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Cultural evolution of birdsong

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Introduction

From evolutionary viewpoint, culture is a part of the phenotypic variance that is transmitted through social learning and this process allows the populations fast adaptation to their environment. It is essential to explore the mechanisms of inheritance, the source of variance and the selection processes in the cultural systems to understand the cultural evolution. A feasible way to study the animal cultural processes is to investigate the acoustic communication based on social learning, because the elements of culture are well defined (e.g. syllables or songs in birds) and on the other hand, these elements can be relatively easy to record, modify and use in experiments.

In this project we studied mainly the song of collared flycatcher (*Ficedula albicollis*), a model species of the sexual selection, the reproduction and the signalling mechanisms. My objectives were (1) to identify the elements of culture, explore the within- and between-individual diversity of them and based on that reveal the individuality of bird song; (2) to study the within-individual changes in the repertoires; (3) to study the role of cultural elements in the sexual selection; and finally (4) to explore the large scale cultural patterns and its influencing factors. For that we extended and analysed our long-term song library of collared flycatcher and conducted several field experiments.

To further investigate these biological questions, we extended our analyses on other model species in which enough data was available from the field or laboratory: Japanese quails (*Coturnix japonica*), common cuckoos (*Cuculus canorus*), canaries (*Serinus canaria*) and blackcaps (*Sylvia atricapilla*).

Objective 1. Cultural elements, diversity and individuality

To study the evolution of acoustic signals it is essential to reveal the discrete elements of the repertoire (Task 1) and study the within- and between-individual variances (Task 2). The source and existence of individuality of the signals is essential to be explored because sexual selection can only act on signals exhibiting consistent individual differences (Task 2).

First, it was necessary to develop a computer program that makes our acoustic analyses efficient. I developed the Ficedula Toolbox in Matlab environment to help in cutting out the songs from recordings, to segment and then cluster the syllables within and across individuals. The program was validated on inter-observer repeatability, and were applied it to build our universal syllable database that was used in most of the tasks of this project (paper published: Zsebők et al 2018a).

It was important to identify the elements of culture on which the cultural evolutionary mechanisms can act. It was already known that the smallest elements, the syllables can be found in the repertoire of different individuals. However, it was not known whether individuals organize their syllables in

sequences, and these phrases can be transmitted between individuals. I showed that individuals use repeated phrases in their songs up to 7 syllables; these phrases can survive even years in the individual repertoires making it possible to be transmitted to other individuals; and the probability of sharing these phrases between individuals are significantly higher than to find these phrases by chance. The results were showed in conference (MET, Zsebők et al 2016) and one manuscript is under preparation from this topic.

The copying and selection mechanisms depending on the frequency of cultural elements on individual and population levels. First, I showed that the frequency of cultural elements follows a "power law probability distribution" within-individually (published paper: Zsebők et al 2018b). Second, I found similar pattern on population level (showed in conference: IBAC, Zsebők et al 2017). Third, I proved that individuals differ in the composition of their repertoires both on syllable and phrase levels, and that individuality is maintained even between years (showed in conference: ECBB, Zsebők et al 2018). From this topic one more manuscript is under preparation.

In a cooperation, we also studied the individuality of the signals and their recognition in laboratory to eliminate the confounding factors that is always presented in the field. Working on Japanese quails, we recorded male mating calls, the crows, analysed the individual differences and conducted operant conditional (GO/NOGO) experiments to characterize the capacity of female quails to discriminate and recognize these calls from different individuals. We found that male crows are individually distinct in time and frequency parameters and that female quails managed to discriminate the crows from each other and were able to recognize individuals based on a multiple parameter analysis. We suggest that such recognition process may be adaptive in the contexts of courtship and mate recognition (results were shown on conference: IBAC, Courvouisier 2017, manuscript in submission, Zsebők et al).

In another cooperation, we also explored the individuality of the advertisement calls of male common cuckoos) based on the description of their within- and between-individual variance. We collected calls from a Hungarian cuckoo population and conducted discriminant function analysis on acoustic parameters to distinguish individuals. We show that individuals differ in both the frequency and time parameters of their calls, most importantly in maximum frequency of the first syllable. Our discrimination of the calls of 26 male individuals was almost 100 % accurate, and even when the number of samples was reduced to five calls per individual, and the number of acoustic parameters was decreased to five variables, accuracy still exceeded 90 %. Our acoustic individual discrimination technique is applicable to a wide range of ecological and behavioural studies because it is easy to perform and can be readily automated (paper published: Zsebők et al 2017b).

Objective 2. Within-individual variation and the mechanism of copying

The repertoire of the individuals changes through the social interactions by modifying their existing repertoire (Task 3) or build new cultural elements into their repertoire (Task 4) that can determine between-individual variance (Task 5). My goal was to reveal these mechanisms based both on correlative and experimental approaches.

Based on observational data, we showed that the repertoire size of individuals is under continuous change throughout their life and it is influenced by the social environment (published paper: Zsebők et al 2017a). I also showed that not only repertoire size but repertoire content changes in the same way, manifested in the change of within-individually rare cultural elements. Also, we found a strong trend that the repertoire similarity is negatively related to the distance both on syllable and phrase levels in a population (showed in conferences: IBAC, Zsebők et al 2017 and ECBB, Zsebők et al 2018).

Additionally, we conducted an experiment related to social context dependent repertoire changes to support the correlative results. By simulating new neighbours in a playback setup, we showed that flycatcher males change their repertoire according to the social context (results showed in conference: ECBB, Zsebők et al. 2018). We plan to publish the observational and experimental results together in one paper (manuscript under preparation).

We also investigated whether flycatcher males can learn new cultural elements in the reproduction area in spring time. For that we conducted a separate experiment, by simulating neighbours with playing back songs containing repertoire elements from populations more than 700 km far from our population. We thoroughly checked and ensured that these new syllables were not found previously in the Pilis Mts in the last 18 years. Altogether, we involved 10 individuals in this experiment, played back the songs for 3.6 ± 1.9 days, recorded and analysed more than 3500 songs. We found that at least one out of 10 birds build one of our new syllable types into its own repertoire, and from the second day of the experiment used it frequently. Based on that we proved that collared flycatcher learns new cultural elements through acoustic territorial interactions in the reproduction area. We plan one manuscript from these results.

In collared flycatcher, it was not studied yet how the genetic inheritance, beside of the cultural processes, influence song parameters. By using diverse and sophisticated statistical methods, we tested the relationships between several song features (including classical acoustic parameters and the repertoire content) and the molecular characteristics of MHC alleles, but we found no statistical proof of that relationship. These findings further support the significance of cultural inheritance in the song of collared flycatcher (Garamszegi et al 2018).

In a cooperation, we also studied in a laboratory experiment, how the learnt cultural elements can be recalled after non-singing periods. It is a fundamental mechanism not studied so far, but essential in all bird species with culturally inherited song. In canaries, we quantified the recurring development of vocal motor skills related to cultural elements and the accompanying changes in synaptic connectivity in the brain of a songbird, while manipulating skill performance by consecutively administrating and withdrawing testosterone. We demonstrated that a songbird with prior singing experience can significantly accelerate the re-acquisition of the learnt cultural elements. We further demonstrated that an increase in vocal performance is accompanied by a pronounced synaptic pruning in the forebrain's vocal motor area HVC, a reduction that is not reversed when birds stop singing. These results provide evidence that lasting synaptic changes in the motor circuitry are associated with the savings of motor skills, enabling a rapid recovery of motor performance under environmental time constraints (manuscript in submission to *ELife*, Vellema et al.).

Objective 3. Selection mechanisms

To investigate the evolution of cultural diversity in the bird song, it is essential to reveal the role of cultural elements in sexual selection. For that we used correlative approach based on our long term database (Task 6) and also conducted field experiment (Task 7) in collared flycatcher.

First, we tested whether syllable types can influence mating success. By determining the relationship between all syllable types and the pairing latency (as standard mating success proxy), we could identify the biologically important syllable types. The significance of these cultural elements can be tested in playback experiments in the future. We also tested whether the acoustic characteristics of the cultural elements are related to the male traits and paring success. We have found that the mean

frequency, the tempo and the repertoire size significantly related to the age of males, however no direct relationship with the mating success was revealed (results published in Garamszegi et al 2018).

We also investigated the significance of the syllable sequence organisation in the song of collared flycatcher. By using network analysis, we studied the potential encoding capacity of signal sequences on different time scales (within-day, between-days, between years), and the relationship with the age, condition and arrival date of males, and with the males' survival, pairing probability and pairing speed. We have found general, non-random tendencies for syllable sequential organization rules on population level, large between-individual differences and time-scale dependent potential encoding capacity of sequence organizations on individual level. We found strong relationships between sequence organization and the age of the individuals, however our results showed no direct relationship with the fitness traits (showed in conference: MET, Tamás et al 2016, manuscript ready for submission, Zsebők et al).

Furthermore, we also tested whether the distribution of cultural elements in the parameter space defined by their acoustic characteristics can potentially encode male quality and relate to the mate choice. We applied functional diversity measures (richness, evenness, divergence and dispersion) first time in bioacoustics to describe four different aspects of the distribution of syllables in the parameter space. We found different and time-scale dependent potential encoding capacity of this measures, relationship with the age and arrival date of males. Also we found indication that functional dispersion may be related to the survival of the animals and functional richness with pairing latency (manuscript ready for submission, Zsebők et al).

We also conducted a field experiment to test whether population dependent content of the songs influence the territorial aggression. Before the experiment, we proved that collared flycatcher has geographic distance-depending between-population dialects (see in Objectives 4). We simulated new rival at the border of the focal male's territory by playing back songs originated from populations with different distances (local, 50 km, and more than 500 km) from the site of the experiment. By conducting the experiment on 24 individuals, we found statistically significant differences between the aggression levels of the response in the focal males depending on the dialects, interestingly reacting strongest to the dialect from the middle distance population. This result shows the significance of culturally inherited content of the song in intra-sexual selection in collared flycatcher (manuscript under preparation).

Objective 4. Large-scale patterns in cultural evolution

The mechanisms of cultural processes generate large scale cultural patterns. We explored how the culture changes in a population based on our 17-year long universal syllable library (Task 8). Furthermore, we investigated the spatial distribution of cultural elements based on samplings from different populations in collared flycatcher and blackcaps.

We analysed the temporal cultural changes in our population on different levels: on the acoustic characteristics of syllable types, the abundance of syllables types, the repertoire content and repertoire diversity. We have found statistically significant changes in the frequency and time parameters of several syllable types through the 17 years studied. However, we found no general patterns would be valid for all syllable types. We also showed, that the survival of the syllable types in our population significantly depends on their relative abundance in the culture. While the common syllable types are constantly present, the rare syllable types' survival is only 50% in the studied 17-year period. We also found that in general, the cultural diversity is stable in our population, and that is maintained by the balanced processes of immigration and extinction of the syllable types. Furthermore, we showed that

the rare new syllable types are introduced in the population by the immigrant young males. Results were shown on conferences: IBAC, Zsebők et al 2017, and MET, Zsebők et al 2017. Preliminary results were published (Vaskuti et al 2016). We plan at least one publication in high-ranked journal from these results, the manuscript is under preparation.

To reveal the spatial cultural patterns, we sampled 7 further populations with different distance from our central population (from 5 to 200 km) by recording and analysing the syllable repertoire of 15 individuals from each population. By using automatic clustering technique, we revealed that the populations differed significantly in their repertoire contents, and it shows distance dependent repertoire differences. Preliminary results were showed on conference: ECBB, Vaskuti et al 2018 and in a paper: Vaskuti et al 2016. We plan one more paper published from this topic, manuscript under preparation.

In a cooperation, we also investigated the large-scale between-population cultural patterns in blackcap. We studied a migratory population (two groups near Paris) and a sedentary population (three groups in Corsica). All of the birds were ringed and blood sampled to investigate genetic relatedness using 17 microsatellite loci. A detailed song analysis showed that this species has a complex repertoire (> 100 syllables), which required the development of a semi-supervised method to classify different categories of syllables and compare sequences of syllables. Our analysis showed no genetic structuring among populations: individuals belonging to the same group were not genetically closer than those from different groups. In contrast, we showed that geographical variations in songs rely on both syllable and sequence content (published paper: Linossier et al 2016).

Changes in the project

While we maximally fulfilled all the four main objectives (with 8 Tasks all in all), one of the original tasks (Task 7) had to be changed. Because of the weather conditions in spring of 2017, we were not able to develop and conduct an experiment on female choice. In 2018, to ensure to have experimental results about revealing the role of the cultural elements in sexual selection, we conducted a successful, previously developed playback experiment on males' territory aggression (one manuscript is under preparation from the results, see above in Obj. 3). Based on that, we got experimental based answer too for our main question related to Objective 3, hence, we already know that cultural elements may be important in sexual selection. In the future, we'd like to test our original female choice hypothesis too.

Also, originally, we planned to involve only one person (György Blázi) in the acoustic data processing, however, because of the large amount of work, and the unavailability of this dedicated person, we included several other persons too in this phase of work: Mónika Jablonszky, Csaba Koszta, Nóra Magonyi, Miklós Laczi, Katalin Krenhardt, Gergely Nagy, Levente Prébli, Melitta Tamás, Péter Szabó, Éva Vaskuti, Ákos Víg. All of them was equally well trained and their contribution was essential for the project.

Miscellaneous

In further cooperations, we also studied the acoustic behaviour of mammals, and these studies are only loosely related to this project. We studied how the acoustic behaviour of mice is influenced by the stress level, and revealed the sex-dependent role of some neuro-transporters in stress regulation (Balázsfi et al 2016).

The other study however, emphasize the importance of cultural transmission to cope with the anthropogenic challenges. We showed that bats have an innate, orientation dependent perception of smooth surfaces, and according to that they try to drink from horizontal smooth surfaces and collide with the vertical smooth surfaces. Our results may be extremely important in the conservation of bats in the future, taking into account that their interpretation about the environment is not culturally learnt and therefore their adaptation potential to anthropogenic environment is low (Greif et al 2017).

In a further flycatcher study, we also investigated, how plastic the risk-taking behaviour of collared flycatcher, and we found age-related expression of this behaviour, that may be a consequence of acquiring general experience with age (Krenhardt 2016). In the future, it might be important to reveal the contribution of social learning in this phenomenon.

Summary, and future prospects

Altogether, the funded project was a success. We have already published 7 strongly and 3 loosely related papers. 4 more manuscripts are already in the submission phase and 7 more manuscripts are expected to be finished and published in the near future. Additionally, 1 informal paper was also published. Most of the results are already shown in several national and international conferences hold in Germany, United Kingdom and India.

As a consequence of our successful investigations, I was invited to a financially fully covered "Bio-acoustic Structure Workshop" hold in Knoxville (Tennessee, USA) in the summer of 2018, which was dedicated to the acoustic investigations related to the within- and between-population investigations of the animal acoustic communication. As a result, beside of disseminating our project results and scientific networking, I'm contributing to a review paper written about the modern bioacoustics methods.

The project was also successful from educational viewpoint: 5 BSc students (Melitta Tamás, Csaba Koszta, Zsófia Baróthi, Katherina Kasper [Germany], Karis Douglas [UK]) and 3 MSc students (Éva Vaskuti, Melinda Szilágyi, Karola Bartha) were involved in the project, and Éva Vaskuti started as PhD student in the second year of this project. Two of our students (Melitta Tamás and Éva Vaskuti) participated on OTDK conference and Melitta Tamás won the 1st price in the Behavioural Ecology section.

In the near future, as a consequence of the results of our intensive data collection and diverse analytic approaches, we plan to cooperate several other research groups to open up new directions in cultural evolutionary studies including comparative investigations and mathematical modelling.

Published papers

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Zsebők, S., Herczeg, G., Blázi, G., Laczi, M., Nagy G., Vaskuti, É., Hargitai, R., Hegyi, G., Herényi, M., Markó, G., Rosivall, B., Szász, E., Szőllősi, E., Török, J., Garamszegi, L.Z. - Sequential organization of bird song: time-scale dependent encoding and relationships with individual quality and fitness traits in collared flycatcher (*Ficedula albicollis*) song. (in submission to *Ethology*)

Zsebők, S., Courvoisier, H., Huetz, C., Hesler, N., Bertin, A., Derégnaucourt, S., Aubin T. - Individual vocal recognition of males by female quails (Coturnix japonica) in a GO/NOGO experiment. (in submission to *Journal of Experimental Biology*)

Conferences

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Informative paper

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