

Sex-linked inheritance of hearing and song in the Belgian Waterslager canary

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Belgian Waterslager canaries have less sensitive hearing at high frequencies and produce songs with more energy at low frequencies than wild-type canaries. A backcross pedigree between Belgian Waterslager canaries and a domestic strain with wild-type song revealed inheritance patterns consistent with a factor of major effect located on the Z sex chromosome affecting both poor high-frequency hearing at 4 kHz and the relative energy in the spectra of the learned songs of males. Hearing thresholds at 4 kHz were significant predictors of the relative amount of song energy at 4 kHz for individual males. One hypothesis for the mechanistic basis of this correlation between hearing and song abnormalities is that a reduction in the ability to hear higher-frequency songs biases males towards learning lower-frequency songs.

Keywords: *Serinus canarius*; hearing; song learning; sex linkage; Z chromosome; sensory linkage

1. INTRODUCTION

Hearing thresholds in birds are typically correlated with the frequency spectrum of their song (Dooling *et al.* 1971; Wright *et al.* 2003), but the source of this correlation is not well understood, particularly for species that learn their song. Domestic canaries (*Serinus canarius*) that have been artificially selected for specific song traits offer an ideal system in which to examine this correlation. One domestic strain, the Belgian Waterslager (BWS) canary, exhibits a shift in the peak energy of its learned song spectrum to lower frequencies than typically found in either wild canaries or other domestic strains (Dooling *et al.* 1971; Guttinger 1985). This strain also exhibits poor high-frequency hearing relative to other canaries; while hearing thresholds below 2 kHz are normal, those above 2 kHz are 20–40 dB higher than those of wild-type canaries (Okanoya & Dooling 1987). This high-frequency hearing loss is apparent in chicks as early as 20 days post-hatching (E. F. Brittan-Powell and R. J. Dooling, unpublished data) and stems from missing and damaged hair cells on the basilar papilla of the inner ear (Gleich *et al.*

1997). The behavioural consequence of this anatomical abnormality is a shift in the region of best hearing from 2–4 kHz in wild-type canaries to 1–2 kHz in BWS canaries.

Strain-specific changes in both song and hearing in canaries may be sex-linked (Okanoya *et al.* 1990; Mundinger 1995, 1999). In birds, sex determination is reverse-heterogametic with ZZ males and ZW females. Recent work has suggested that genes controlling sexually selected traits (such as birdsong) commonly occur on the Z chromosome (Iyengar *et al.* 2002; Price 2002), despite the fact that in birds it comprises only 7–10% of the total genome (Bloom *et al.* 1993). Here, we report the effect of the Z chromosome on hearing sensitivities and male song frequency in a backcross pedigree between the BWS strain and the Border, a domestic canary with wild-type hearing and song.

2. MATERIAL AND METHODS

(a) Pedigree breeding

The pedigree was founded with reciprocal crosses between a BWS female and a Border male and between a Border female and a BWS male (see electronic Appendix A for a pedigree diagram and methodological details). Female F₁ offspring from these reciprocal crosses were backcrossed to the BWS male to create backcross offspring with an average autosomal complement of 25% Border and 75% BWS. Male backcross offspring had either two BWS Z chromosomes or one Border Z and one BWS Z depending on the origin of their F₁ mothers. If either song or hearing is affected by a dominant Z-linked factor, then any individual carrying a BWS Z chromosome would show BWS traits, while a recessive Z-linked factor would require a full complement of two BWS Z chromosomes for trait expression. Results from autosomally linked factors would be mixed (Mundinger 1999). Pure strain offspring were also produced by within-strain matings of the BWS or Border founders.

(b) Song tutoring and analysis

Offspring were raised in acoustic chambers isolated from adult males. Males were tape tutored in their first autumn with three low-pitched canary songs and three high-pitched or wild-type songs, each from different adult males. We recorded 5–10 entire songs from 14 tutored males, created long-term power spectra and measured the peak spectral amplitude in the one-third octave bands centred at 1 kHz and 4 kHz (figure 1a). We then calculated the difference between peak amplitudes in the two one-third octave bands to give a single measure of the relative distribution of energy in songs between low and high frequencies.

(c) Hearing thresholds

We measured hearing thresholds of the 23 pedigree birds (14 males and nine females) using the auditory brainstem response (ABR), a sound-evoked potential from the peripheral auditory system that provides a measure of hearing threshold. Frequency-specific ABRs were evoked at 1 kHz and 4 kHz by stimulus trains consisting of nine tone bursts of increasing intensity presented at a rate of 4 s⁻¹ (Brittan-Powell *et al.* 2002). Thresholds were defined as the lowest intensity at which a response was visible in the ABR waveform (figure 1b).

3. RESULTS

(a) Hearing thresholds

Hearing thresholds in the parental generation (figure 2a,b, black symbols) were higher for pure BWS than for pure Border canaries at 4 kHz but not at 1 kHz (table 1). Backcross hybrids (figure 2a,b, grey symbols) with a full complement of BWS Z chromosomes had higher thresholds than individuals with one Border Z chromosome at both frequencies. When all generations were combined, individuals with a full complement of BWS Z chromosomes had higher thresholds than those with at least one Border Z chromosome at both 1 kHz and 4 kHz (table 1).

A nonlinear regression modelling a completely recessive Z chromosome effect explained 87% of the variation in hearing threshold at 4 kHz ($Y = 23.9Z_{\text{add}} - 23.9Z_{\text{dom}}$

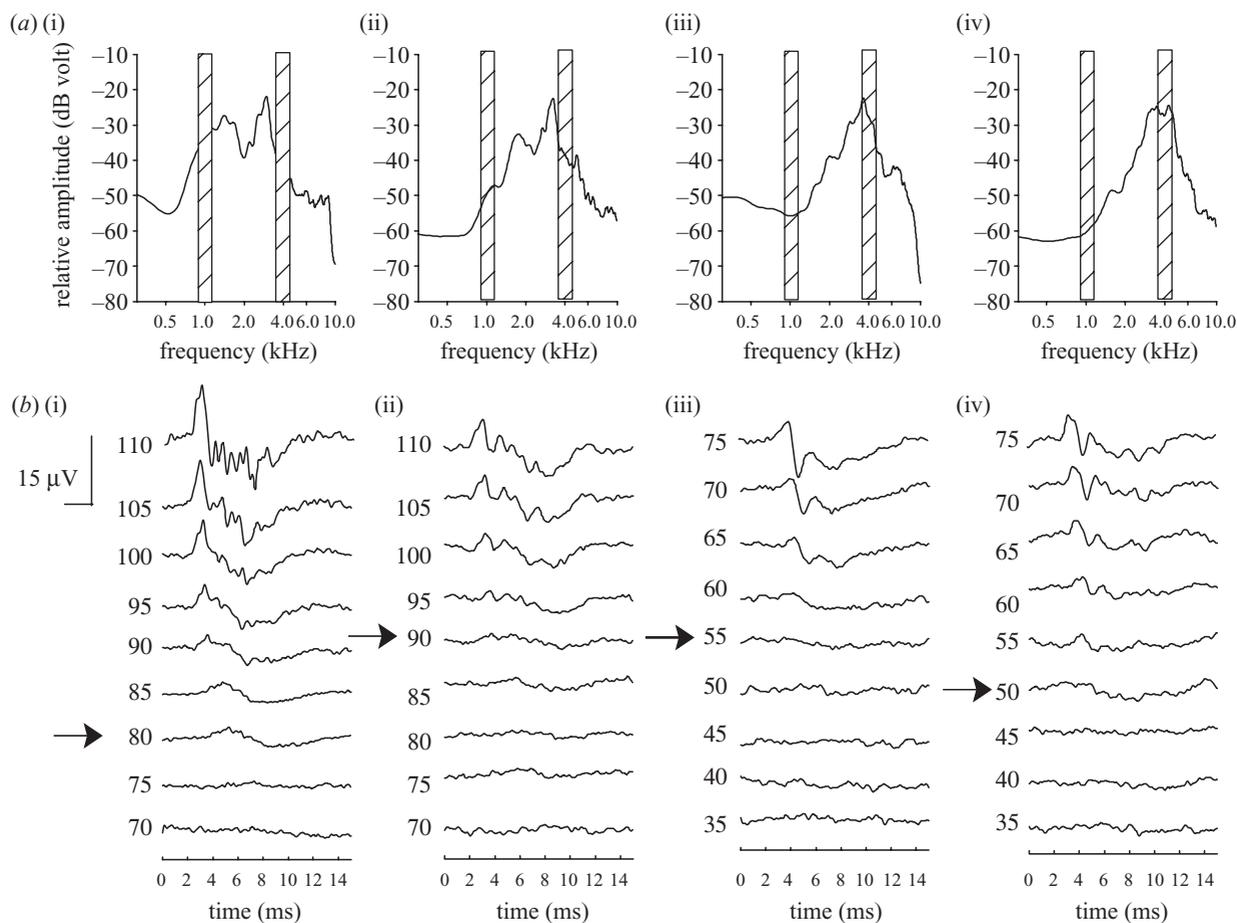


Figure 1. (a) Averaged frequency spectra of 10 songs from two parental strain canaries and two backcross hybrids with the one-third octave bands at 1 kHz and 4 kHz shown by hatched bars. (i) BWS parent; (ii) backcross with a full complement of BWS Z chromosomes; (iii) Border parent; and (iv) backcross with a partial complement of BWS Z chromosomes. (b) ABR waveforms evoked by a 4 kHz tone delivered at nine intensities (in dB); hearing thresholds are indicated by arrows. (i)–(iv) as in (a).

+ 42.7, corrected $r^2 = 0.87$, where Z_{add} and Z_{dom} are the additive and dominance components of the Z chromosome effect, respectively). The model allowing additive and dominant sex chromosome components to vary, independently explained a slightly higher amount of the variation ($Y = 21.2Z_{\text{add}} - 30.6Z_{\text{dom}} + 45$, corrected $r^2 = 0.91$), but the difference between the two models was not significant ($2(\log\text{-likelihood ratio}) = 3.4$, d.f. = 1, χ^2 -test $p > 0.05$).

(b) Song spectral characteristics

Backcross hybrids (figure 2c, grey symbols) showed no difference in song spectral amplitude at 1 kHz minus 4 kHz between males with two BWS Z chromosomes versus individuals with a mixed Z chromosome complement (table 1). When all three generations were combined, however, males with two BWS Z chromosomes had significantly larger values for song amplitude difference than males with one or zero BWS Z chromosomes.

A recessive Z chromosome effect explained 79% of the variation in song peak frequency ($Y = 3.7Z_{\text{add}} - 3.7Z_{\text{dom}} - 7.1$, corrected $r^2 = 0.79$), whereas allowing additive and dominant Z chromosome effects to vary independently explained 86% of the variation in this variable ($Y = 4.5Z_{\text{add}} - 2.2Z_{\text{dom}} - 8.7$, corrected $r^2 = 0.86$). The

difference between the two models was not significant ($2(\log\text{-likelihood ratio}) = 3.2$, d.f. = 1, χ^2 -test $p > 0.05$).

(c) Correlation between hearing and song

Linear regressions of the song spectral amplitude difference on hearing thresholds of males were significant and positive for thresholds at 4 kHz (figure 2d; $n = 12$, $y = 0.083x - 9.14$, $r^2 = 0.49$, $p = 0.01$) but not at 1 kHz ($n = 12$, $r^2 = 0.17$, $p = 0.2$).

4. DISCUSSION

(a) Inheritance of hearing and song spectrum

Our results demonstrate that the high-frequency hearing deficit in BWS canaries is associated with a genetic factor of major effect located on the Z chromosome. Hearing thresholds at 4 kHz were significantly higher in birds with a full complement of BWS Z chromosomes than in individuals with at least one Border Z chromosome, regardless of autosomal complement. The BWS Z chromosome is estimated to be responsible for 87% to 91% of the difference between the two strains in hearing at 4 kHz depending on the degree of recessive expression assumed. The non-significant difference between the two models indicates that the Z-linked factor is largely recessive.

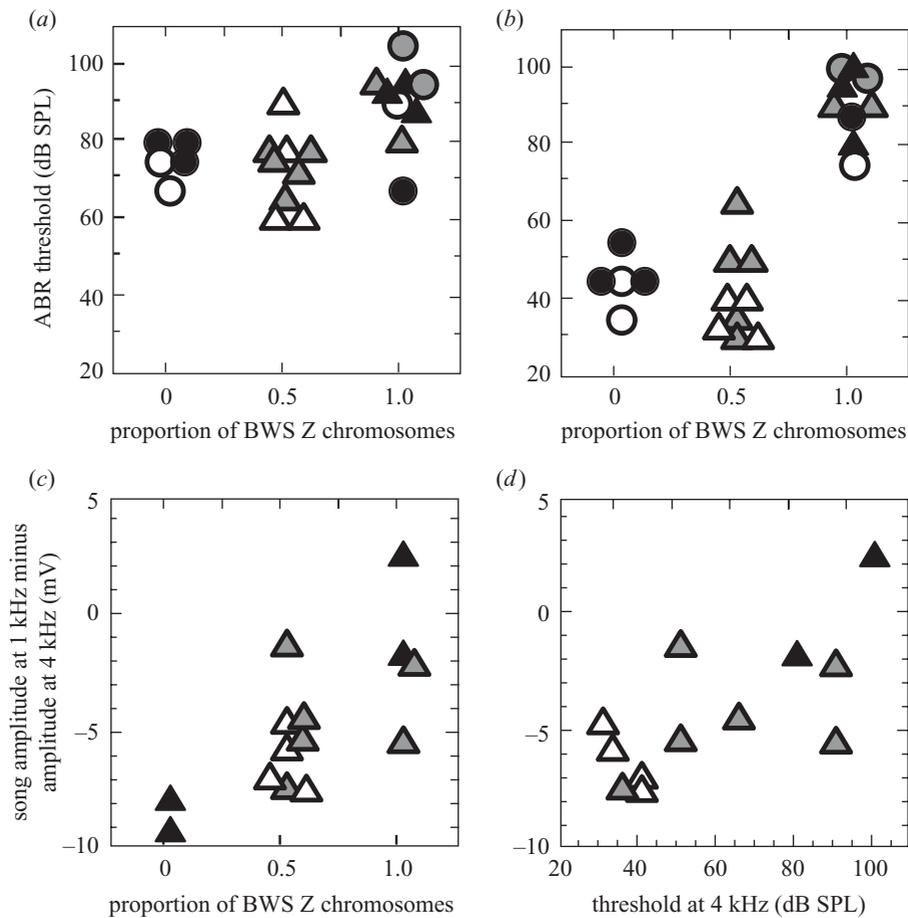


Figure 2. Hearing thresholds at (a) 1 kHz and (b) 4 kHz for individuals plotted by the proportion of Z chromosomes of BWS origin (0 equals no BWS Z chromosomes; 0.5 equals hybrid males with one Z chromosome from each strain; 1 equals females with one or males with two BWS Z chromosomes). (c) The difference in the song spectrum amplitude at 1 kHz and 4 kHz plotted by Z chromosome complement. (d) The regression of the difference in song spectrum amplitude against hearing threshold at 4 kHz for males. Black triangles, parental males; black circles, parental females; white triangles, F₁ males; white circles, F₁ females; grey triangles, backcross males; grey circles, backcross females.

Table 1. Comparisons of hearing and song spectra between pedigree birds with a full complement of Belgian Waterslager Z chromosomes versus those with at least one Border Z chromosome.

measure	pedigree generation compared	n	tied Z-value ^a	tied p-value ^a	higher in birds with a full complement of BWS Z chromosomes
hearing threshold at 1 kHz	parental	7	1.1	0.284	no
	backcross	9	2.5	0.014	yes
	all	23	3.1	0.019	yes
hearing threshold at 4 kHz	parental	7	2.1	0.032	yes
	backcross	9	2.5	0.014	yes
	all	23	4.0	< 0.001	yes
song amplitude at 1 kHz minus amplitude at 4 kHz	backcross	6	0	> 1.000	no
	all	14	2.0	0.048	yes

^a Mann–Whitney tests.

The inheritance pattern of song spectral energy was also consistent with a recessive Z-linked factor; nonlinear regression models explained 79% to 86% of the variation in song spectral energy. The songs of pure BWS males had more energy at 1 kHz than at 4 kHz, while the reverse was true for the songs of wild-type Border males. Hybrid

males were generally intermediate in song spectral energy when compared with the two parental strains.

(b) Correlations between hearing and song

The hearing threshold at 4 kHz for individual males was a significant predictor of the frequency spectrum of their

song, explaining 49% of the variation in song energy. One potential explanation for this association is that it arises during song learning and is driven by poor perception of the high-frequency elements of wild-type song. Biased perception could affect both the memorization phase, when young BWS canaries first acquire their auditory templates, and the motor phase, when they attempt to match their songs to those of other birds. This bias need not be absolute; for example, some learning of less-perceptible song elements could have occurred under the controlled conditions of our tutoring regime where masking noise was minimized. Another possibility, albeit less likely, is that the observed association between hearing and song arises from a genetic correlation through pleiotropy (one gene affecting multiple traits) or physical linkage of different genes.

(c) *Sensory linkage*

The apparent linkage of hearing and song traits through sensory perception in BWS canaries may have general implications for the evolution of sexually selected traits. Learned male song in oscine songbirds is important in mate choice (Searcy & Yasukawa 1996), and in many species, females learn their sexual preferences for male song through early exposure (Nagle & Kreutzer 1997; Irwin & Price 1999). Thus, changes in traits such as auditory perception that affect song learning could potentially alter the expression of song in males and the preference of females simultaneously. Such 'sensory linkage' could provide a mechanism for rapid trait evolution and reproductive isolation through runaway sexual selection (Fisher 1930; Lande 1981; West-Eberhard 1983), wherein the learned male trait and learned female preference would be linked via a common dependence on sensory input rather than through physical linkage of separate genetic loci.

We have demonstrated a correlation between sensory perception and male song in hybrids of two canary strains that probably arises during the learning process. Investigations of variation in male song, female preferences and hearing sensitivities among wild canary populations could be used to test the hypothesized sensory linkage.

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