Plumage colour signals: dynamic and static mechanisms (K124443)
Grant closing report

Below we describe the new results we obtained in the last three months of the grant, since the last report. **The sections of these most recent developments are indicated with boldface.** For the topics that had been addressed earlier, we provide a brief summary of our main findings. The report structure follows the four main aims of the original research plan.

1) How does feather nanostructure contribute to the colour of achromatic white plumage areas?
We first had to refine electron microscopy methods for the description of colour-producing feather nanostructures at the anatomy department of our university. During this refinement, we described in detail the nanostructural basis of blue tit blue crown reflectance (Hegyi et al. 2018 J Exp Biol) and great tit black crown reflectance (Laczi et al. 2019 Biol J Linn Soc). The novel findings of these studies took us in directions that made the blue tit nanostructural study an important part of a separate, recently started research project (K143222). When we measured the nanostructure of feather barbs in the white wing patch of collared flycatchers, we found great within-feather variation in structural variables, which suggested that individual-specific reflectance attributes must be due to macrostructural and not nanostructural differences.

We then described the white wing patch macrostructure with two simple measures (barb angle and outer vane width). We resampled the patch macrostructure and reflectance of individual females in two different stages of the same breeding and found that within-individual change in macrostructure explained change in reflectance (Laczi et al. 2021 Ibis). In a two-year cross-sectional study of males and females, we focused on the late nestling rearing period and found that a point sample of macrostructure adequately predicts reflectance only in males but not females. This can be because in females most abrasion happens before this regular capture period (Laczi et al. 2022 Behav Ecol Sociobiol).

2) Is the feather nanostructure of achromatic white plumage areas condition-dependent?
Following our structural findings detailed above, this question was reformulated for the feather macrostructure and the associated phenomena (reflectance and information content). Along this line, we first described relationships of white patch sizes with plasma lipid metabolite levels as dynamic measures of body condition. Among others, the findings suggested that individuals subject to nutritional stress during one reproductive season grow forehead patches for the next year which are more susceptible to structural degradation. This indirectly confirms a long-term component of white area condition dependence (Hegyi et al. 2019 Behav Ecol Sociobiol).

As a more direct but still correlative test of the condition-dependence of macrostructure, we examined male macrostructure in the two-year cross-sectional study described above. In males, wing patch structural state not only predicted reflectance (see above), but it also reflected previous year reproductive effort (negative relationship) and indicated current year offspring rearing capacity (positive relationship), thereby presenting white feather macrostructure as an indicator of condition and reproductive quality (Laczi et al. 2022 Behav Ecol Sociobiol).

As a direct experimental test of condition-dependence, as we originally planned, we used the next-year recaptures of parents from the brood size manipulation experiment. We

ultimately collected enough recaptures by 2023 to do this analysis. We found that manipulation did not significantly affect the feather macrostructure or reflectance characteristics of the plumage of adult birds by the next breeding season. It did, however, affect the size of the ornamental white wing patch in both sexes, while there was no significant effect on the white forehead patch size of males. Moreover, the same effect on wing but not forehead patch size appeared when looking at the adult (two years old or older) male recruits (returning offspring) from the experiment. In a Swedish population of the species, the reverse pattern has been demonstrated, with a significant effect of brood size manipulation on male forehead but not wing patch size in adults and recruits. We can therefore interpret our results as the first experimentally demonstrated between-population "crossover" in the information content of two sexual ornaments. This result is highly publishable because even experimental studies of the between-year condition-dependence of single sexual traits are very rare (Hegyi et al. 2024 manuscript).

Signal integration is an important aspect of signal information content, and integration is often due to shared condition dependence. Nearly all species display multiple different (putative or real) signal traits, and very often multiple traits of the same type (e.g. white colour patches). Whether these similar traits are different signals or a single integrated signal is rarely examined. Therefore, we formulated hypothetical scenarios for the functioning of signal coherence in sexual selection, suggested an analytical approach to test these, and applied this framework to our collared flycatcher plumage reflectance system. Our study provided the first evidence for threshold-based signal filtering by receivers in a natural population, with fundamental implications for future observational and experimental studies (Hegyi et al. 2022 Am Nat). We also applied the concept of signal integration to temporal integration at two time scales: within-year and long-term. We described year-round condition-indicator value in a pigmented plumage trait not prone to abrasion (Hegyi et al. 2020 Ecol Evol), and the dependence of a nutritionally based plumage colour trait on large-scale climatic shifts (Laczi et al. 2020 Ibis). Finally, as another aspect of signal integration, an accidental observation led us to consider a visually homogeneous plumage area (the black abdominal stripe of great tits) as the composite of three patches of different reflectance attributes. Spectrometric measurements found drastic sexual dichromatism, with the central (breast) area of the bib being more reflective than the surroundings in males, and less reflective in females. This may contribute to rapid sex recognition by distant conspecifics, or form a "hidden" sexual signal (Laczi et al. 2023 Front Ecol Evol).

3) Is plumage colour change of an individual during breeding used as a signal by the partner?
4) Is rapid plumage colour change during breeding state-dependent?
We addressed these two questions in a single brood size manipulation experiment. We increased and reduced brood size after hatching by two nestlings and sampled male and female wing patch macrostructure and reflectance at the manipulation and before fledging of the young, and we also monitored feeding rates of the parents in the pre-fledging stage. Male and female reproductive effort (feeding rate and nestling biomass) consistently reacted to brood size reduction but not brood size enlargement. **The same was the case when we recently considered the oxidative stress levels of parents from blood samples taken during the experiment: oxidative stress reacted to the brood size manipulation in parents but not in the nestlings.** However, no matter whether we measured the experimental effects by the manipulation itself or by the effort of the parent, we found a significant effect on male breast UV chroma and female breast brightness. This confirms experimentally for the first time that short-term change in white patch degradation can indicate reproductive effort

(and possibly other aspects of body condition). Moreover, in both sexes, the feeding rate of the partner was significantly related to the degree of the experimentally induced colour degradation, thereby presenting rapid plumage colour degradation as a signal used by the partner. **These findings have now been published (Hegyi et al. 2023 Sci Rep) and the oxidative stress results are currently being prepared for publication.** Finally, our study of parental care also detected and described a case of non-conventional parental care sharing among male and female flycatchers (Laczi et al. 2021 Ecol Evol).

Publications

Hegyi G, Laczi M, Kötél D, Csizmadia T, Lőw P, Rosivall B, Szöllősi E, Török J (2018) Reflectance variation in the blue tit crown in relation to feather structure. Journal of Experimental Biology 221: jeb176727

Hegyi G, Jenni-Eiermann S, Boross N, Garamszegi LZ, Laczi M, Kötél D, Krenhardt K, Jablonszky M, Markó G, Nagy G, Rosivall B, Szász E, Török J (2019) Ornaments and condition: plumage patch sizes, nutritional reserve state, reserve accumulation and reserve depletion. Behavioral Ecology and Sociobiology 73:89.

Laczi M, Hegyi G, Kötél D, Csizmadia T, Lőw P, Török J (2019) Reflectance in relation to macro- and nanostructure in the crown feathers of the great tit (*Parus major*). The Biological Journal of the Linnean Society 127:113-124.

Hegyi G, Laczi M, Kötél D, Csörgő T (2020) Melanin-based ornament darkness positively correlates with across-season nutritional condition. Ecology and Evolution 10: 13084-13097

Laczi M, Hegyi G, Nagy G, Pongrácz R, Török J (2020) Yellow plumage colour of great tits Parus major correlates with changing temperature and precipitation. Ibis 162: 232-237

Laczi M, Balogh J, Nardou X, Török J, Hegyi G (2021) The meaning of purely structural colour: white plumage reflectance indicates feather condition. Ibis 163:407-416

Laczi M, Kopena R, Sarkadi F, Kötél D, Török J, Rosivall B, Hegyi G (2021) Triparental care in the collared flycatcher (*Ficedula albicollis*): Cooperation of two females with a cuckolded male in rearing a brood. Ecology and Evolution 11:10754-10760.

Hegyi G, Laczi M, Herényi M, Markó G, Nagy G, Rosivall B, Szász E, Török J (2022) Functional integration of multiple sexual ornaments: signal coherence and sexual selection. American Naturalist 200:486-505.

Laczi M, Jablonszky M, Markó G, Nagy G, Szabó G, Zsebők S, Török J, Hegyi G (2022) White plumage color as an honest indicator: feather macrostructure links reflectance with reproductive effort and success. Behavioral Ecology and Sociobiology 76:125

Hegyi G, Laczi M, Szabó G, Sarkadi F, Török J (2023) Plumage color degradation indicates reproductive effort: an experiment. Scientific Reports 13:18770

Laczi M, Herczeg G, Szabó G, Gyarmathy H, Sarkadi F, Török J, Hegyi G (2023) The great tit abdominal stripe contains a sexually dichromatic colour patch hidden from the human eye. Frontiers in Ecology and Evolution 11:1263974

Hegyi G, Laczi M, Szabó G, Sarkadi F, Török J (2024) Experimental demonstration of between-population crossover in sexual trait information content. Manuscript