**K-139992 (formerly 129215): FINAL REPORT 01.10.2018-30.09.2023**

The primary aim of this project was to investigate the cultural systems in association with birdsong across various hierarchical levels, and uncover the factors that determine the structure and stability of these cultural systems. The foundation of our project was the idea that syllables (the smaller components of birdsong) could be considered as socially learned units, akin to cultural elements that could be objectively studied using computerized acoustic processing. Our main objectives were connected to the following levels:

Obj. 1. – Individual capacity of signal acquisition and plasticity

Obj. 2. – Dyadic interactions: short- and long-term effects

Obj. 3. – Communication networks: the interplay between network position and song

Obj. 4. – Population level: spatial and temporal variation of culture

To accomplish our objectives, we mainly studied the song of collared flycatcher (*Ficedula albicollis*) in different correlational and experimental frameworks. In each year of the project, we sampled a flycatcher population in the central nest-box plots in Pilis-Visegrádi Mts. following a uniform sound recording and behaviour-testing protocol. We established a unique long-term database which contains information of the song, personality, and life-history traits of individually marked flycatcher males (Obj. 1-4). Additionally, in a set of field experiments, we performed hypothesis-driven behavioural tests with stimulus birds and playback techniques to study the learning capacity (Obj. 1) and dyadic interactions (Obj. 2). We built geo-referenced nesting, acoustic and personality databases to support the communication network investigations (Obj 3). Additionally, we sampled several other natural flycatcher populations in multiple years to study the between population differences (Obj 4).

According to our research plan, we intended to study animal culture in a complex and unifying way with the main focus on bird song. However, we discovered that other behavioural traits can also be socially learned units (Obj. 3) thus these traits can be shaped by cultural evolution as well. Accordingly, we have extended our research focus by also investigating some of the proposed hypotheses in relation to risk taking and exploratory behaviours, which vary consistently among individuals but are also loaded with considerable plasticity to respond to social environmental cues similarly to bird song. Individuals may copy behavioural elements that incur fitness benefits from each other, which may rise specific spatial and temporal patterns in the population like in other cultural systems. Furthermore, these behavioural traits can influence the spread and use of culturally inherited song elements. Therefore, we expanded our analyses to explore the relationship between song and other behaviours, and also described the variability of these behavioural traits within our population along some of the predictions we originally formulated for song traits.

**Achievements**

*Methodological achievements*

- For effective song processing, we developed a new automatic song and syllable detection framework based on deep neural network techniques (Zsebők et al., 2019). Using this method, we processed over 500 sound recordings, resulting in approximately 30,000 fully processed songs. This dataset served as the foundation for our analyses of all our objectives.
- Furthermore, we have introduced a novel technique for quantifying the diversity of cultural elements within an individual's repertoire. This approach is grounded in community ecology methods and can also be applied to characterize the structural composition of culture at the population level (Zsebők et al., 2021a).
- Song (and other behavioural) traits are loaded with considerable within-individual variations and plasticity, which may pose difficulties when one aims at determining the genetic components of these traits based on animal modelling (see Obj. 1). Therefore, in a simulation study, we determined the optimal sampling at the within- and between-individual levels that could result in the unbiased and powerful estimation of heritability of song traits (Jablonszky and Garamszegi submitted to Behavioral Ecology and Sociobiology).

Related papers:

Zsebők, S., Nagy-Egri, M. F., Barnaföldi, G. G., Laczi, M., Nagy, G., Vaskuti, É., & Garamszegi, L. Z. (2019). Automatic bird song and syllable segmentation with an open-source deep-learning object detection method – a case study in the collared flycatcher (*Ficedula albicollis*). Ornis Hungarica, 27(2), 59–66. https://doi.org/10.2478/orhu-2019-0015

Zsebők, S., Schmera, D., Laczi, M., Nagy, G., Vaskuti, É., Török, J., & Zsolt Garamszegi, L. (2021a). A practical approach to measuring the acoustic diversity by community ecology methods. Methods in Ecology and Evolution, 12(5), 874–884. https://doi.org/10.1111/2041-210X.13558

Jablonszky, M. & Garamszegi, L. Z. (2023). The effect of repeated measurements and within-individual variance on the estimation of heritability: a simulation study. Behavioral Ecology and Sociobiology, submitted.

*Objective 1*

100% achievement. Several papers were published and further manuscripts are still expected to be published in the near future. We explored the relationship between genetics, individual quality, behaviour and birdsong from several aspects. As we hypothesized that the expression and the spread of cultural elements depends on the individual quality and personality of the singing males, we also investigated the different aspects of the personality and courtship behaviour.

- We explored the link between the song of individuals and their genetic relatedness and showed that the main characteristics of the flycatcher song have little heritability which further supports that the crystallized adult bird song is largely shaped by the cultural mechanisms (Jablonszky et al. 2022a). Further analyses failed to reveal a clear relationship

between song content and genetic distance within the population (Canal et al., manuscript in prep).

- We showed that the sequential organization of syllables in the songs, thus the presentation of cultural elements is not random and largely depends on the age of the singing males (Zsebők et al., 2021b).
- We demonstrated that individual-specific elements of behaviour can correlate with dispersal propensity indicating that consistent within-individual variation in behaviour can signal an individual characteristic that can potentially determine the spread of cultural elements in the population (Jablonszky et al. 2020).
- We quantified the plasticity of the main characteristics of birdsong, including its individuality and social context dependency (Jablonszky et al., 2022b).
- We also characterized the relationship between the personality and the song in flycatchers, and showed that both aggression and risk-taking behaviour were related to the mean frequency and length of the song, which study resulted in a BSc report (Ortega 2021) and a potential future paper.
- We found a temporal trend in the relationship between risk taking behaviour and the survival of the males over a 14 year-long study period the study years, which draws the attention to a population-level changes in the behaviour and its fitness consequences that can be especially important in the survival and spread of cultural traits (Krenhardt et al., submitted to Journal of Evolutionary Biology).
- In a meta-analysis we showed that predictability (environment-independent within-individual variation) and behavioural type (mean behaviour) are not independent suggesting that the evolution of these components might be constrained in a system-specific way as resulted from local adaptations (Horvath et al. 2023). These constraints, albeit may be important, are largely neglected in relation to cultural traits.
- In two collaborations, we also studied the learning aspects of the individual capacity. We investigated the domestic chicken (*Gallus gallus*), in which we examined how vocal activity develops in different social conditions (Zachar et al 2019), and the canaries (*Serinus canaria*), in which we revealed the mechanisms between song memorisation, testosterone and neural development (Vellema et al. 2019).

*Related papers:*

*Canal, D., Lao, O., Jablonszky, M., Krenhardt, K., Markó, G., Nagy G., Szász E., Török, J., Zsebők, S., Garamszegi, L.Z. Genetic similarity and song similarity in a wild migratory passerine songbird [manuscript in preparation]*

*Horváth G., Garamszegi, L. Z. & Herczeg, G.(2023) Phylogenetic meta-analysis reveals system-specific behavioural type–behavioural predictability correlations. R. Soc. open sci.10230303230303, http://doi.org/10.1098/rsos.230303*

*Jablonszky, M, Krenhardt, K, Markó, G, Szász, E, Hegyi, G, Herényi, M, Koetel, D, Laczi, M, Nagy, G, Rosivall, B, Török, J, Garamszegi, LZ 2020: A behavioural trait displayed in an artificial novel environment correlates with dispersal in a wild bird. ETHOLOGY 10.1111/eth.13005*

*Jablonszky, M., Canal, D., Hegyi, G., Herényi, M., Laczi, M., Lao, O., Markó, G., Nagy, G., Rosivall, B., Szász, E., Török, J., Zsebõk, S., & Garamszegi, L. Z. (2022a). Estimating heritability of song considering within-individual variance in a wild songbird: The collared flycatcher. Frontiers in Ecology and Evolution, 10(October), 1–9. https://doi.org/10.3389/fevo.2022.975687*

*Jablonszky, M., Canal, D., Hegyi, G., Krenhardt, K., Laczi, M., Markó, G., Nagy, G., Rosivall, B., Szász, E., Zsebők, S., & Garamszegi, L. Z. (2022b). Individual differences in song plasticity in response to social stimuli and singing position. Ecology and Evolution, 12(5), 1–15. https://doi.org/10.1002/ece3.8883*

*Krenhardt, K., Martínez-Padilla, J., Canal, D., …. Garamszegi, L.Z. The effect of environmental variation on the selection of risk-taking behaviour in a migratory [submitted to Journal of Evolutionary Biology]*

*Ortega, Tirso Moreno (2021). Relationships of risk-taking tendency and aggression with song traits in a population of Collared flycatcher. Vrije Universiteit Amsterdam, Student Report 1-31.*

*Vellema, M., Diales Rocha, M., Bascones, S., Zsebők, S., Dreier, J., Leitner, S., Van der Linden, A., Brewer, J., & Gahr, M. (2019). Accelerated redevelopment of vocal skills is preceded by lasting reorganization of the song motor circuitry. ELife, 8, 1–46. https://doi.org/10.7554/elife.43194*

*Zachar, G., Tóth, A. S., Gerecsei, L. I., Zsebők, S., Ádám, Á., & Csillag, A. (2019). Valproate Exposure in ovo Attenuates the Acquisition of Social Preferences of Young Post-hatch Domestic Chicks. Frontiers in Physiology, 10(July). https://doi.org/10.3389/fphys.2019.00881*

*Zsebők, S., Herczeg, G., Laczi, M., Nagy, G., Vaskuti, É., Hargitai, R., Hegyi, G., Herényi, M., Markó, G., Rosivall, B., Szász, E., Szöllősi, E., Török, J., & Garamszegi, L. Z. (2021b). Sequential organization of birdsong: relationships with individual quality and fitness. Behavioral Ecology, 1–12. https://doi.org/10.1093/beheco/araa104*

*Objective 2*

100% achievement. Several papers were published and further manuscripts are expected to be published in the near future.

- We have shown that males of the collared flycatcher may be able to learn and incorporate new song elements into their repáűb ertoires during simulated counter-singing context – which is an important assumption of cultural evolution to act – but such incidents occur at very low frequencies and loaded with learning mistakes (Vaskuti et al., 2022).
- We experimentally studied the listener-specific effect on the birdsong which revealed a hidden variance-generating mechanism that may contribute to cultural diversity (Jablonszky et al. 2021) and that males can differently sing to different listeners suggesting a role for male choice (Jablonszky et al., submitted to Animal Behaviour).
- Based on simulated social context and playback experiments, we also showed that different social contexts (counter-singing, male intrusion, approaching female, and solo singing) induce

different expression of the repeated syllable sequences (Zsebők et al., under review in Animal Behaviour).

- In a cooperation, another paper was published about the mechanism of social learning and recognition of individual-specific birdsong that can help to understand the inter-individual interactions and the role of the individuality in bird song culture (Geberzahn et al. 2021).
- We also explored the determinants of the courtship behaviour and so the intensity of expression of cultural elements, in which we showed that the identity of the female visitors can influence the intensity of the courtship behaviour and this behaviour varied consistently between males (Canal et al. 2022).
- We also published a paper about testing the effect of social environment on risk taking behaviour in collared flycatcher (Krenhardt et al. 2021), which is also relevant to Obj. 1 and 2, because it tested the same predictions on a behavioural trait that is highly plastic and correlates with some features of birdsong.

*Related papers:*


Canal, D., Jablonszky, M., Krenhardt, K., Markó, G., Nagy, G., Szász, E., Török, J., Zsebők, S., & Garamszegi, L. Z. (2022). Male and female identity and environmental contexts influence courtship behaviour in a songbird. Animal Behaviour, 186, 11–19. https://doi.org/10.1016/j.anbehav.2022.01.006

Geberzahn, N., Zsebők, S., & Derégnaucourt, S. (2021). Auditory perception of self and others in zebra finches : evidence from an operant discrimination task. Journal of Experimental Biology, 224, jeb233817. https://doi.org/10.1242/jeb.233817

Jablonszky, M., Zsebők, S., Laczi, M., Nagy, G., Vaskuti, É., & Garamszegi, L. Z. (2021). The effect of social environment on bird song: listener-specific expression of a sexual signal. Behavioral Ecology, 32(3), 395–406. https://doi.org/https://doi.org/10.1093/beheco/araa132

Jablonszky, M., Barta, K., Krenhardt, K., Nagy, G., Zsebők, S., Garamszegi, L.Z. Individuality and the effect of social environment on bird song [submitted to Animal Behaviour]

Krenhardt, K., Markó, G., Jablonszky, M., Török, J., and Garamszegi, L.Z. 2021: Sex-dependent risk-taking behaviour towards different predatory stimuli in the collared flycatcher. Behavioural Processes, 186: 104360. https://doi.org/10.1016/j.beproc.2021.104360

Vaskuti, É., Zsebők, S., & Garamszegi, L. (2022). Tutoring new song elements to male birds in the wild. Ornis Fennica, 99(2-3), 52-59. https://doi.org/10.51812/of.120666

Zsebők, Sándor; Vaskuti, Éva; Laczi, Miklós; Nagy, Gergely; Jablonszky, Mónika; Barta, Karola Anna; Canal, David; Derégnaucourt, Sébastien; Garamszegi, László Zsolt; Context-dependent organisation of birdsong - experimental evidence from collared flycatcher (*Ficedula albicollis*) [under review in Animal Behaviour]

*Objective 3*

90 % achievement. We have compiled the database and conducted the necessary network and statistical analyses, one manuscript is under writing.

- One of our goals was to assess the strength of the association between the proximity of males and their quality and characteristics of their songs. We performed a meta-analysis on the association results of 17 years of data collection, and we found that there is no strong relationship between male proximity and the content of their repertoires. However, we did observe a significant relationship between male proximity and certain acoustic features, such as the minimum frequency of the songs. We also showed that the association between the neighbourness and such acoustic characteristics as minimum frequency, song length, and repertoire size were influenced by the population density and juvenile rate of males in the studied population. We are currently in the process of writing of the manuscript based on these results (Jablonszky et al. manuscript in prep).
- We have performed a similar analysis for other behavioural traits using data for 14 years to investigate potential consequences of copying behaviour via a cultural process. We found that the distribution of males in the social network is not independent from their quality and personality indicated by the positive association between the neighbourness as well as the age and risk-taking of males (Jablonszky et al. manuscript in prep).
- We also studied how the non-social environment influences the within-population spatial song patterns. By taking detailed assessment of the microhabitat around the singing males, we explored and identified some important factors. We showed that age of the neighbouring trees, the percent of the canopy coverage, and the height of the nest hole can influence such acoustic characteristics like the mean frequency, tempo, complexity, and song length. The manuscript writing from this material is under process (Krenhardt et al., manuscript in prep).

*Related papers:*

Jablonszky, M., Barta, K. A., Tóth, Z., Vaskuti, É., Zsebők, S., Garamszegi, L. Z. Communication network in birdsong: spatial patterns of individual quality and song features revealed by distance-based network analysis [manuscript in preparation]

Jablonszky, M., Tóth, Z., Krenhardt, K., Marko, G., Zsebők, S., Garamszegi, L. Z. Spatial patterns of risk taking and exploration behaviour in the collared flycatcher based on distance-based network analysis [manuscript in preparation]

Krenhardt, K., Barta, K.A, Jablonszky, M., Nagy, G., Zsebők, S., Garamszegi, L.Z. Exploring the environmental factors that shape the complex birdsong – evidences from the collared flycatcher (Ficedula albicollis) [manuscript in preparation]

*Objective 4*

90% achievement. One paper is published and further manuscripts are being prepared. Based on our long-term database of our study population in the Pilis-Visegrádi Mts., we characterized the change of the flycatcher song on different levels. We analyzed the long-term acoustic changes within syllable types, the change in the frequency of occurrence of syllable types, and the repertoire content of the whole population.

- We published a combined study based on the field data and computer simulation (Barta et al., 2023), in which we showed that related to the relative frequency of the syllable types in the population the flycatcher song culture is continuously changing, and we also revealed that in the presence of immigration, mutation and conformist learning, cultures similar to flycatcher song culture can be evolved.
- We also studied the cultural evolution of syllable types in association with their acoustic characteristics. We showed that the acoustic features of the syllable types are changing with a higher rate than by chance indicating a long-term cultural evolution within the cultural elements. Furthermore, we demonstrated that the acoustic characteristics, the pairing success related to the syllables, and demographic features as population density and immigration influence the evolution of syllable types. The manuscript is under writing (Zsebők et al., manuscript).
- We collected song recordings from 10 different populations in Hungary to investigate the between-population cultural differences. We showed that while the main acoustic characteristics of the songs are similar in the different populations, the repertoire content shows distance-dependent differences. The results are detailed in the PhD dissertation of Éva Vaskuti (2023), and one manuscript is expected from these results.
- Currently further agent-based mathematical simulations are running to study the effect of the size, density and shape of the population on the song cultures with multiple repertoire characteristics to obtain generally applicable results in animal cultures that can be essential from conservation viewpoint. We predict one further manuscript from this sub-project.
- Focusing on antipredator traits, we investigated how interaction with humans can shape the expression of traits in long-term. We used a phylogenetic meta-analysis to determine how the mean and variability in risk taking behaviour change as a function of the number of generations spent in contact with humans under 3 different contexts: urbanization, captivity, and domestication. We found that any contact with humans leads to a rapid reduction in mean antipredator responses, while the variance among individuals over time observed a short-term increase followed by a gradual decrease. A potential explanation for the rapid decrease in the means of antipredator traits relies on phenotypic plasticity, in which it is also plausible that individuals quickly learn the optimal behavioural responses from each other in the human environment (Gefffroy et al. 2020).

*Related papers:*

Barta, K. A., Garamszegi, L. Z., Scheuring, I., & Zseb, S. (2023). Effects of positive frequency-dependent learning , learning mistakes , and immigration on complex cultures – Validation on the song of collared flycatcher (*Ficedula albicollis*) by individual-based modeling. Frontiers in Ecology and Evolution, 11:1040550. https://doi.org/10.3389/fevo.2023.1040550

Geffroy, B., Sadoul, B., Putman, B. J., Berger-Tal, O., Garamszegi, L. Z., Møller, A. P. & Blumstein, D. T. (2020). Evolutionary dynamics in the Anthropocene: Life history and intensity of human contact shape antipredator responses. Plos Biology 18: e3000818, https://doi.org/10.1371/journal.pbio.3000818

Vaskuti, É. (2023). A madárének kulturális evolúciója [Cultural evolution of birdsong]. PhD doctoral dissertation. Eötvös Loránd University. 1-132.

Zsebők, S., Vaskuti, É., Laczi, M., Nagy, G., Jablonszky, M., Barta, K.A., Canal, D., Hegyi, G., Herényi, M., Markó, G., Rosivall, B., Szász, E., Szöllősi, E., Török, J., Garamszegi, L.Z., Temporal variation in cultural elements: acoustic characteristics and relative frequency shifts in the song of a collared flycatcher population (*Ficedula albicollis*) [manuscript]

Overall, we consider that the project was successful. So far, we have published 16 scientific papers closely related to our objectives (with 11 focusing on bird song and 5 focusing on other behavioural traits) that include K-139992/129215 as funding ID. We expect at least 10 additional publications related to this project in the near future, which are under review (4 manuscripts) or in drafts at present (6 manuscripts). These future papers will appropriately acknowledge the current project as main funding.

Based on the correlational and experimental field studies and computer simulations, we now understand better the cultural systems embedded in birdsong. Our knowledge has been broadened about the inheritance, the structure, and the individual quality-dependence of birdsong. We deepened our knowledge about the plasticity of song in the dyadic interactions in different social contexts. We learned the role of communication networks in the observed within-population spatial patterns. Finally, we described the large-scale spatial and temporal patterns on population level and the contribution of the main culture-influencing mechanisms. We also believe that our studies opened up new directions to study birdsong and cultural systems in general.

**Difficulties**

During the course of the project, we experienced some difficulties concerning the field work. The nature of the studying natural population means that it largely depends on the weather conditions. In several years, the low temperature and frequent rain experienced in the early spring, decreased the number of the successful sampling days. Consequently, we delayed one of the planned experiments in relation to Obj. 3, in which we intended to actively influence the settling of males in the territories with a playback approach.

Additionally, the Covid-19 pandemic made the logistic arrangements in the field and personal meetings difficult. We had to be cautious about the social distance, thus we had to organize the field work by minimizing the number of contacts among researchers (i.e. we had to use separate cars to get to the field station) that decreased the effectiveness of our work.

To compensate for the unpredictability of the weather conditions and the effectiveness of the field work in the focal species, we have initiated cooperations with other researcher groups to study other systems for cultural evolution and to test the predictions in different models (Geberzahn et al., 2021, Vellema et al., 2019, Zachar et al., 2019).

**Training**

Several PhD students contributed to the project. Éva Vaskuti helped in the field and lab work, and prepared her doctoral dissertation on the topic of cultural evolution of birdsong focusing on all four levels of our grant, and prepared to defend her PhD in the near future. Karola Barta is still an active PhD student, beside the field work, she focuses on the programming and evaluation of the agent-based computer simulations. Mónika Jablonszky defended her PhD in the beginning of our grant project, and since then heavily contributed with several publications as a leading author to the success of the project. Katalin Krenhardt defended her PhD in 2023, focused mainly on the personality-related studies and the environment-dependent song expression. All of these young researchers are still working on manuscripts that are related to the project and which are expected to turn into scientific papers in the near future.

We also had a visiting MSc student, Tirso Moreno, from Vrije University of Amsterdam on a sub-project in relation to association between song characteristics and personality in the flycatcher. After his training period under the supervision of Mónika Jablonszky, he successfully produced a scientific report.

**Outreach**

We participated in all the annual Conferences of the Hungarian Ethological Society in the period of the grant, the International Bioacoustic Congress in 2019 in Brighton (England), Winter Meeting of the Association for the study of Animal Behaviour in 2019 in London (England), the 12th Meeting of the European Ornithological Union in 2019 in Cluj (Romania), where we presented several talks (including a plenary) about our work in relation to the project.

Beside of the scientific publications, we presented our results to the general public mainly through the online media (more than 50 popular articles, videos and radio reports). We were also regularly organized bird song related interactive games in the public events of “Madarak és fák napja” and a round table talk related strongly to our birdsong research in „Kert a köbön fesztivál” in the Vácrátót Botanical Garden. As a part of our outreach program, we have also presented a talk at the “Kutatók Éjszakája” (Scientists’ Night). We have also written two popular science papers about the animal cultural evolution (Barta et al., 2022a,b).

*Published popular science papers*

Barta, K.A., Scheuring, I., Zsebők, S. (2022a) Állati kultúrák és sokféleségük - Eszközhasználat és bálnaének. ÉLET ÉS TUDOMÁNY 77:40 pp. 1286-1288.

Barta, K. A., Scheuring, I., Zsebők, S. (2022b) Állati kultúrák és sokféleségük - Mire jó a madárének? ÉLET ÉS TUDOMÁNY 77:41 pp. 1327-1329.