Simulated infection induced changes in DNA methylation differ between introduced and native house sparrow (Passer domesticus)

Aaron Schrey[1], Oluremi Ige[1], Daniella Ray[2], M. Ellesse Lauer[1], Danielle Dawkins[1], Natalie Schrey[3], Elizabeth Sheldon[4], Kailey McCain[5], J Maddox[6], Kevin D. Kohl[7], Mark Ravinet[8], J. Briskie[9], Kate Buchanan[10], Roi Dor[11], Henrik Jensen[12], Blanca Jimeno[13], Kimberley Mathot[14], Phuong Ho[15], Melissah Rowe[16], Jorgen Soraker[17], Massamba Thiam[18], Vu Tien Thinh[19], Cedric Zimmer [20], and Lynn B. Martin[21]

[1]Georgia Southern University - Armstrong Campus
[2]Georgia Southern University
[3]Savanah Technical College
[4]Sorbonne Université
[5]University of South Florida Health
[6]Field Museum of Natural History
[7]University of Pittsburgh
[8]University of Oslo
[9]University of Canterbury
[10]Deakin University
[11]The Open University of Israel
[12]Norwegian University of Science and Technology, Centre for biodiversity dynamics
[13]Pyrenean Institute of Ecology
[14]University of Alberta
[15]vietnam national university of forestry
[16]Netherlands Institute of Ecology
[17]Norwegian University of Science and Technology
[18]Cheikh Anta Diop University of Dakar
[19]Vietnam National University of Forestry
[20]Université Sorbonne Paris Nord
[21]University of South Florida

February 14, 2025

Abstract

DNA methylation, which can change within-individuals over time and regulate gene expression, is important to many aspects of avian biology. It is particularly important in avian responses to various stressors associated with introductions, such as infection and environmental changes. However, it remains unclear whether native and invasive bird species differ in their epigenetic responses to stress, and how DNA methylation may contribute to the success of invasive species because of the limited availability of longitudinal epigenetic studies. To address this knowledge gap, we used epiRADseq to investigate changes in DNA methylation within-individual house sparrows (Passer domesticus) over an eight hour period in response to simulated infection. We compare wild-caught house sparrows from introduced populations with those from native populations, assessing the number

of genomic locations that exhibit changes, the magnitude of those changes, and the variance among individuals. Our results show that individuals from introduced populations experience more widespread changes in DNA methylation, with greater magnitude and higher variance, compared to their counterparts from native populations. Together, these findings suggest that DNA methylation plays a significant role in an individual's response to infection. They also indicate that individuals from introduced populations may exhibit distinct epigenetic responses compared to their native counterparts, consistent with the concept of epigenetic buffering.

Title Simulated infection induced changes in DNA methylation differ between introduced and native house sparrow *(Passer domesticus)*

Abstract

DNA methylation, which can change within-individuals over time and regulate gene expression, is important to many aspects of avian biology. It is particularly important in avian responses to various stressors associated with introductions, such as infection and environmental changes. However, it remains unclear whether native and invasive bird species differ in their epigenetic responses to stress, and how DNA methylation may contribute to the success of invasive species because of the limited availability of longitudinal epigenetic studies. To address this knowledge gap, we used epiRADseq to investigate changes in DNA methylation within-individual house sparrows (*Passer domesticus*) over an eight hour period in response to simulated infection. We compare wild-caught house sparrows from introduced populations with those from native populations, assessing the number of genomic locations that exhibit changes, the magnitude of those changes, and the variance among individuals. Our results show that individuals from introduced populations experience more widespread changes in DNA methylation, with greater magnitude and higher variance, compared to their counterparts from native populations. Together, these findings suggest that DNA methylation plays a significant role in an individual's response to infection. They also indicate that individuals from introduced populations may exhibit distinct epigenetic responses compared to their native counterparts, consistent with the concept of epigenetic buffering.

Keywords epigenetics, phenotypic plasticity, response to stress

Introduction

There is a growing body of evidence that epigenetics is important to many aspects of avian biology. There are three molecular epigenetic mechanisms: DNA methylation, histone modification, and chromatin structure. All are critical for the function and survival of multicellular species, and therefore all are important to birds. Of the three, DNA methylation is the most well-studied to date (Schrey et al. 2013; Kilvitis et al. 2014).

In birds, DNA methylation can regulate gene expression (Kilvitis et al. 2019) and vary both among tissues (Siller and Rubenstein 2019) and developmental stages (Sun et al. 2020). DNA methylation is important for an individual bird's response to stress (Taff et al. 2019, 2024; Siller Wilks et al. 2024) and changes in the environment (Sheldon et al. 2018a; Chen et al. 2022; McNew et al. 2024). DNA methylation differs in birds among developmental temperatures (Sheldon et al. 2020), but not in response to all developmental stressors (Sepers et al. 2023). DNA methylation in birds also varies in response to infection (Lundregan et al. 2022), arsenic contamination (Laine et al. 2021), lead pollution (Makinen et al. 2022), and urbanization (Watson et al. 2020). Further, DNA methylation differs with brood size (Sheldon et al. 2018b), among postnatal environments (Sepers et al. 2024) and with early life condition (Rubenstein et al. 2016).

A defining characteristic of DNA methylation is that it can change rapidly and dynamically over time and be correlated to changes in RNA expression (Lindner et al. 2021b). This temporal change can be driven by reproductive behavior (Liebl et al. 2021), the initiation of reproduction (Lindner et al. 2021a), seasonal factors (Viitaniemi et al. 2019), and the myriad environmental changes faced by organisms introduced to areas outside their native ranges (Lauer et al. 2024). Introduced species provide a unique opportunity to ask how rapid changes in DNA methylation occur in populations with different histories. One suggestion is that birds in introduced populations are successful colonizers because they can use methylation to adjust

gene expression rapidly in response to changes in the environment (Chen et al. 2022). Indeed, phenotypic plasticity is one of the best predictors of the ability of a lineage to thrive outside its native range.

The house sparrow (*Passer domesticus*) is one of the world's most successful introduced species (Liebl et al. 2015). This success is likely the result of its ability to rapidly respond to new environments (Anderson 2006; Lima et al. 2012; Martin et al. 2014), including through DNA methylation. DNA methylation is important to the success of introduced populations. DNA methylation varies among house sparrows from different introduced locations (Schrey et al. 2011, 2012; Sheldon et al. 2018a), and it may compensate for decreases in genetic diversity associated with introduction (Liebl et al. 2013). In house sparrows, DNA methylation varies with time since introduction among putative *Toll-like Receptor* (*TLR*) promoters (Hanson et al. 2022), and DNA methylation of a specific CpG site (genomic motif where a cytosine is immediately followed by u a guanine) in the putative *TLR-4* promoter is associated with the expression of this gene (Kilvitis et al. 2019). Further, DNA methylation is more variable among individuals from introduced locations compared to native locations (Lauer et al. 2024), a pattern that is consistent with epigenetic buffering (O'dea et al. 2016), a mechanism in which individuals responding to a stressor leverage rapid epigenic-based modifications to facilitate resiliency and suppress transposons (Deniz, et al. 2019).

Our objectives were to investigate the change in DNA methylation within individuals, over time, in response to a simulated infection. We compared patterns of DNA methylation among wild-caught individuals from both the introduced and native range of house sparrows before and after exposure to a highly immunostimulatory element of *E.coli* (i.e., lipopolysaccharide). We characterized the number of CpG sites with significant change in DNA methylation before and after simulated infection, the direction of the change, and the magnitude and variance of the change. We hypothesized that individuals from introduced populations would change DNA methylation at more CpG sites, with greater magnitude, and greater variance, indicative of an "introduced-bird" phenotype of higher reliance on epigenetic mechanisms and supporting epigenetic buffering.

Methods

Sample Collection and Simulated Infection

House sparrows were collected from four locations in their native range: Israel (n = 6), Norway (n = 6), Spain (n = 6), and Vietnam (n = 6), and three locations in their introduced range: Australia (n = 3), Canada (n = 6), Senegal (n = 6; Table 1). We classified each introduced site by their date of first introduction: Australia 1860s (Sheldon et al. 2018), Canada early 1900s (Grinnell 1919; Anderson 2006), and Senegal 1970s (Hanson et al. 2020a; Table 1). We captured adult house sparrows via mist netting from sunrise to 11.00 during the non-breeding seasons of 2020-2023. Upon capture, we took a 50 µl blood sample from the brachial vein of each bird and stored it in a cryovial with 300 µl of DNA/RNA shield (Zymo). Immediately after this, we injected each bird with 100 µl of 1 mg/ml^{-1} LPS (from *E. coli* 055:B5; Fisher L4005) in sterile saline subcutaneously over the breast muscle. Post injection, we housed birds individually in wire songbird cages (35.6 x 40.6 x 44.5) with food and water *ad libitum* . Although individually housed, the birds could hear and see one another. Eight hours post-injection, we took an additional 10 µl of blood from the brachial vein. All animal research procedures adhered to local animal research guidelines and were approved in advance by both the USF IACUC (IS00011653) and the relevant authorities in the country of origin. We extracted DNA samples using the DNeasy Kit (Qiagen, Valencia CA USA). Thus, we had paired 0- and 8-hour samples for each individual to screen changes in DNA methylation.

Data Collection

We used epiRADseq (Schield et al. 2016) to screen variation in DNA methylation among house sparrows on the Ion Torrent PGM platform (Thermo Fisher Scientific, Waltham, MA). epiRADseq is a ddRADseq protocol, developed for species without well-annotated genomes. This method uses a DNA methylation sensitive restriction enzyme, *HpaII* , which fails to cut when its CCGG restriction site is modified by DNA methylation at the internal CG. The enzyme thus generates a variable fragment library among individuals based on the DNA methylation state of the *HpaII* restriction site. If the site is methylated, no fragments are

generated to be sequenced. Thus, variation in DNA methylation is assayed as read count variation among individuals, which estimates the differences in DNA methylation of the screened CCGG sites. epiRADseq generates data in which zero read count result for an individual is meaningful, and therefore, we did not use cutoffs for differences in methylation.

We followed a genotype-by-sequencing (GBS) protocol developed for the Ion Torrent platform (Mascher et al. 2013), substituting the DNA methylation sensitive restriction enzyme *HpaII* for *MspI* (New England Biolabs, Ipswich, MA) to construct the epiRADseq library. After restriction digestion, we ligated Ion Torrent IonXpress barcoded adaptors and y-adapters. We ran emulsion polymerase chain reactions (PCR) following manufacturer protocols of the Ion PGM-Hi-Q-View OT2-200 kit on the Ion Express OneTouch2 platform. We sequenced resultant fragments following manufacturer protocols of the Ion PGM-Hi-Q-View Sequencing 200 Kit using an Ion 316v2 BC Chips.

The epiRADseq technique is a vast improvement on MS-AFLP (Schrey et al. 2013), yet it maintains many of the same limitations (i.e., anonymous CCGG sites, analysis focused on variable sites among individuals) and benefits (not requiring a reference genome, using standard RNA-seq analysis methods, and being economical) of MS-AFLP. We believe that epiRADseq is best used to ask questions about *variation* in DNA methylation among experimental units, rather than to address specific questions about the functional role of DNA methylation at the molecular level. Importantly, epiRADseq is not comparable to bisulfite- or enzymatic-methyl sequencing-like approaches. As such we have intentionally maintained a separation of our analysis to that typically expected of these techniques to avoid confusion or overinterpretation of our results.

Data Analysis

We demultiplexed runs and conducted quality control with Torrent Suite version 4.4.3. We retained bases above the AQ20 confidence threshold. We trimmed sequences to 100 bp targeting the higher quality sequence at the 5' end. We performed a *de novo* assembly and constructed a pseudo-reference using Geneious Prime v. 2022.1.1 (Dotmatics). We mapped individual sequences with BWA Galaxy Version 0.7.17.4 (Li and Durbin 2009, 2010). We used featureCounts Galaxy Version 1.6.4+galaxy1 (Liao et al. 2013) to determine read counts of fragments within 100 bp bins spanning the pseudo-reference. The 100 bp bins were used to count fragments among individuals ultimately to represent variation in DNA methylation among the CCGG sites screened. For a fragment to be sequenced, it had to have a non-methylated CCGG site. Counting matches to the bins across the pseudo-reference equates to variation in DNA methylation among the CCGG sites. As epiRADseq generates data with the zero read count result indicating DNA methylation, we used two approaches to control for sequencing coverage differences. First, we only analyzed individuals with 5,000 sequencing reads or higher. Second, we standardized all statistics by sequence read count at the individual sample level.

We used edgeR, Galaxy Version 3.24.1+galaxy1 (Robinson et al. 2010), to detect differently methylated regions (DMR), between the 0- and 8-hour samples, with a False Discovery Rate (FDR) of 0.05. We first compared all samples between 0- and 8-hour; we then repeated the comparison separately for individuals from native and introduced populations. We determined the number of DMRs in each comparison and identified DMR that were shared or unique to a particular comparison.

For every house sparrow, we calculated the change in DNA methylation between the 0- and 8-hour sample for all bins with significant differences as identified by the EdgeR analyses. We standardized each count for each bin by sequencing depth as (observed count for bin x / total read count) x 1,000. We compared methylation estimates among introduced and native birds using *t-tests* , *f-tests* , and Pearson's correlations. Statistical tests used alpha = 0.05 and were corrected by the sequential Bonferroni method when appropriate (Rice 1989).

Results

Screening DNA methylation using the epiRADseq method on the Ion Torrent PGM in house sparrows generated a pseudo-reference of 17,532,684 bases. At the individual-level, between 5,095 and 89,777 CCGG

sites were resolved. We constructed a dataset of all individuals at 0- and 8-hours, and a dataset of the magnitude of change between temporally paired individual samples at 0- and 8-hours.

House sparrows from introduced populations had more significant differences in DNA methylation between 0- and 8-hour samples, with a stronger magnitude of change, compared to house sparrows from native populations (Table 1, Figure 1). We detected 9 differently methylated CpG sites among all samples with a magnitude of change ranging between -2.01 and 4.37 (Figure 2). In this comparison, the individuals from introduced populations had a magnitude of change ranging between -5.24 and 11.41, and the individuals from native populations had a magnitude of change ranging between -0.63 and 0.08 (Figure 2). The individuals from introduced populations had significantly higher mean change in DNA methylation (introduced 4.61, native -0.1; *t-test* $P = 0.01$) and significantly greater variance in change of DNA methylation (introduced 32.10, native 0.07; *f-test* $P < 0.001$).

We detected a qualitatively similar, but quantitively stronger, pattern when we analyzed individuals from introduced and native populations separately. We detected 35 differently methylated CpG sites among only individuals from introduced populations with a magnitude of change ranging between -9.31 and 11.41 (Figure 1). We detected only 1 differently methylated CpG site among only individuals from native populations with a magnitude of change of -0.38 (Figure 1). We were not able to directly compare the numerical results between native and introduced, due to the separate analyses. However, we detected more significant CpG sites in the individuals from introduced populations and found higher magnitude and variance in change among the individuals from introduced populations.

Further, house sparrows from introduced populations had more uniquely differently methylated CpG sites. Among all significant tests, there were 4 differently methylated CpG sites shared between the "all individuals" and the "introduced" analysis; and there were 5 unique differently methylated CpG sites for "all individuals" analysis, 31 unique differently methylated CpG sites for the "introduced" analysis, and only 1 unique differently methylated CpG site for the "native" analysis.

Discussion

House sparrows from the introduced range had a stronger and more variable epigenetic response to simulated infection within 8-hours compared to individuals from the native range. Between 0- and 8-hours post simulated infection, DNA methylation differed at more genomic locations, with a greater magnitude of change, in introduced than native house sparrows. These changes in DNA methylation occurred in both directions, with some sites gaining methylation and others losing methylation. Introduced individuals had more unique genomic locations (n = 35) that were differently methylated, while only a single unique genomic location was differently methylated in native individuals. Introduced individuals had a higher variance in the magnitude of change in DNA methylation in response to simulated infection compared to native individuals. It is possible that the change in DNA methylation we observed over the 8 hour time series might not reflect an immune response per se, but could also reflect response to the stress of being brought into captivity, or a combination of the two. These results support the hypothesis of an epigenetically mediated invader phenotype present among introduce house sparrows, which provided a mechanism for plasticity in response to novel stressors (Sheldon et al. 2023). Further, it supports the hypothesis that epigenetic buffering likely plays a role in the manner of this response (O'dea et al. 2016; Lauer et al. 2024).

Our results expand previous research on the importance of DNA methylation in the response to infection in supporting the immune response of house sparrows in multiple contexts. In response to a parasite infection, DNA methylation differed between infected and non-infected house sparrows, and among individuals sampled temporally after infection, at the nestling stage (10-14 days old) and at the fledged juvenile stage (26-37 days; Lundregan et al. 2022). Here, the DNA methylation patterns of a selected marker gene, NR1D1, differed between infected and uninfected individuals and was correlated to recruitment. Our findings are congruent in detecting DNA methylation changes post infection, yet over a much shorter timeframe. Further, introduced house sparrows had higher expression of pathogen surveillance genes and cytokine responses genes, to the simulated bacterial infection investigated in the present study (McCain et al 2025). In introduced house

sparrows, as *TLR-4* expression increased, IL-1β and IL-10 responses decreased, which was not detected in native sparrows. Our results suggest that the observed differences in immune response in introduced house sparrows are at least in part mediated by changes in DNA methylation. In addition, introduced house sparrows with higher epigenetic potential (estimated by the number of CpG sites in the promoter of *TLR-4*) had higher resistance to infection by*Salmonella enterica* compared to individuals with lower epigenetic potential (Sheldon et al. 2023). As epigenetic potential measures the genetic potential for an individual to adopt different DNA methylation states, our results suggest that both an individual's inherent capability to be methylated differently, and it actually being methylated differently, are important factors in the response to infection. Integrating these findings demonstrates that both epigenetic potential, and the actual changing of DNA methylation state is important in the response to infection. Also, these studies indicate that is highly likely that the difference in change of DNA methylation we detected between introduced and native individuals would ultimately support introduction success.

Our results also provide new context for previous findings of the role of DNA methylation in the success of introduced house sparrows, by finding supporting results in how individuals change over time. Differences in DNA methylation occur within and among introductions of house sparrow (Liebl et al. 2013; Sheldon et al. 2018a) and these differences manifest across the edge-core axis of introduction (Hanson et al. 2020b). Further, DNA methylation differs between introduced and native individuals, with those from more recent introductions having greater variance in DNA methylation (Lauer et al. 2024). The present temporal study found congruent results within individuals over time: detecting differences in DNA methylation among introductions, and between introduced and native individuals. The individual-level response to simulated infection, suggests that the larger patterns detected may, in part, be shaped by individual responses.

Our results also provide new information in the study of how DNA methylation changes over time in birds. We document substantial changes in DNA methylation state in response to simulated infection within 8 hours, which, to our knowledge, is the shortest time frame studied. In aviary-controlled conditions, temporal changes in DNA methylation were detected in great tits (*Parus major*) among three time points, 21 days apart, across a breeding season. Time points targeted the initiation of gonadal development, nest building, and initiation of egg laying (Linder et al. 2021). Changes in DNA methylation in liver and blood were correlated, and DNA methylation near transcription start sites was correlated to decrease in gene expression. In captive great tits from aviary conditions, changes in DNA methylation in blood were detected both between temperature treatments and temporally across four selected time points, which roughly spanned reproductive behaviors of initiation of reproduction, through 50% of individuals laying eggs (Viitaniemi et al. 2019). A relatively large number of small magnitude changes in DNA methylation were detected and there was a large variation in the change over time given a relative low level of methylation, with a large amount of among individual variation. In wild collected chestnut-crowed babblers (*Pomatostomus ruficeps*), DNA methylation in blood differed among individuals sampled at hatching, fledgling, and 1-year (Liebl et al. 2021). In this cooperative breeder, first year dispersers had a greater number of loci that changed DNA methylation state between hatchling and fledgling, and had lower DNA methylation, compared to non-dispersers before fledgling but not as hatchlings or adults. Together, these studies show within individual change in DNA methylation is critically important to the response to environment, and coordination of temporally variable behaviors.

While we demonstrate a clear individual response in DNA methylation to simulated infection, it is important to note that DNA methylation is active in multiple different contexts within individuals, and even within cells (see Chen et al. 2022; Sheldon et al. 2022). Thus, not all individuals in all introduced areas are expected to show identical change in DNA methylation, or even that the response in DNA methylation would be expected to be directional in general. Rather, it is likely that maintaining, or increasing, the ability to change, is of primary importance to introduced species. The potential for change and the response to immediate local stressors might best be detected in variance of DNA methylation among introduced individuals, or in targeted analysis of the regulation of specific genes. Also, it is highly likely that histone modification is another critically important epigenetic mechanism to this process (Ray et al. 2024). We encourage investigations in all three areas, to provide additional insights into the response of individuals to stress and to the success of the house sparrows as introduced species.

References

Anderson, T.R., 2008. *Biology of the ubiquitous house sparrow: from genes to populations* . Oxford University Press.

Chen, Y., Ni, P., Fu, R., Murphy, K.J., Wyeth, R.C., Bishop, C.D., Huang, X., Li, S. and Zhan, A., 2024. (Epi) genomic adaptation driven by fine geographical scale environmental heterogeneity after recent biological invasions. Ecological Applications 34(1), p.e2772.

Deniz, Ö., Frost, J.M. and Branco, M.R., 2019. Regulation of transposable elements by DNA modifications. Nature Reviews Genetics 20: 417-431.

Grinnell, J., 1919. The English sparrow has arrived in Death Valley: An experiment in nature. American Naturalist 53: 468–472.

Hanson, H.E., Zolik, J.E., and Martin L,B., 2020a. "House Sparrow (*Passer domesticus* Linnaeus, 1758)." In *Invasive Birds: Global trends and impacts* , pp. 85-96. Wallingford UK: CABI, 2020.

Hanson, H.E., Kilvitis, H.J., Schrey, A.W., Maddox, J.D., Martin, L.B.,*2020b* . Epigenetic potential in native and introduced populations of house sparrows (*Passer domesticus*). Integrative and Comparative Biology icaa060, doi.org/10.1093/icb/icaa060

Hanson, H.E., Wanga, C., Schrey, A.W., Liebl, A.L., Jianga, R.H.Y., Martin, L.B., 2022. Epigenetic potential and DNA methylation in an ongoing house sparrow (*Passer domesticus*) range expansion. American Naturalist 200: 662-674.

Kilvitis, H., Alvarez, M., Foust, C., Richards, C.L., Robertson, M., and Schrey, A.. 2014. Ecological epigenetics. *In* "Ecological and Evolutionary Genomics". Edited by Landry, C. and N. Aubin-Horth.

Kilvitis, H.J., Schrey, A.W., Ragsdale, A.K., Berrio, A., Phelps, S., and Martin, L.B., 2019. DNA methylation predicts immune gene expression in introduced house sparrows (*Passer domesticus*). Journal of Avian Biology 50. https://doi.org/10.1111/jav.01965

Laine, V.N., Verschuuren, M., van Oers, K., Espín, S., Sánchez-Virosta, P., Eeva, T. and Ruuskanen, S., 2021. Does arsenic contamination affect DNA methylation patterns in a wild bird population? An experimental approach. Environmental Science & Technology 55: 8947-8954.

Lauer, M.E., Kodak, H., Albayrak, T., Lima, M., Ray, D., Simpson, E., Tevs, D., Sheldon, E., Martin, L., and Schrey, A.W., *2024* . Introduced house sparrows (*Passer domesticus*) have greater variation in DNA methylation than native house sparrows. Journal of Heredity 115:11-8. p.esad067.

Li, H., and Durbin, R., 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25: 1754-1760.

Li, H., and Durbin, R., 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatic 26: 589-595.

Liao, Y., Smyth, G. K., and Shi, W., 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30: 923-930.

Liebl, A., Schrey, A., Richards, C., and Martin, L., 2013. Patterns of DNA methylation throughout a range expansion of an introduced songbird. Integrative and Comparative Biology 53: 351-358.

Liebl, A.L., Schrey, A., Andrews, S.C., Sheldon, E., and Griffith, S.C., 2015. Invasion genetics: lessons from a ubiquitous bird, the house sparrow (*Passer domesticus*). Current Zoology 61: 465–476.

Liebl, A.L., Wesner, J.S., Russell, A.F., and Schrey, A.W., 2021. Methylation patterns at fledgling predict dispersal in a cooperative breeding bird. PLOS One https://doi.org/10.1371/journal.pone.0252227

Lima, M., Macedo, R., Martins, T., Schrey, A., Martin, L., and Bensch, S., 2012. Genetic and morphometric divergence of an invasive bird: the introduced house sparrow (*Passer domesticus*) in Brazil. PLoS ONE 7(12): e53332. doi:10.1371/journal.pone.0053332.

Lindner, M., Laine, V.N., Verhagen, I., Viitaniemi, H.M., Visser, M.E., van Oers, K. and Husby, A., 2021a. Rapid changes in DNA methylation associated with the initiation of reproduction in a small songbird. Molecular Ecology 30:3645-3659.

Lindner, M., Verhagen, I., Viitaniemi, H.M., Laine, V.N., Visser, M.E., Husby, A. and van Oers, K., 2021b. Temporal changes in DNA methylation and RNA expression in a small song bird: within-and between-tissue comparisons. BMC Genomics 22: 1-16.

Lundregan, S.L., Mäkinen, H., Buer, A., Holand, H., Jensen, H. and Husby, A., 2022. Infection by a helminth parasite is associated with changes in DNA methylation in the house sparrow. Ecology and Evolution 12(11), p.e9539.

Martin, L., Coon, C., Liebl, A., and Schrey, A., 2014. Surveillance for microbes and range expansion in house sparrows. Proceedings of the Royal Society B 281: 20132690.

McCain, K.M., Mansilla, G., Sheldon, E.L., Zimmer, C., Schrey, A.W., Rowe, M., Dor, R., Kohl, K., Soraker, J., Jensen, H., Mathot, K., Vu, T., Phoung, H.T., Jimeno, B., Buchanan, K., Thiam, M., Briskie, J., Martin, L.B., 2025. Microbial surveillance verses cytokine responsiveness in native and non-native house sparrows Biology Letters 21: 2024031.

McNew, S.M., Taff, C.C. and Vitousek, M.N., 2024. Manipulation of a social signal affects DNA methylation of a stress-related gene in a free-living bird. Journal of Experimental Biology 227(15), p.jeb246819.

Mäkinen, H., van Oers, K., Eeva, T., Laine, V.N. and Ruuskanen, S., 2019. The effect of experimental lead pollution on DNA methylation in a wild bird population. bioRxiv 851998.

O'Dea, R.E., Noble, D.W., Johnson, S.L., Hesselson, D., and Nakagawa, S., 2016. The role of non-genetic inheritance in evolutionary rescue: epigenetic buffering, heritable bet hedging and epigenetic traps. Environmental Epigenetics 2(1), dvv014.

Ray, D., Sheldon, E., Zimmer, C., Martin, L.B., and Schrey, A., 2024. Screening H3 histone acetylation in a wild bird, the house sparrow (*Passer domesticus*) IOB 2024 Feb 27:obae004

Rice, W. R., 1989. Analyzing tables of statistical tests. Evolution, 43(1), 223-225.

Robinson, M.D., McCarthy, D.J., and Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139-140.

Rubenstein, D.R., Skolnik, H., Berrio, A., Champagne, F.A., Phelps, S. and Solomon, J., 2016. Sex-specific fitness effects of unpredictable early life conditions are associated with DNA methylation in the avian glucocorticoid receptor. Molecular Ecology 25: 1714-1728.

Schield, D.R., Walsh, M.R., Card, D.C., Andrew, A.L., Adams, R.H., and Castoe, T.A., 2016. Epi RAD seq: scalable analysis of genomewide patterns of methylation using next-generation sequencing. Methods in Ecology and Evolution 7: 60-69.

Schrey, A., Grispo, M., Awad, M., Cook, M., McCoy, E., Mushinsky, H., Albayrak, T., Bensch, S., Butler, L., Fokidis, H., Jensen, H., Imboma, T., Kessler-Rios, M., Marzal, A., Stewart, I., Westerdahl, H., Westneat, D., Zehtindjiev, P., and Martin, L., 2011. Broad-scale latitudinal patterns of genetic diversity among native European and introduced house sparrow (*Passer domesticus*) populations. Molecular Ecology 20: 1133-1143.

Schrey, A., Coon, C., Grispo, M., Awad, M., McCoy, E., Mushinsky, H., Richards, C., and Martin, L., 2012. Epigenetic variation may compensate for decreased genetic variation with introductions: a case study

using house sparrows (*Passer domesticus*) on two continents. Genetics Research International.Volume 2012 Article ID 979751.

Schrey, A., Alvarez, M., Foust, C.M., Kilvitis, H.J., Lee, J.D., Liebl, A.L., Martin, L.B., Richards, C.L., and Robertson, M., 2013. Ecological Epigenetics: beyond MS-AFLP. Integrative and Comparative Biology 53: 340-350.

Sepers, B., Mateman, A.C., Gawehns, F., Verhoeven, K.J. and van Oers, K., 2023. Developmental stress does not induce genome-wide DNA methylation changes in wild great tit (*Parus major*) nestlings. Molecular Ecology 32: 3960-3974.

Sepers, B., Verhoeven, K.J. and van Oers, K., 2024. Early developmental carry-over effects on exploratory behaviour and DNA methylation in wild great tits (*Parus major*). Evolutionary Applications 17(3), p.e13664.

Sheldon, E., Ragsdale, A.K., Schrey, A.W., and Griffith, S., 2018a. Epigenetic and genetic variation among three separate introductions of the house sparrow (*Passer domesticus*) into Australia. Royal Society Open Science 5: 172185.

Sheldon, E., Schrey, A.W., Ragsdale, A.K., Griffith, S., 2018b. Brood size influences genome-wide DNA methylation levels in wild zebra finches (*Taeniopygia guttata).* Auk.135: 1113-1122.

Sheldon, E., Schrey, A., and Griffith, S.. 2020. Dynamic changes in DNA methylation during postnatal development in zebra finches (*Taeniopygia guttata*) exposed to different temperature*. Journal Avian Biology 51 doi.org/10.1111/jav.02294*

Sheldon, E.L., Ton, R., Boner, W., Monaghan, P., Raveh, S., Schrey, A.W., and Griffith, S., 2022. Associations between DNA methylation and telomere length during early life: insight from wild zebra finches (*Taeniopygia guttata*). Molecular Ecology 31: 6261-6272.

Sheldon, E., Zimmer, C., Hanson, H., Koussayer, B., Schrey, A., Reese, D., Wigley, P., Wedley, A.L., and Martin, L.B., 2023. High epigenetic potential protects songbirds against pathogenic Salmonella enterica infection. Journal of Experimental Biology jeb-245475.

Siller, S.J. and Rubenstein, D.R., 2019. A tissue comparison of DNA methylation of the glucocorticoid receptor gene (Nr3c1) in European starlings. Integrative and Comparative Biology 59: 264-272.

Siller Wilks, S.J., Heidinger, B.J., Westneat, D.F., Solomon, J. and Rubenstein, D.R., 2024. The impact of parental and developmental stress on DNA methylation in the avian hypothalamic–pituitary–adrenal axis. Molecular Ecology 33: 17291.

Sun, D., Layman, T.S., Jeong, H., Chatterjee, P., Grogan, K., Merritt, J.R., Maney, D.L. and Yi, S.V., 2021. Genome-wide variation in DNA methylation linked to developmental stage and chromosomal suppression of recombination in white-throated sparrows. Molecular Ecology 30: 3453-3467.

Taff, C.C., Campagna, L. and Vitousek, M.N., 2019. Genome-wide variation in DNA methylation is associated with stress resilience and plumage brightness in a wild bird. Molecular Ecology 28: 3722-3737.

Taff, C.C., McNew, S.M., Campagna, L. and Vitousek, M.N., 2024. Corticosterone exposure is associated with long-term changes in DNA methylation, physiology and breeding decisions in a wild bird. Molecular Ecology 33(15), p.e17456.

Viitaniemi, H.M., Verhagen, I., Visser, M.E., Honkela, A., Van Oers, K. and Husby, A., 2019. Seasonal variation in genome-wide DNA methylation patterns and the onset of seasonal timing of reproduction in great tits. Genome Biology and Evolution 11L 970-983.

Watson, H., Powell, D., Salmon, P., Jacobs, A. and Isaksson, C., 2021. Urbanization is associated with modifications in DNA methylation in a small passerine bird. Evolutionary Applications 14: 85-98.

Tables and Figures

Table 1. Summary of house sparrow samples screened for change in DNA methylation eight hours after lipopolysaccharide injection. The country of origin, date of introduction, number of 0- and 8-hour pairs screened, with the mean change in DNA methylation and the variance in change of DNA methylation.

Country	Date of Introduction	N pairs	Mean Change	Variance Change
Introduced				
Australia	1860s	3	0.059	0.002
Canada	1900s	6	-1.511	13.179
Senegal	1970s	6	12.994	388.840
Native				
Israel		6	0.055	0.013
Norway		6	-0.455	1.783
Spain		6	0.006	0.023
Vietnam		6	-0.012	0.017

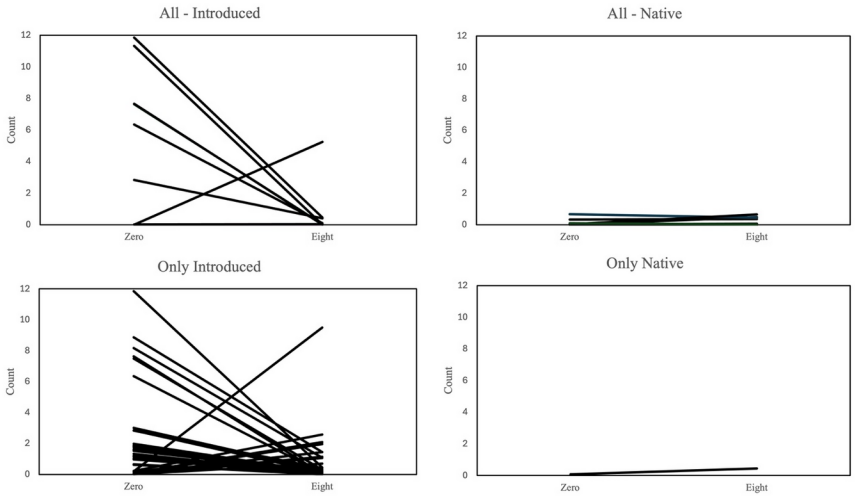

Figure 1. Introduced house sparrows had more sites with significant change in DNA methylation and a larger magnitude of change in DNA methylation between 0- and 8-hours after lipopolysaccharide injection. Change in DNA methylation estimated via standardized change in count data of epiRADseq data for house sparrows. Results from three separate analysis between 0- and 8-hour samples are presented, 1) all individuals presented for introduced and native samples, 2) analysis for only introduced individuals, and 3) analysis for only native individuals.

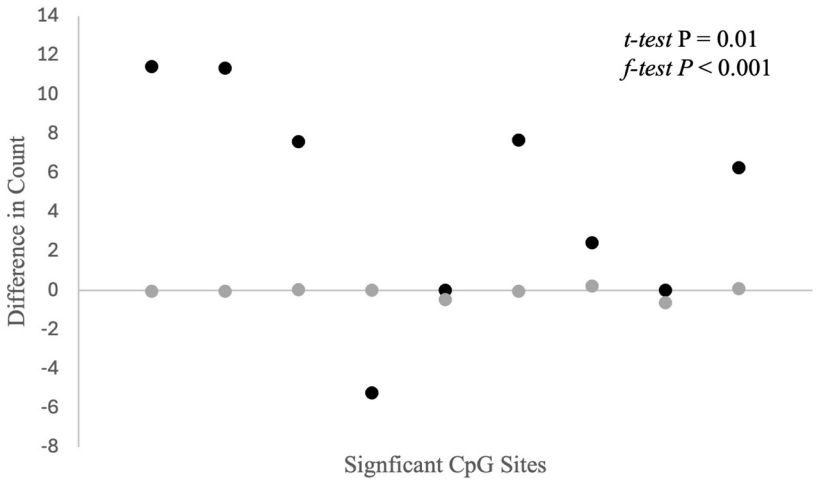

Figure 2. Introduced house sparrows (black) had greater mean change in DNA methylation (*t-test* $P = 0.01$) and greater variance in change of DNA methylation (*f-test* $P < 0.001$) among the nine significantly differently methylated CpG sites compared to native (gray) house sparrows in an analysis including all screened individuals.