

Song repertoire is not affected by stress in an adult male songbird, the Whitethroat *Sylvia communis*

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(Accepted: 29 May 2007)

Summary

It is believed that bird song has evolved as a reliable signal of quality of displaying individuals. Recent research has focused on costs of development of complex song. In the present paper we test if the acquired repertoire size is costly to maintain. We compared changes in song structure in male Whitethroats (*Sylvia communis*) after 48 h exposure to a stressor (5% body mass weight attached to the tail feathers) vs. changes observed within the same time interval in the control group. The strophe length was marginally significantly shorter in the handicapped males comparing to controls. However, the repertoire size (i.e., a measure of diversity of different song elements) remained intact in both groups. We concluded that the song repertoire in Whitethroats is a static secondary sexual trait. A review of literature has revealed no convincing examples of decreasing repertoire size in adult male songbirds. Further research is needed to improve our understanding of evolutionary and proximate mechanisms maintaining the stability of song repertoires.

Introduction

It is believed that in songbirds the song produced by the male provides females with information about its phenotypic and genetic quality (Gil & Gahr, 2002). Given that interests of both sexes are conflicting to some extent (i.e., it is to the male's advantage to exaggerate his quality), the stable signalling system must impose some costs on advertising males (Zahavi & Zahavi, 1997). The reliability of male signals is maintained by a set of physiological and en-

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vironmental constraints such as energy requirements, predation risk or social aggression (Gil & Gahr, 2002). In particular, it is hypothesised that the complex song is costly because neural structures underlying its learning develop early in life, when young birds may experience developmental stress due to malnutrition. Thus the nutritional stress hypothesis (Nowicki et al., 1998, 2002) proposes that “by choosing males based on song features that reflect the outcome of song learning, females really are choosing males that fared better in the face of nutritional (and other) stresses early in life” and “such males are likely to be phenotypically or genetically superior”. The hypothesis has received support from experimental studies manipulating the nutritional environment of young (Nowicki et al., 2002; Buchanan et al., 2003; Spencer et al., 2003; Soma et al., 2006; but see Gil et al., 2006).

The nutritional stress hypothesis focuses on costs of development of the song repertoire and assumes that it is a static indicator trait (*sensu* Hill et al., 1999). Another issue is if the song variability already acquired is costly to maintain and sensitive to environmental stressors. There is a growing body of evidence that stress might impair long-term memory retrieval and it appears that this mechanism might be triggered by hormonal reaction involving corticoids (Rozen daal, 2002; Podos et al., 2004). Additionally the disturbance in immune function caused by the prolonged stress may suppress testosterone production (Folstad & Karter, 1992). To conclude, we might expect that the repertoire size is sensitive to changes in condition of displaying males and, consequently, can carry reliable information about the present phenotypic quality of signallers.

In our study species, the Whitethroat, sexual advertising involves singing from a perch and song flights (Balsby, 2000a, b). In a previous paper (Halupka & Borowiec, 2006) we demonstrated that 48 h exposure to a stressor (5% body mass weight attached to the tail feathers) limited the elaborated song flight made by males which in turn lowered their mating success. Here we report results of an experiment in which we tested if such a handicap also affected the repertoire size, another sexually selected trait in Whitethroats (Balsby, 2000a). We expected that the prolonged stress would influence the expression of song repertoire in handicapped males.

Methods

Data were collected between 1998 and 2002 in a 6 km² study plot in SW Poland. All field work was carried out in the morning (4.30-10.30) between

April and June. Colour-ringed individuals were randomly divided into two groups: treatment ($N = 8$) and control ($N = 9$). In both groups song produced by males was recorded twice with a 48 h break between sessions (we used a Sony TCD-D10 Pro II DAT recorder with a Telinga parabolic microphone). We selected unmated males which had established territories and songs were recorded during 'normal' behaviour (i.e., without intense counter singing with another males or advertising to a newly arrived female). Males in the experimental group were mist-netted immediately after the first recording of song, and we attached a 0.7 g lead weight (about 5% of body mass; range 4.4-5.5%) to the base of their tail feathers using 'Super Glue'. Such a method of handicapping is recommended since it neither increases the mortality nor causes excessive distress (Cuthill, 1991). The experimental males were released after 5 min and immediately resumed their normal activity.

Repertoire sizes and strophe lengths were estimated twice for each male, separately for the initial recording and the one made 48 h later. Researchers who analysed recordings did not know which group the given male belonged to. Each repertoire size estimation was based on the analysis of 20 subsequent songs. We did not discriminate between the song types (Balsby, 2000a, b) because the handicap did not influence their number (Halupka & Borowiec, 2006).

We analyzed 48 kHz/16 bit PCM WAV sound files with Avisoft SASLab Pro 4.3× software and the following set of parameters: 1024 FFT length, Frame (%) = 25, Window = Hamming and Temporal Overlap = 87.5%. This gave a 244 Hz bandwidth with 42 Hz frequency and 2.9 ms time resolution of sonograms (Specht, 2002).

For all recordings we measured length of each strophe (in seconds). The mean value for each recording was calculated for the same twenty songs used in repertoire size estimation (see below). The following terminology was used to define repertoire components: an element consisted of any sound showing no gaps in a sonogram in the time domain; a song consisted of any unique assemblage of elements preceded and followed by at least 1 s of silence (Figure 1).

We categorized song element types by visual inspection of sonograms. Categorization was initially done by one author (KŁ) and then verified independently by the second one (TSO). As the element repertoire size of the Whitethroat can be very large (Balsby & Dabelsteen, 2001), we used different sets of codes (letter or letter and number combination) denoting particu-

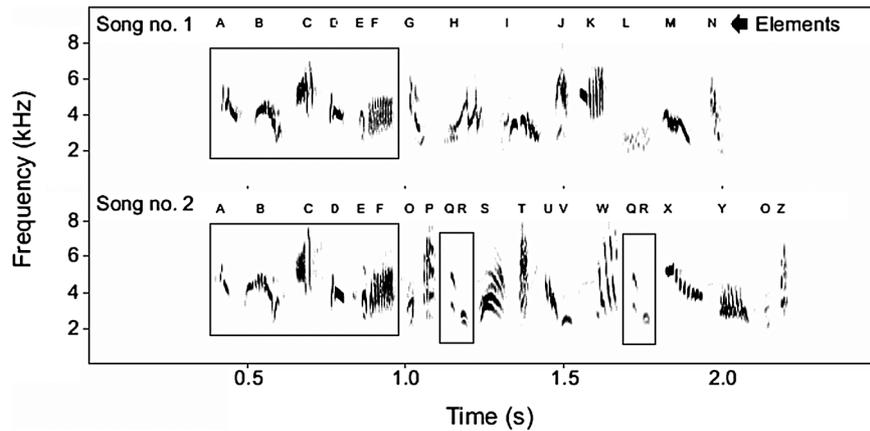


Figure 1. The two following songs of the Whitethroat male. Letters indicate elements and boxes show some shared sequences of elements.

lar elements for each individual. This was to make the process of elements categorization technically and perceptually easier.

We applied capture-recapture method for song element repertoire size estimation based on first 20 songs from each recording. Twenty songs was the minimal number of consecutive, good quality songs we had for all males and this value seems to be sufficient for the estimation of repertoire size of the species (Garamszegi et al., 2005). We used ComDyn4 — Community Dynamics software (by Jim Hines, www.mbrpwr.usgs.gov), which was developed to estimate parameters associated with community dynamics using presence-absence data from two locations or time periods (Boulinier et al., 1998). In our case, we estimated an equivalent of species richness, i.e., a song element repertoire size, and for each male we had data on song element presence or absence for two time periods separated by a 48 h break. Consequently, our analysis focused on compositional changes in the repertoire between these two time periods. The basic estimator for species richness in ComDyn4 is the jack-knife estimator proposed by Burnham & Overton (1978, 1979), which was shown to be reasonably robust to potential departures from model assumptions and has performed well in several studies (Garamszegi et al., 2002, 2005; Bell et al., 2004).

Statistical analysis was performed using StatsDirect software. For each male we calculated the percentage change in the repertoire size and strophe

length within 48 h, with the initial repertoire size as the base. All tests are two-tailed. *P*-values for the Mann-Whitney test and confidence intervals for medians are exact (i.e., without normal approximations).

Results

At the beginning of the experiment (i.e., before handicapping the experimental birds) the median repertoire size across both groups ($N = 17$) equalled 22.9 (interquartile range 20.9-44.5) and the strophe length 1.47 s (1.19-1.61). The repertoire size and strophe length were not correlated ($r = -0.209$, $N = 17$, $p = 0.421$).

We compared median percentage changes in the repertoire size and strophe length within 48 h in the treatment group with the corresponding statistic calculated for the controls. The difference calculated for the repertoire size was not significant (Mann-Whitney: $U = 26$, $p = 0.369$; Figure 2) with its 95% confidence interval widely extended around the zero value, ranging from -55.1 to 33.3 . This suggests that the insignificant result reflects a negligible effect size and cannot be attributed to problems with achieving sufficient power of the test. In contrast, the strophe length changes calculated with the same method were marginally significant ($U = 16$, $p = 0.059$; 95% confidence interval for the difference: -1.29 to 40.0 ; Figure 2) and we conclude that males in the treatment group, when compared to the control, tended to reduce the average length of the strophe.

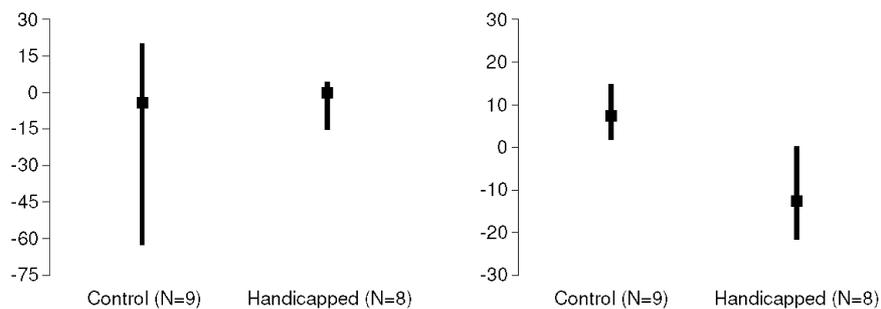


Figure 2. Median percentage change (\pm interquartile ranges) in repertoire size (left plot) and strophe length (right plot) within 48 h in the experimental group (birds handicapped after the first measurement of the repertoire size) and the control group.

Discussion

The repertoire size in Whitethroats is not sensitive to minor changes in condition of the male and not costly to maintain. This conclusion is generally in line with results of another study (Garamszegi et al., 2004) in which an experimental challenge of the immune system with a novel antigen did not influence the song structure in Collared Flycatcher (*Ficedula albicollis*) males, but significantly reduced the song rate. To sum up, in both species studied additional costs imposed on advertising males significantly affected song output but the song repertoire apparently remained intact.

It is puzzling why and how the acquired repertoire size is so resistant to degradation. Longterm memory in vertebrates is prone to slow disintegration and this process may be facilitated by prolonged stress (Luine et al., 1994; de Quervain et al., 1998, 2000; Roozendaal, 2002). However, even though the literature on song acquisition is voluminous (reviewed in Nowicki et al., 2002; Hultsch & Todt, 2004), the possibility of song modification by forgetting some elements is not discussed. The term 'forgetting' appears in the literature on the development of song repertoires, but it is used therein to describe selective dropping of some learned song elements which allows the singer to fit its social environment (Nottebohm, 1984; Nelson, 2000; Nordby et al., 2000). Actually, we found only one, and rather peculiar, account that the acquired repertoire size may decrease: deafened Canaries (*Serinus canarius*) gradually forget their song (Nottebohm et al., 1976, after Nottebohm, 1984). It is difficult to judge whether such a literature bias reflects a deficit in our knowledge or a rather unusual durability of memorized song elements and a stability of the song repertoire.

Hill and co-workers (1999) proposed that static signals of male quality, in contrast to dynamic ones, were free from noise generated by current environmental conditions and thus could carry more reliable information about the genetic background of the male (Kipper et al., 2004). We might expect that in socially monogamous birds the dynamic traits are used by females in the selection of socio-sexual partners which contribute costly parental effort, in addition to genes. Song repertoire might be more important in the selection of extra-pair partners, which contribute only genes (e.g., Hasselquist et al., 1996; Forstmeier et al., 2002). Indeed, it appears that the song repertoire may have a genetic component (Reid et al., 2005).

To conclude, our study demonstrates that the song repertoire in White-throats is a static secondary sexual trait. This finding corresponds with re-

sults of another study (Garamszegi et al., 2004) and with data in the literature in which no convincing example of the song repertoire deterioration is described. Further research is needed to improve our understanding of the mechanisms maintaining the stability of song repertoires in birds.

Acknowledgements

We thank Bartek Sklepowicz and Krzysztof Zając for helping in the fieldwork and Laszlo Garamszegi for advice in using ComDyn software for repertoire size estimation and comments on this manuscript. We also thank Ben D. Bell for useful comments on this manuscript and improving the English. The project was supported by the Polish State Committee for Scientific Research (PO4C 092 14) and the University of Wrocław (2020/W/IZ/1998-2004).

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