Differential response of males of a subarctic population of Bluethroat *Luscinina svecica svecica* to playbacks of their own and foreign subspecies

Lucia Turčaková · Václav Pavel · Bohumír Chutný · Adam Petrusek · Tereza Petrusková

Received: 25 July 2010 / Revised: 10 January 2011 / Accepted: 7 March 2011
© Dt. Ornithologen-Gesellschaft e.V. 2011

Abstract Differences in vocalization between subspecies of songbirds may influence intraspecific interactions including mate choice and territorial behavior, and contribute to their further divergence by strengthening pre-mating reproductive isolation. We have recently shown that songs of two Bluethroat subspecies, *Luscinia svecica svecica* and *L. s. cyanecula*, differ in spectral characteristics and structure. Here, in a first experimental test of whether these differences are biologically relevant, we compared responses of *L. s. svecica* males to both types of songs. Altogether 19 males from a subarctic population in Abisko, Sweden, were exposed to playbacks of songs from the two subspecies, accompanied by display of a stuffed Bluethroat lacking subspecies-specific colour marks. These stimuli were presented in random order, separated by over 1 h. We evaluated two largely uncorrelated components of male responses: non-vocal territorial behavior, and singing activity. A significant difference in reaction to subspecies playbacks was observed in aggressive behavior: independently of the song order, males generally responded more strongly to playback of their own subspecies. In contrast, playback order rather than subspecies identity significantly influenced the singing activity, which usually increased in the second trial. A more detailed analysis nevertheless suggested that vocal responses of birds exposed first to playback of their own subspecies were stimulated in the subsequent trial but not vice versa. Our results show that Bluethroats clearly discriminate the two song types, indicating the potential for subspecies recognition. Further experimental work is needed to assess the general validity of these patterns.

Keywords *Luscinia svecica* · Subspecies recognition · Song variation · Aggressive behavior · Playback experiments


Introduction

Vocal cues in birds are considered a major component of species recognition (Catchpole and Slater 1995). However, noticeable differences in songs among conspecific populations are often observed (e.g., Koetz et al. 2007; Petrusková et al. 2010). Song divergence has evolved under the pressure of either ecological selection and/or social interactions (Payne 1996; Nordby et al. 2000). In some cases, differential responses to divergent acoustic signals may result in reproductive isolation (Slabbekoorn and Smith 2002; Edwards et al. 2005), as species-specific vocalization may contribute to pre-mating reproductive barriers (den Hartog et al. 2007). Song divergence therefore could be an important step in the speciation of birds (Irwin and Price 1999). Additionally, consistent differences in songs may contribute to recognition of phenotypically cryptic species, as recently shown for example by Blyth’s Leaf Warbler and White-tailed Leaf Warbler complexes (Phylloscopus reguloides, P. davisoni), in which differences in territorial songs reflect genetic divergence (Päckert et al. 2009).

A number of studies have shown that song divergence is also distinguishable among populations belonging to different bird subspecies (Nelson 2000; Ruegg et al. 2006; Ribot et al. 2009). They may diverge in various song features, including temporal (Dingle et al. 2008; Ribot et al. 2009), structural (Tubaro and Segura 1995; Matessi et al. 2000) and frequency parameters (Ruegg et al. 2006), or a combination thereof. In some taxa, subspecies differ in morphology or coloration as well as in song (Matessi et al. 2000; Liu et al. 2008). However, in many cases, subspecies are very similar in external morphology and only clearly differ in their vocalization (Irwin 2000; Brambilla et al. 2008).

In various taxa, playback experiments have confirmed the recognition ability of birds at the subspecies level (e.g., Petrino and Pattson 1981; Lampe and Baker 1994; Martens and Steil 1997; Liu et al. 2008), which may strengthen reproductive barriers among populations of different subspecies. If subspecies recognition through acoustic signaling effectively prevents hybridization, it may suggest that such taxa have already diverged to the point when they should be treated as distinct biological species (Leger and Mountjoy 2003; Brambilla et al. 2008). Even in phenotypically distinct subspecies, song divergence and subsequent recognition ability based on this divergence may play an important role, as acoustic signals may be more useful than visual ones for communication over long distances (Sorjonen 1986a) or in dense habitats (Morton 1975).

Subspecies complexes and their diversification offer insight into recent evolutionary events and early stages of speciation (Sol et al. 2005; Seddon et al. 2008). The Bluethroat (Luscinia svecica) is a suitable model for studying subspecies divergence. This Palaearctic taxon, which probably diverged over the last 15,000 years (Zink et al. 2003), is a complex of several distinct morphs that are usually given subspecies status; 10–11 subspecies are recognized that differ to various extents morphologically (Glutz von Blotzheim and Bauer 1988; Eybert et al. 1999; Arizaga et al. 2006), in throat ornamentation (Johnsen et al. 2006) and in breeding habitat preferences (Cramp 1988). Microsatellite genotyping (Johnsen et al. 2006) has supported the genetic divergence of some but not all tested putative subspecies. However, it has also confirmed that subspecies with more southern distribution, carrying either a white or plain blue throat spot, are well differentiated from each other as well as from northern populations with a red throat spot. Splitting of these red-spotted populations into several taxa seems to be more problematic (Johnsen et al. 2006).

Bluethroat males have complex vocalization (Sorjonen 1986b; Cramp 1988), but the variation in their songs has not yet been thoroughly studied. In our pilot study on Bluethroat songs (Turčoková et al. 2010), we compared two populations of L. s. svecica (subspecies belonging to the red-spotted northern group) and two populations of L. s. cyanecula (white-spotted subspecies from the southern group). Apart from among-population variation, we observed significant differences in some spectral and structural parameters of songs that were consistent between the two subspecies. Although such differences are not necessarily the result of divergent selection on acoustic cues themselves (e.g., the variation in frequency parameters seems to be related to differences in male body size;
Turčoková et al. 2010), the resulting divergence in songs may be useful in subspecies recognition.

In this study, we conducted behavioral experiments to investigate the recognition ability of males from a subarctic population of Luscinia s. svecica, in which we measured the intensity of behavioral responses (aggressive reactions and vocalizations) to the playback of songs from both studied subspecies. We hypothesized that differences in the characteristics of songs from these two subspecies were sufficient to allow discrimination by the tested males, and that a song from their own, familiar, subspecies would be considered a stronger threat than a song from an unfamiliar subspecies. Therefore, we predicted that the males’ reactions should be stronger and more aggressive to the former, and weaker to the latter.

**Methods**

**Study species**

The Bluethroat Luscinia svecica (Passeriformes, Muscicapidae) is a small migratory ground-breeding passerine bird (Cramp 1988), with conspicuous coloration and a noticeably complex song (Sorjonen 1986b). There are clear differences in phenotype, particularly color patterns, among subspecies, which are distributed throughout the Palaearctic (Cramp 1988). In our experiments, we used recordings of two subspecies that differ in phenotype, habitat selection, and song structure (Turčoková et al. 2010). The nominate subspecies Luscinia svecica svecica has a red-chestnut spot, and breeds in arctic and boreal latitudes and mountainous regions of Scandinavia and northern Russia (Cramp 1988), though isolated populations are also known from Central European mountains (Johnsen et al. 2006; Chutný and Pavel 2009). Such populations may occasionally come into contact and hybridize with the local subspecies Luscinia svecica cyanecula (Chutný and Pavel 2009). L. s. cyanecula has a white throat spot and breeds mostly in European lowlands, particularly in reedbeds or other dense vegetation close to water.

**Playback experiments**

The individuals tested belonged to the arctic population of L. s. svecica that breeds in Abisko, Sweden (68°21’N, 18°48’E, 370 m a.s.l.), ca. 200 km north of the Arctic Circle. Males of this population never encounter other subspecies of L. svecica on their breeding grounds. The local habitat is typical arctic tundra—bogs partially covered by low birch trees and other shrubs.

The experiments were conducted during two breeding seasons (May–June) in 2006 and 2007. All experiments were carried out during fair weather conditions, between 0700 and 2100 hours (Central European time), following the natural activity of Bluethroat males. We tested mated males during the pre-laying period, when males defending their mates are willing to respond to experimental “intrusions”, as has been shown in other species (e.g., Nowicki et al. 2002; Petrusková et al. 2008).

We compared the reactions of territorial males to song playbacks from their own subspecies (L. s. svecica) as well as from the foreign subspecies (L. s. cyanecula). Altogether 19 color-ringed males defending their territories were tested, each of them by a playback session consisting of three different song stimuli (trials) separated by 30-min pauses: playbacks of song of L. s. svecica (Fig. 1a), L. s. cyanecula (Fig. 1b) and Willow Warbler Philloscopus trochilus (as control), accompanied by the presentation of a stuffed bird of the respective species (Bluethroat or Willow Warbler). The use of a dummy bird as visual stimulus provided a clear target for any aggressive actions (see Petrusková et al. 2008), potentially allowing a more precise evaluation of the males’ reactions. To make sure birds did not respond to color pattern rather than songs, a stuffed Bluethroat completely lacking subspecies-specific coloration (the reddish or white spot), i.e., with a plain blue throat, was presented during song playbacks of both Bluethroat subspecies. Each stuffed bird was attached to a remotely controlled reversible lid of a wooden box, and could be either completely hidden from view or visible, apparently perching on top of the box.

We placed a loudspeaker and boxes with stuffed birds (one Bluethroat and one Willow Warbler) near the center

---

**Fig. 1** Examples of the songs used as acoustic stimuli in the experiments: 8-s fragments of songs of two different males of Luscinia svecica cyanecula (a, b) and L. s. svecica (c, d). Note that each male was tested by a different recording.
of the territory of each male bird tested, and built a shelter approximately 25 m away, from which we controlled the experiment and observed the tested male during the whole experiment. The first playback trial started when the male occupying the territory habituated to the experimental equipment, approximately 20 min after the set-up. At that moment, the tested male was always present in the territory, and could be seen or heard. The singing response of the male was recorded on a Marantz PMD 222 tape recorder through a Sennheiser MKH 50 P48 microphone placed approximately 2 m from the playback equipment. Two persons always conducted the experiment. One operated the equipment (playback, remote control of stuffed bird models, and tape recorder) and the second noted the behavior of the bird and its timing.

Each experiment, lasting altogether 90 min, was initiated by the synchronous emergence of a stuffed bird from the box and playback of the respective song. These stimuli (i.e., both acoustic and visual) lasted for 5 min; afterwards, the stuffed bird was exposed for 3 more minutes without playback (visual stimulus only). After hiding it from view, we recorded the behavior of the tested male for 2 more minutes. The subsequent stimulus was separated by a 30-min “habituation pause”. The order of Bluethroat stimuli was chosen randomly, ten males were tested first by L. s. svecica stimulus, nine males by L. s. cyanecula stimulus. These two trials were always separated by the playback of the Willow Warbler song (accompanied by the respective stuffed bird). This species, common in the study area, was used as a control to check whether males excited by conspecific stimuli show any territorial behavior towards a non-competitor species (as observed in Meadow Pipits Anthus pratensis by Petruskova et al. 2008). No response to the Willow Warbler dummy was observed for any tested Bluethroat male; therefore we did not include these control experiments in further analyses.

For each playback trial, a different song recording was used as an experimental stimulus. Songs of L. s. svecica came from Abisko and were recorded during the previous year or far away (at least 3 km) from tested males, in order to avoid the neighbor effect (e.g., Stoddard et al. 1990; Molles and Vehrencamp 2001). L. s. cyanecula songs came from populations breeding in the Czech Republic, while Willow Warbler songs came from both Abisko and the Czech Republic.

During the experiment, we verbally described and recorded on a dictaphone the behavior of the tested male; these recordings were afterwards analyzed to evaluate the timing and strength of behavioral responses. We categorized various male actions into several response levels, ordered for further analysis by their apparent strength (modified from Petrusková et al. 2007): interest (an approach to <10 m from the dummy, erratic movements around, sometimes with a brief approach to a closer distance), attentive observation (long-time observation from a post, occasional flights directly over the dummy), flight attack (a distinct dive in flight, approaching the dummy to closer than 1 m), and physical contact with the dummy. Each action of the bird was classified into one of these categories, which allowed subsequent assessment of the maximal strength of the male response (ranging from 0, no interest, to 4, physical contact). We also estimated the distance of the closest approach of the tested male to the dummy, and quantified the time the male expressed any interest in the dummy. The singing response was evaluated from recordings taken during each trial. We noted the time when the male started to sing, counted the number of songs sung by the male, and noted the length for each of them.

Data analysis

To summarize the reactions of tested males in each subspecies trial, we calculated two behavioral scores from six variables by principal component analysis (PCA) with varimax rotation. Three variables summarized the non-vocal territorial behavior: (1) the minimal distance to which the male approached the dummy during a particular 10-min trial with one stimulus; (2) interest: the proportion of the trial time when the male clearly showed interest in the “intruder” (including time spent by aggressive actions); and (3) the maximal intensity of male aggressive reactions during the trial. Other three variables represented the acoustic response, i.e., the vocalization of the males: (1) the number of songs sung by the male during the whole trial; (2) the song rate (number of songs per minute since the first vocal response to the end of the trial); and (3) the song output (proportion of time spent by singing since the first vocal response).

The first two components of the PCA represented 84.9% of the variation in the original variables. After varimax rotation, the first factor (that explained 47.9% of variation) was tightly correlated with the three variables describing the singing activity, and the second factor (37.0%) was correlated with the remaining three variables describing non-vocal behavioral responses (Fig. 2). This shows that the vocal and non-vocal responses of tested males were largely uncorrelated, so we further analyzed the two types of response separately, using the respective PCA factors from each playback experiment as summary scores (further labeled as “singing” and “aggressiveness” scores). These PCA scores were not normally distributed; therefore, we consistently applied non-parametric statistical tests throughout the analyses.

As the response of territorial males to playback may depend on a preceding stimulus (Petrusková et al. 2008), we compared not only the reactions of individual males to
stimuli representing the two subspecies but also their reactions to the first and to the second stimulus regardless of the subspecies identity. These differences were analyzed by the Wilcoxon matched pairs test.

Furthermore, based on the results of previous analyses, we tested the potential effect of playback order on the vocal response of males to playback from the same subspecies (this was tested for both \textit{L. s. svecica} and \textit{L. s. cyanecula}). In this comparison, four individuals that did not respond by singing to either of the two playbacks were excluded from the analysis. The remaining males were split into two groups, differing in the order of presentation of subspecies stimuli, and the singing scores in the same subspecies trial between males of both groups were compared by the Mann–Whitney $U$ tests. In these comparisons, males in one group were exposed to the respective playback first (with no preceding stimulus), while males in the other group may have been potentially affected by the previous playback. All statistical analyses were conducted in the software package Statistica v.6.1 (StatSoft, Tulsa, USA).

**Fig. 2** Factor loadings of six variables characterizing the behavior of tested males included in the factor analysis (principal component analysis with varimax rotation). \textit{Factor 1} (“singing”) was used as the behavioral score describing the vocal response of the tested male during the trial; \textit{Factor 2} (“aggressiveness”) characterized its non-vocal response. As all three variables representing singing activity are highly correlated in the factor plane, they are not labeled individually. Note that higher values of the minimal distance to the dummy reflect lower aggressiveness of the focal male.

**Results**

The non-vocal aggressive behavior responses of tested males to two different subspecies stimuli differed significantly, but the singing activity did not (Fig. 3a). Males reacted more strongly to the playback of \textit{L. s. svecica} (i.e., to their own subspecies) than to \textit{L. s. cyanecula} (Wilcoxon matched pairs test, $n = 19$, $Z = 2.09$, $p = 0.036$); the overall acoustic response did not differ significantly between the two types of playback ($Z = 0.52$, $p = 0.60$).

On the other hand, the singing activity was clearly affected by the order of the stimuli (Fig. 3b); males sang significantly more during the second trial, irrespective of what subspecies it was (Wilcoxon matched pairs test, $n = 19$, $Z = 2.41$, $p = 0.016$). This was not the case for aggressive responses, which did not differ significantly between the first and the second stimuli ($Z = 0.28$, $p = 0.78$).

An interesting underlying pattern emerged when the responses to the two subspecies playback were evaluated separately (Fig. 4): while the response to the \textit{L. s. svecica} playback did not differ significantly whether it was played as the first or the second stimulus (Mann–Whitney $U$ test, $n_1 = 7$, $n_2 = 8$, $Z = 0.46$, $p = 0.64$), the response to the playback of \textit{L. s. cyanecula} was significantly higher for those males to whom it was played as the second stimulus (Mann–Whitney $U$ test, $n_1 = 7$, $n_2 = 8$, $Z = -2.08$, $p = 0.037$).

**Discussion**

Our experiments conducted on \textit{Luscinia s. svecica} males confirmed that the individuals we tested responded with
stronger aggressive behavior to songs of their own subspecies than to those of *L. s. cyanecula*. As we only tested a single population of the arctic subspecies, it cannot yet be concluded with certainty whether the observed differences in behavior reflect a general ability to discriminate between local and foreign songs, or whether this really implies distinct responses on the subspecies level. In several bird species, responses to playbacks are stronger to local songs than to songs of foreign conspecific populations; this has been observed for example in the Corn Bunting *Emberiza calandra* (McGregor 1983), Song Sparrow *Melospiza melodia* (Searcy et al. 1997) and Orange-Fronted Conure *Aratinga canicularis* (Vehrencamp et al. 2003). Our observations are consistent with these studies. However, as our previous analysis confirmed significant differences between subspecies for several song features, in particular spectral parameters (Turčoková et al. 2010), we hypothesize that the weaker aggressive behavioral reaction of Bluethroat males to another subspecies’ song may be a more general pattern. Several studies have documented stronger responses by males when presented with a song of their own rather than foreign subspecies. The taxa for which this has been observed include, for example, the Chiffchaff (*Phylloscopus collybita*; Salomon 1989) and Lesser Whitethroat (*Sylvia curruca*; Martens and Steil 1997).

We observed a significantly stronger aggressive behavioral response by tested males towards their own subspecies’ song playback, independently of the song order. Interestingly, the acoustic response was different: the playback order had a significant effect while the stimulus type did not. Overall, the singing reaction of tested males to songs of their own and foreign subspecies did not differ significantly. However, it was apparently affected by the order of stimuli, usually increasing in the second trial. This suggests that the apparent similarity of the acoustic response between subspecies stimuli may be an experimental artefact.

Experiments in Meadow Pipits have shown that males excited by a conspecific playback reacted aggressively to subsequent heterospecific stimulus, although when the order of stimuli was reversed, there was no heterospecific playback response (Petrusková et al. 2008). In our experiments here, such a carryover effect, increasing the reaction to songs of foreign Bluethroat subspecies, may have obscured the acoustic response patterns: only when the *cyanecula* (i.e., foreign) song was played as the second stimulus did the tested male react with increased singing intensity. Such excitation elicited by a preceding song was not observed when a *svecica* (i.e., own subspecies) song was played second, i.e., the preceding cyanecula playback apparently did not arouse them sufficiently to cause an observable carryover effect.

Additionally, our evaluation of the acoustic response may have been influenced by another methodological issue. Bluethroat males can produce specific low-volume “soft songs”, which are often used during aggressive encounters (Anderson et al. 2007, 2008). In territorial contexts, such behavior usually predicts a subsequent attack by the singing male (Searcy and Nowicky 2006). This type of singing activity to deter rivals has also been observed in *L. s. svecica* populations breeding in the Central European Giant Mountain range and in Abisko (Chutný, Pavel, and Turčoková, personal observation). These soft songs were performed mostly from low vegetation, commonly when males interacted over short distances. As these vocal interactions are very inconspicuous and poorly audible (being usually directed at a single receiver, particularly when in an aggressive context; Dabelsteen et al. 1998; Searcy and Nowicky 2006), they would not have been recorded at the distance used in the present experiments. Thus, it is possible that variations in soft song performance rather than loud singing may correlate with the aggressive responses observed.

Our playback experiments confirm the ability of Bluethroat males to discriminate between songs of different populations, and did not falsify the hypothesis that they are able to specifically discriminate their own subspecies. The tested males apparently considered the song from an intruder of the different subspecies to be a threat not requiring a particularly strong aggressive response. They may also have evaluated the song as being sung by a relatively smaller male, as the frequency characteristics of cyanecula songs are consistent with the slightly smaller body size of that subspecies (Turčoková et al. 2010). The presence of a stuffed dummy bird nevertheless allowed the
tested male to evaluate the size of an intruder visually, without the need to rely solely on acoustic cues. Experimental evidence for a relationship between the level of territorial aggression in birds and the apparent quality of intruding males is conflicting (see, e.g., Pryke et al. 2001; Alonso-Alvarez et al. 2004). Aggressive encounters between males substantially differing in body size or other characteristics indicating quality are generally less likely to be escalated than encounters between rivals of similar quality (Riechert 1998). However, this applies to conditions when both rivals interact, and threats such as displays are sufficient to repel a weaker individual. In cases when an apparently weaker intruder does not react to threatening displays, territorial owners should be more likely to attack the rival to chase it away. We therefore suggest that the generally weaker reactions to cyanecula songs are more likely related to their different origin rather than to indications of a smaller body size. Additional experimental work is required to confirm whether this is a general pattern.

Several approaches might improve our understanding of factors that influence the response of Bluethroat males to subspecies song. First, testing males from other populations and other subspecies should strengthen or weaken the evidence of subspecies recognition. Second, placing the experimental equipment inside the territory but closer to the edge rather than the center would emulate a less threatening territorial intrusion, possibly provoking more graded singing responses from the tested males. Finally, an experiment that would force the bird to make a parallel discrimination between alternating stimulus pairs from different parts of the territory may further elucidate the recognition abilities of the species.

The Bluethroat subspecies we tested diverged not only in song features (Turčoková et al. 2010) but also in morphology, throat ornamentation and preferences for breeding habitat. During secondary contact, all these differences may contribute to some degree of reproductive isolation. The response of territorial males to individuals of another subspecies is nevertheless only one side of the coin. As different sexes may have different preferences for songs of their own subspecies or their local dialect (Salomon 1989; Ratcliffe and Otter 1996; Nelson and Soha 2004), individuals from other populations might become unable to recognize each other as potential sex partners. This means that differential preferences of females for specific motives of male songs (Vanechouette 1997; O’Loghlen and Beecher 1999) could cause increased signal differences between populations and reinforce species recognition through female learned preferences (Clayton 1990; Irwin and Price 1999). Female choice could therefore play a central role in the gradual isolation of the subspecies (Searcy and Brenowitz 1988). It is therefore necessary to take into account the recognition ability of females and their reaction to the songs of foreign subspecies.

Acknowledgments The study was supported by the Czech Ministry of Education (projects no. MSM0021620828 and MSM6198959212), the fieldwork in Abisko was funded by ATANS (projects no. FP6 506004). The experiments complied with all relevant Swedish and Czech laws. The authors declare no conflict of interest. We thank Nils Ake Andersson and Karel Weidinger for field assistance, two anonymous referees for valuable comments that improved the manuscript, and David Hardekopf for language corrections.

References

Deng Hartog PM, de Kort SR, ten Cate C (2007) Hybrid vocalizations are effective within, but not outside, an avian hybrid zone. Behav Ecol 18:608–614


Molles LE, Vehrencamp SL (2001) Neighbour recognition by resident males in the banded wren, Thryothorus pleurostictus, a tropical songbird with high song type sharing. Anim Behav 61:119–127


Salomon M (1989) Song as a possible reproductive isolating mechanism between two parapatric forms. The case of the chiffchaffs Phylloscopus c. collybita and P. c. brehmii in the Western Pyrenees. Behaviour 111:270–290


Vanecekoue MT (1997) Bird song as a possible cultural mechanism for speciation. J Memetics 1:130–139
