# Supplementary material for: Brief increases in corticosterone result in immediate and lasting changes to DNA methylation in a wild bird

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## ABSTRACT

*Keywords: reduced representation bisulfite sequencing, stress, endocrinology, carryover effects*

## INTRODUCTION

Even during adulthood, brief challenges can prime changes (Vitousek et al., 2018).

## METHODS

We studied tree swallows breeding at field sites in and around Ithaca, New York, U.S.A. from April to July of 2015-2016. This population of tree swallows has been continuously studied since 1986 and we followed established monitoring protocols (Winkler et al., 2020). In the first year of this study, adult females were captured on day **X** after the beginning of incubation and again on day **XX** of incubation. In the second year of the study, any

returning females were captured on day **XX** of incubation. In this study, we only report data from a single blood sample taken within three minutes of capture from each of the (up to) three captures. However, we took a series of three blood samples over the course of one hour along with a set of standardized morphological measurements at each capture [see xxx]. All birds also received a unique USGS aluminum band and passive integrated transponder (PIT) tag if they were not previously banded.

Between the first and second capture in year one, females were randomly assigned to either a control or experimental treatment group. In the experimental group, we simulated a brief spike in corticosterone on 5 days between the two captures. To accomplish this, we applied a 60 $\mu$l dose of corticosterone gel (xx DMSO, xx corticosterone) to a fake egg anchored in the nest cup at a randomly chosen time during the day when females were absent from the nest. Upon returning, females incubated the clutch and absorbed a dose of corticosterone across the brood patch.

We previously validated that this dosing method results in a brief ($<$ 180?? minutes) increase in corticosterone within the range of natural acute corticosterone responses (Vitousek et al., 2018). Control nests received either no manipulation or a sham control in which they were dosed as described above but with DMSO gel only with no corticosterone added. We previously found no difference in physiology, behavior, reproductive success, or survival between control and sham control birds receiving this treatment [cite] and we combine both control groups in the analyses described here.

*Sample processing*

Blood samples collected in the field were immediately stored on ice in a cooler and processed in the lab within 3 hours of capture. Red blood cells were separated from plasma by centrifugation and added to 1 mL of ice cold cryopreservation buffer [90% newborn calf serum, 10% DMSO; Haussmann & Mauck (2008)]. Samples were then frozen at a constant cooling rate in a Mr. Frosty container with isopropyl alcohol and stored at -80∘ C until further

processing.

Cryopreserved blood samples were thawed and DNA was extracted using the DNeasy Blood & Tissue spin column extraction kits according to the manufacturer's protocol, except that we used a 50$\mu$l final elution volume. Extracted DNA samples were assayed by nanodrop and Qubit for DNA quality and concentration. Any samples with **XXX** were re-extracted.

*Reduced representation bisulfite sequencing*

We prepared our samples for reduced representation bisulfite sequencing (RRBS) using the Diagenode Premium RRBS Kit and closely following the manufacturer's protocol (Veillard, Datlinger, Laczik, Squazzo, & Bock, 2016). Briefly, samples were diluted to **XX** and **XX** of sample was used for library preparation. The process included enzymatic digestion with Mspl and size selection to increase coverage of CpG-rich regions, such as CpG islands and enhancers. Individual samples received a unique barcode and were pooled in groups of 6 before bisulfite conversion. We also included a methylated and unmethylated spike in control with each sample to confirm the efficiency of bisulfite conversion.

From the available samples, we selected 120 samples to process. These samples were chosen to maximize the power of our planned comparisons (i.e., preferentially birds with all 3 samples then birds with just two samples). Prior to RRBS processing, these 120 samples were assigned random numbers for processing order to account for any batch effects. Libraries were prepared with the Diagenode kit in two batches (one set of 24 and one of 96) and sequencing was performed on 5 lanes with 24 samples per lane.

*RRBS bioinformatics pipeline*

*Tree swallow reference genome assembly*

For this study, we improved upon a previously published reference genome for tree swallows sequenced from this population (Taff, Campagna, & Vitousek, 2019). We extracted fresh DNA from the same individual previously sequenced and submitted to Duke(?) for xxx. We

combined the new sequence data with the xxx from previous work. List of tools and process for assembly. List of some quality reports and access to genome...

*Data analysis*

# RESULTS

# DISCUSSION

# ACKNOWLEDGMENTS

# AUTHOR CONTRIBUTIONS

# ETHICAL NOTE

# FUNDING

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