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GENERAL INFORMATION:

1. Title of Dataset: Data from: Relating wing morphology and immune function to patterns of partial and differential bat migration using stable isotopes.
2. Author Information

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1. Date of data collection: May 2018
2. Geographic location of data collection: New Mexico, USA
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DATA & FILE OVERVIEW

1. Description of dataset:

These data were collected to investigate intraspecific variation in wing morphology and immune function in silver-haired bats (*Lasionycteris noctivagans*), a species that follows both partial and differential migration patterns. Using stable hydrogen isotope techniques, we estimated breeding latitude of spring migrants from fur samples collected from each bat. Based on estimated breeding latitude, we classified bats as residents (n=18), southern origin migrants (n=12), northern long-distance migrants (n=14), and northern short-distance migrants (n=30). We took photos of each bat with both wings fully extended and used ImageJ to take wing measurements. Using blood smears and collected plasma, we quantified immune function as absolute and differential white-blood cell count, bacteria killing ability, and IgG concentration. Sample sizes for immune assays varied due to limited volumes of blood or plasma.

1. File list:

File 1 Name: rogers\_et\_al\_2022\_DRYAD.csv

File 1 Description: bat capture data, bat sex, bat morphometrics, immune function metrics, fur stable hydrogen isotope values, migration groups

METHODOLOGICAL INFORMATION

*Capture and sampling*

We captured bats (n = 80) in the southwest U.S. (Sandoval County, New Mexico, 35.22°N 106.30°W) during vernal migration in May 2018 using mist nets. We measured body mass (± 0.1 g) using a digital scale, forearm length (± 0.1 mm) using calipers, and lean mass and fat mass using quantitative magnetic resonance body composition analysis (QMR; EchoMRI-B; Echo Medical Systems, Houston, TX), a noninvasive method for body composition analysis (McGuire & Guglielmo, 2010). We calculated body fat as a percentage of total body mass.

For stable hydrogen isotope analysis, we collected small fur samples from the anterior dorsal region of each bat using surgical scissors. For wing morphometrics, we took photographs of the bats following Senawi & Kingston (2019). Bats were positioned on a graph mat with both wings fully extended, using transparent cellophane tape to briefly (< 1 min) restrain the wings and tail membrane while the photographs were taken with a digital camera mounted to a tripod above the bat. For immune function quantification, we collected up to 70 µL of blood in heparinized capillary tubes following puncture of the antebrachial vein with a sterile 26-gauge needle. We prepared blood smears on glass slides, fixed the smears with methanol, and later stained them with buffered Wright-Giemsa (Camco Quik Stain II). We divided plasma from blood cells by centrifuging for 10 min at 10,000*g* and stored samples at –80° C until further analysis.

*Stable isotope analysis*

Stable hydrogen isotope analyses of fur samples were conducted at the Laboratory for Stable Isotope Science at the University of Western Ontario in London, Canada. Fur samples were soaked overnight in a 2:1 chloroform: methanol solution and then rinsed with more solution and left to dry in a fume hood for at least 48 hours. About 0.35–0.37 mg of each sample and keratin standards were weighed into individual silver capsules that were then crimped loosely closed. Sample analyses included a 10% duplication rate. To account for the presence of exchangeable hydrogen in the fur samples, all samples were equilibrated to the laboratory atmosphere for > 4 days alongside keratin standards that have known non-exchangeable stable hydrogen isotope compositions. The samples and standards were then fully folded shut on the morning of isotopic analysis. All samples and standards were pyrolyzed at 1450°C using a Thermo ScientificTM High Temperature Conversion Elemental Analyzer (TC/EA) equipped with a glassy carbon reactor. The hydrogen gas was then He-swept in continuous flow mode to a Thermo ScientificTM Delta VTM Plus isotope ratio mass spectrometer (IRMS).

The hydrogen isotope results are reported using the normal *δ*-notation in parts per thousand (‰) relative to the VSMOW-SLAP scale, as calibrated using two internationally accepted homogenized and powdered keratin standards, including Caribou Hoof Standard and Kudu Horn Standard (CHS and KHS; US Geological Survey, Reston, VA, USA; –157.0 ± 0.9‰, –35.3 ± 1.1‰) (Soto et al., 2017). The accuracy of the calibration curve was tested using an internal laboratory standard, Spectrum-1. It returned a value (–56.1 ± 1.9‰, SD; n = 10) that compares well with its accepted value (–57‰). The average reproducibility of CBS and KHS were 1.1‰ (SD; n = 9) and 0.5‰ (SD; n = 9), respectively. The average reproducibility of samples was 1.9‰ (SD; n = 8). The hydrogen isotope data for all samples are provided in Table S1.

*Isoscape generation and origin analysis*

The accepted values of the non-exchangeable stable hydrogen isotope compositions of the CBS and KHS reference materials were revised recently (Soto et al., 2017), so we recalibrated previously published fur reference materials and known-origin samples of *L. noctivagans* fur (Table S2; Fraser et al., 2017) and created a new isoscape. To do so, we followed the procedure in Fraser et al. (2017) to generate estimates of *δ*2Hprecip values (IsoMAP job key = 75701) and then calculated a revised isoscape equation using Standardized/Reduced Major Axis Regression (‘SMA’ function in R package, ‘SMATR’; Pylant et al., 2014; Campbell et al., 2020; Smith, 2009). We used the R package ‘Isocat’ (Campbell et al., 2020), the *δ*2Hprecip isoscape, and the revised *δ*2Hfur/*δ*2Hprecip equation to create a probability of origin surface for each individual bat in the study using the function ‘isotopeAssignmentModel’.

Because we analyzed fur samples, our assumption was that we were estimating locations of individual bats during their previous period of fur growth (*i.e.,* the preceding summer; Fraser et al., 2013). We then used the function ‘makecumsumsSurface’ to use a cumulative sum technique to quantify the probability of origin for each bat and to identify a threshold of 0.40 probability of origin. We selected this threshold in an effort to balance the accuracy and precision of our origin assignments (Campbell et al*.*, 2020). We then calculated the minimum distance for each bat from the location of capture to the closest raster cell with a cumulative sum probability above the threshold using the ‘distancefrompoints’ function in the ‘raster’ package.

*Wing photograph analysis*

Six wing measurements were taken from each bat using ImageJ (v.152a; Rasband 1997-2018) following Norberg & Rayner (1987), including “armwing” (plagiopatagium) length, “handwing” (dactylopatagium) length, “armwing” area, “handwing” area, wingspan (measured from wing tip to wing tip), and total surface area of one half of the bat’s body with wings outstretched, including the wing and tail membranes and a lateral half of the body excluding the head. All measurements were combined using a Principal Component Analysis, using the ‘prcomp’ function from the ‘stats’ package. All variables were zero-centered and scaled. Wing and mass measurements were used to further calculate wing loading, wing aspect ratio, and tip shape (Norberg & Rayner, 1987).

*Immunoglobulin G concentration*

To quantify adaptive humoral immunity, we measured immunoglobulin G (IgG) levels using a protein G ELISA (Moore et al., 2013; Schneeberger et al., 2014). We coated a microtiter plate (Nunc MaxiSorp flat-bottom 96-well plates, Invitrogen eBioscience) with 100 µL duplicate plasma samples diluted 1:20,000 in 50mM NaHCO3 at pH 9.5. We incubated the plate for 1h at 37°C then washed the plate twice with Tris-buffered saline Tween-20 solution (TBS-T) and added 200 µL blocking buffer (Bethyl Laboratories, E104) to each well to block non-specific binding. After incubating for 30 minutes at 37°C, we washed the plate once and added 100 µL protein-G horseradish peroxidase conjugate (Invitrogen) diluted 1:48,000 in TBS-T to each well. Subsequently, we incubated the plate for 30 min at room temperature, washed the plate five times, and then added 100 µL substrate (TMB One Component HRP Microwell Substrate, Bethyl Laboratories, E102) to each well. After 15 min, we stopped the reaction with 1M HCl and immediately measured OD at 450 nm. As antibody concentration is directly proportional to OD based on the Beer-Lambert Law, we used mean IgG OD for analysis.

*Leukocyte profiling*

We conducted both absolute and differential leukocyte counts using 1–2 blood smears (Becker et al., 2017; Schneeberger et al., 2013). We manually estimated total white blood cell (WBC) count by quantifying the mean number of leukocytes per 100 fields of view (FOV) at 400x magnification. To determine differential WBC counts we counted 100 leukocytes at 1000x magnification (oil immersion) and recorded the number of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. We multiplied differential and total WBC counts to obtain absolute leukocyte counts per FOV. In general, total white blood counts were low (0.19 ± 0.09 white blood cells per FOV) and because many individuals had fewer than 100 white blood cells represented per slide, we were unable to determine absolute leukocyte counts for all bats. As elevated glucocorticoid hormones can alter vertebrate leukocyte profiles (Davis et al., 2008), we calculated the ratio of neutrophils to lymphocytes (NL ratio) for individuals as a measure of chronic stress.

*Bacterial killing assay*

We quantified the *ex vivo* bacterial killing ability (BKA) of plasma against *Escherichia coli* following the microplate method of French & Neuman-Lee (2012) with modifications described by Becker et al. (2017). In duplicate, we challenged 22 *µ*L plasma samples diluted 1:16 in phosphate-buffered saline (PBS) in 96-well plates with 5 *µ*L of *E. coli* (ATCC 8739; Microbiologics Inc.)reconstituted in PBS at a working concentration of 104 bacteria/mL. We added 125 *µ*L tryptic soy broth (Millipore-Sigma) to each well and measured background optical density (OD) at 300 nm. After incubating plates for 12 h at 37°C, we measured OD again and calculated BKA as 1 minus the mean change in sample OD relative to the positive control (no plasma). Samples with BKA values slightly below or above the expected range of 0–1 (n = 8; actual range −0.07–1.07) were reported as 0 and 1, respectively.

DATA-SPECIFIC INFORMATION FOR: rogers\_et\_al\_2022\_DRYAD.csv

1. Number of variables: 34
2. Number of cases/rows: 80
3. Missing data codes: None
4. Abbreviations used: NA; not applicable
5. Variable list:

ID – bat identification code

capture\_date – date of bat capture

sunset\_time – sunset time on the date of capture

capture\_time – time of bat capture

bleed\_time – time of blood sample collection

sex – bat sex (M-male; F-female)

forearm – forearm length (mm)

mass – total body mass (g)

fat – fat mass (g) measured using quantitative magnetic resonance

fuel\_load – calculated by dividing ‘fat’ by ‘mass’ and converting to a percentage

IgG – the mean optical density (OD) of the IgG-protein G ELISA at 450 nm, which is directly proportional to the concentration of immunoglobulin G in the plasma

BKA – bacteria killing ability; the proportion of bacteria killed in a plasma challenge assay

BKA\_reported - samples with BKA values slightly below or above the expected range of 0–1 (n = 8; actual range −0.07–1.07) were reported as 0 and 1, respectively

WBC – total white blood cell count; the mean number of leukocytes per 100 fields of view (FOV) at 400x magnification

lymphocytes – the proportion of lymphocytes in 100 FOV multiplied by the total WBC count

neutrophils – the proportion of neutrophils in 100 FOV multiplied by the total WBC count

monocytes – the proportion of monocytes in 100 FOV multiplied by the total WBC count

eosinophils – the proportion of eosinophils in 100 FOV multiplied by the total WBC count

basophils – the proportion of basophils in 100 FOV multiplied by the total WBC count

NL\_ratio – the ratio of neutrophils to lymphocytes

*δ*2H\_VSMOW – stable hydrogen isotope composition of the fur, reported using the normal *δ*-notation in parts per thousand (‰) relative to the VSMOW-SLAP scale

dist\_trav – the minimum distance for each bat from the location of capture to the estimated location of individual bats during their previous period of fur growth using a threshold of 0.40 probability of origin

migrat\_group – ‘1’ = long-distance northern migrant; ‘2’ = short-distance northern migrant; ‘3’ = resident; ‘4’ = southern origin migrant

length\_plagio – plagiopatagium length (mm).

length\_dactylo – dactylopatagium length (mm).

wingspan – length from wing tip to wing tip (mm).

area\_halfbody – the total surface area of one half of the bat’s body with wings outstretched, including the wing and tail membranes and a lateral half of the body excluding the head (mm2).

area\_plagio – the surface area of the plagiopatagium (mm2).

area\_dactylo – the surface area of the dactylopatagium (mm2).

tip\_length – the length of the wing tip, calculated as the length of the dactylopatagium divided by the length of the plagiopatagium (Norberg and Rayner 1987).

tip\_area – the tip surface area, calculated as the surface area of the dactylopatagium divided by the surface area of the plagiopatagium (Norberg and Rayner 1987).

tip\_shape – the tip shape index, calculated as the tip surface area divided by the difference between the tip length and tip surface area (Norberg and Rayner 1987).

aspectratio – the aspect ratio of the wing, calculated as wingspan2/total surface area of the bat’s body.

wingloading – the wingloading value, calculated as: mass(g)/total surface area of the bat’s body.