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# Sex differences in auditory filters of brown-headed cowbirds (*Molothrus ater*)

**Megan D. Gall · Jeffrey R. Lucas**

**Abstract** Receiver sensory abilities can be influenced by a number of factors, including habitat, phylogeny and the selective pressure to acquire information about conspecifics or heterospecifics. It has been hypothesized that brood-parasitic brown-headed cowbird (*Molothrus ater*) females may locate or determine the quality of potential hosts by eavesdropping on their sexual signals. This is expected to produce different sex-specific pressures on the auditory system to detect conspecific and heterospecific acoustic signals. Here, we examined auditory filter shape and efficiency, which influence the ability to resolve spectral and temporal information, in males and females at center frequencies of 2, 3 and 4 kHz. We found that overall, cowbirds had relatively wide filters (lsmean ± SE: 619.8 ± 41.6 Hz). Moreover, females had narrower filters (females: 491.4 ± 66.8, males: 713.8 ± 67.3 Hz) and greater filter efficiency (females: 59.0 ± 2.0, males: 69.8 ± 1.9 dB) than males. Our results suggest that the filters of female cowbirds may allow them to extract spectral information from heterospecific vocalizations. The broader auditory filters of males may reflect limited spectral energy in conspecific vocalizations in this frequency range, and hence, weaker selection for high resolution of frequency in the range of 2–4 kHz.

**Keywords** Auditory filter · Brood parasitism · Brown-headed cowbird · Auditory evoked potentials · Sender–receiver mismatch

## Introduction

In many species, signal design and signal reception are closely linked (Endler 1992). For instance a number of songbird species are most sensitive to the dominant frequencies in their own vocalizations (Dooling et al. 2000; Henry and Lucas 2008) and the upper limit of frequency sensitivity correlates well with the maximum frequencies of vocalizations of frogs and birds (Feng et al. 2006; Konishi 1970). Additionally, sensitivity to harmonics correlates well with the use of harmonics in vocalizations (Dooling et al. 2002). There is also evidence that some fish and birds upregulate auditory processing during the breeding season (Lucas et al. 2002, 2007; Henry and Lucas 2009; Sisneros 2009), which improves the processing of conspecific vocalizations.

However, not all species have sensory systems that reflect the signals that conspecifics produce. For instance, predators are commonly sensitive to the signals or cues of their prey. Bats that eat tungara frogs have specialized low frequency hearing to localize the "chuck" call of their prey (Bruns et al. 1989). Additionally, barn owls phase-lock to high frequencies (Köppl 1997) which is thought to be largely a result of detecting prey, rather than conspecific vocalizations. Barn owls also have excellent temporal resolution, which allows them to acoustically detect and localize their prey (Dent et al. 2002). Parasites are also sensitive to the signals of their hosts and may have sex-specific sensory specializations if hosts are used for reproduction. For instance, ormia flies, which deposit their eggs on crickets, have auditory specializations that allow them to localize potential hosts using the sexual signals of their hosts (Robert et al. 1992, 1996).

Brown-headed cowbirds (*Molothrus ater*) may have unique selective pressures on their sensory systems due to

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their brood-parasitic breeding strategy. It has been hypothesized that female cowbirds eavesdrop on the sexual signals of hosts in order to detect, locate, or determine the quality of potential hosts (Clotfelter 1998; Banks and Martin 2001; Parejo and Avilés 2007), although this hypothesis has remained relatively unexplored. If the reception of host signals provides a fitness benefit, then cowbirds should have sensory systems that are capable of processing host signals. However, sensory specializations for host detection should be found in females but not males, because female cowbirds alone search for nests.

Female cowbirds parasitize a wide range of species (Friedmann and Kiff 1985), but generally prefer hosts at woodland edges (Lowther 1993). Species in this habitat generally produce vocalizations that range from relatively tonal to slowly modulated, with frequencies between 1 and 4 kHz (Wiley and Richards 1978; Richards and Wiley 1980). In contrast, male cowbirds produce songs with peak spectral energy above 4 kHz (Lowther 1993). Therefore, we would expect a release from selective pressure on the auditory system at or below 4 kHz based on conspecific vocalizations, but strong selective pressure based on host vocalizations. This could lead to a mismatch between conspecific signal design and signal reception in females, which attend to host vocalizations (Gall and Lucas 2010).

This mismatch between conspecific signals and reception may be reflected in the auditory filters, which perform frequency analysis of sounds in the inner ear. The shape of the auditory filters creates a trade-off between the ability of the auditory system to resolve frequencies versus the ability to process rapid modulation. Sharp, or narrow, filters improve frequency resolution because different frequencies are more likely to be processed in separate channels (Moore 1993). Wide filters are better able to pass amplitude and frequency modulated stimuli, because frequency sidebands are more likely to be processed in a single channel (Viemeister and Plack 1993; Sek and Moore 2000; Thyer and Mahar 2006). Indeed, Henry and Lucas (2010) found that songbird species in open habitats that favor rapid modulations had wider filters than species in closed habitats that favor pure tone vocalizations.

Filter shape can be estimated using thresholds determined in a notched-noise masker (Patterson 1976; Glassberg and Moore 1990). In this method, a tone is played at the frequency of interest in a broadband white noise, which prevents the listener from using information gathered outside the filter to detect the tone (off-frequency listening). Here, the tone is difficult to detect because the filter centered at the tone frequency is also processing noise. A notch of silence is then added to the noise, centered at the frequency of interest. As the notch is widened, the tone should become easier to detect because there is less noise entering the filter. The shape of the threshold by notch width function dictates the shape of the filter: steep functions indicate narrow filters and shallow functions indicate wide filters. Filter shape can then be described by the bandwidth of the filter or by the quality of the filter (the ratio of the center frequency to bandwidth). The auditory filter model also fits an efficiency parameter, or the signal to noise ratio of the detection threshold.

Here, we explore the hypothesis that auditory filters' shape is influenced by selective pressure for signal reception in the brood-parasitic brown-headed cowbird. We used auditory evoked potentials, which are minute electrical potentials measured from the scalp (Hall 2007), to determine the auditory filter shape at 2, 3, and 4 kHz. Male brown-headed cowbirds were expected to have auditory filters adapted for processing conspecific vocalizations, while female brown-headed cowbirds were expected to have auditory filters that were adapted for processing both conspecific and heterospecific vocalizations. Auditory filters generally increase in bandwidth outside of the frequency range of vocal signals in other songbirds (see for instance the dark-eyed junco, Henry and Lucas 2010). Male brown-headed cowbird songs contain little spectral energy from 2 to 4 kHz; therefore, we expected that there would be little selective pressure from conspecific communication on tuning and relatively broad filters in males at 2, 3, and 4 kHz. The vocalizations of the preferred brown-headed cowbird hosts predominately have spectral energy in the 2–4 kHz range. This should favor relatively narrow filters in brown-headed cowbird females at 2, 3, and 4 kHz. Additionally, females are expected to have greater filter efficiency than males, to enhance the long distance detection of host vocalizations.

## Methods

### Animal capture and housing

Brown-headed cowbirds were trapped with baited walk-in traps at a USDA APHIS station in May and early June of 2009. Adult males and females were identified by plumage. The brown-headed cowbirds were considered to be in breeding condition as males engaged in song and females responded to male song by adopting a copulatory position. Individuals were housed individually in 1-m³ cages at Purdue University and provided ad libitum with mixed seed, mealworms, and water. Brown-headed cowbirds were transferred to Purdue University Animal Care and used

Committee protocol # 08-012 upon completion of the auditory experiments.

## Auditory evoked potentials

We recorded a class of auditory evoked potentials called auditory brainstem responses (ABR). ABRs are onset responses generated by the auditory nerve and brainstem within the first 10–12 ms following stimulus onset (Hall 2007). In songbirds, the ABR is dominated by a biphasic deflection that usually occurs within 5 ms of onset and thought to be generated primarily by the auditory nerve (Brittan-Powell et al. 2002). ABRs are generally insensitive to the effects of anesthesia (Hall 2007), which allows rapid assessment of peripheral auditory processing in species that do not lend themselves well to behavioral auditory assessments. ABRs have been used in wild-caught songbirds to evaluate frequency sensitivity, frequency selectivity and temporal processing (Henry and Lucas 2008, 2010).

A total of 17 brown-headed cowbirds (9 males and 8 females) were tested. Auditory evoked potentials were recorded from individuals anesthetized with a combination of ketamine (40–60 mg kg$^{-1}$) and midazolam (6–8 mg kg$^{-1}$) injected into the breast muscle. Subjects were placed on a microwavable heating pad wrapped in towels at the center of a sound-proof chamber lined with Sonex acoustic foam (Acoustic Solutions, Richmond, VA, USA). We added or removed towels to maintain a temperature of 39 ± 2°C between the heating pad and the bird. A non-inverting needle electrode (Nicolet Biomedical, Fitchburg, WI, USA) was placed under the scalp at the vertex of the head, an inverting electrode beneath the skin of the auditory meatus, directly behind the ear opening, and a ground electrode below the skin on the nape of the neck. We periodically evoked responses to 100 μs broadband clicks to monitor anesthesia level. Sessions were ended and data discarded if ABR amplitude or latency changes were greater than 20% of the initial response. Males and females appeared to respond similarly to anesthesia.

Auditory stimuli (tone bursts and notched noise) were created in SigGen32 and presented using a computer running TDT BioSig32 software and a TDT II rack-mounted system (Tucker Davis Technologies, Gainesville, Florida). White noise was created with two analogue waveform generators (TDT WG1). Subsequently, the notched noise was created by passing one noise source through a high-pass filter and one noise source through a low-pass filter with corner frequencies set to the notch width (TDT programmable filter PF1). The roll-off of the filters was approximately 156 dB per octave based on 26 poles. The noise had a spectrum level of 15.3 ± 2 dB re: 20 μPa$^2$ outside the notch (Fig. 1). Tone bursts created in SigGen32
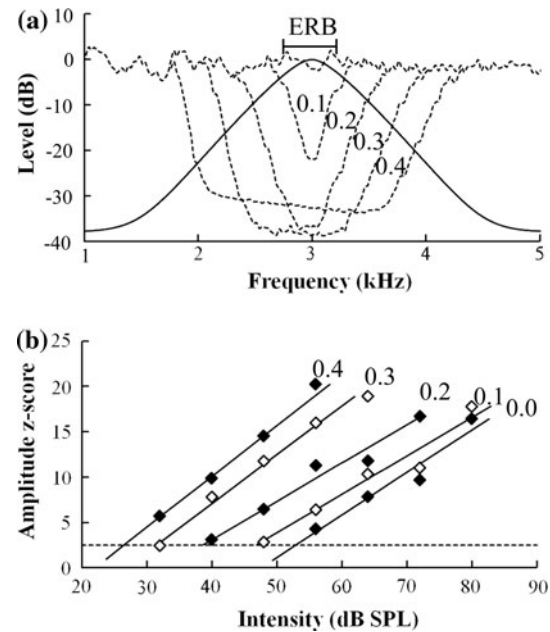


**Fig. 1** **a** The *solid line* represents an auditory filter centered at 3 kHz derived from the auditory evoked responses to tones in notched noise. The amount of attenuation provided by the filter (in relative dB) increases with distance from the center frequency. The equivalent rectangular bandwidth (ERB) is equal to the bandwidth of a rectangle with the same height and area as the auditory filter. The *dashed lines* represent the average long term power spectrum of the noise (25 Hz bins) for each normalized notch width. The spectrum level of the noise is also presented in dB (relative to the noise level outside of the notch). **b** The amplitude *z* score by intensity functions used to extrapolate thresholds at each normalized notch width from a single female. The amplitude *z* score was created by cross-correlating a frequency specific template (response at 72 dB SPL) to subsequent responses at lower intensities. The *dashed line* represents the upper bound of the physiological background noise. *Solid lines* represent the fit of the generalized linear model. Thresholds were extrapolated from intersection of the amplitude *z* score by intensity function with the upper bound of physiological background noise

were passed through a TDT DA1 digital to analogue converter and equalized at every third-octave band with a 31 band equalizer (Behringer Ultragraph model FBQ6200).

Stimuli were presented through a Crown D75 amplifier and a magnetically shielded speaker (RCA Model 40-5000; 140–20,000 Hz frequency response) suspended 30 cm above the bird's head. Auditory evoked responses were recorded using the TDT BioSig32 software and a TDT II after being passed through a DB4 biological amplifier (amplification = 200,000×) and AD1 analogue to digital converter. We recorded a class of auditory evoked responses called ABRs. ABRs are electrical potentials that are generated within 5–10 ms after the onset of the acoustic stimulus. Early ABR peaks are relatively unaffected by anesthetic or sedative drugs, therefore ABRs allow for relatively rapid testing of peripheral hearing responses in non-human animals (Hall 2007).

Auditory evoked potential threshold

Auditory filter shape was estimated at center frequencies of 2, 3, and 4 kHz using the cross-correlation technique and filter fitting procedure outlined in Henry and Lucas (2010). Previous studies suggest that auditory evoked potentials are robust estimates of behavioral auditory thresholds at these frequencies in brown-headed cowbirds (Gall and Lucas 2010). Thresholds were estimated at five normalized notch widths (half the notch bandwidth divided by the center frequency) ranging from 0 to 0.4 at each center frequency (Fig. 1). Auditory responses were evoked with 8 ms tone bursts with 2 ms $\cos^2$ onset–offset ramps and alternating phase (90° and 270°). Frequency presentation was randomized. Tone bursts were presented sequentially at intensities ranging from 72 to 16 dB SPL in 8 dB SPL steps at each frequency–notch width combination. The stimuli were presented at 51.1 stimuli per second with an interstimulus interval of 11.6 ms. Two sets of ABRs were recorded at each intensity, center frequency, and notch width. Each ABR was the averaged response to 500 stimuli presentations. ABRs were sampled at a rate of 40 kHz for 12 ms. All responses were bandpass filtered from 0.1 to 10 kHz prior to averaging.

Auditory thresholds were determined using a cross-correlation technique in PRAAT (Boersma and Weenink 2009). First, we created a template from the averaged 72 dB response at each frequency–notch width combination. The subsequent responses (64–16 dB SPL) at that frequency–notch width combination were then cross-correlated with the template. The template was also cross-correlated with species-specific physiological background noise recorded in quiet. The cross-products of the physiological background noise were approximately normal with a mean of 0. The cross products were then divided by the standard deviation of the physiological background noise to determine the ABR amplitude $z$ score. Amplitude $z$ scores greater than 2.56 were significantly different than the physiological background noise at the 99.5% confidence level.

We then extrapolated the ABR amplitude $z$ score by intensity function to estimate the threshold at the upper limit of physiological background noise. The extrapolation was performed separately for each notch width using PROC GLM in SAS 9.2 (Fig. 1). An intensity squared term was included in the model to account for slight non-linearity. Thresholds were excluded from further analysis if the $R^2$ value was less than 0.8. If two or more thresholds were excluded at a single frequency all responses at that frequency were discarded for that bird. Auditory filter shape, therefore, was determined for a total of 13 cowbirds (7 males and 6 females).

Auditory filter shape

Auditory filter shape was determined from the threshold by notch width functions. As the notch width increases the threshold should decrease, because the signal to noise ratio in the filter increases. This is expressed as:

$$P_s = K \int_0^\infty N(f)W(f)\mathrm{d}f$$

where $N(f)$ is the long term average power spectrum of the masking noise in $Pa^2/Hz$ and was measured directly from the stimulus. $K$ is a constant representing the signal to noise ratio that is required to evoke a response, $W(f)$ is a weighting function and $P_s$ is the masked threshold. $W(f)$ and $K$ were solved for using an iterative Gauss–Newton polynomial fitting procedure in SAS (Proc NLIN; SAS Institute Inc., v. 9.2). $W(f)$ was modeled following the two parameter rounded exponential model [roex($p,r$)] of Patterson et al. (1982) where the $p$ parameter represents the slope of the filter near the center frequency. The roex model does not fit data well when the masked threshold approaches the absolute threshold, so the $r$ parameter constrains the dynamic range of the filter to improve fit (Moore 1993). However, the $r$ parameter has little effect on the shape of the filter near the center frequency (Popov et al. 2006). We constrained the $r$ parameter to be less than the contribution of the $p$ parameter when $g$ was within 0.35. Here, $g$ represents the normalized frequency deviation from the center frequency. The weight of the filter then becomes

$$W(g) = (1 - r)(1 + pg)\mathrm{e}^{-pg} + r.$$

To solve $K$, $p$ and $r$, the above equations were combined:

$$P_s(nw)' = K' + 10 \, \log_{10} \sum_{i=1}^N \frac{\mathrm{PSD}_{nw(i)} \times \int_i \mathrm{roex}(p,r)}{4 \times 10^{-10}}$$

Here, $P_s(nw)'$ is the masked ABR threshold in dB SPL in noise with notch width nw. $K'$ is the efficiency constant $K$ expressed in decibels ($10 \log_{10}K$). $N$ is the number of 25 Hz bins in the largest notch width, $\mathrm{PSD}_{nw(i)}$ is the power spectral density in $Pa^2/Hz$ of the noise in each bin and

$$\int_i \mathrm{roex}(p,r) = -(1-r)p^{-1}(2+pa)\mathrm{e}^{-pa}$$
$$+ (1-r)p^{-1}(2+pa)\mathrm{e}^{-pb} + r(a-b)$$

where $a$ and $b$ are the upper and lower frequency limits of each bin, respectively. We calculated the equivalent rectangular bandwidth (ERB), which describes a rectangle with the same height and area as the filter. ERB is equal to $4/p$ times the center frequency of the filter when expressed in Hz.

## Statistical design

All data were checked for normality and homoscedacity in PROC UNIVARIATE. Repeated measures ANOVAs were conducted using Proc MIXED in SAS 9.2 with bird ID as a subject variable. Several covariance structures were modeled (first-order autoregressive, compound symmetry, unstructured). The final covariance structure (compound symmetry) was chosen based on the AIC value, however, the results were not qualitatively different among the different models. We first examined thresholds as a function of frequency, notch width, sex, and their interactions. We then investigated filter parameters. Dependent variables were the ERB, $Q_{ERB}$ (the quality of the filter expressed as center frequency/ERB) and $K'$. Independent variables were sex, frequency and their interaction. Significant effects were examined post-hoc with the LSMEANS procedure.

## Results

As predicted there were significant main effects of sex ($F_{1,11.3} = 23.44$, $p < 0.001$), frequency ($F_{2,129.6} = 39.9$, $p < 0.001$), and notch width ($F_{4,126.3} = 131.3$, $p < 0.001$) on ABR threshold. Females were on an average $15.1 \pm 3.1$ dB more sensitive than males. Thresholds at 2 and 4 kHz were higher than at 3 kHz and decreased with increasing notch width (Fig. 2). There were also significant interaction effects of sex $\times$ notch width ($F_{4,126.3} = 3.7$, $p = 0.007$) and frequency $\times$ notch width ($F_{8,126.3} = 4.94$, $p < 0.001$) on ABR thresholds, suggesting that the shape of the filters changes with both sex and frequency. The slice
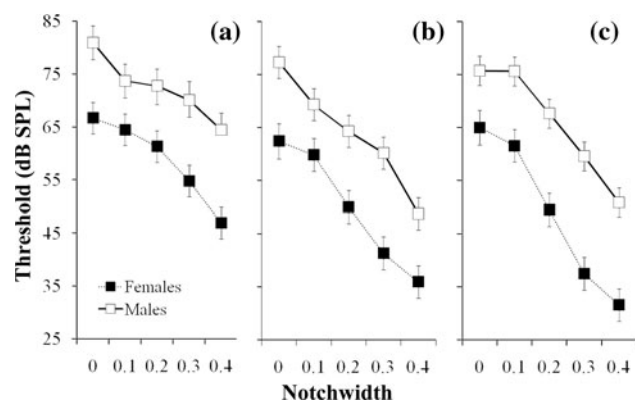
procedure indicated that females had lower mean thresholds than males at all notch widths ($F_{1,14} > 4.9$, $p < 0.045$). The slopes of the threshold by notch width function also tended to be steeper in females than in males, particularly at 4 kHz, suggesting narrower filters in females (Fig. 2). Steepness of the threshold by notch width slope decreased with increasing frequency, suggesting an increase in filter width with increasing center frequency (Fig. 2).

There was a significant main effect of sex ($F_{1,8.1} = 5.6$, $p = 0.04$) on ERB; however, there was no main effect of frequency ($F_{2,6.9} = 0.3$, $p = 0.78$), nor was there a significant interaction between sex and frequency. As predicted, females (lsmean $\pm$ SE = $491.4 \pm 66.8$) had significantly smaller ERBs than males (lsmean $\pm$ SE = $713.8 \pm 67.3$) across all frequencies (Fig. 3a).

There were significant main effects of sex ($F_{1,8.8} = 7.4$, $p = 0.024$) and frequency ($F_{2,5.7} = 24$, $p < 0.001$) on the quality of the filter. Females filter quality increased more sharply than males with increasing center frequency (Fig. 3b). Filter quality also increased with increasing center frequencies ($t > 3.7$, $p < 0.003$; Fig. 3b). There was not a significant sex $\times$ frequency interaction ($F_{2,5.7} = 3.7$, $p = 0.09$).

There was a significant main effect of sex ($F_{1,10.9} = 15.2$, $p = 0.002$) but not frequency ($F_{2,15.7} = 2.7$, $p = 0.1$) or the sex $\times$ frequency interaction ($F_{2,15.7} = 0.05$, $p = 0.96$) on filter efficiency. Females were on an average $10.8 \pm 2.8$ dB better at detecting a tone against a constant noise background than males (Fig. 3c).



**Fig. 2** Auditory thresholds as a function of notch width at **a** 2 kHz **b** 3 kHz and **c** 4 kHz for male and female brown-headed cowbirds. Steeper slopes indicate narrower auditory filters, while shallow slopes indicate wider auditory filters. Males generally had shallower slopes than females. The slope of the function was also influenced by frequency, with the steepest slopes at 3 kHz. Estimates are least squares means ($\pm$SE) generated using the LSMEANS procedure in PROC MIXED
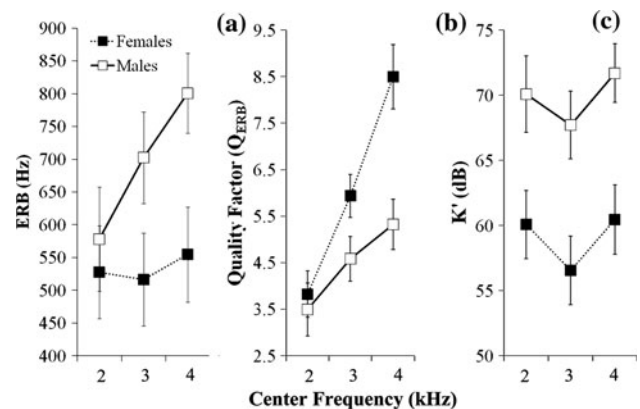


**Fig. 3** **a** Equivalent rectangular bandwidth (ERB) in Hz as a function of center frequency for male and female brown-headed cowbirds. **b** Quality factor ($Q_{ERB}$) of the auditory filters as a function of center frequency for male and female brown-headed cowbirds. $Q_{ERB}$ is the ratio of the center frequency to the ERB. Higher values of $Q$ indicate sharper auditory filter tuning. **c** Filter efficiency ($K'$) of the auditory filters as a function of center frequency for male and female brown-headed cowbirds. $K'$ is the signal to noise ratio expressed in dB at the auditory threshold. Lower values of $K'$ indicate a better ability to detect tones in noise. Estimates are from the LSMEANS procedure ($\pm$SE)

## Discussion

Overall, we found that auditory evoked potential estimates of auditory filter shape in brown-headed cowbirds were similar to other songbird species. Filter quality increased with increasing center frequency for both sexes. However, there was little effect of frequency on ERB, particularly in females. This suggests that brown-headed cowbirds have an auditory system that is closer to constant bandwidth than to constant Q from 2–4 kHz, which is different from that of most mammals (Popov et al. 2006) and birds (Dooling et al. 2000).

An increase in the quality of the filter with center frequency should result in increasing frequency resolution with increasing center frequency. Indeed, Sinnott et al. (1980) found that a single male cowbird could detect changes of $\sim$20 Hz at a center frequency of 2 kHz and $\sim$25 Hz at a center frequency of 4 kHz. Relative discrimination ability, therefore, increases with increasing center frequency, as does the quality of filters in this frequency range. However, discrimination ability decreased above 4 kHz (Sinnott et al. 1980), suggesting decreasing filter quality above 4 kHz. The male cowbird was not as sensitive to frequency changes in this range as humans, but slightly more sensitive than pigeons (Sinnott et al. 1980). Estimates of human auditory filters (Moore 1993) are generally narrower than those found in birds thus far. These relatively small discriminable changes in frequency suggest that there are additional mechanisms in the ascending auditory pathway that improve the frequency resolution provided by the peripheral filters.

We also found that thresholds in noise with no notch (notch width = 0) were approximately 10–15 dB above previously reported critical ratios estimated with behavioral protocols, but followed the same general pattern (Hienz and Sachs 1987). This is likely due to the use of ABRs to estimate thresholds. In general, thresholds estimated with ABRs tend to follow the same general patterns, but are usually at least 15–20 dB above behavioral estimates of auditory thresholds (Brittan-Powell et al. 2002, 2005). The thresholds we obtained in noise with the largest notch were also higher than our previous estimates of ABR thresholds in quiet (Gall and Lucas 2010). This could be due to the longer rise–fall time of the stimuli used in this experiment, which evoke less robust ABRs, but improve frequency specificity.

### Sex differences

Overall, we found that females had smaller bandwidths and higher quality filters than males. Filter quality increased more rapidly in females than in males and was sharpest at 4 kHz. Females also had greater filter efficiency than males, suggesting that females may be under greater selective pressure for frequency discrimination and the detection of tones in noise.

Differences in auditory tuning in brown-headed cowbirds may be due to differential expression of sex hormones, such as estrogen. Estrogen receptors have been found in the inner ears of fish (Forlano et al. 2005), birds (Noirot et al. 2009) and mammals (Stenberg et al. 1999, 2001). Additionally, estrogen has been implicated in a variety of sex-correlated differences in the auditory periphery (Charitidi et al. 2009). For instance, implanting ovariectomized non-breeding female plainfin midshipman (*Porichthys notatus*) with 17β-estradiol or testosterone, which is converted to 17β-estradiol by aromatase, induces upregulation of responses to frequencies in male courtship vocalizations (Sinsneros et al. 2004).

A number of recent studies also suggest that auditory tuning can vary seasonally and is often correlated with seasonal changes in vocalizations in a variety of taxa. For instance, the plainfin midshipman fish mentioned above, upregulate the processing of frequencies in male songs during the breeding season (Sisneros 2009). Neurons in the auditory midbrain of male leopard frogs show improved phase-locking to the envelopes of synthetic advertisement calls during the breeding season (Goense and Feng 2005). In birds, house sparrows (*Passer domesticus*) show enhanced suprathreshold auditory evoked responses to tone bursts in the range of male song during the breeding season (Henry and Lucas 2009). Seasonality in auditory evoked responses to clicks and tones have also been shown in Carolina chickadees, tufted titmice, downy woodpeckers and white-breasted nuthatches (Lucas et al. 2002, 2007).

Seasonal changes and sex differences in auditory tuning, as well as the presence of aromatase and estrogen receptors in the sensory epithelium of songbirds (Noirot et al. 2009), suggest a role for estrogen in auditory tuning in songbirds. However, further studies are needed to explicitly link circulating estrogen with seasonal or sex-specific differences in the auditory tuning of songbirds. Additionally, without a detailed study of seasonal changes it is unclear whether sex differences in brown-headed cowbird auditory tuning is a result of auditory plasticity or fixed developmental trajectories.

### Songbird auditory filters

Although differences exist among species, most songbirds are capable of detecting changes in frequency of less than 1% (reviewed in Dooling et al. 2000), suggesting some conservation in auditory filter shape. Similarity among species could be due to either phylogenetic conservation or convergent signal design. Auditory filter shape could also be related to habitat-related signal propagation—favoring

wide filters in open habitats and narrow filters in closed habitats (Henry and Lucas 2010). The unique vocalization of the brown-headed cowbird makes it unlikely that convergent auditory tuning is a result of similar vocal repertoires, due both to the high frequencies and relatively tonal vocalizations. Therefore, filter similarity is more likely to reflect shared evolutionary history or habitat. Auditory filter shape has been estimated with auditory evoked potentials for species in the superfamilies *Sylvioidea* (tufted titmouse), *Muscicapoidea* (white-breasted nuthatch), *Passeroidea* (house sparrow, white-crowned sparrow, dark-eyed junco) and behaviorally (Marean et al. 1998) in the superfamily *Muscicapoidea* (European starling). Brown-headed cowbirds are in the superfamily *Passeroidea*, and are in a sister clade to the American sparrows (Jønsson and Fjeldså 2006). So we would expect them to be most similar to white-crowned sparrows and dark-eyed juncos, as well as house sparrows.

Indeed, in brown-headed cowbirds, the average auditory filter width was most similar to other species in the superfamily *Passeroidea*, which have relatively wide filters (see Fig. 4a). However, when segregated by sex, females had narrow filters, while males had wide filters (Fig. 4b) compared to other species. When we look at the pattern of ERBs across frequencies, males fall out with house sparrows and white-crowned sparrows, but female ERBs are more similar to the dark-eyed junco, which has a nearly constant ERB (Fig. 5). However, there is currently no information on sex differences in auditory filters for other songbirds, so it is unclear whether sex differences themselves represent a specialization of the brown-headed
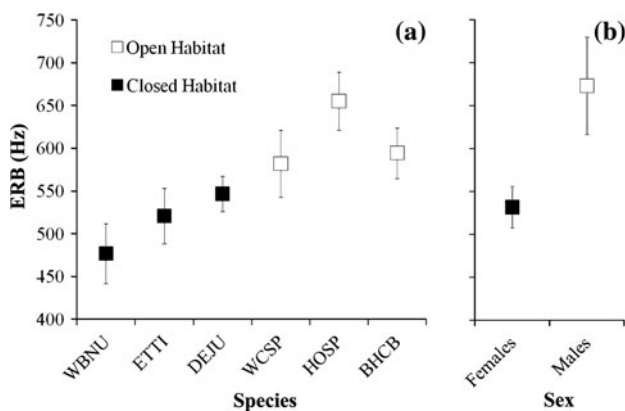


**Fig. 5** Equivalent rectangular bandwidths ± SE (ERB) in Hz of the auditory filters as a function of center frequency for songbird species in open habitats [white-crowned sparrow (WCSP), house sparrow (HOSP)], closed habitats [white-breasted nuthatch (WBNU), tufted titmouse (ETTI), the dark-eyed junco (DEJU)] and brown-headed cowbird (BHCB) males and females. Additional data are from Henry and Lucas 2010

cowbird, or are commonplace in sexually dimorphic songbirds.

These patterns could also be driven by similarity in habitat. Brown-headed cowbirds use relatively open habitats, although females preferentially parasitize species in field-woodland edges. Among the species investigated thus far, the white-crowned and house sparrow live in relatively open areas, titmice and nuthatches live in relatively closed habitats, and dark-eyed juncos live in mixed habitats (Henry and Lucas 2010). It could be, then, that male brown-headed filter width is related to the relatively open habitats they use and are thus most similar to other open habitat species. On the other hand, female filter width is more similar to the pattern seen in dark-eyed juncos. This may be because brown-headed cowbirds preferentially parasitize species at woodland-field ecotones and dark-eyed juncos breed in coniferous forests, which may impose intermediate acoustic limitations because vegetation density influences signal attenuation and reverberative decay rates (Padgham 2004).

Implications for vocal processing

Cowbirds had relatively wide filters at 2, 3, and 4 kHz. An extrapolation of filter width to higher frequencies (most likely a conservative estimate of bandwidth) suggests that peripheral filtering will result in poor resolution of high frequency elements of male song, particularly in males. Brown-headed cowbirds' vocalizations are also accompanied by visual displays and male-directed visual displays are more intense than female directed displays (O'Loghlen and Rothstein 2010). This may reduce the pressure on males to evaluate opponent quality acoustically and this in



**Fig. 4** Mean ± SE equivalent rectangular bandwidth (ERB) in Hz for **a** songbird species in open habitats [white-crowned sparrow (WCSP), house sparrow (HOSP)], closed habitats [white-breasted nuthatch (WBNU), tufted titmouse (ETTI), the dark-eyed junco (DEJU)] across center frequencies of 2, 3, and 4 kHz and **b** for male and female brown-headed cowbirds (BHCB). Only species that have had their auditory filters estimated with auditory evoked potentials are presented. Additional data are from Henry and Lucas 2010
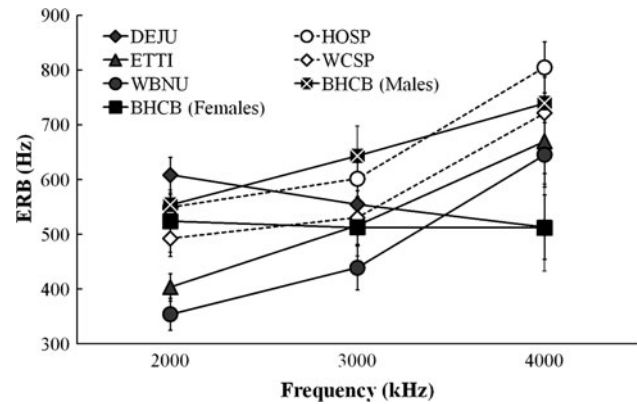
turn could relax selection on male cowbird filter width. Additionally, female brown-headed cowbirds communicate with males using frequency-modulated contact calls, with energy primarily in the 2–6 kHz frequency range. These calls exhibit tremendous variation in temporal and spectral features among females that is likely to provide information about individual identity (Burnell and Rothstein 1994). The improved temporal resolution of wider filters may allow males to identify and locate females.

Species in a variety of taxa use frequency-specific attenuation as a distance cue (Wilczynski and Brenowitz 1988; Brenowitz 1989; Naguib 1995; Nelson 2000). Narrow filters in female cowbirds may be important for determining the distance of potential hosts. This is because the attenuation of high frequency components in vocalizations provides ranging, or distance estimation information (Naguib and Wiley 2001) and narrow filters are likely to improve the ability to resolve spectral differences in degraded stimuli. Finally, male cowbird perched song contains low amplitude elements at low frequencies. Narrow filters in females may enhance the discrimination of these low amplitude elements.

## Conclusions

Overall, we found a mismatch between conspecific vocal signals and receiver physiology. This mismatch could be due to a relaxation of selection pressure on males in this frequency range and females to detect or range potential hosts. However, further work is needed to determine whether this mismatch is adaptive or the result of phylogenetic conservation within the family *Icteridae*. Analysis of auditory filters at higher frequencies could provide a more complete picture of the auditory processing capabilities of the brown-headed cowbird. Additionally, sex-specific differences suggest a role for estrogen in mediating tuning in the auditory periphery. Future work should explicitly examine the link between seasonal hormonal changes and auditory reception.

## References

Banks AJ, Martin TE (2001) Host activity and the risk of nest parasitism by brown-headed cowbirds. Behav Ecol 12:31–40

Boersma P, Weenink D (2009) Praat: Doing Phonetics by Computer version 5.1.17. Computer program retrieved September 2008. http://www.praat.org/

Brenowitz EA (1989) Neighbour call amplitude influences aggressive behaviour and intermale spacing in choruses of the Pacific treefrog (*Hyla regilla*). Ethology 83:69–79

Brittan-Powell EF, Dooling RJ, Gleich O (2002) Auditory brainstem responses (ABR) in adult budgerigars (*Melopsittacus undulates*). J Acoust Soc Am 112:999–1008

Brittan-Powell EF, Lohr B, Hahn DC, Dooling RJ (2005) Auditory brainstem responses in the eastern screech owl: an estimate of auditory thresholds. J Acoust Soc Am 118:314–321

Bruns V, Burda H, Ryan MJ (1989) Ear morphology of the frog-eating bat (*Trachops cirrhosus* Family: Phyllostomidae): apparent specializations for low-frequency hearing. J Morphol 199:103–118

Burnell K, Rothstein SI (1994) Variation in the structure of female brown-headed vocalizations and its relation to vocal function. Condor 96:703–715

Charitidi K, Meltser I, Tahera Y, Canlon B (2009) Functional responses of estrogen receptors in the male and female auditory system. Hear Res 252:71–78

Clotfelter ED (1998) What cues due brown-headed cowbirds use to locate red-winged blackbird host nests? Anim Behav 55:1181–1189

Dent ML, Klump GM, Schwenzfeier C (2002) Temporal modulation transfer functions in the barn owl (*Tyto alba*). J Comp Physiol A 187:937–943

Dooling RJ, Lohr B, Dent ML (2000) Hearing in birds and reptiles. In: Dooling RJ, Popper AN, Fay RR (eds) Comparative hearing: birds and reptiles. Springer-Verlag, New York, pp 308–359

Dooling RJ, Leek MR, Gleich O, Dent ML (2002) Auditory temporal resolution in birds: discrimination of harmonic complexes. J Acoust Soc Am 112:748–759

Endler JA (1992) Signals, signal conditions, and the direction of evolution. Am Nat 139:S125–S153

Feng AS, Narins PM, Xu C, Lin W, Yu Z, Qiu Q, Xu Z (2006) Ultrasonic communication in frogs. Nature 440:333–336

Forlano PL, Deitcher DL, Bass AH (2005) Distribution of estrogen receptor alpha mRNA in the brain and inner ear of a vocal fish with comparisons to sites of aromatase expression. J Comp Neurol 483:91–113

Friedmann H, Kiff LF (1985) The parasitic cowbirds and their hosts. Proc West Found Vertebr Zool 2:225–304

Gall MD, Lucas JR (2010) Sex difference in frequency-specific auditory processing in the brown-headed cowbird (*Molothrus ater*): implications for vocal processing (in review)

Glassberg BR, Moore BCJ (1990) Derivation of auditory filter shapes from notched-noise data. Hear Res 47:103–138

Goense JBM, Feng AS (2005) Seasonal changes in frequency tuning and temporal processing in single neurons in the frog auditory midbrain. J Neurobiol 65:22–36

Hall JW III (2007) New handbook of auditory-evoked responses. Allyn and Bacon, Boston

Henry KS, Lucas JR (2008) Coevolution of auditory sensitivity and temporal resolution with acoustic signal space in three songbirds. Anim Behav 76:1659–1671

Henry KS, Lucas JR (2009) Vocally correlated seasonal auditory variation in the house sparrow (*Passer domesticus*). J Exp Biol 212:3817–3822

Henry KS, Lucas JR (2010) Habitat-related differences in auditory filter bandwidth of songbirds. Funct Ecol 24:614–624

Hienz JD, Sachs MB (1987) Effects of noise on pure-tone thresholds in blackbirds (*Agelaius phoeniceus* and *Molothrus ater*) and pigeons (*Columba livia*). J Comp Psychol 101:16–24

Jønsson KA, Fjeldså J (2006) A phylogenetic supertree of oscine passerine birds (Aves: Passeri). Zoologica Scripta 35:149–186

Konishi M (1970) Comparative neurophysiological studies of hearing and vocalizations in songbirds. Z Vgl Physiol 66:257–272

Köppl C (1997) Phase locking to high frequencies in the auditory nerve and cochlear nucleus magnocellularis of the barn owl, *Tyto alba*. J Neurosci 17:3312–3321

Lowther PE (1993) Brown-headed cowbird (*Molothrus ater*). In: Poole A (ed) The birds of North America online Cornell Lab of Ornithology, Ithaca

Lucas JR, Freeberg TM, Krishnan A, Long GR (2002) A comparative study of avian auditory brainstem responses: correlations with phylogeny and vocal complexity, and seasonal effects. J Comp Physiol A 188:981–992

Lucas JR, Freeberg TM, Long GR, Krishnan A (2007) Seasonal variation in avian auditory evoked responses to tones: a comparative analysis of Carolina chickadees, tufted titmice, and white-breasted nuthatches. J Comp Physiol A 192:201–215

Marean GC, Burt JM, Beecher MD, Rubel EW (1998) Auditory perception following hair cell regeneration in European starling (*Sturnus volgaris*): frequency and temporal resolution. J Acoust Soc Am 103:3567–3580

Moore BCJ (1993) Frequency analysis and pitch perception. In: Yost WA, Popper AN, Fay RR (eds) Human psychophysics. Springer-Verlag, New York, pp 56–115

Naguib M (1995) Auditory distance estimation of singing conspecifics in *Carolina wrens*: the role of reverberation and frequency-dependent attenuation. Anim Behav 50:1297–1307

Naguib M, Wiley RH (2001) Estimating the distance to a source sound: mechanisms and adaptations for long-range communication. Anim Behav 62:825–837

Nelson BS (2000) Avian dependence on sound pressure level as an auditory distance cue. Anim Behav 59:57–67

Noirot IC, Adler HJ, Cornil CA, Harada N, Dooling RJ, Balthazart J, Ball GF (2009) Presence of aromatase and estrogen receptor alpha in the inner ear of zebra finches. Hear Res 252:49–55

O'Loghlen AL, Rothstein SI (2010) Multimodal signaling in a songbird: male audiovisual displays vary significantly by social context in brown-headed cowbirds. Anim Behav (in press)

Padgham M (2004) Reverberation and frequency attenuation in forests—implications for acoustic communication in animals. J Acoust Soc Am 115:402–410

Parejo D, Avilés JM (2007) Do avian brood parasites eavesdrop on heterospecific sexual signals revealing host quality? A review of the evidence. Anim Cogn 10:81–88

Patterson RD (1976) Auditory filter shape derived with noise stimuli. J Acoust Soc Am 59:640–654

Patterson RD, Nimmo-Smith I, Weber DL, Milroy R (1982) The deterioration of hearing with age: frequency selectivity, the critical ratio, the audiogram, and speech threshold. J Acoust Soc Am 72:1788–1803

Popov VV, Supin AY, Wang D, Wang K (2006) Nonconstant quality of auditory filters in the porpoises, *Phocoena phocoena* and *Neophocaena phocaenoides* (Cetacea Phocoenidae). J Acoust Soc Am 119:3173–3180

Richards DG, Wiley RH (1980) Reverberations and amplitude fluctuations in the propagation of sound in a forest: implications for animal communication. Am Nat 115:381–399

Robert D, Amoroso J, Hoy RR (1992) The evolutionary convergence between a parasitoid fly and its cricket host. Science 258:1135–1137

Robert D, Miles RN, Hoy RR (1996) Directional hearing by mechanical coupling in the parasitoid fly, *Ormia ochracea*. J Comp Physiol A 179:29–44

Sek A, Moore BCJ (2000) Detection of quasitrapezoidal frequency and amplitude modulation. J Acoust Soc Am 107:1598–1604

Sinnott JM, Sachs MB, Hienz RD (1980) Aspects of frequency discrimination in passerine birds and pigeons. J Comp Physiol Psychol 94:401–415

Sinsneros JA, Forlano PM, Deitcher DL, Bass AH (2004) Steroid-dependent auditory plasticity leads to adaptive coupling of sender and receiver. Science 305:404–407

Sisneros JA (2009) Adaptive hearing in the vocal plainfin midshipman fish: getting in tune for the breeding season and implications for acoustic communication. Integr Zool 4:33–42

Stenberg AE, Wang H, Sahlin L, Hultcrantz M (1999) Mapping of estrogen receptors a and b in the inner ear of mouse and rat. Hear Res 136:29–34

Stenberg AE, Wang H, Fish J III, Schrott-Fischer A, Sahlin L, Hultcrantz M (2001) Estrogen receptors in the normal adult and developing human inner ear and in Turner's syndrome. Hear Res 157:87–92

Thyer N, Mahar D (2006) Discrimination of nonlinear frequency glides. J Acoust Soc Am 119:2929–2936

Viemeister NF, Plack CJ (1993) Time analysis. In: Yost WA, Popper AN, Fay RR (eds) Human psychophysics. Springer-Verlag, New York, pp 116–154

Wilczynski W, Brenowitz EA (1988) Acoustic cues mediate inter-male spacing in a neotropical frog. Anim Behav 36:1054–1063

Wiley RH, Richards DG (1978) Physical constraints on acoustic communication in atmosphere—implications for evolution of animal vocalizations. Behav Ecol Sociobiol 3:69–94