**Electronic Supplementary Material**

**Revisiting a classic hybrid zone: rapid movement of the northern flicker hybrid zone in contemporary times**

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**Text S1. Detailed methods for multispectral photography**

We collected multispectral images of all contemporary CUMV flicker specimens, plus a handful of examples of allopatric individuals from both taxa (4 yellow-shafted and 8 red-shafted; Table S2), to obtain a more quantitative assessment of phenotypic traits following (Ligon et al. 2018). We took RAW format images under standardized conditions using a Canon 7D camera (Tokyo, Japan) with full-spectrum quartz conversion and fitted with a Novoflex Noflexar 35 mm lens. Specimens were illuminated by two eyeColor arc lamps (Iwasaki, Tokyo, Japan) that simulate CIE-recommended daylight (D65) and were diffused through 0.5 mm polytetrafluoroethylene sheets. The lamps include a UV-blocking coating, which was removed prior to image collection. To capture the important aspects of flicker plumage, each specimen was photographed from three viewing angles: ventral (flat on its back), dorsal (flat on its belly), and lateral (on its left side). Additionally, for each viewing angle, we used filters (Baader, Mammendorf, Germany) to take two photographs: one capturing visible light between 400-700 nm and one capturing only UV light between 300-400 nm. All specimens were photographed against a blank white background and included size and color standards. For visible light photographs, we used a shutter speed of 1/6” and ISO of 400, and for UV photographs those parameters were increased to 4” and 3200, respectively.

Visible and UV photographs were used to create standardized multispectral image files for each specimen and viewing angle using the Multispectral Image Calibration and Analysis Toolbox (micaToolbox; Troscianko and Stevens 2015) in ImageJ (Schneider et al. 2012). Standardized multispectral images combine, equalize, and linearize the different color channels from the two photographs (Stevens et al. 2007). We identified regions of interest (ROI) within each flicker as follows: throat and shaft with the ventral viewing angle; crown and nuchal patch with the dorsal viewing angle; and ear coverts and malar stripe with the lateral viewing angle. We then used the micaToolbox to estimate the color sensitivity of our camera/lens combination and generate a custom mapping function to convert colors in the image to stimulation values corresponding to an avian visual space (the Eurasian blue tit, *Cyanistes caeruleus*). We used the Batch Multispectral Image Analysis option in the micaToolbox to output values for each color channel (red, green, blue, UV) and luminance within each ROI, as well as the overall area of the ROI (important only for the nuchal patch).

**References**

Ligon, R. A., C. D. Diaz, J. L. Morano, J. Troscianko, M. Stevens, A. Moskeland, T. G. Laman, et al. 2018. Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. PLOS Biology 16:e2006962.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671–675.

Stevens, M., C. A. Párraga, I. C. Cuthill, J. C. Partridge, and T. S. Troscianko. 2007. Using digital photography to study animal coloration. Biological Journal of the Linnean Society 90:211–237.

Troscianko, J., and M. Stevens. 2015. Image calibration and analysis toolbox—a free software suite for objectively measuring reflectance, colour and pattern. Methods in Ecology and Evolution 6:1320–1331.

**Table S1.** Details on the scoring of six plumage trait differences between red-shafted and yellow-shafted flickers. This method is slightly adapted from Short (1965).

|  |  |
| --- | --- |
| **Plumage score** | **Description** |
| *Crown color* | |
| 0 | Gray, as in yellow-shafted |
| 1 | Gray with brown traces in forehead and crown |
| 2 | Mixed gray and brown (crown half brown with more gray on hind neck) |
| 3 | Crown brown with hind neck gray toward back |
| 4 | Brown, as in red-shafted |
|  |  |
| *Ear covert color* | |
| 0 | Tan, as in yellow-shafted |
| 1 | Tan with gray traces |
| 2 | Mixed gray and tan |
| 3 | Gray with tan traces (especially below eye) |
| 4 | Gray, as in red-shafted |
|  |  |
| *Malar stripe color (males only)* | |
| 0 | Black, as in yellow-shafted |
| 1 | Black with <20% red |
| 2 | Mixed black and red |
| 3 | Red with <20% black |
| 4 | Red, as in red-shafted­­­­­ |
|  |  |
| *Nuchal patch presence* | |
| 0 | Present and broad, as in yellow-shafted |
| 1 | Present and restricted in width (less than one-half of normal width) |
| 2 | Present and broken in one or more places |
| 3 | Traces present, usually at sides of nape |
| 4 | Absent, as in red-shafted |
|  |  |
| *Shaft color* | |
| 0 | Bright yellow, as in yellow-shafted |
| 1 | Yellow-orange |
| 2 | Orange |
| 3 | Red-orange |
| 4 | Deep salmon red, as in red-shafted |
|  |  |
| *Throat color* | |
| 0 | Tan, as in yellow-shafted |
| 1 | Tan with gray traces (usually on lower throat) |
| 2 | Mixed gray and tan |
| 3 | Gray with tan traces (usually near chin) |
| 4 | Gray, as in red-shafted |

**Table S2.** Information on samples included in this study. Locality ID/Name correspond to values in Figure 1 and Table S3. Plumage scores range from 0 (yellow-shafted) to 4 (red-shafted). The overall hybrid index has been transformed to range from 0 to 1 to allow comparisons between the sexes. *Table included as separate excel file*

**Table S3.** Sampling localities used in the geographic cline analyses and shown in the inset map in Figure 1.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Locality ID** | **Locality Name** | **Latitude** | **Longitude** | **Distance** | **Sampling Period** |
| 0 | Allopatric RSFL |  |  | -100 | photos only |
| 1 | Western | 40.8830559 | -105.51687 | 0 | contemporary |
| 2 | Greeley | 40.346603 | -104.82279 | 58.4 | historic |
| 3 | Kersey | 40.3693671 | -104.44526 | 90.1 | contemporary |
| 4 | Orchard | 40.351842 | -104.06838 | 121.8 | historic and contemporary |
| 5 | W Fort Morgan | 40.267982 | -103.94052 | 132.6 | historic |
| 6 | Morrill | 41.9594945 | -103.92463 | 133.9 | contemporary |
| 7 | E Fort Morgan | 40.2825194 | -103.70253 | 152.6 | contemporary |
| 8 | Brush | 40.3272657 | -103.57831 | 163.0 | contemporary |
| 9 | Minatare | 41.8984992 | -103.42772 | 175.7 | contemporary |
| 10 | Merino | 40.4047694 | -103.39182 | 178.7 | contemporary |
| 11 | Bridgeport | 41.6895056 | -103.16821 | 197.5 | contemporary |
| 12 | Crook | 40.8444414 | -102.77278 | 230.7 | historic and contemporary |
| 13 | Lisco | 41.4499137 | -102.53066 | 251.1 | contemporary |
| 14 | Sedgwick | 40.932974 | -102.48678 | 254.8 | contemporary |
| 15 | Big Springs | 40.9935125 | -102.17377 | 281.1 | historic and contemporary |
| 16 | Lewellen | 41.2998817 | -102.02722 | 293.4 | contemporary |
| 17 | Ogallala | 41.22806 | -101.74345 | 317.3 | contemporary |
| 18 | Sutherland | 41.1451911 | -101.11715 | 369.9 | historic and contemporary |
| 19 | North Platte | 41.17314 | -100.78951 | 397.5 | contemporary |
| 20 | Halsey | 41.903358 | -100.31039 | 437.7 | historic |
| 21 | Gothenberg | 40.9061571 | -100.16424 | 450.0 | historic and contemporary |
| 22 | Elm Creek | 40.686218 | -99.347335 | 518.7 | historic and contemporary |
| 23 | Burwell | 41.756348 | -99.282391 | 524.1 | historic |
| 24 | Grand Island | 40.853711 | -98.406833 | 597.7 | historic and contemporary |
| 25 | Silver Creek | 41.2795413 | -97.680686 | 658.7 | historic and contemporary |
| 26 | Schuyler | 41.4080137 | -97.031092 | 713.3 | historic and contemporary |
| 27 | Eastern | 41.3335076 | -96.238837 | 779.8 | historic and contemporary |
| 28 | Allopatric YSFL |  |  | 880 | photos only |

**Table S4.** Model results for the geographic clines from plumage scoring in the historic and contemporary sampling periods.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Trait** | **Center** | **Center 95% CI** | **Width** | **Width 95% CI** |
| *Historic sampling period* | | | | |
| hybrid index | 208.3763 | 190.0316, 226.8876 | 251.1938 | 188.8206, 328.1764 |
| crown | 277.0663 | 206.6787, 351.9533 | 462.8924 | 149.3879, 826.9237 |
| ear coverts | 153.8192 | 144.0973, 163.6192 | 267.7168 | 232.0703, 307.8535 |
| malar stripe | 218.0839 | 203.8221, 231.8209 | 175.5512 | 122.5301, 227.6591 |
| nuchal patch | 228.9754 | 205.2576, 251.3624 | 173.7698 | 62.07621, 268.8413 |
| shaft | 207.8027 | 184.1068, 231.5219\* | 211.4893 | 134.074, 294.1099\* |
| throat | 191.2107 | 173.0066, 209.2131 | 337.3793 | 275.8701, 409.57 |
|  | | | | |
| *Contemporary sampling period* | | | | |
| hybrid index | 135.6074 | 108.7064, 156.7343 | 274.5806 | 179.1994, 408.213 |
| crown | 114.2603 | 74.6308, 140.5735 | 237.7157 | 109.6608, 422.9463 |
| ear coverts | 128.313 | 99.8488, 148.052 | 179.0388 | 103.3117, 303.813 |
| malar stripe | 186.9072 | 132.0964, 235.1586 | 332.3642 | 173.667, 677.4881 |
| nuchal patch | 115.918 | 77.6389, 141.2332 | 263.3228 | 147.1721, 429.0008 |
| shaft | 116.5658 | 38.8284, 158.1011 | 300.6879 | 28.15649, 670.1835 |
| throat | 179.5272 | 151.5103, 205.1583 | 261.3114 | 162.6929, 427.2188 |

\* Confidence intervals based on bootstrapping.

**Table S5.** Comparison of the model results for geographic clines from plumage scoring and multispectral photography for the two image parameters with well-formed clines.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Data source** | **Center** | **Center 95% CI** | **Width** | **Width 95% CI** |
| *Nuchal patch, area* | | | | |
| Scoring | 115.918 | 77.6389, 141.2332 | 263.3228 | 147.1721, 429.0008 |
| Photos | 162.6336 | 106.6577, 208.6296 | 384.7626 | 183.28, 861.3652 |
|  |  |  |  |  |
| *Malar stripe, long wave (red channel)* | | | | |
| Scoring | 186.9072 | 132.0964, 235.1586 | 332.3642 | 173.667, 677.4881 |
| Photos | 166.8599 | 130.9951, 197.7631 | 258.4683 | 145.8842, 464.7625 |

**Table S6.** Results from multiple regressions comparing the plumage score with values from the multispectral photography for each plumage trait. For the nuchal patch, we compared the plumage score to the patch area in the image. For all other traits, we compared the plumage score to the values for the four color channels and luminance.

|  |  |  |
| --- | --- | --- |
| **Trait** | **Adjusted R2** | **p-value** |
| crown | 0.4501 | 2.653 × 10-12 |
| ear coverts | 0.732 | < 2.2 × 10-16 |
| malar stripe | 0.8815 | < 2.2 × 10-16 |
| nuchal patch | 0.7136 | < 2.2 × 10-16 |
| shaft | 0.9203 | < 2.2 × 10-16 |
| throat | 0.872 | < 2.2 × 10-16 |

**Figure S1.** Linear relationships between the six plumage traits and the image parameters from the multispectral photography. Image parameters are indicated by different colors (legend at bottom). Significant relationships are indicated with an asterisk on the right side of the plot.



**Figure S2.** Geographic clines of the six plumage traits for the (**A**) historic and (**B**) contemporary sampling periods. Traits are indicated by different colors and points indicate the average trait score at each sampling locality (jittered for visualization). Corresponding model outputs are available in Table S4.



**Figure S3.** Geographic clines for image parameters from the multispectral photography across the six plumage traits. Image parameters are indicated by different colors (legend at bottom) and points indicate the average trait score at each sampling locality.

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**Figure S4.** Geographic clines for the two well-formed image parameter clines: (**A**) area of the nuchal patch and (**B**) red channel of the malar stripe. Points indicate the average image parameter value at each sampling locality and shading represents the 95% bootstrap confidence intervals. Corresponding model outputs are available in Table S5.



**Figure S5.** Estimated cline centers with confidence intervals for the geographic clines estimated from plumage scoring (black) and multispectral photography (red) in the contemporary sampling period.

