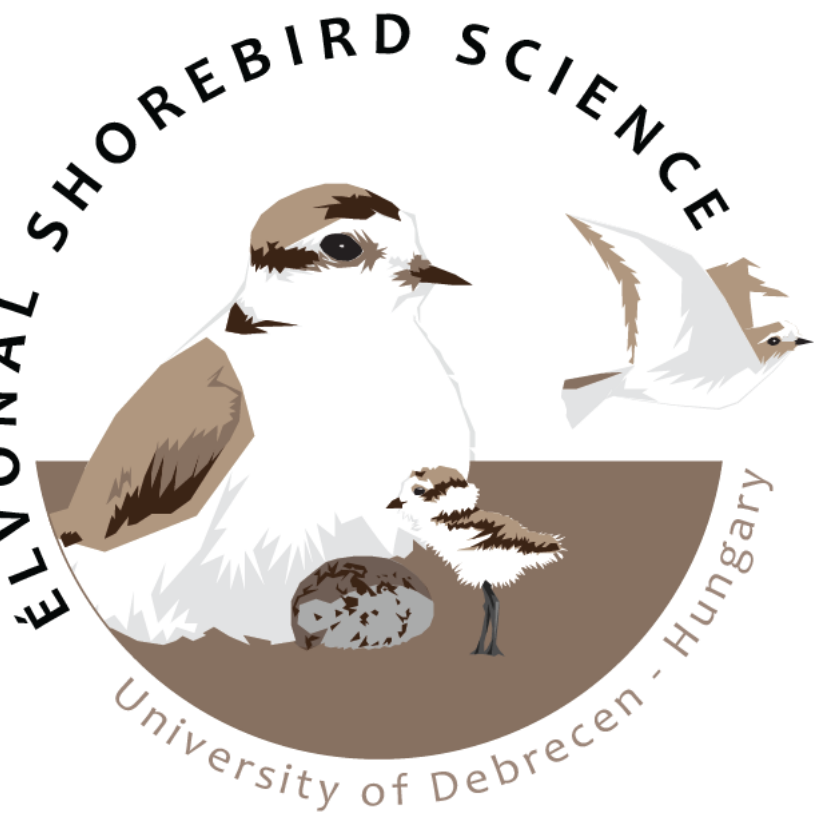
Final report - ÉLVONAL KKP-126949 project 2018 – 2023

Sex role evolution: testing the impacts of ecology, demography and genes



Prof Tamás Székely, University of Debrecen

28 February 2024

Executive summary

Sex roles are some of the most diverse social behaviours because courtship, pair-bonding and parenting exhibit immense variation across the tree of life. Understanding the origins and maintenance of sex roles is one of the core objectives in evolutionary biology since sex differences (often termed sexual dimorphism) underlie much of ecology, behaviour and life history of organisms. Importantly, understanding sex differences is essential for combating biodiversity loss, management of wild and captive animal populations and projecting the future responses of animals to human-induced pressures such as habitat loss and climate change. The overarching aim of this ÉLVONAL project was to investigate the causes and consequences of sex role variation using shorebirds (sandpipers, plovers and allies) as model organisms. We made substantial progress toward this aim.

Firstly, we collected an unprecedented amount of data on sex roles from wild shorebirds across the globe that included 97 populations of 29 species in 41 locations. We captured 16,000 individuals, took 16,485 behavioural observations and recorded 120,096 hours of incubation behaviour. Secondly, we developed new theory to understand how demography and population sex ratios interactively shape sex roles. Thirdly, we initiated demographic data collection and analysed demographic changes in wild populations with the ultimate objective of testing theoretical predictions regarding the demographical drivers of sex role variation. Fourthly, to test the causes of sex-specific moralities and thus sex ratio variation, we investigated parasites, diseases and immune systems. Finally, we published reviews and meta-analyses that synthetise current understanding of sex roles and breeding systems. Taken together, these theoretical and empirical studies represent the most comprehensive cross-disciplinary analyses of sex roles in any organism to date.

The project produced significant impact in science and beyond. (i) We published over 40 peer-reviewed papers that include top interdisciplinary journals such as Science, Nature, PNAS and Nature Communications. (ii) The results of these publications were well represented in the national and international media: blogs, newspapers and media interviews. (iii) The ÉLVONAL research involved over 25 teams globally and attracted talented young scientists to Hungary. It facilitated the career of 17 early-career scientists (i.e., PhD students, post-docs and research fellows) of which 7 were female. (iv) We organised annual ÉLVONAL conferences in Debrecen that were attended by 25-45 students, young scientists and collaborators from Hungary and abroad. In addition to these conferences, we organised training workshops that targeted postgraduate students. (v) The ÉLVONAL project stimulated successful grant applications and research projects in Hungary with total value of 3.5 M EUR. Importantly, it led to the creation of a new research institute, the Debrecen Biodiversity Centre. (vi) The project made an impact on biodiversity conservation by fostering successful conservation stories in collaboration with two Hungarian national parks: Hortobágy National Park and Kiskunság National Park. In addition, the ÉLVONAL project continued collaborating with a biodiversity-conservation NGO in Cape Verde and led to the foundation of a new conservation NGO in Madagascar. (vii) Finally, an important spin-off of the project was education and public outreach. We initiated a new MSc course at Debrecen University on Nature Conservation and currently restructuring the biology doctoral school that will benefit the research and career prospects of biodiversity-related postgraduate students of Debrecen University. As part of our education programme, we also launched a series of lectures, seminars and field courses for primary and secondary schools in Debrecen and its surrounding, and run a successful field training workshop for biology teachers in Hortobágy National Park. Taken together, the ÉLVONAL project made substantial impact both in Hungary and abroad, and the project outputs went beyond academia, research and conservation, and reached the general public both nationally and internationally. Importantly, the core concept developed by the ÉLVONAL project will continue beyond the life-time of the grant by linking discovery-based science to training and education, and ultimately to direct conservation actions (see www.szekelylab.com)

Rationale

When it comes to reproduction, males and females have immense variation in their courtship rituals, mating antics and parenting devotions. These behaviours (termed sex roles) attracted the attention of evolutionary biologists ever since Darwin (1871), although they have remained surprisingly controversial since even after 150 years of research because we still lack a solid understanding how variations in these behaviours emerge.

There are three main theories explaining how divergent sex roles may emerge (Fig. 1, Székely et al. 2023). First, differences between male and female behaviour may originate in gamete size differences (anisogamy), because males produce small and many gametes (sperm) whereas females produce nutrient-rich but few gametes (eggs). The cascade initiated by anisogamy is presumed to lead to a bias in the number of males vs females ready to mate (i.e, biased operational sex ratio) with a knock-on effect on sexual selection and therefore sex differences in both mating competition and parenting (Darwin-Bateman paradigm, Janicke et al. 2016, Mokos et al. 2021).

Second, sex differences may emerge from selection driven by the ecological environment (Slatkin 1984). Frequency-dependent or density-dependent competition for shared ecological resources may generate disruptive selection in resource acquisition traits between males and females that lead to sexual dimorphism in body size, and then to sex different mating and parenting behaviours (ecological sexual dimorphism, De Lisle 2019).

Third, the social environment may influence sex roles and breeding system variations (Székely et al. 2014, Schacht et al. 2022) since traits associated with sexual selection are expected to depend on the ratio of sexes in the population, termed the adult sex ratio (ASR). The core idea here is frequency dependence: when adult males are more abundant in a population than adult females, this provides the upper hand for females in choosing new mate and in retaining their mate for future pair-bonds. Consequently, in male-skewed ASR males are more likely to care for the young than females given their bleak chances of remating (demography-based sex roles, Székely et al. 2000, Kokko & Jennions 2008, Schacht et al. 2022).

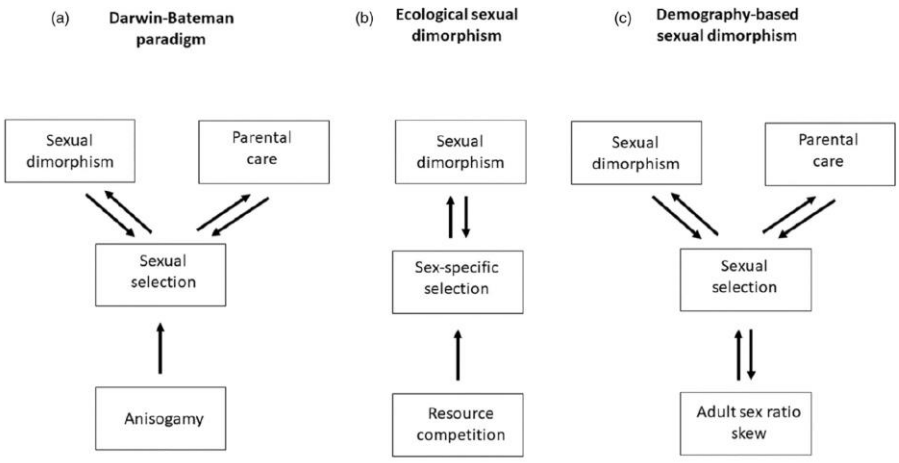


Fig. 1. Schematic illustration of three hypothetical scenarios for the emergence of sex roles. (a) Darwin–Bateman paradigm, (b) ecological sexual dimorphism and (c) demographic origins of sex roles (Székely et al. 2023).

In this ÉLVONAL project we used shorebirds (sandpipers, plovers and allies) as model organisms to investigate sex role variation. First, we used shorebirds because they have an unusually diverse display behaviour, pair bonding and parenting behaviour, and this variation has been used by several venerable scientists to illustrate breeding system variations by venerable scientists including Charles Darwin, Niko Tinbergen and Frank Pitelka (Fig 2). Second, shorebirds are widely distributed and they

breed on all continents including Antarctica (Székely et al. 2023). Third, our research group has over 30 years of experience working with various shorebird populations in Europe, Asia, Africa and Latin America. These studies demonstrated that shorebird ecology, behaviour and evolution can be studied efficiently in the field, laboratory and comparatively (Székely et al. 2004, Eberhart-Phillips et al. 2018, Székely 2019).

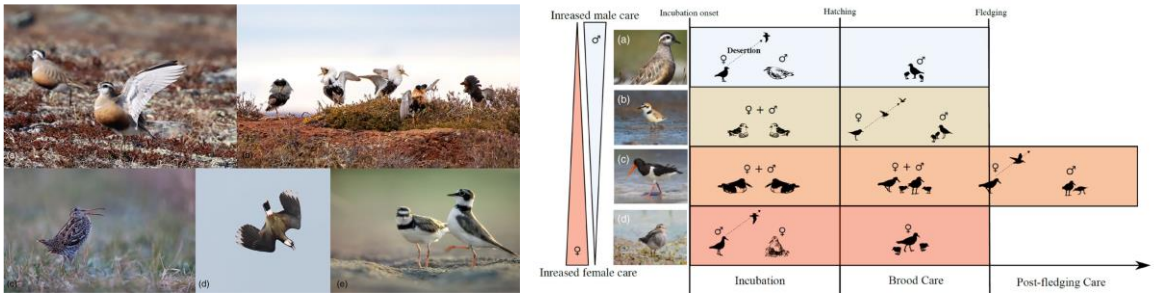


Fig. 2. Diversity of courtship behaviour (left) and parental care (right) in shorebirds (Székely et al. 2023).

Main achievements

General project structure

The proposed project was an unusually cross-disciplinary work cutting through several different research fields: behavioural ecology, population biology, evolutionary genomics and theoretical biology. In addition, we planned to work with several dozen research teams internationally. Therefore, the first major task was to develop a project organisation and management structure that was going to deliver the results.

We made five main steps to setup and manage the ÉLVONAL project.

(i) New database. Since no scientist has attempted to work across all major aspects of breeding behaviour using multiple species, we developed standardised protocols for cross-species comparative research in order to record data in the field and defined the data structure for uploading data onto a computer server. These protocols are now widely used by the shorebird community, and they remained in use beyond the life-time of the ÉLVONAL project:

- Kosztolányi, A., Zs. Tóth, V. Kubelka, F. Takács & T. Székely 2023. File structure and definition of variables for the OBM Global Shorebird Database. Available from https://elvonalshorebirds.com/
- Székely, T. & V. Kubelka 2019. Protocol for collecting behavioural data for ÉLVONAL shorebird project. Available from https://elvonalshorebirds.com/

In addition, the ÉLVONAL data collection protocol was publicised at several conferences including:

- Kubelka, V. & F Takács. 2018 International Wader Study Group Conference, Workum, Netherlands https://onlinelibrary.wiley.com/doi/10.1111/ibi.13277
- Kubelka, V & T. Székely. 2024. International Biogeographic Society Conference, Prague, Czech Republic

(ii) Fieldwork methodology training workshops. We carried out training workshops in the field (Fig. 3). These Workshops were attended by 15-25 students, young scientists and prospective collaborators:

- Cadiz, Spain, 24–28 April 2019
- Turov, Belarus, 9–16 May 2019
- Melbourne, Australia, 21–24 November 2019



(iii) International network of collaborators. As a result, we recruited several dozen collaborators within Hungary and internationally (Fig. 4). Some of these collaborators provided data throughout the project whereas others only provided data in one or two years. The ÉLVONAL project only provided partial financial support for these existing research teams (for instance, we sent them nest cameras (Wyzecams®) for recording incubation behaviour, and/or ringing and bird trapping tools), which made the project cost effective since the total cost of generating comparable data would have been at least 2-3 times higher if all costs were to cover by the grant.

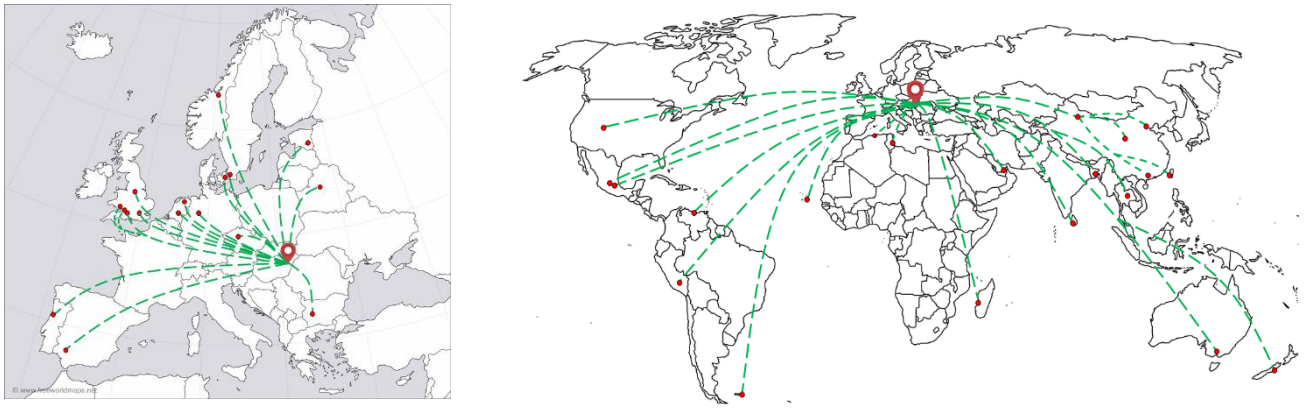
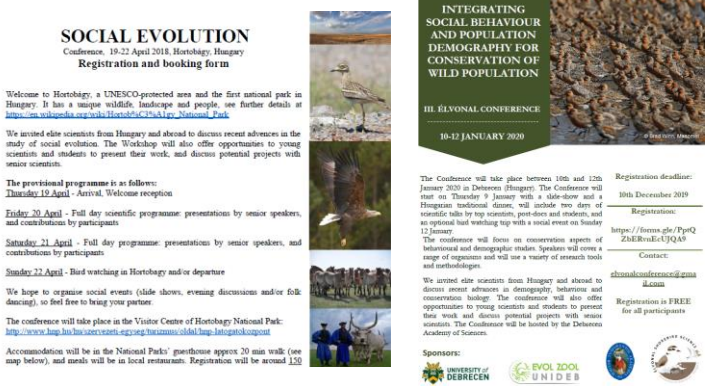


Fig. 4. Collaborators involved in the ÉLVONAL project. From Hungary, the main institutions were the University of Debrecen, University of Pannonia (Veszprém), University of Veterinary Medicine (Budapest) and Hortobágy National Park.

(iv) Regular ÉLVONAL conferences. To provide instructions for the collaborators and keep them in touch throughout the project, we organised a number of conferences. These conferences were attended by 30 - 60 students, young scientists and collaborators (Fig. 5-7).

- Hortobágy, April 2018
- Groningen (Holland), Sept 2018
- Debrecen, January 2019
- Sheffield (UK), September 2019
- Debrecen, March 2019
- Debrecen, January 2020
- Debrecen, August 2020
- Debrecen, January 2021
- Debrecen, July 2021
- Debrecen, September 2021
- Debrecen, January 2022
- Szeged, September 2022
- Debrecen, January 2023
- Ceske Budejovice (Czech Republic), July 2023
- Debrecen, October 2023


SOCIAL EVOLUTION
Conference, 19-22 April 2018, Hortobágy, Hungary
Registration and booking form

Welcome to Hortobágy, a UNESCO-protected area and the first national park in Hungary. It has a unique wildlife, landscape and people, see further details at https://en.wikipedia.org/wiki/Hortob%C3%A1gy_National_Park

We invited elite scientists from Hungary and abroad to discuss recent advances in the study of social evolution. The Workshop will also offer opportunities to young scientists and students to present their work, and discuss potential projects with senior scientists.

The provisional programme is as follows:
Thursday 19 April - Arrival, Welcome reception

Friday 20 April - Full day scientific programme: presentations by senior speakers, and contributions by participants

Saturday 21 April - Full day programme: presentations by senior speakers, and contributions by participants

Sunday 22 April - Bird watching in Hortobagy and/or departure

We hope to organise social events (slide shows, evening discussions and/or folk dancing), so feel free to bring your partner.

The conference will take place in the Visitor Centre of Hortobagy National Park: http://www.hnp.hu/hu/szervezeti-egyseg/turizmus/oldal/hnp-latogatokozpont

Accommodation will be in the National Parks' guesthouse approx 20 min walk (see map below), and meals will be in local restaurants. Registration will be around 150

INTEGRATING SOCIAL BEHAVIOUR AND POPULATION DEMOGRAPHY FOR CONSERVATION OF WILD POPULATION

III. ÉLVONAL CONFERENCE

10-12 JANUARY 2020

The Conference will take place between 10th and 12th January 2020 in Debrecen (Hungary). The Conference will start on Thursday 9 January with a slide-show and a Hungarian traditional dinner, will include two days of scientific talks by top scientists, post-docs and students, and an optional bird watching trip with a social event on Sunday 12 January.
The conference will focus on conservation aspects of behavioural and demographic studies. Speakers will cover a range of organisms and will use a variety of research tools and methodologies.

We invited elite scientists from Hungary and abroad to discuss recent advances in demography, behaviour and conservation biology. The conference will also offer opportunities to young scientists and students to present their work and discuss potential projects with senior scientists. The Conference will be hosted by the Debrecen Academy of Sciences.

Registration deadline:
10th December 2019

Registration:
https://forms.gle/PpzQZhEBvuEcUJQA9

Contact:
elvonalconference@gmail.com

Registration is FREE for all participants

Sponsors:

Fig. 5. Conference invitations. The conferences were advertised internationally and nationally, and participation was open to all.

Fig. 6. ÉLVONAL shorebird conferences. From left to right: Hortobágy National Park Visitor Centre (April 2018), Debrecen Academy of Sciences (January 2019) and Debrecen Academy of Sciences (January 2020).

During pandemic or when travelling of key participants were limited, the conferences were run online or were hybrid.

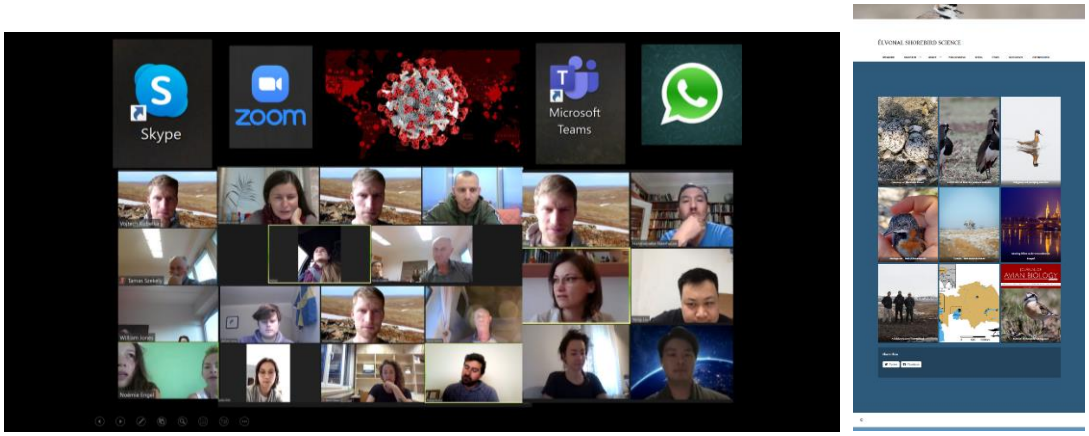


Fig. 7. ÉLVONAL shorebird conference, January 2022 (left). ÉLVONAL shorebird science website (right).

(v) Dedicated website for the project. To keep in touch with collaborators we established a website https://elvonalshorebirds.com/ The website not only holds the list of collaborators and key project-related documents, but was regularly updated with news from the project teams from all over the world (Fig. 7).

To summarise, the ÉLVONAL project established the methodology for investigating sex roles in the field and it created an international community of scientists and conservationists. The network of these students, academics and environmental scientists will remain active beyond the life-time of the ÉLVONAL project.

Objective_1. To investigate the association between components of sex roles.

To achieve this objective:

(i) Our team, with the help of external collaborators gathered data from 97 populations of 29 shorebird species in 41 locations. The data in total represent 16,000 captured individuals, 16,485 behavioural records that represent 5,495 pairs and 5,004 days of incubation videos (approx. 120,096 hours). From 33 populations we have data from three of more years.

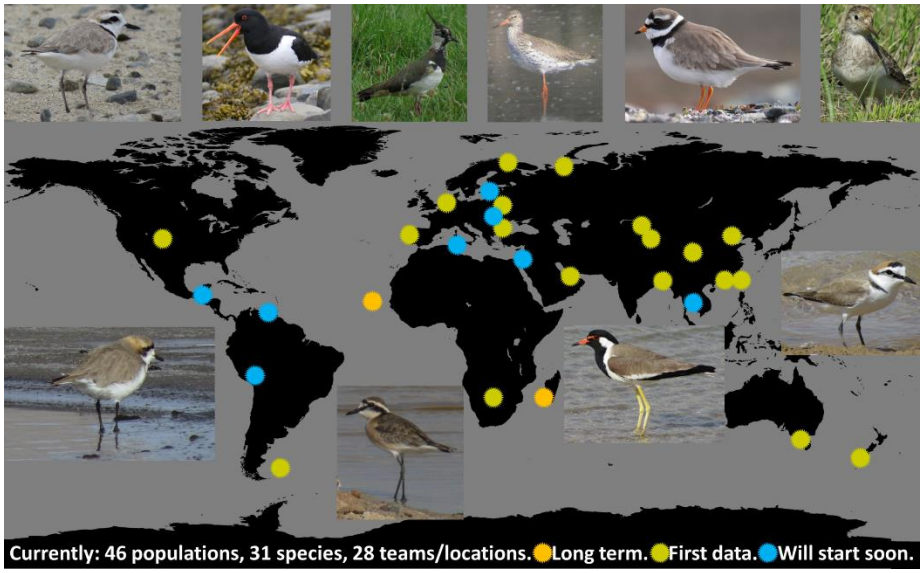


Fig. 8. An in-progress map illustrating the sites where the ÉLVONAL team and our collaborators have collected data.

(ii) To characterise courtship behaviour, mating system and parenting of specific shorebirds, we published the following papers:

- D'Urban Jackson, J, S. Zefania & T. Székely. 2021. Madagascar jacana, *Actophilornis albinucha*. Goodman, S. M. (ed.) (in press). The new natural history of Madagascar. Princeton, Princeton University Press.
- Fresneau, N., Y. Lee, W-C Lee, A. Kosztolányi, T. Székely & A. Liker. 2021. Sex role reversal and high frequency of social polyandry in the pheasant-tailed jacana (*Hydrophasianus chirurgus*). Frontiers in Ecology & Evolution 9:742588.
- Halimubieke, N, J. O. Valdebenito, P. Harding, M. Cruz-López, M. A. Serrano-Meneses, R. James, K. Kupán & T. Székely. 2019. Mate fidelity in a polygamous shorebird, the snowy plover (*Charadrius nivosus*). Ecology & Evolution 9: 10734-10745.
- Halimubieke, N., X. Lin, M. Almalki, Q. Huang, Y. Liu, T. Zhang, Z. Zhang, T. Székely, Y. Liu. 2024. Breeding ecology of a high-altitude shorebird in the Qinghai-Tibetan Plateau. J. Ornithology (accepted).
- Kupán, K., T. Székely, M. Cruz-López, K. Seymour & C. Küpper. 2020. Offspring desertion with care? Chick mortality and plastic female desertion in Snowy Plovers. Behavioral Ecology 32: 428-439.
- McDonald, G.C, N. Engel, S. Ratão, T. Székely & A. Kosztolányi. 2020. The impact of social structure on breeding strategies in an island bird. Scientific Reports 10: 13872.
- McDonald, G. C., I. C. Cuthill, T. Székely & A. Kosztolányi. 2023. Remating opportunities and low costs underlie maternal desertion. Evolution 77: 97-109.
- Rice, R, J. O. Valdebenito, M. Ottensmann, N. Engel, A. Adrião & T. Székely. 2019. Breeding ecology of the Cream-coloured Courser in Cape Verde. Ostrich 91: 65-73.
- Wanders, K., M. Almalki, O. Heggøy, T. Lislevand, C. McGuigan, G. Eichhorn, G. W. Gabrielsen, V. Azarov, L. Khasyanova & T. Székely. 2023. Incubation behaviour of the Common Ringed Plover *Charadrius hiaticula* at different latitudes. Journal for Ornithology 164: 825–833.
- Zefania, S., & Székely T., 2021. Charadrius, plovers. Goodman, S. M. (ed.) The new natural history of Madagascar. Princeton, Princeton University Press. - (in press).

Further publications are near completion:

- Kubelka, V. & T. Székely. Courtship behaviour in Eurasian dotterels (in prep).
- McDonald, G.C, Z. Barta, T. Székely A. Kosztolányi. Sex roles in parental care in a species with precocial offspring and frequent brood desertion. Animal Behaviour (submitted)

(iii) We started to explore the associations among components of sex roles. These analyses are challenging since they require detailed observations from individually marked birds from several components of sex role behaviour:

- Kubelka, V., E. Faki, T. Székely & M. Slavek. Behavioural consistency across sex role components in red-wattled lapwing (in prep).
- Frauendorf, M., B Ens, T. Székely & V. Kubelka. Consistemncy in courtship, incubation and brood care in Eurasian oystercatchers (in prep).

(iv) We also analysed the emergence of sex roles across shorebirds and birds as a whole:

- Gonzalez-Voyer, A., G. H. Thomas, A. Liker, O. Krüger, J. Komdeur & T. Székely. 2022. Sex roles in birds: phylogenetic analyses of the influence of climate, life histories and social environment. Ecology Letters 25:647–660.
- Székely, T., M. C. Carmona-Isunza, N. Engel, N. Halimubieke, W. Jones, V. Kubelka, R. Rice, C. E. Tanner, Z. Tóth, J. O. Valdebenito, K. Wanders & G. C. McDonald. 2023. The causes and implications of sex role diversity in shorebird breeding systems. Ibis (accepted).

These phylogenetically controlled analyses have revealed that across shorebirds, the association among sex role components were statically significant (Fig. 9), although across birds as whole, there was no association. We believe, the discrepancy between the shorebird results and those emerged across all birds is partly due to higher resolution of data from shorebirds. Therefore, as far as shorebirds are concerned, it is legitimate to call sex roles a "syndrome" since some of these components appear to co-evolve (Székely et al. 2023).

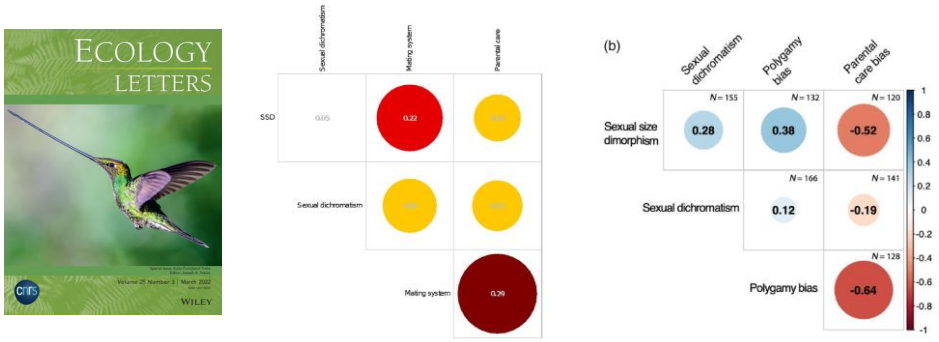


Fig. 9. Correlations among sex role components in birds (left, Gonzalez-Voyer et al. 2022) and shorebirds (right, Székely et al. 2023). Numbers inside circles indicate the value of the phylogenetically controlled correlation, N = number of species.

Objective_2. To test the roles of ecology driving sex role variation.

We made three major steps toward achieving this objective.

(i) We published papers on how ecological and life history predictors influence sex role behaviours. These studies have shown that successful breeding predicts divorce (Halimubieke et al. 2020). In addition, the social environment (i.e. ASR) is emerging as a major predictor of sex role behaviour (Gonzalez-Voyer et al. 2022).

- Halimubieke, N., K. Kupán, J. O. Valdebenito, V. Kubelka, M. C. Carmona-Isunza, D. Burgas, D. Catlin, J. J. H. St Clair, J. Cohen, J. Figuerola, M. Yasué, M. Johnson, M. Mencarelli, M. Cruz-

López, M. Stantial, M. A. Weston, P. Lloyd, P. Que, T. Montalvo, U. Bansal, G. C. McDonald, Y. Liu, A. Kosztolányi & T. Székely. 2020. Successful breeding predicts divorce in plovers. Scientific Reports 10: 15576.

- Long, X, Y. Liu, A. Liker, F. J. Weissing, J. Komdeur & T. Székely. 2022. Does ecology and life history predict parental cooperation in birds? A comparative analysis. Behavioral Ecology & Sociobiology 76: 92.

(ii) Importantly, we revised one of the core ideas that lead to the ÉLVONAL application – i.e., the hypothesised association among mating system, parental care and adult sex ratio in shorebirds (Fresneau et al. in revision). The latter recent work fully supported previous analyses of Liker et al. (2013). In addition, Fresneau et al. (in revision) used a substantially revised and augmented dataset to show that an ecological factor (breeding density) and ASR together influence sex role variation. Furthermore, using advanced phylogenetic methods Fresneau et al. (in revision) showed that mating system and parental care were evolutionary responses to ASR, rather than *vice versa*.

- Fresneau, N., I. Pipoly, D. Gigler, A. Kosztolányi, T. Székely & A. Liker. The evolution of sex roles: the importance of ecology and social environment. Proc Nat Acad Sci US (in revision).

(iii) Analysing the amount of comparative data we collected (see above) raises challenges in data cleaning and the statistical analyses needed to control for both phylogenetic and spatial non-independence. We have developed a data cleaning protocol to incorporate datasets from multiple species and populations (Fig. 10; given the novelty of this approach and the sheer amount of raw data from 90+ populations we anticipate this will lead to a data-paper) and expect to finish data cleaning by June 2024. The cleaned data will underpin 4 major planned publications on components of sex roles: courtship (Carmona-Isunza et al. in prep), pair-bonding (Halimubieke et al. in prep), incubation (Fresneau et al. in rep) and brood care (McDonald et al. in prep).

The theoretical bases of these publications have been established by Székely et al. (2006) and Székely et al. (2023), and the analytic tools will be upgraded based on Kubelka et al. (2018).

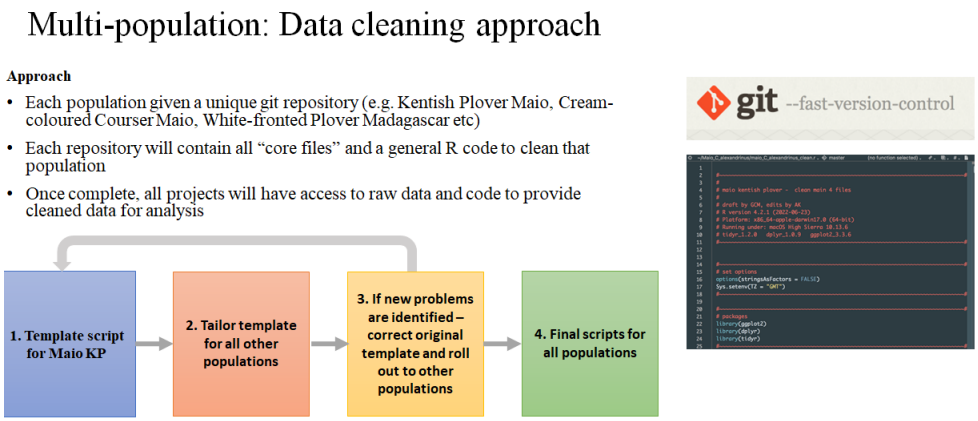


Fig. 10. Data cleaning structure. We anticipate we will publish a paper describing the data and the data cleaning approach.

Objective_3. To investigate the demographic causes of ASR and sex role variation.

We made substantial progress toward this objective.

(i) We collected demographic data from 33 wild populations. However, for robust statistical estimates we need several years of data preferably from large number of individuals. To this end, we focused publications on those populations we already have over 10 years of data: the Kentish plover from Cabo Verde (Engel et al. 2023) and three plover species from Madagascar (Jones et al. 2021).

- Engel, N, B. K. Sandercock, A. Kosztolányi, A. Adrião, A. Tavares, R. Rice & T. Székely. 2024. Climatic variation influences annual survival of an island-breeding tropical shorebird. Journal of Avian Biology (accepted).
- Jones, W., L. J. Eberhart-Hertel, R. Freckleton, J. I. Hoffman, O. Krüger, B. K. Sandercock, O. Vincze, S. Zefania & T. Székely. 2021. Exceptionally high apparent adult survival in three tropical species of plovers in Madagascar. Journal of Avian Biology 1-10.

(ii) We analysed nest survival of Kentish plovers in Maio, and showed that over a 10-year time period, nest survival declined significantly (Engel et al. 2023).

- Engel, N., G. McDonald, B. K. Sandercock, R. Rice, R. Moreno, S. Ratão & T. Székely. 2023. Long-term decline in nest survival of a ground-nesting shorebird on a tropical island. Global Change Biology (accepted).

(iii) We also analysed nest survival data using published information, and showed that nest survival declined recently in shorebirds, especially in those that breed in the Arctic (Kubelka et al. 2018). Based on these findings, we proposed that changes in nest survival could influence migratory behaviour if the Arctic is no longer a safe breeding ground (Kubelka et al. 2021).

- Kubelka, V., M. Šálek, P. Tomkovich, Zs. Végvári, R. Freckleton & T. Székely. 2018. Global pattern of nest predation is disrupted by climate change in shorebirds. Science 362: 680-683.
- Kubelka, V., B. K. Sandercock, T. Székely & R. P. Freckleton. 2021. Animal migration to northern latitudes: environmental changes and increasing threats. Trends in Ecology & Evolution 37: 30-41.

(iv) We estimated ASR using a novel demographic approach in 6 plover populations and showed that ASR is related to parental behaviour (Eberhart-Phillips et al. 2018).

- Eberhart-Phillips, L. J., C. Küpper, M. C. Carmona-Isunza, O. Vincze, S. Zefania, M. Cruz-López, A. Kosztolányi, T. E. Miller, Z. Barta, I. C. Cuthill, T. Burke, T. Székely, J. I. Hoffman & O. Krüger. 2018. Demographic causes of adult sex ratio variation and their consequences for parental cooperation. Nature Communications 9:1651.

(v) We extended the demographic approach to other taxa, and quantified sex different mortalities across mammals (Lemaitre et al. 2020).

- Lemaître, J-F, V. Ronget, M. Tidière, D. Allainé, V. Berger, A. Cohas, F. Colchero, D. Conde, M. Garratt, A. Liker, G. A.B. Marais, A. Scheuerlein, T. Székely & J-M. Gaillard. 2020. Sex differences in adult lifespan and aging rates of mortality across wild mammals. Proceedings of The National Academy of Sciences US 117: 8546-8553.

Objective_4. To use genomic tools to explore causes of differential mortalities.

To understand the genomic/physiological/immunological causes of sex different mortalities is a huge undertaking, and we made substantial progress toward this challenging objective.

(i) We investigated the mortality costs of sex-specific parasitism in wild birds using meta-analysis and a theoretical model. We also explored seasonal variation in sex-specific immunity in wild birds.

- Halimubieke, N., A. Pirrie, T. Székely & B. Ashby. 2021. How do biases in sex ratio and disease characteristics affect the spread of sexually transmitted infections? Journal of Theoretical Biology 527: 110832.
- Valdebenito, J.O., A. Liker, N.Halimubieke, J. Figuerola & T. Székely. 2020. Mortality cost of sex-specific parasitism in wild bird populations. Scientific Reports 10: 20983.
- Valdebenito, J. O., N. Halimubieke, Á. Z. Lendvai, J. Figuerola, G. Eichhorn & T. Székely. 2021. Seasonal variation in sex-specific immunity in wild birds. Scientific Reports 11: 1349.

(ii) We investigated the prevalence of avian malaria – a common protozoan parasite in birds – and showed that inland-breeding shorebirds show surprisingly high level of malaria infections (Jones et al. 2024). Importantly however, the malaria prevalence was not different between males and females.

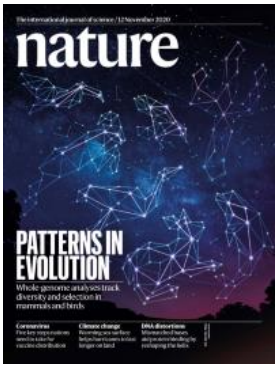
- Jones, W., Zs. Tóth, V. Khursanov, A. Kuzmiankova, O. Krüger, T. Székely, N. Karlionova, P. Pinchuk & N Chakarov. 2024. Haemosporidian infections are more common in breeding shorebirds than migrating shorebirds. Ibis (accepted).
- Valdebenito, J. O., J. Martínez-de la Puente, M. Castro, A. Pérez-Hurtado, G. Tejera, T. Székely, N. Halimubieke, J. Schroeder, J. Figuerola. 2020. Association of insularity and body condition to cloacal bacteria prevalence in a small shorebird. Plos One 15: e0237369.

(iii) Also, we compared the immune gene expression among male and female Kentish plovers (Valdebenito et al. 2022). This work showed that immune genes are more heavily expressed in male plovers than in female plovers.


Polygamy and purifying selection in birds

- Valdebenito, J. O., K. H. Maher, G. Zachar, Q. Huang, Z. Zhang, L. J. Young, T. Székely, P. Que, Y. Liu & A. O. Urrutia. 2022. Sex differences in immune gene expression in the brain of a small shorebird. Immunogenetics 74:487-496.

(iv) In a conceptually novel paper (Wanders et al. 2023), we explored the implications of mating system variation for genome evolution using data from across birds and showed that polygamy was strongly associated with more efficient purifying selection. In a follow-up publication we showed that sex-role reversed shorebirds have an unusually high rate of genomic changes on the Z chromosome (Wanders et al. in revision). Based on our shorebird-genome expertise, we were invited to join the B10k consortium and contribute to two papers in Nature (Feng et al. 2020, Stiller et al. 2024).



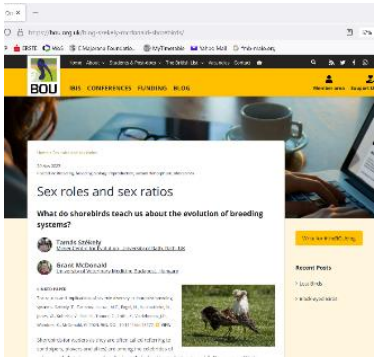
- Feng, S., J. Stiller …. A. Urrutia , T. Székely, Y. Liu, …… F. Lei, C. Rahbek, M. T. P. Gilbert, G. Graves, E. Jarvis, B. Paten & G. Zhang. 2020. Dense sampling of bird diversity increases power of comparative genomics. Nature 587:252-257.

- Stiller, J…… T. Székely, ….M. T. P. Gilbert, E. D. Jarvis, S. Mirarab & G. Zhang. 2024. Complexity of avian evolution revealed by family-level genomes. Nature (accepted).
- Wanders, K., G. Chen, S. Feng, G. Zhang, T. Székely, M. W Bruford, Zs. Végvári, G. Eichhorn, A. Urrutia. 2023. Polygamy and purifying selection in birds. Evolution 77:276-28.
- Wanders, K., G. Chen, S. Feng, T. Székely & A. O. Urrutia. Role-reversed polyandry is associated with faster fast-Z in shorebirds. Proc Roy Soc B (in revision).

Objective_5. To develop a new theoretical model to analyse the feedbacks between sex roles, environment and demography.

To achieve this theoretical objective, we carried out 3 main steps.

(i) With contributions by key ÉLVONAL team members, we published an overview on sex role variation in shorebirds (Székely et al. 2023). This review made publicly available the research agenda that underpinned the ÉLVONAL project. In an accompanying blog, we explored the significance of the sex role review in an accessible style https://bou.org.uk/blog-szekely-mcdonald-shorebirds/



(ii) We explored the association among gametic anisogamy using meta-analytic techniques and found no support for this association (Mokos et al 2021). In addition, we published a general review on sex roles and sex ratio variation by overviewing the empirical and theoretical research in studies of humans and non-human animals (Schacht et al. 2022). We also published reviews explaining the significance of sex role studies for evolutionary biology (Székely 2019, 2024).

- Mokos, J., I Scheuring, A. Liker, R. P. Freckleton & T. Székely. 2021. Degree of anisogamy is unrelated to the intensity of sexual selection. Scientific Reports 11: 19424.
- Schacht, R., S. R. Beissinger, C. Wedekind, M. D. Jennions, B. Geffroy, A. Liker, P. M. Kappeler, F. J. Weissing, K. L. Kramer, T. Hesketh, J. Boissier, C. Uggla, M. Hollingshaus & T. Székely. 2022. Adult sex ratios: causes of variation and implications for animal and human societies. Communications Biology 5: 1273
- Székely, T. 2019. Why study plovers? The significance of non-model organisms in avian ecology, behaviour and evolution. Journal of Ornithology 160: 923-933.
- Székely, T. 2024. Evolution of reproductive strategies: sex roles, sex ratios and phylogenies. Biologia Futura thematic volume "A lifetime devoted to studying evolution and biodiversity – honorary issue dedicated to Prof Zoltán Varga" (Editors: G. Sramkó, Z. Barta and T. Székely).

(iii) We developed a mathematical model to investigate the potentially complex feedbacks between sex roles, demography and environment (Long et al. submitted). The analyses showed that sex-specific mortalities, parental behaviour and sex ratios have complex associations, and multiple evolutionarily stable outcomes can be expected as a response to a single set of demographic parameters.

- Long, X., T. Székely, J. Komdeur & F. J. Weissing A life-history perspective on the evolutionary interplay of sex ratios and parental sex roles. American Naturalist (submitted).

Taken together, our team made substantial progress in regard to all five objectives. One sub-objective (Objective 2ii Experimental manipulation in the field), however, has not been accomplished. There were two reasons for this. First, as we reported in the project interview (March

2020), the hospitalisation of Prof Székely in 2019 and the COVID pandemic in 2020 made fieldwork in China impossible. Second, we re-evaluated the feasibility and potential benefit of the experiment and decided that the *a priori* designed field experiment (i.e., running parallel experiments in 4 different field sites simultaneously), is beyond the ÉLVONAL team's logistic and financial possibilities.

Further achievements

In addition to the aforementioned achievements, the ÉLVONAL shorebird project made additional impacts and spin-offs.

(i) Database. The project produced a database on ecology, behaviour, life history and demography of shorebirds. This Open BioMaps Database https://openbiomaps.org/projects/ will continue beyond the life-time of the project. Some parts of the database have been made open access already, and we are planning to make more data open access as we progress with publications.

Thanks to volunteer collaborators and limited funding for future projects, we will continue augmenting the database with new information.

(ii) Biodiversity conservation. The ÉLVONAL project made substantial impact on protection of shorebirds and their habitats. First, jointly with Hortobágy National Park, we run a conservation action to boost the breeding population of collared pratincole. This species used to be a characteristic breeder in alkaline grasslands called puszta, although since 1990's their numbers dramatically declined. Thanks to dedicated efforts by farmers, the national park and scientists, the pratincole population is now bouncing back (Kiss et al. 2023).

- Kiss, Á, Z. Végvári, V. Kubelka, Á. Monoki, I. Kapocsi, S. Gőri & T. Székely. 2023. Breeding in an agricultural landscape: conservation actions increase nest survival in a ground-nesting bird. Oryx (accepted).

Second, in collaboration with HUN-REN Aquatic Research Institute and Kiskunság National Park, we are restoring the breeding habitats of several shorebird species in Miklapuszta, Central Hungary with the support of EU-LIFE program. Shorebirds had high breeding density in Miklapuszta in the 1990'ies, however, with the decline of sheep grazing the breeding shorebirds rapidly declined. The project aims to restore habitats to boost the numbers of breeding shorebirds (Kentish plover, lapwing, redshank and avocets).

Third, we carried out waterbird populations and these data support basic conservation monitoring in several locations, which contribute to population estimates, e.g. Kazakhstan, Cabo Verde and India (McDonald et al. 2022, Jain et al. submitted, Roast et al. in prep), and shared these data with conservation organisations.

- Jain, S, E. Hsu-Hsun, T. Szekely. Waterbird surveys in wetlands of four states in India. Journal of Bombay Natural History Society (submitted).
- McDonald, G. C., Á. Bede-Fazekas, A. Ivanov, L. Crecco, T. Székely & A. Kosztolányi. 2022. Landscape and climatic predictors of Kentish Plover (*Charadrius alexandrinus*) distributions throughout Kazakhstan. Ibis (accepted).
- Roast, M, G. McDonald, N. Engel, A Kosztolányi & T. Székely. Population trends of Kentish plovers in a tropical island (in prep).

Fourth, we are closely collaborating with a local NGO, the Maio Biodiversity Foundation in Cape Verde to protect breeding shorebirds: Kentish plovers and Cream-coloured coursers (Rice et al. 2020, Engel et al. 2023).

- Rice, R, J. O. Valdebenito, M. Ottensmann, N. Engel, A. Adrião & T. Székely. 2019. Breeding ecology of the Cream-coloured Courser in Cape Verde. Ostrich 91: 65-73.

Fifth, in Madagascar, we established a new conservation NGO - Madagascar Nature Conservation - to protect breeding shorebird habitats, and currently we are building up the NGO.

(iii) Higher education. The ÉLVONAL team works closely with University of Debrecen (UD) academics, and we jointly proposed a new MSc course in Nature Conservation. In addition, we joined the UD doctoral school with a new doctoral programme "Biodiversity and Climate Change", and we are actively involved in shaping the doctoral school's policy.

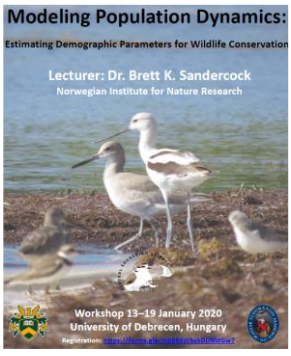
(iv) Mentoring early-career scientists. We are proud that numerous Hungarian and international young scientists joined our project, so that our project is an excellent example of multi-partner international collaborations that benefit science, higher education and biodiversity conservation.

First, 10 PhD students were involved in the ÉLVONAL project: Zsófia Tóth (Debrecen), Fanni Takács (Debrecen), Romy Rice (Bath), Noemie Engel (Bath), Wondimu Ersino (Debrecen), Hela Boughdiri (Debrecen), Jose Valdebenito (Bath), Narhulan Halimubieke (Bath), Claire Tanner (Bath), Kees Wanders (Bath).

Second, 7 post-doctoral researchers participated in the project: Dr Vojtěch Kubelka, Dr Grant McDonald, Dr William Jones, Dr Nolwenn Fresneau, Dr Jose Valdebenito, Dr Narhulan Halimubieke, Dr Cristina Carmona-Isunza. Some of these were based in Debrecen (Kubelka, Jones), whereas others were based in Budapest (McDonald), Veszprém (Fresneau), Bath (Valdebenito, Halimubieke) or Mexico (Carmona-Isunza). Two core members (Dr Vojtěch Kubelka and Dr Grant McDonald) secured permanent positions in Ceske Budejovice (Czech Republic) and Budapest, respectively. Further post-doctoral scientists remain involved in Debrecen-based projects (Dr Jose Valdebenito, Dr Noemie Engel) and thus contribute to brain-gain for Hungary.

(v) Conferences and workshops. As part of the ÉLVONAL project, we organised international workshops and conferences:

- In 2019 we organised an international symposium on Reproductive Strategies in Debrecen that was attended by 120 foreign and Hungarian students, young scientists and senior scientists;
- We organised a workshop on population demography in Debrecen 2020 which was attended by 20 students and young scientists;
- Our group has organised an International Wader Study Group Conference in 2022 in Szeged which was attended by 120 delegates from Hungary and abroad;
- We initiated and co-organising a Workshop on "Sexual selection a changing world" that will take place in Erice, Italy in May 2024.

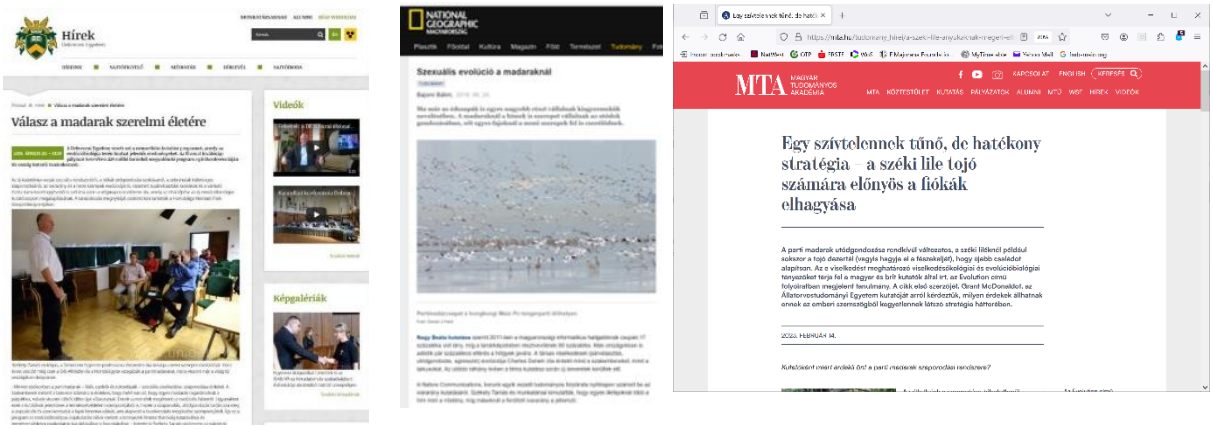


(iv) Outreach. Every year we contribute to Debrecen University's outreach "Researcher's Night" when approx. 150-180 primary school and secondary school students come to the university to learn about research. In 2021 for instance, 250 pupils joined the discission of our research – much of it revolved around ÉLVONAL-funded themes.

(vi) Training teachers in field biology. In 2021 we initiated a summer school for biology teachers in Hajdiu-Bihar province. At the first event we had 18 teachers joined the summer school while for the

second such event in 2022 we had 25 biology teachers. Jointly with Hortobágy National Park, we are currently planning a field-based accreditation programme for biology teachers.

(vii) Publicity and media. ÉLVONAL-related news were often in the media. First, Debrecen University and national news reported the launch of ÉLVONAL shorebird science project, and project-related news were also on the MTA website, Nat Geog Magyarország and various other outlets.



Second, the press release on Kubelka et al (2018) had an unusually wide coverage that included 30+ national and international newspapers with total readership estimated to be 7.7 M people.

Third, with the help of ex-film producers of BBC, we produced a short feature-film on some of the main achievements of the project (Fig. 11). For further details, see https://www.szekelylab.com/

Fig. 11. A feature-film focused on project-related achievements directed by Dan Freeman and Nigel Aschroft (Green Umbrella Productions).

(viii) Follow-up funding success. The ÉLVONAL project led to 8 new research projects in Hungary:

- EU-LIFE NAT HU 001404 (PI: Prof Lengyel, 3,425,659 EUR)
- NKFIH -NN 125642 (PI: Dr Kosztolányi, 44 M Ft)
- NKFIH -KH130430 (PI: Prof Liker, 20 M Ft)
- NKFIH- FK134741 fiatal kutatói kiválósági pályázat (PI: Dr McDonald, 40 M Ft)
- NKFIH poszt- doktori pályázat (PI: Dr Fresneau, 18 M Ft)
- NKFIH poszt- doktori pályázat (PI: Dr Vági, 18 M Ft)
- NKFIH ANN 22-143995 (PI: Dr Kosztolányi, 48 M Ft)
- HUN-REN-DE Reproductive strategies (PI: Prof Székely, 200 M Ft)

- Stipendium Hungaricum scholarship for 1 MSc student (Oscar Garcia Miranda) and 5 PhD students (Wondimu Ersino, Hela Boughdiri, Ahmed Awad, Vishakha Gupta, Khawla Bouali, PIs: Prof Székely & Prof Végvári, 70 M Ft)

In total, the value of new research grants generated by ÉLVONAL is approx. 4,530,000 EUR.

(ix) New conservation institute. Importantly, the University of Debrecen created a new biodiversity-focused institute in 2022 that is based (in part) on the success of the ÉLVONAL project. The new institute (Debrecen Biodiversity Centre, DBK) is formed to create a centre of biodiversity-focused activities in Debrecen and its surroundings. Prof Székely was appointed to be first director of the DBK, see https://hirek.unideb.hu/sokfeleseg-es-fenntarthatosag-szolgalataban

Closing statement

The ÉLVONAL-funded shorebird sex role project targeted fundamental evolutionary questions, and with the help of many teams worldwide we achieved substantial success. New field methodology, new data analytic approaches and novel theoretical and empirical insights have emerged from this research. We believe these achievements put our team in the international forefront of sexual dimorphism research. In addition, the project contributed to biodiversity conservation and education at three levels (primary schools, secondary schools and higher education), and had significant outreach and media output. Importantly, the project contributed to the career progression of young scientists in Hungary and abroad, and generated significant funding for follow-up studies. We believe that the overwhelming success of our work delivering a legacy will endure beyond the lifetime of the project and will provide a foundation for future research and conservation.

References (only those not provided above)

Darwin, C. 1871. The descent of man and selection in relation to sex. Murray, London.

De Lisle, S. P. 2019. Understanding the evolution of ecological sex differences: Integrating character displacement and the Darwin-Bateman paradigm. Evol. Lett. 3, 434–447.

Janicke, T, I. K. Häderer, M. J. Lajeunesse & N. Anthes. 2016. Darwinian sex roles confirmed across the animal kingdom. Sci. Adv. 2016; 2 : e1500983

Kokko, H. & M. Jennions. 2008. Parental investment, sexual selection and sex ratios. J Evolutionary Biology 21: 919–948.

Slatkin, M. 1984. Ecological causes of sexual dimorphism. Evolution 38:622–630.

Székely, T., R. P. Freckleton & J. D. Reynolds. 2004. Sexual selection explains Rensch's rule of size dimorphism in shorebirds. Proc. Natl. Acad. Sci. US 101: 12224 - 12227.

Székely, T., G. H. Thomas & I. C. Cuthill. 2006. Sexual conflict, ecology and breeding systems in shorebirds. BioScience 56: 801-808.

Székely, T., J.N. Webb, & I.C. Cuthill. 2000. Mating patterns, sexual selection and parental care: an integrative approach. IN Vertebrate mating systems, M. Apollonio, M. Festa-Bianchet & D. Mainardi (eds), World Scientific Press, Singapore, pp 194-223.

Székely, T., F. J. Weissing & J. Komdeur. 2014. Adult sex ratio variation: implications for breeding system evolution. Journal of Evolutionary Biology 27: 1500-1512.