overall, values for response measures were lower during the preplayback period than during the playback of the 2 test stimuli (Figure 4 and Table 2). Neither the order in which test stimuli were presented nor the first principal component of the lure playbacks had a significant effect; these factors were therefore removed from the final models. The crucial test of our hypothesis was the comparison of responses between different test stimuli: It took females longer to approach the loudspeaker when low & long than when normal stimuli were broadcast, they were less likely to approach closer than 5 m, and there was also a trend for them to spend less time within

20 m of the loudspeaker (Figure 4 and Table 3). Otherwise, the females' responses to low & long stimuli were as strong as to the normal stimuli (Figure 4 and Table 3). Thus, females responded strongly to both types of stimuli, but they did not approach the loudspeaker as quickly and as closely when the low & long stimuli were broadcast in comparison to when normal stimuli were broadcast.

DISCUSSION

Territorial female black coucals responded to the playback of conspecific song that was broadcast from the center of their own territory and was simulating a territorial intrusion: When exposed to stimulus songs containing either only whoots or only k'tucs, they vocalized more and spent more time close to the speaker than during the preplayback period. When exposed to stimulus songs with normal pitch and duration of elements or stimulus songs with low pitch and long duration of elements, females vocalized more, flew more often, and spent more time flying and more time in the vicinity of the speaker than during the preplayback period. This comparison with the preplayback period demonstrated that the playback was effective in simulating a territorial intrusion, as it elicited responses in female black coucals that in studies on male birds are considered typical territorial reactions (review in McGregor 1991).

Table 2

Test statistics and parameter estimates of post hoc comparisons using Tukey contrasts for differences in response variables during the periods: preplayback and either test 1 or test 2

Playback	Period compared	Response measure	Parameter estimate	Standard error	z Value	Adjusted P
Composition	0 % whoots versus preplayback	No. of whoots	10.78	2.79	3.87	<0.001
		No. of flights	0.49	0.16	3.00	0.008
		Time spent within 5 m	44.63	7.07	6.31	<0.001
		Time spent within 20 m	35.95	7.96	4.52	<0.001
		Time spent within 40 m	39.22	7.01	5.60	<0.001
	100 % whoots versus preplayback	No. of whoots	10.55	2.79	3.79	<0.001
		No. of flights	0.46	0.16	2.84	0.01
		Time spent within 5 m	44.42	7.07	6.28	<0.001
		Time spent within 20 m	37.64	7.96	4.73	<0.001
		Time spent within 40 m	41.32	7.01	5.89	<0.001
Pitch/duration	Normal versus preplayback	No. of whoots	11.03	2.67	4.14	<0.001
		No. of flights	0.64	0.17	3.76	<0.001
		Time in air	4.33	1.34	3.22	0.004
		Time spent within 5 m	27.82	6.65	4.19	<0.001
		Time spent within 20 m	48.01	8.06	5.96	<0.001
		Time spent within 40 m	39.20	8.37	4.68	<0.001
	Low & long versus preplayback	No. of whoots	7.53	2.67	2.83	0.01
		No. of flights	0.43	0.17	2.54	0.03
		Time in air	1.66	1.32	1.26	0.42
		Time spent within 5 m	8.17	6.50	1.26	0.42
		Time spent within 20 m	31.26	7.89	3.96	<0.001
		Time spent within 40 m	31.68	8.20	3.86	<0.001

P values adjusted using single-step method.

We were interested in the question whether female black coucals would pay attention to vocal parameters that we have previously shown to covary with the context: When experimentally challenged by a playback, females altered their vocalizations by increasing the proportion of whoots, by lowering the pitch, and by enhancing the duration of song elements. We also showed that when females were challenged, the song characteristics correlated with their body size (Geberzahn et al. 2009), and hence expected that a signal that mimicked a larger and/or more aggressively motivated individual would be perceived as more threatening.

When confronted to songs varying in the composition of whoots and k'tucs (100% whoots vs. 0% whoots), females responded equally strong to both types of stimuli, that is, their vocal and locomotor behavior was of similar intensity, suggesting that different proportions of whoots did not correspond to different levels of territorial threat. An alternative explanation is that ceiling effects might have obscured differences in responses as we have placed the loudspeaker in the center of a territory. Experiments with loudspeakers placed just outside of the territorial border would be necessary to rule out this possibility. Also, we cannot exclude that some effects might

Table 3

Test statistics and parameter estimates of post hoc comparisons using Tukey contrasts for differences in response variables during the playback periods test 1 and test 2

Playback	Compared playback period	Response measure	Parameter estimate	Standard error	z Value	Adjusted P
Composition	0% versus 100% whoots	No. of whoots	−0.23	2.37	−0.10	0.99
		No. of flights	−0.03	0.15	−0.17	0.98
		Time spent within 5 m	−0.21	6.48	−0.03	1.00
		Time spent within 20 m	1.69	7.30	0.23	0.97
		Time spent within 40 m	2.10	6.44	0.33	0.94
		Latency to sing[a]	28.92	42.71	0.68	0.51
		Latency to approach[a]	−9.78	6.31	−1.55	0.32
Pitch/duration	Normal versus low & long	No. of whoots	3.50	2.56	1.37	0.36
		No. of flights	0.22	0.16	1.31	0.39
		Time in air	2.67	1.29	2.07	0.10
		Time spent within 5 m	19.65	6.34	3.10	0.006
		Time spent within 20 m	16.76	7.71	2.18	0.08
		Time spent within 40 m	7.52	8.01	0.94	0.62
		Latency to sing[a]	−62.45	47.23	−1.32	0.22
		Latency to approach[a]	−57.60	20.87	−2.76	0.04

P values adjusted using single-step method.
[a] Latencies analyzed using one sample *t*-tests (*t* values reported instead of *z* values, *P* values adjusted using sequential Bonferroni procedure).
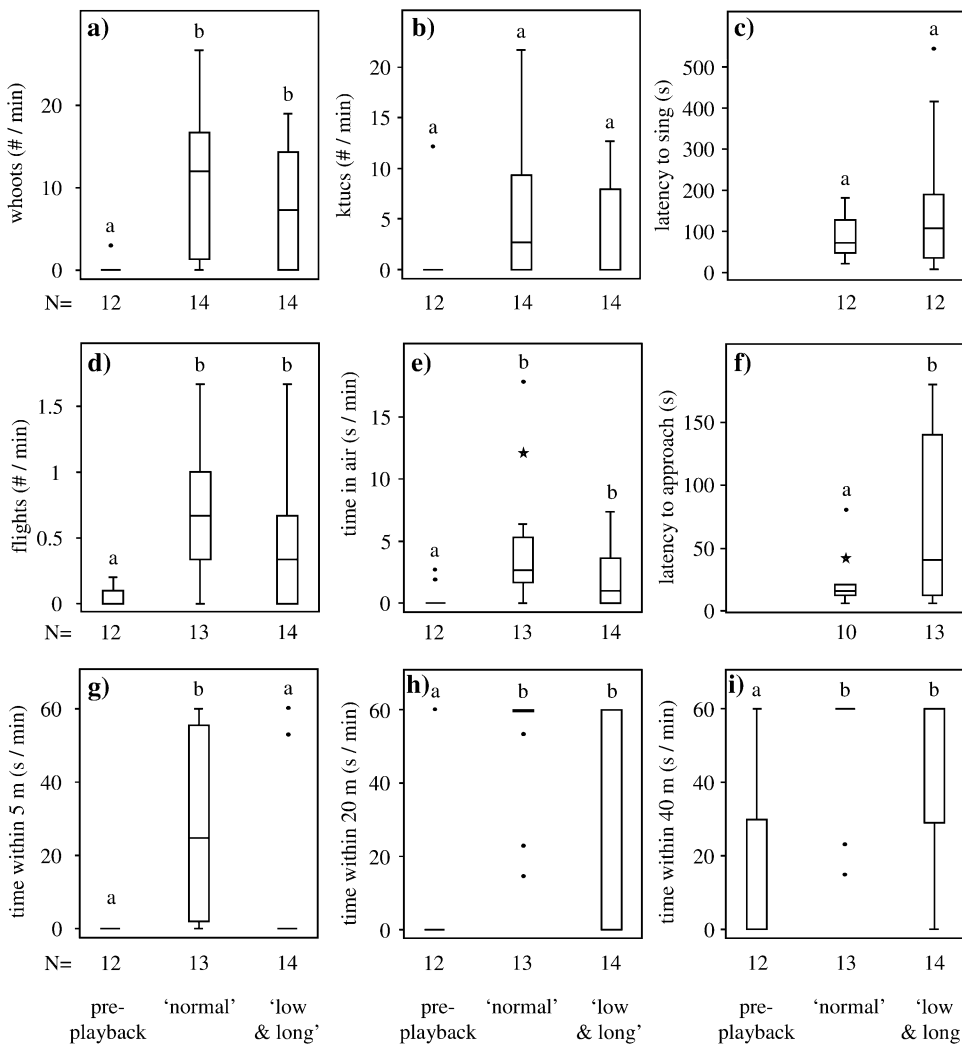
Figure 4
Response measures of 14 females for the preplayback period and the test stimuli that varied in the pitch and duration of song elements (pitch/duration playbacks): (a) number of whoots per minute, (b) number of k'tucs per minute (c) latency to sing, (d) number of flights per minute, (e) seconds in air per minute, (f) latency to approach, (g) time spent within 5 m of the loudspeaker, (h) time spent within 20 m of the loudspeaker, and (i) time spent within 40 m of the loudspeaker. Boxes indicate the interquartile range (IQR), black horizontal bars indicate medians, asterisks represent values within 1.5 times the IQR from the end of the box, and circles represent values within 3 times the IQR from the end of the box. Note that during the preplayback period, only a few females approached or sang; therefore, latencies are not indicated. Different lower case letters indicate significant differences (N is indicated below box plots, it may deviate from 14 because of missing values due to females not moving or being out of sight; for tests statistics, see Tables 1–3).

have been obtained with a larger sample size. However, when confronted to songs that vary in pitch and duration of song elements, females' approach behavior was different: It took them longer to approach the loudspeaker when low & long stimuli were broadcast, and they were less likely to spend time close to the speaker. Otherwise, their responses were equally strong to both types of stimuli, that is, their vocal and locomotor behavior was of similar intensity, suggesting that both stimuli were effective as territorial threats. Our findings suggest that females may not have approached as quickly and as closely when the low & long song stimuli were broadcasted because they perceived them as a greater threat.

Vocally, our subjects responded equally strong to the different types of test stimuli, that is, they produced a similar number of vocalizations. Like female black coucals, male green frogs (*Rana clamitans*) lower the pitch of their voice during simulated territorial intrusions (Bee and Perrill 1996). However, in contrast to black coucals, green frogs differentiated their vocal responses depending on the pitch of the calls that simulated either a large or a small competitor (Bee et al. 1999). The fact that vocally female black coucals responded equally strong irrespective of the type of stimulus suggests that their vocal response is not conditional on the perceived level of threat.

The low & long song elements were transformed digitally in pitch and duration during the stimulus preparation. In contrast, in normal songs, elements were not transformed in pitch

and duration. The transformation in pitch and duration potentially may have resulted in subjects not recognizing the stimulus as conspecific song and accordingly to respond less intensely toward it. Whereas we cannot completely rule out this possibility, it seems an unlikely explanation: Vocally, female black coucals responded equally strong to the transformed and to the untransformed stimuli as there were no differences in the amount of songs and in the latency to sing. The same is true for their locomotor activity (flying) and the time spent within 40 m of the loudspeaker. Also, it should be kept in mind that all test stimuli were digitally edited (band or high-passed filtered) and artificially constructed, and this manipulation did not keep the subjects from responding to the stimuli. Furthermore, the parameter range of the transformed stimuli stayed within the natural range of the species. Thus, we are confident that interpreting the differences in responses of females to normal and low & long stimuli as a sign of intimidation is the more likely explanation. Such an interpretation is in line with findings on male birds as well as male toads and frogs: For example, male territorial scops owls (*Otus scops*) exposed to playbacks responded less intensely, that is, they displayed less, when they heard low-pitched hoots simulating heavier intruders (Hardouin et al. 2007), male red-winged blackbirds (*Agelaius phoeniceus*) displayed less, when exposed to high-performance trills, a trait reflecting vocal proficiency (Cramer and Price 2007), and banded wrens (*Thryophilus pleurostictus*) were less likely to approach

high-performance stimuli, reflecting more competitive singers (Illes et al. 2006; de Kort et al. 2009). Male toads and frogs of several species were more likely to respond strongly when confronted with high-pitched calls simulating small competitors, whereas they were more likely to retreat when confronted to low-pitched calls simulating large competitors (Davies and Halliday, 1978; Arak, 1983; Wagner, 1989). In all of those studies, the intimidating signal seems to reliably reflect competitive abilities, as performance limits are reached, with high trill rates constrained by sound production mechanisms and low-frequency sounds constrained by body size. Although in black coucals, the underlying mechanisms are not yet understood, it is likely that the production of low-frequency sounds is constrained by body size as well: When females were territorially challenged, the frequency of their k'tuc elements showed a negative correlation with body size. In contrast, such a correlation could not be detected in spontaneously singing females (Geberzahn et al. 2009). Thus, perhaps the fact that females go to a performance limit reveals to the receiver not only their body size but also their motivation to fight.

We showed that the variation in pitch/duration parameters conveyed a signal to the females, whereas different proportions of whoots seemed not to signal different levels of territorial threat. This raises the question, why then female black coucal alter the proportion of whoots if such variation is not meaningful to the rival. Whoots are usually produced at lower amplitude than k'tucs (e.g., see Figure 1 in Geberzahn et al. 2009), and k'tucs can usually be heard from a further distance of the singing female than whoots (Goymann W, Geberzahn N, personal observation). Thus, one possibility is that by increasing the proportion of whoots females may aim at reducing the active space of vocal communication to avoid neighbors to eavesdrop on their vocal interaction. This hypothesis was originally proposed to explain why many songbirds use soft song during close-range escalated agonistic interactions (e.g., Dabelsteen 2004). Whether this might play a role in vocal interactions of female black coucals could be tested in future studies.

In conclusion, we could demonstrate that female black coucals paid attention to acoustic properties in their song that were likely indicators of competitive abilities. Vocal territorial interactions in female birds of sex-role reversed species seem to follow the same rules as in male birds with typical sex roles: Signals that are produced at physical limits are perceived as the strongest threat. Our study helps confirming the general validity of hypotheses on the territorial function of song in the competing sex, be it males or females.

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