Song and the city : a comparison between urban and forest blackbirds

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Do blackbirds signal motivation to fight with their song?

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Conflicts about resources can be solved with physical fights, but animals will in general try to avoid them due to the costs and risks associated with fighting. Fight outcome depends on the fighting ability as well as the motivation to fight, which means that individuals should try to assess both during a pre-escalation phase. In the present study, we investigated whether blackbirds (*Turdus merula*) acoustically signal their motivation to fight with their advertisement song, which consists of a motif part with pure-toned low frequency elements followed by a twitter part with more complex and higher-pitched elements. We tested (1) whether the temporal and spectral characteristics of spontaneous songs broadcasted by a territorial male could predict its subsequent aggressive behaviour to a playback and (2) if the song of territorial males changed in case they responded aggressively to the playback. We found no effects in the temporal song structure, which contradicts results from a previous study involving playback experiments with blackbirds. We did however find an interesting pattern in the spectral characteristics of the twitter, since aggressively responding males significantly increased the twitter frequency after the playback. The post-playback songs of aggressive responders were nevertheless indistinguishable from spontaneous pre-playback songs of non-aggressive responders. Therefore, we conclude that changes in twitter frequency, rather than absolute twitter frequencies, are advertising information about motivation to fight.

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Introduction

Competition amongst conspecifics is common in the animal kingdom, because valuable resources such as food and partners are often limited. Conflicts over access to resources can be solved with physical fights although most animals try to avoid them. This is because both for the winner and the loser there are costs involved with fighting in terms of time and energy as well as the risk of getting injured. Competitors can make a decision about whether or not to engage in a physical fight during a pre-escalation phase by assessing their opponents fighting ability. An assessment of fighting ability is in most cases based on signals that are correlated with body size or body condition (e.g. Clutton-Brock & Albon 1979; Enquist & Jakobsson 1986; Panhuis & Wilkinson 1999).

However, fighting ability alone does not always correctly predict the outcome of a fight, since motivation to fight can also play an important role. Motivation to fight can differ between animals due to an asymmetry between opponents in the potential fitness benefits of winning a conflict, which may be caused by differences in for example age, hormone levels or social status (Dzieweczynski *et al.* 2005; Elwood *et al.* 1998; Kotiaho *et al.* 1999). Animals in conflict should therefore not only attempt to assess the fighting ability, but also the motivation to fight of their opponents, as both factors can influence the fight outcome.

Many studies have addressed whether and how fighting ability is encoded in acoustic signals (e.g. ten Cate *et al.* 2002), but far fewer have explored motivational signals. Nevertheless, in birds it is known that the amount of song matching and overlapping can play an important role in signalling motivation to escalate into a fight (e.g. Dabelsteen *et al.* 1997; Naguib & Kipper 2006; Vehrencamp 2001). Furthermore, intra-individual variation in temporal and spectral characteristics of song can also carry such a motivational message. For example, more aggressive behaviours are preluded by songs of relatively few notes in bobolinks (*Dolichonyx oryzivorus*: Capp & Searcy 1991) and by song components of relatively long duration in hoopoe (*Upupa epops*: Martin-Vivaldi *et al.* 2004), great tits (*Parus major*: McGregor & Horn 1992), and pigeon guillemot (*Cepphus columba*: Nelson 1984), while in the latter species a relatively high minimum frequency also predicts a subsequent attack (Nelson 1984).

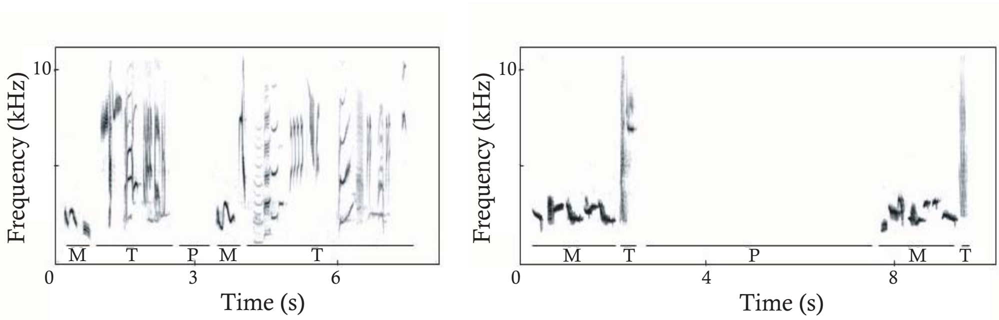

Figure 3.1. Spectrograms of blackbird song from the extreme ends of a continuum to illustrate the large variation in temporal structure. On the left an example of blackbird song with relatively short motifs (M), long twitters (T) and a short pause (P), and on the right an example of blackbird song with relatively long motifs, short twitters and a long pause.

One of the most advanced model systems in the context of motivational signalling seems to be the Eurasian blackbird (*Turdus merula*) (Dabelsteen 1984a; Dabelsteen & Pedersen 1990). This species is known to signal their motivation to fight by using two acoustically distinct types of song. "Strangled song" is a short-distance signal of high aggressive arousal, accompanied by visual threat displays, and often directly preceding physical attack. It consists of high frequency elements, produced in bouts of irregular duration, and is of distinct low amplitude. In contrast, the "regular advertisement song" is a loud, long-distance signal, potentially audible from more than a hundred metres away. It has a stereotypic song structure in which a motif part consisting of pure-toned elements of relatively low frequency is followed by a twitter part with more complex and higher-pitched elements (Dabelsteen & Pedersen 1985; Todt 1970a). There is large variation within individuals in the relative duration of the motif and twitter parts as well as in the pause duration between these advertisement songs (Figure 3.1), which has been suggested to reflect the motivational state of an individual from low to moderately aggressive (Dabelsteen 1984a). Territorial male blackbirds respond more aggressively to songs with a high proportion of twitter and short pauses, typical for songs sung in the morning hours with most territorial interactions (Dabelsteen & Pedersen 1990). This indicates that the temporal variation in song produced by an intruder influences the aggressive response of a territorial male, but it does not show whether male blackbirds singing in their own territory signal their own motivation to fight in a similar way.

In the current study, we aimed at testing the hypothesis that the structure of the advertisement song of Eurasian blackbirds indeed acts as a signal of their motivation to fight. We conducted experiments in which we tested if temporal song characteristics predict a male's aggressive response to subsequent playback. We also tested whether temporal characteristics change within individuals as a result of the playback-induced aggressive response. Furthermore, we explored whether spectral characteristics of the motif or twitter part differ in either of these two comparisons. Based on the pioneer studies on this system (Dabelsteen 1984a; Dabelsteen & Pedersen 1990), we expected that males are more likely to react aggressively when they produce songs with a relatively large proportion of twitter and short pauses between songs, and that an aggressive response causes an increase in the proportion of twitter and a decrease in the pause duration. We had no explicit expectations for spectral differences in these comparisons.

Material & Methods

Experimental design

We tested the possibility that acoustic variation within individuals in the advertisement songs serves as a signal of motivation to fight in two ways. Firstly, we recorded songs of spontaneously singing blackbirds (i.e. not stimulated by playback) and compared the song characteristics of those that responded aggressively to a subsequent playback to those that did not. We defined aggressive responders based on whether they performed strangled song or not during the playback. Strangled song can be easily distinguished from regular advertisement song as it has a much lower amplitude, an irregular temporal structure, only high frequency elements and it is frequently accompanied with visual threat signals (e.g. bow posture, tail up posture) (Dabelsteen 1981; Dabelsteen & Pedersen 1990; Snow 1958; personal observations). Secondly, we recorded songs of the aggressive responders directly after their agonistic response and compared these with their own songs before the playback to see if a change in agonistic behaviour was reflected in their songs. As a control we also compared the songs of non-aggressive responders before and after playback.

Playback design

Playback experiments were conducted in the morning between 0600 and 1030 hours and in the evening between 2030 and 2230 hours in the period from 25 May to 27 June 2005 in the urban area between Leiden and Leidschendam (52°15N, 4°45E). Spontaneously singing territory owners were selected as the focal male in 23 playback experiments. An experiment consisted of the following three phases: (1) two minutes of audio recording before playback, (2) one minute of playback and (3) a period of audio recording that continued until the focal male had sang another two minutes after the playback had stopped. Recordings were made with a Sennheiser ME67/K6 directional microphone attached to a Marantz PMD670 digital recorder.

Stimulus songs

The stimulus songs were collected from 23 different males between 18 March and 16 of May 2005 in the same area where the playback experiments were conducted. Recordings were high-pass filtered at 1 kHz and normalised in Avisoft-SAS Lab-Pro version 4.3, resulting in 23 playback recordings consisting of a variable number of stimulus songs (mean ± SD = 9.2 ± 2.7) with a high signal to noise ratio and equal peak amplitudes. Each set of stimulus songs was used only once. Focal males were never confronted with stimulus songs of their own or those of direct neighbours.

The stimulus songs were played back with a remote-controlled Sony CDX-S2000 player connected to a Blaupunkt CB4500 100W speaker. At the start of a playback experiment these were placed inside a territory at a distance of approximately 10 to 12 metres from the singing focal male, thereby minimising the effect the position of the loudspeaker might have on a male's response (i.e. the centre edge-effect: e.g. Giraldeau & Ydenberg 1987). The amplitude of playback was ~74 dB(A) as measured with a Pulsar Model 30 SPL meter at 1 meter from the speaker using a 1 second averaging window.

Song analysis

From each test bird we analysed 15 songs that were sung directly before the playback started as well as the first 15 songs that were produced after the playback had finished. Songs were analysed in Avisoft-SAS Lab-Pro version 4.3 (Avisoft Bioacoustics, Berlin, Germany). The sampling rate of the recordings was 48 kHz. We used a Fast Fourier Transformation size of 512 with a Hamming window type and a temporal overlap of 50 %, which led to a frequency resolution of 94 Hz and a temporal resolution of 5.3 ms for each measurement. The following three temporal characteristics were measured for each song: motif, twitter and pause duration (Figure 3.1). The proportion of twitter per song was calculated from the motif and twitter duration. There were also six spectral characteristics measured: Peak, minimum and maximum frequency of both the motif and twitter part. The peak frequency was the frequency with the loudest amplitude and the minimum and maximum frequencies were defined as the lowest and highest frequency within 15 dB of the peak frequency respectively.

Statistical analysis

Statistical analyses were performed in R version 2.4.1 (Ihaka & Gentleman 1996). Linear mixed models (LMM) were used, since this made it possible to account for the intra-individual variation by entering the variable "individual" as a random effect. Each song parameter was tested separately as a dependent variable in a linear mixed model. The variable "reaction" (aggressive vs. non-aggressive) was included as a fixed parameter in models assessing whether song characteristics differed between aggressive and non-aggressive responders. Similarly, the variable "phase" (pre- or post playback) was entered as a fixed effect to determine if the song of males differed as a result of the stimulation by playback. The latter model was done for aggressive and non-aggressive responders separately, whereby the group of non-aggressives was meant as a control.

A principal component analysis was performed on all spectral song characteristics to reduce the number of variables, since this would decrease the probability of a type I error by lowering the number of statistical comparisons (Rice 1989). We tested the two temporal parameters directly, because previous studies have explicitly suggested that the proportion of twitter in a song and the pause duration signal a male's motivation to fight (Dabelsteen 1984a; Dabelsteen & Pedersen 1990). The principal component analysis was done with a Varimax rotation and resulted in two factors explaining 56.9% of the variance in the dataset on spectral characteristics. The first principal component (PC1) had high loadings for the variables of the twitter part and low loadings for the variables of the motif part (Table 3.1). For the second principal component (PC2) this was the other way around (Table 3.1). Therefore a higher value for PC1 reflects a higher frequency of the twitter and a higher value for PC2 reflects a higher frequency of the motif.

Table 3.1. Loading values from a principal component analysis on the spectral variables. The first and second principal component explained respectively 29.8 and 27.1% of the total variance in the dataset.

		Principal components	
	Spectral variables	1	2
Motif part	Minimum frequency	0.06	0.83
	Peak frequency	-0.03	0.67
	Maximum frequency	0.15	0.69
Twitter part	Minimum frequency	0.89	0.07
	Peak frequency	0.77	0.03
	Maximum frequency	0.61	0.07

Results

Behavioural response

We classified the 23 males into two groups based on the production of strangled song after the start of the playback phase. The first group consisted of nine males that did not sing any strangled song after the playback. They will be referred to as the non-aggressive responders. In the second group, the aggressive responders, there were 12 males that sang one or more strangled songs (mean ± SD = 7.9 ± 10.9) within one minute after the playback had stopped. Two males were not included in any of the groups, because they produced their first strangled song 3.5 and 5.5 minutes after the playback had finished and therefore it was unclear whether the strangled songs were still a reaction to the playback. None of the blackbirds sang a strangled song during the two minutes before the playback. Non-aggressive responders resumed producing normal advertisement song shortly after the playback (mean ± SD = 53.8 ± 84.6 s) and aggressive responders did the same after their last strangled song (mean ± SD = 38.8 ± 88.6 s).

Song features predicting an aggressive response

Although aggressive responders had as predicted on average shorter pauses and larger twitter proportions than non-aggressive responders before playback, these average differences were marginal in comparison to the large variation in these parameters and hence, no significant differences in temporal characteristics were detected (LMM: proportion of twitter; $F_{1,19}$ = 1.17, p = 0.43, Figure 3.2a and pause duration; $F_{1,19}$ = 1.17, p = 0.29, Figure 3.2b). Similarly, PC1 and PC2 of aggressive and non-aggressive responders did not differ in the pre-playback phase (LMM: PC1; $F_{1,19}$ = 2.29, p = 0.15, Figure 3.2c and PC2; $F_{1,19}$ = 0.05, p = 0.83, Figure 3d). The stimulus songs played to aggressive and non-aggressive responders did not differ in any of the measured song characteristics (LMM: $F_{1,19}$, p > 0.1).

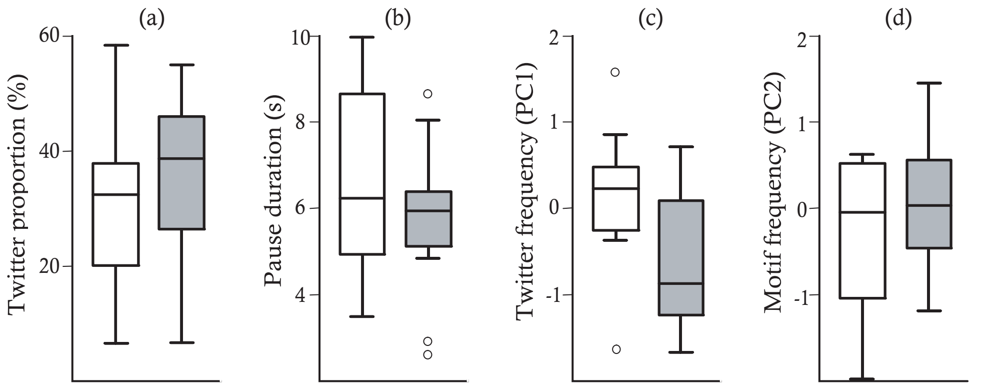

Figure 3.2. Comparison of song characteristics before playback of non-aggressive responders (white bars, N = 9) and aggressive responders (grey bars, N = 12). The boxplots show the median (line), interquartile range (box), 95% range (whiskers) and outliers (circles) for the (a) proportion of twitter (b) pause duration, (c) frequency of the twitter (PC1) and (d) frequency of the motif (PC2).

Song changes after an aggressive response

We compared the song of aggressive responders before and after playback to investigate if their songs changed during an aggressive response. As predicted, aggressive responders had on average shorter pauses and larger twitter proportions after playback, but again the average differences were not significant considering the large variation present in these parameters (LMM: proportion of twitter; $F_{1,10} = 0.14$, $p = 0.71$, Figure 3.3a and pause duration; $F_{1,10} = 1.57$, $p = 0.21$, Figure 3.3b). The PC2 did also not change (LMM: $F_{1,10} = 1.44$, $p = 0.23$, Figure 3.3d), but there was a highly significant increase in PC1 after playback (LMM: $F_{1,10} = 17.52$, $p < 0.0001$, Figure 3.3c). The change of PC1 was also apparent in two of the six separate variables; the minimum- and peak frequency of the twitter significantly increased after the playback with 0.45, and 0.62 Khz, respectively (LMM: minimum frequency twitter; $F_{1.10} = 19.0$, $p < 0.0001$ and peak frequency twitter; $F_{1,10} = 13.46$, $p < 0.0001$). In the control group of non-aggressive responders none of the song characteristics differed between the period before and after playback (LMM: $F_{1,7}$, $p > 0.1$). Furthermore, there was no significant difference between the PC1 of the aggressive responders after playback and the non-aggressive responders before playback (LMM: $F_{1,19} = 1.58$, $p = 0.22$).

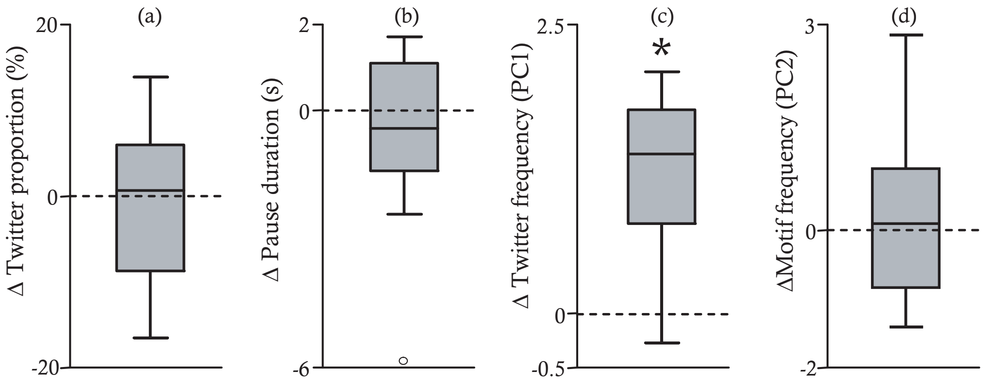

Figure 3.3. Comparison of song after playback of 12 aggressive responders relative to their own songs before playback. Dotted lines represent individual values before playback. The boxplots show the median (line), interquartile range (box), 95% range (whiskers) and outliers (circles) for the difference in (a) proportion of twitter (b) pause duration, (c) twitter frequency (PC1) and (d) motif frequency (PC2). * indicates $p < 0.05$.

Discussion

We tested a hypothesis originally postulated by Dabelsteen (1984a) in which temporal features in blackbird song were put forward to signal motivation to fight. We found no differences in either the proportion of twitter or the pause duration between blackbirds that responded aggressively to a playback and those that did not. Furthermore, the proportion of twitter and the pause duration did not change in response to playback. In contrast, the comparison of spectral characteristics revealed an interesting pattern in particular for the twitter part. Aggressive responders to playback showed a highly significant increase in the frequency of the twitter, which was most apparent in the minimum and peak frequency of the twitter.

Playback experimental design

Although we were inspired by the pioneer studies of Dabelsteen (1984a) and Dabelsteen & Pedersen (1990), the lack of a relationship between the temporal structure and the aggressive response to playback in the current study is contradicting the conclusions from their earlier playbacks with blackbirds. Dabelsteen & Pedersen (1990) investigated the aggressive reaction of territorial males to playback of two stimulus variants and found stronger responses to song with a relatively long twitter proportion and relatively short pause duration. They interpreted these findings as a confirmation of the hypothesis that blackbirds signal their motivation to fight by modifying the temporal structure of their songs. However, although a stronger response to a more aggressive opponent may be in line with the expectations, it is an indirect way to infer intentions of the sender of a signal by analysing the behaviour of the receiver of a signal. Our study investigated the behaviour of the sender following its broadcasting of song, but our findings do not support the hypothesis of a signal on motivation to fight in temporal song features.

Not only the distinction between sender and receiver is important, but also the distinction between resident and intruder seems relevant to reconsider in the context of comparing current and previous playback results. Dabelsteen & Pedersen (1990) investigated the signal of a simulated intruder, while we analysed the signal of a resident blackbird. Male singing behaviour is commonly used to defend a territory against neighbours and non-territorial floaters, but not typically to take over a territory. Playback results are therefore more likely to reflect natural signalling conditions when investigating the relationship between a territory owner's song and its own aggressive response to a subsequent playback rather than when investigating the relationship between an intruder's song and the response of a territory owner (c.f. Searcy & Nowicki 2000). In addition, signals of residents and intruders are especially difficult to compare with respect to their motivation to escalate into a fight. Territory owners generally defeat intruders due to the asymmetry in the cost and benefits of winning or loosing the territory (Johnsson & Forser 2002; Krebs 1982; Mathis *et al.* 2000), which presumably affects their motivation to fight. This discrepancy between territory owners and intruders may confound the relationship between acoustic features and intra-individual variation in the relative willingness to escalate a fight.

Considering the above, it is not surprising that the current and previous playback experiments with blackbirds have resulted in different findings concerning a possible relationship between the temporal song structure and motivation to fight. Our playback experiment addressed the topic of vocal signalling of motivation to fight more directly and reflected a more natural song context. Therefore, we conclude that it is unlikely that the temporal song structure of blackbirds contains a message about the motivation to fight, as despite large variation between individuals in both the twitter proportion and pause duration there was no indication that this variation was related to either the likelihood of escalating into a fight or to changes in motivational state.

Signalling aggressiveness via twitter frequency

An important finding of the current study is that aggressive responders sang with a significantly increased frequency of the twitter part after the playback. This shows that the twitter frequency in blackbirds differs with a change in social context and consequently potentially with a change in motivational state.

Playback experiments generally provoke an aggressive response in territorial male birds (see Searcy & Nowicki 2000; ten Cate *et al.* 2002), including blackbirds (Dabelsteen 1982; Dabelsteen 1984b; Dabelsteen & Pedersen 1990). Additional evidence that the response to an intruder or a territorial dispute may increase the motivation to fight in birds is provided by endocrinological studies. For example, territorial male song sparrows (*Melospiza melodia*) were removed from their territory after which their territories were reoccupied within 12-72 hours. In the replacement males as well as their neighbours the plasma levels of testosterone were significantly elevated (Wingfield 1985). This indicates that agonistic interactions or other behavioural stimuli from challenging males stimulates the secretion of testosterone, a hormone that is related to aggressive territorial behaviour in many species (Balthazart 1983; Harding 1983; Oliveira 1998). Moreover, a study on red-winged blackbirds (*Agelaius phoenecius*) demonstrated that hormones related to aggressive behaviour can fluctuate within minutes, since males caught during a short aggressive interaction, which was triggered by placing a caged male accompanied with playback in their territory, already had altered hormone concentrations compared to foraging males (Harding & Follett 1979). Considering the above, a change in motivational state in blackbirds from before to after playback would most likely concern an increase in aggressiveness. The increase in frequency of the twitter by aggressive responders due to stimulation by playback therefore probably reflects an elevated motivation to fight.

The frequency of the twitter was not significantly different between post-playback songs of aggressive responders and pre-playback songs of non-aggressive responders. This shows that a judgement of the motivation to fight should be based on changes in twitter frequencies rather than absolute values. Blackbirds might do this by assessing alterations in twitter frequency during a territorial conflict. Additionally, it is likely that neighbours recognise each others song characteristics (e.g. Stoddard 1996), maybe allowing them to decipher the motivational message encoded in the absolute twitter frequency of a familiar neighbour.

In conclusion, this study confirms that blackbirds acoustically signal about their motivational state with relatively subtle alterations in their advertisement song (c.f. Dabelsteen 1984a; Dabelsteen & Pedersen 1990). However, our data do not support the details of the original hypothesis, since we found that spectral characteristics of the twitter, rather than the temporal features, are the potential carriers of motivational information. Furthermore, it appears that changes within individuals contain a message about motivation to fight, but not absolute differences between individuals. We think blackbird singing interactions have a high potential to yield more insight in how motivation to fight can play a role in communication during animal conflicts in general.

Acknowledgments

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