Shift of song frequencies in response to masking tones

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Animal communication signals enroute from sender to receiver are often subject to interference. For example, loud ambient noise for acoustic signals, or murky media for visual signals, can reduce the ability of receivers to detect those signals, a phenomenon known as ‘masking’ (Klump 1996). Signals that minimize masking in their respective habitats have generally been favoured over evolutionary time because of their greater efficacy (Ryan & Cummings 2005). In the acoustic realm, signal frequency and timing features evolve to minimize habitat-specific degradation (acoustic adaptation hypothesis; Morton 1975; Wiley 1991). Masking interference from other vocalizing animals provides another source of selection on vocal behaviour. In chorus settings, taxa as diverse as frogs, cicadas and birds reduce masking interference by partitioning their signals in time and structure among species (Garcia-Rutledge & Narins 2001; Sueur 2008; Luther 2009), and by accentuating signal differences when ranges overlap (character displacement, e.g. Schluter 2000).

While acoustic signals are typically adapted for transmission efficacy, they can still be masked when the sound environment is dynamic or unpredictable. Two such scenarios that have received particular attention in recent years are when signalers are masked by vocalizing conspecifics (Todt & Naguib 2000) or by anthropogenic noise (Brumm & Slabbekoorn 2005). Available evidence suggests that animals faced with either scenario are indeed able to adjust their vocal output, at least to some extent. Frogs (Grafe 1996) and birds (Wasserman 1977; Popp et al. 1985) may avoid masking by conspecifics by actively alternating the timing of their signals, by switching to other signal types (if they have a signal repertoire, Catchpole & Slater 2008), or by altering spectral characteristics of their signals (Mennill & Ratcliffe 2004a). Birds faced with anthropogenic noise, such as in urban environments, appear able to avoid acoustic masking by increasing frequency (Slabbekoorn & Peet 2003; Wood & Yezierinac 2006; Hu & Cardoso 2009), increasing amplitude (Brumm & Todt 2002; Brumm 2004; Nemeth & Brumm 2010), or by altering signal timing (Fuller et al. 2007).

Most prior studies on animals’ responses to acoustic masking have been correlative. However, several recent studies on urban noise effects in birds have taken an experimental approach, presenting individuals with transient noise through loudspeakers and documenting subsequent vocal behaviour. This approach holds the promise of offering direct insights into how quickly and completely individuals may adjust to masking interference. Great tits, Parus major, played simulated city noise were found to switch to song
types with more high-frequency elements, and when played an inverse high-frequency noise, they switched to song types with more low-frequency elements (Halfwerk & Slabbekoorn 2009). Reed buntings, Emberiza schoeniclus, responded to simulated traffic noise by singing with increased minimum frequencies, a pattern not found when the subjects were played heterospecific song (Gross et al. 2010). Chiffchaffs, Phylloscopus collybita, exposed to pre-recorded traffic noise in an otherwise quiet habitat sang at higher minimum frequencies, and returned to lower frequencies after cessation of playback (Verzijden et al. 2010). Finally, captive house finches, Carpodacus mexicanus, played urban noise also shifted the frequencies of their song upwards in response (Bermudez-Cuamatzin et al. 2011). All of these species thus show some evidence of vocal plasticity in the face of transient masking noise, although much remains to be learned.

In the present study, we assessed vocal plasticity in response to masking noise in black-capped chickadees, Poecile atricapillus. This species is known to sing at higher frequencies in noisy environments (Propp et al. 2012), to transpose the starting frequencies of their songs under natural conditions (Horn et al. 1992) and to match frequencies during social interactions (Horn et al. 1992; Mennill & Ratcliffe 2004a; Foote et al. 2008). These lines of evidence suggest that chickadees are especially attentive to the structure of conspecific vocalizations and other environmental sounds, and we hypothesized that chickadees would thus also be highly responsive to the presence of masking noise. In contrast to previous experimental studies on songbirds’ responses to acoustic masking (cited above), which used broadband noise as experimental stimuli, here we present masking stimuli in the form of pure tones targeted to chickadees’ song frequencies. Broadband masking stimuli might elicit different kinds of responses than narrow-frequency masking stimuli as they are structurally similar to a portion of the chickadee’s song, a possibility that has yet to be tested. Moreover, the wealth of information already available on vocal communication and chickadee social behaviour (e.g. Smith 1991; Otter 2007) provides a useful context for interpreting data on the effects of masking. In our study, we compared birds’ reactions to targeted pure-tones and to control pure-tones that did not overlap the singers’ vocal frequencies. We predicted that, if chickadees respond to masking, then they should shift song frequencies more rapidly in the presence of masking pure-tones than in the presence of nonmasking control tones.

METHODS

Study System and Site

Black-capped chickadees are common resident birds across the central and northern extent of the U.S. and Canada. They are generalist foragers that thrive at woodland edges, and are common visitors at feeding stations (Foote et al. 2010). Both males and females produce a variety of vocalizations (Ficken et al. 1978; Smith 1991), but only males produce a two note ‘fee-bee’ song (Fig. 1), which functions in mate attraction and territory defence. Across most of their geographical range, the fee-bee song is highly stereotyped in relative note frequencies, exhibiting less than 2% variation in frequency ratios between the start and the end of the ‘fee’ note, and between the end of the ‘fee’ and the ‘bee’ note (Weisman et al. 1990; Kroodsma et al. 1999). By contrast, individual males often vary considerably in the absolute frequencies of the fee-bee song, naturally transposing the entire song such that the bee note varies up and down a continuous range of about 2700–3600 Hz (Horn et al. 1992). Males typically shift their song frequency, under normal conditions, once every 30–40 songs (Horn et al. 1992; Christie et al. 2004).

We studied populations of chickadees in western Massachusetts in state and local parks across Hampshire and Franklin County (Quabbin Reservoir 42°17′45″N, 72°19′14″W; Mount Holyoke Range State Park 42°18′27″N, 72°30′55″W; Audubon Society’s Arcadia Wildlife Sanctuary 42°17′30″N, 72°38′58″W; Mount Toby State Forest 42°29′35″N, 72°31′50″W; Amherst Brook Conservation Area 42°22′42″N, 72°28′60″W; and Groff Park 42°21′30″N, 72°31′3″W). Focal males were separated by at least 500 m to minimize the risk of recording the same individual twice (Wilson & Mennill 2011). Our research was conducted between 28 April and 5 June 2010, and between 26 April and 30 May 2011. During these months, winter flocks disperse and males establish and defend territories with song (Smith 1991). Song output peaks during the dawn chorus, which begins about 30 min before sunrise and continues for about 30 min past sunrise. During this chorus, males sing at a nearly continuous rate and often from a single perch, facilitating the localization of individual males and our ability to conduct full experimental trials.

Experimental Trials

Prior to our study, we generated a library of ‘masking’ tones to be used for playback, using the tone generator function in Audacity 1.3.12 (http://audacity.sourceforge.net). We created nine pure tones at frequencies of 2900–3700 Hz in increments of 100 Hz, corresponding to the range of natural variation in the bee portion of chickadee song. We also generated a single control tone at 5000 Hz, a frequency well above observed frequencies in chickadee songs but presumably still within their capacity to hear (e.g. Dooling 1982; Henry & Lucas 2010).

For each trial, we first located a singing male within 1 h before dawn each morning, and positioned our playback equipment within 10 m. Each trial lasted 10 min and consisted of five sequential 2 min blocks (Fig. 2), which included both a 2 min masking tone and the 2 min control tone. Focal males were recorded for 2 min, then presented either a 2 min masking tone or control tone followed by 2 min of silence, then the other tone, again followed by 2 min of silence. Presentation order of the control tone and masking tone were balanced across trials. While the bird was singing in the 2 min time block preceding the masking treatment, we inspected real-time spectrograms of the male’s song using Syrinx (Burt 2001) and a Dell Inspiron 600m laptop computer receiving input from a second Sennheiser MK 66 microphone. From these real-time spectrograms we were able to measure the frequency of the bee note of the focal bird’s songs and to select the masking tone (from the nine available) that best matched the focal bird’s bee frequency (Fig. 3). We verified the match between the tone selected and the birds own song in our trial recordings. In the
masking treatment, tones closely matched the last bee note, with an average difference of $37.36 \pm 6.18$ Hz between the masking tone and the song. We played back tones using an iPod Nano connected to a portable speaker (Radio Shack, Cat. No. 40-1434). We standardized the amplitude of tones to 80 db at 0.5 m as measured with a handheld sound level meter (Radio Shack, Cat. No. 33-2050). If a focal male stopped singing for more than 3 min during the trial, or flew from his perch such that we could no longer be certain we were recording the same male, we terminated the trial. During the entire trial, the focal bird was recorded using a Marantz PMD 660 solid-state recorder and a Sennheiser MK 66 microphone.

**Ethical Note**

Tones were played to chickadees at amplitudes approximating their natural singing levels. While this may have interfered with chickadees’ singing behaviour, the effect was likely transient and of no lasting consequence. Our methods were approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee (IACUC No. 2010-002).

### Vocal Behaviour and Analyses

From recordings of each trial, we extracted measures of vocal behaviour across the control and masking tone playback (2 min), as well as from the 2 min pretrial recording. We measured latency (s) to the first song frequency shift, the number of songs sung before the first frequency shift, song rate (songs/s), pause length in song after initiating playback (measured from tone onset up to the first song), the birds’ last singing frequency before a frequency shifted song, the direction of the first frequency shift (up or down) and the magnitude of the shift in Hz. All song measurements were estimated in RavenPro 1.3, using an FFT size of 5000 for frequency measures, corresponding to a frequency resolution of 8.8 Hz with a sampling rate at 44.1 kHz (Charif et al. 2008). We measured the frequency of each song using power spectra to determine the frequency with the greatest amplitude on the second half of the bee note for each song. If a bird did not shift frequency during the tone playback or the following 2 min, we cored its latency measure with the maximum value possible, 120 s, and we cored the total number of songs before a shift as the total number of songs during the tone.

All data were analysed in R (R Development Core Team 2012). We first checked for correlations among our variables of central interest, latency (s) to shift frequency and the number of songs before a frequency shift. We identified strong correlations in these variables (Spearman rank correlation: $r_s = 0.75$, $P < 0.0001$); therefore, we consider only latency to shift frequency in further analysis. We constructed a generalized linear model (GLM, family = quasi, link = identity) to evaluate how latency to shift song frequency varied in response to treatment group (control or masked tone), the order of presentation and the tone used. We then evaluated how other song behaviours varied by treatment type, using a MANOVA and the following response variables: the duration (s) of the pause after tone initiation, the bird’s last singing frequency before a shift, the direction of the shift (up or down) and the magnitude of the shift. We then used Friedman tests and post hoc Wilcoxon signed-ranks tests to examine how latency and song rate varied by treatment type. All means are reported $\pm 1$ SE.

### RESULTS

Although we initiated trials on 64 males, males often moved or stopped singing (as a result of the experiment or the end of the dawn chorus). Therefore, we only recorded 20 complete trials. Because we wished to restrict our results to those trials where a male received a complete treatment, we limit our data analyses to those 20 trials.

Before the onset of any playback, males shifted frequency every $26.6 \pm 7.5$ songs or every $90.62 \pm 9.47$ s, slightly below the range

**Figure 2.** Timeline of experimental trial. Focal males were recorded for 2 min without playback and for 2 min following playback of a masking tone and then the control tone (upper panel), or vice versa (lower panel). Each playback tone was followed by 2 min of silence.

**Figure 3.** Spectrograms depicting (a) the control treatment, where the playback tone was above the frequency range of the chickadee song (here an example of the male continuing to sing at the same frequency), and (b) the masking treatment, where the playback tone masked the bee portion of the last song (here an example of a rapid shift downward from a masking tone). FFT = 256.
observed for other studies (30–40 songs; Horn et al. 1992; Christie et al. 2004). Eight males shifted song frequencies during control playback, whereas 14 males shifted song frequencies during masking playback. Five of the males did not shift across either treatment.

After controlling for the order of presentation and the tone used during playbacks, latency to shift song frequency varied only in response to treatment type (GLM: $F_{1,38} = 5.19, P = 0.029$). Other measures of song behaviour were invariant across control and masking tones. Pause duration after tone onset, the bird’s own song frequency before the first frequency shift, shift direction and shift magnitude were all similar across control and masked treatments (MANOVA: Hotelling’s trace = 0.42, $F_{1,4} = 1.78, P = 0.179$). Song rates across the 2 min ambient recording and the tone playback periods were also similar (Friedman test: $\chi^2 = 2.33, P = 0.314$). Latency to shift song frequency, however, did vary by treatment type (Friedman test: $\chi^2 = 7.43, P = 0.024$; Fig. 4). Birds shifted their song frequency more rapidly after the onset of masking tones ($66.47 \pm 10.48$ s latency to shift) than after the onset of the control tone ($95.78 \pm 7.78$ s; Wilcoxon signed-ranks test: $T = 104, N = 20, P = 0.013$). Additionally, chickadees shifted song frequencies at similar rates during pretrial and control tone treatments ($90.62 \pm 9.47$ s; Wilcoxon signed-ranks test: $T = 23, N = 20, P = 0.398$), yet shifted more slowly during the pretrial treatment than during the masking treatment (Wilcoxon signed-ranks test: $T = 100, N = 20, P = 0.025$).

**DISCUSSION**

Our main finding was that male chickadees presented with masking tones shifted song frequencies more quickly than when presented with a nonmasking tone. The higher rate of frequency shifting in the masking treatment was not a correlated effect of differences in song output, as birds sang at similar rates across treatment types. Furthermore, birds did not vary in a suite of other song behaviours measured during control and masking playbacks. Thus, we can conclude that chickadees attend to and respond quickly to masking noise by shifting their song frequencies. Our findings accord with and build upon a growing body of literature illustrating individual vocal plasticity in response to acoustic interference (Halfwerk & Slabbeekoom 2009; Gross et al. 2010; Bermudez-Cuamatzin et al. 2011).

Prior studies of frequency shifting in chickadees have focused on this behaviour’s potential role in social interactions. In particular, male chickadees engaged in song contests or bouts of countersinging have been observed to sometimes shift their songs to match the song frequencies of rivals, presumably as a signal of aggression (Horn et al. 1992; Mennill & Ratcliffe 2004a; Foote et al. 2008). Additionally, rival chickadees sometimes overlap each other such that one song begins before a rival’s song ends, producing a temporarily highly masked sound environment, especially when song frequencies of rivals are matched. As with matching, overlapping might serve as a signal of a male’s aggression: chickadees that overlap sometimes alter their own singing behaviour by shortening their songs (Mennill & Ratcliffe 2004a) and, in a two-speaker design, in which one speaker was timed to overlap the other, high-ranking males were more likely to approach the overlapping speaker (Mennill & Ratcliffe 2004b). However, the question of whether overlapping serves as a signal of aggression remains unresolved, especially because some responses to presumably aggressive, overlapping signals are behaviours that might also be expected to avoid signal jamming (Seary & Beecher 2009, 2011; Naguib & Mennill 2010). Our study contributes to this discussion by confirming that frequency shifting can be driven not only by social context but also by attempts to maintain signal efficacy. In our experiment, the masking tone was a continuous narrow frequency band that overlapped and matched only one portion of the song (the bee), and thus did not approximate the structure of natural chickadee song. The masking tones did, however, simulate the same masking effect experienced in overlapping and matched contests, and thus frequency shifting documented here can be viewed as a mechanistic response to the sudden change in the sound environment. The extent to which frequency shifting during natural contexts can be attributed to social functions versus attempts to maintain signalling efficacy remains to be determined.

One open question raised by our study is whether some chickadees show greater plasticity in their singing behaviour than others. In winter, chickadees form flocks with linear hierarchies with stable social ranks (Smith 1976, 1991), and social rank is positively correlated with a number of song parameters including song output at dawn (Otter et al. 1997), the ability to maintain consistent amplitude between fee and bee notes (Hoeschele et al. 2010), and the ability to maintain a consistent internote ratio between the fee and bee frequency (Christie et al. 2004). Might social dominance also correlate with the tendency to shift song frequency? Available data argue against this possibility: both high- and low-ranking individuals shift frequencies at similar rates and with similar magnitudes (Christie et al. 2004), and rank is not correlated with the proportion of songs that are matched to the frequencies of neighbours (Fitzsimmons et al. 2008). However, matched bouts of disparately ranked individuals are shorter than those of closely ranked rivals (Foote et al. 2008), and contest-induced frequency shifting by subordinate males would provide a mechanism to explain this pattern. High-ranking males that lose singing contests face considerable loss of paternity (Mennill et al. 2002), and, therefore, may be particularly reluctant to shift
frequency in social contexts. We were unable to collect dominance data on our study populations, but we suggest that the relation between plasticity in frequency-shifting behaviour and dominance status warrants further study.

Beyond conspecific interactions in chickadees, our results bear more generally on how vocalizing individuals may respond to transient masking interference. In urban environments, acoustic communication is disrupted by anthropogenic noise, a source of interference that will continue to compound as human infrastructure expands (Vitousek et al. 1997). Studies of urban birds have revealed that populations in areas with high-amplitude, low-frequency noise tend to sing at higher minimum frequencies, releasing them from masking (i.e. great tits: Slabbekoorn & Peet 2003; nightingales, Luscinia megarhynchos: Brumm & Todt 2002; Brumm 2004; song sparrows, Melospiza melodia: Wood & Yezernic 2006; house finches: Fernández-Juricic et al. 2005). Several studies have also documented altered patterns of abundance in noisy areas (Reijnen & Poppen 1995; Reijnen et al. 1995, 1996, 1997; Forman et al. 2002), and two studies have linked these abundance patterns to song structures that may be differentially affected by masking noise (Rheindt 2003; Goodwin & Shriver 2011). That populations in noisy areas differ in their acoustic signals is well established, although the timing of signal adaptation remains understudied. With songbirds in particular, several hypotheses to explain vocal adaptations to urban noise are plausible and not mutually exclusive: habitat assortment, such that individuals that sing at higher frequencies selectively occupy louder habitats; learning bias, whereby individuals disproportionately hear and thus learn higher-frequency songs; adaptive evolution, whereby urban populations diverge genetically, and thus vocally, from quieter rural populations; or individual vocal plasticity, as described herein. Our results, and other similar recent results in other species, support the role for vocal plasticity in observed population differences in acoustic signals among urban and rural populations.

Yet we also note that individual vocal plasticity can be limited. One limitation is how quickly individuals can respond to acoustic interference. In our study, chickadees shifted song frequencies on average more than 1 min into the masking playback tone. During the lag time between the onset of the masking playback and the shift in frequency of the first song, signal efficacy was probably very low. Our results here echo those of Halfwerk & Slabbekoorn (2009), who measured spectral characteristics of the song types sung before and after presentation of broadband masking noise. While those birds shifted to song types that reduced masking, switches were not instantaneous: the more masked bouts continued between approximately 100 and 600 s before a switch to a different song type. This evidence, combined with our results herein, suggest the observed flexibility in shifting song frequencies may be constrained or limited, perhaps in relation to the length of the bout at a certain frequency. In support of this hypothesis, in some birds, song type switching is correlated with the length of the bout, rather than the number of songs delivered (Riebel & Slater 1999), which could lead to inflexibility in switching even if the signals are masked. Another limitation to vocal plasticity is the specific morphology of the species in question. Vocalizing animals are constrained to certain frequency ranges as a result of their size, structure and vocal apparatus (Fletcher & Tarnopolsky 1999; Podos & Nowicki 2004). Although noisy environments may favour the production of specific frequencies or frequency ranges as a release from masking, some species may be mechanistically unable to produce those frequencies. With such limitations and constraints in mind, we might expect noise-induced changes in signal design to be complemented by eventual geographical shifts away from habitats with masking noise, especially for those species most constrained in their ability to modify song structure in response to noise (Rheindt 2003; Goodwin & Shriver 2011).

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