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**Manipulation of a social signal affects DNA methylation of a stress-related gene in a free-living bird**

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**Abstract:**

Social status directly effects the health of social animals. Low status individuals receive more antagonistic encounters, have fewer supportive relationships, and have overall worse health outcomes. However, in most wild species, it is unclear what physiological mechanisms mediate the effects of the social environment on health and fitness. Epigenetic regulation of the HPA-axis, the neuroendocrine pathway that activates in response to stressors, may be one process that is sensitive to the social environment. Here, we experimentally manipulated plumage, a key social signal in female tree swallows (*Tachycineta bicolor*) and quantified methylation of four genes in the HPA axis before and after treatment. We found that dulling the white breast plumage affected methylation in one gene, CRHR1; however, the effect depended on the original brightness of the bird. Methylation in this gene was correlated with baseline corticosterone levels, indicating that DNA methylation of CRHR1 helps regulate glucocorticoid production in this species. Methylation in two other genes, FKBP5 and GR, changed over the course of the experiment, independent of treatment. These results show that methylation of these genes is labile into adulthood and suggest that epigenetic mechanisms are important regulators of the HPA axis in free-living birds.

**Introduction:**

The social environment affects the health of humans and other social animals. Lower status individuals generally have shorter lifespans and are more susceptible to disease (Alwin & Wray, 2005; Razzoli et al., 2018; Singh-Manoux et al., 2003). One potential mechanism that may mediate the effects of social interactions on health is the hypothalamic—pituitary—adrenal (HPA) axis, the neuroendocrine pathway that regulates the production of glucocorticoid hormones (Creel et al., 2013). The production of glucocorticoid hormones is part of a suite of physiological and behavioral changes that help individuals respond to adverse events (Monaghan, 2014; Sapolsky et al., 2000; Wingfield et al., 1998). Although the stress response enhances immediate survival, sustained HPA axis activation can have negative health effects (Clinchy et al., 2004; Korte et al., 2005). Social status can interact with the stress response in both positive and negative ways. Social bonds between high-ranking individuals can reduce glucocorticoid levels in the face of adverse events (Engh et al., 2006; Young et al., 2014). For instance, male Barbary macaques (*Macaca sylvanus*) with strong social bonds had lower fecal glucocorticoid levels in response to social stressors (aggressive encounters) as well as environmental stressors (cold temperatures) (Young et al., 2014). On the other hand, low status individuals may receive frequent antagonistic attacks from conspecifics, leading to chronic stress (Snyder-Mackler et al., 2019).

Epigenetic changes to genes involved in the HPA axis may underlie the connection between the social environment and stress-related disorders (Lee & Sawa, 2014; Snyder-Mackler et al., 2019; Turecki & Meaney, 2016). Epigenetic modifications, such as DNA methylation, are sensitive to the environment and can affect DNA expression and physiology (Sotnikov & Markt, 2014). A robust body of literature from primates and lab rodents demonstrates that environmental stressors may cause persistent changes in the function of genes involved with the stress response (Schartner et al., 2017; Turecki & Meaney, 2016). For instance, one study found that prenatal trauma exposure in mice led to changes in methylation of two genes in the stress axis, which were accompanied by changes in mRNA levels, corticosterone levels, and behavior (Plank et al., 2021). Changes in the social environment of primates can cause changes in chromatin availability and gene expression, leading to dysregulation in the HPA axis and corresponding negative health effects (Snyder-Mackler et al., 2019).

Many bird species are highly social; however, the epigenetic signatures of their dynamic social landscape have not been well studied. Social interactions between conspecifics can raise glucocorticoid levels (Deviche et al., 2014; Landys et al., 2010; Potticary & Duckworth, 2020). For example, female bluebirds (*Sialia mexicana*) living in dense populations where competitive interactions are common have higher circulating levels of glucocorticoids, which may be transmitted to their eggs, affecting the aggression and dispersal behaviors of their offspring (Potticary & Duckworth, 2020). In addition, a recent study found that increased competition in tree swallows (*Tachycineta bicolor*) has epigenetic and transcriptomic effects in brain tissue (Bentz et al., 2021). Still, it is largely unclear whether epigenetic processes mediate a physiological response to the social environment in birds.

In this study, we experimentally manipulated a key plumage signal in tree swallows and measured its effects on glucocorticoid levels and methylation of four genes involved in the stress response. Plumage is an important social signal in birds, conveying information about condition, parasite load, and social dominance (Mason & Bowie, 2020; Mougeot et al., 2010; Taff, Zimmer, et al., 2019). In female tree swallows, brighter white females have stronger immune function, more social interactions with conspecifics and are less likely to abandon their nests under stressful conditions (Beck et al., 2015; Taff, Zimmer, et al., 2019). Methylation in some areas of the genome is correlated with plumage brightness and stress resilience, suggesting that epigenetic processes connect this plumage signal to physiology (Taff, Campagna, et al., 2019). Experimental manipulation of this plumage alters social interactions, glucose levels, and breeding success (Taff et al., 2021). We correspondingly predicted that experimentally dulling this white plumage would lead to decreased methylation and corresponding activation of the HPA axis. We secondarily predicted that the effects of plumage dulling would be strongest in females who were originally brightest.

**Methods:**

We studied breeding tree swallows in Ithaca, New York, USA, during April to July of 2017 (42 degrees 30’11”N, 76 degrees 26’ 13”W). Females at each nest were captured three times during the breeding season (day 6-7 of incubation, day 3-4 after hatching, and day 7-8 after hatching). At the first capture, females were assigned randomly either to a plumage dulling treatment or to a control treatment after balancing treatments by female age (second year vs. after second year). We dulled plumage by uniformly coloring the feathers from the throat to the legs using a light gray nontoxic marker (Faber-Castell PITT Artist Pen ‘Big Brush’ Warm Grey III 272), following methods in (Taff et al., 2021). Females in the control treatment were marked in the same way with a colorless marker (Prismacolor Premier Colorless Blender PB-121). The marking treatment was re-applied at the second and third captures. We quantified the effects of dulling through spectrophotometry (Appendix). In total, the dulled group included 34 females and the control group included 36 females. Females did not differ significantly in initial brightness (average % reflectance in the control group = 39.85, dulled group = 41.05; *P* = 0.491). Experimental dulling significantly reduced plumage brightness for all individuals in the treatment (Taff et al., 2021).

At the first and third captures (hereafter “pre-treatment” and “post-treatment”) we took a small blood sample within three minutes of capture via brachial venipuncture to measure stress physiology and quantify DNA methylation. Within three hours erythrocytes and plasma were separated by centrifugation and stored separately at -30. Baseline corticosterone was measured in the plasma using commercially available microplate kits that have been validated in this population (Taff et al., 2021; Taff, Zimmer, et al., 2019). Data on the behavior, microbiome, and reproductive success of adults in this experiment have been published previously (Taff et al., 2021). Here, we focus on the epigenetic effects of plumage manipulation and their connection to stress physiology.

We investigated DNA methylation of four genes: Corticotropin Releasing Hormone (CRH), Corticotropin Releasing Hormone Receptor 1 (CRHR1), FKBP Prolyl Isomerase 5 (FKBP5), and Glucocorticoid Receptor (GR, also called NR3C1), all of which form part of the HPA axis. Epigenetic dysregulation of these genes is associated with stress-related pathologies in humans and rodents (Lee & Sawa, 2014). Primer development, assay validation, and pyrosequencing was conducted at EpigenDX. Methylation was quantified using pyrosequencing at between 11 and 19 CpG sites per gene (Appendix).

*Analysis*

We modeled methylation in each gene separately using linear mixed effects models in R (version 4.2.2). Each model predicted per-CpG methylation as a function of capture (pre- or post-treatment), treatment, and initial plumage brightness. We first created an interaction model of the three main effects (i.e. capture\*brightness\*treatment). When interactions were not significant, they were removed, and we present estimates from the additive model instead. Models also included the random effect of CpG site and the random effect of individual. We logit transformed methylation data prior to modeling it following best-practices for percentage data (Stevens et al., 2016; Warton & Hui, 2011). We partitioned the variance in methylation among the fixed effects and random effects using rptR (Stoffel et al., 2017). We tested for a relationship between corticosterone levels and methylation at each gene by modeling methylation as a function of corticosterone, using methylation and corticosterone data from both captures. These models included the random effects of individual and CpG identity. Conceptually, we predicted that DNA methylation controls gene expression in the HPA axis and thus affects blood corticosterone levels (i.e. corticosterone is dependent on DNA methylation). However, due to the hierarchical structure of the data, we found it more appropriate to model DNA methylation as the dependent variable (with corticosterone as a fixed effect and CpG as a random effect).

**Results:**

We quantified methylation data at 56 CpG sites in the four focal genes (CRH, FKBP5, GR, and GRHR1). After filtering, we had methylation data from between 85 and 120 samples at each CpG (Table S1). The mean methylation per site varied from 2.6% to 76.8% (Table S1).

Methylation of the CRH gene did not significantly differ between treatments or between captures and was not associated with initial female brightness (Figure 1, Table S2). Methylation in the FKBP5 gene was significantly higher pre-treatment compared to post-treatment (Figure 1, Table S3). However, there was no significant difference between treatments, and no relationship between initial brightness and methylation. In contrast, methylation in the GR gene was significantly lower pre-treatment compared to post-treatment (Figure 1, Table S4). Again, methylation did not significantly differ between treatments and was uncorrelated with initial brightness. Finally, we found that methylation of the CRHR1 gene depended on the three-way interaction between treatment, capture, and brightness (Figure 2, Table S5). Females in the dulled treatment tended to lose methylation in CRHR1 following experimental dulling. The decrease in methylation post-treatment was strongest for females that were originally bright. In contrast, for females in the control group, methylation tended to increase slightly post-treatment and did not depend on initial brightness.

In all genes the random effects of individual and CpG explained substantially more variation than did the fixed effects of treatment, capture number, and initial brightness (i.e. Conditional R2 >> Marginal R2; Tables S2 – S5). Individual bird identity alone explained between 2.9% and 48% of variation in methylation at each gene (Tables S2 – S5).

Baseline corticosterone levels did not differ significantly between treatments or between captures and were not associated with initial plumage brightness (Table S6). However, methylation of CRHR1 was significantly associated with corticosterone levels. There was a significant negative relationship between baseline corticosterone and methylation in CRHR1 (Figure 2; Table S7). Corticosterone was not associated with methylation of any other of the three genes (Tables S8 – S10).

**Discussion**

In this study we tested the effects of manipulating a key social plumage signal on HPA-axis methylation in tree swallows. We found that experimentally dulling the white breast plumage of tree swallows resulted in changes in the methylation of the corticotropin releasing hormone receptor 1 (CRHR1) gene. The effect of dulling on methylation was strongest for females who were initially bright, suggesting that high-status females experienced the most severe consequences of social environment change.

CRHR1 binds corticotropin releasing hormone (CRH), triggering the release of the adrenocorticotropic hormone which leads to the release of corticosteroids (Schartner et al., 2017). Decreased methylation of CRHR1 and associated upregulation of this gene are associated with anxiety-related phenotypes in humans and rodent models (Plank et al., 2021; Schartner et al., 2017; Sotnikov & Markt, 2014). Baseline corticosterone values in our tree swallows were negatively associated with methylation across this gene. Thus, the decrease in methylation that we observed in females is consistent with the upregulation of this gene and activation of the HPA axis in response to plumage dulling and the concomitant changes to the social environment (Taff et al., 2021).

In contrast, the methylation of the other three genes we studied (GR, FKBP5, and CRH) was not significantly associated with treatment. We chose these candidate genes as targets because they have known epigenetic associations with stress (Lee & Sawa, 2014). Still, we interrogated relatively few sites across these specific genes and so it is possible that we did not detect some of the epigenetic effects of the plumage manipulation. Previous analysis of this experimental manipulation found that plumage dulling had effects on the behavior, microbiome, and glucose levels of the females in our study (Taff et al., 2021). However, many of these effects were dependent on nestling stage and initial female brightness. The fact that we did not detect significant differences in corticosterone levels between treatments or between sampling points further indicates that the effects of our signal manipulation may have been relatively minor, and/or context dependent. More work is needed to understand exactly how manipulation of white plumage in tree swallows affects their social environment.

The methylation of two genes (GR and FKBP5) were not affected by treatment; however, it did change significantly between the two time points in the study (pre-treatment: days 6-7 of incubation and post-treatment: ~ 14 days later). The glucocorticoid receptor (GR, also called NR3C1) is an intracellular transcription factor that mediates the expression of a number of proteins involved in the stress response (Guidotti et al., 2013; Zannas et al., 2016). Methylation of GR increased over the study period in our swallows. Since methylation is typically associated with lower gene expression, this change could also signal a shift towards downregulation or modulation of the HPA axis. A previous study of superb starlings (*Lamprotornis superbus*), found that methylation in the promoter of GR was positively correlated with environmental conditions (rainfall) early in life, suggesting that methylation of this gene could help adaptively prime individuals living in highly variable environments (Rubenstein et al., 2016). Our data could similarly reflect effects of environmental conditions on stress physiology of tree swallows. Moreover, our data show that methylation of this gene is labile into adulthood in this species.

The FK506-binding protein 41 (FKBP5) is a negative regulator of GR signaling (Menke et al., 2013; Zannas & Binder, 2014). Dysregulation of FBKP5 is associated with psychiatric disorders and other stress-related phenotypes in humans and laboratory models (Zannas et al., 2016; Zimmer et al., 2020). Although data from wild organisms are limited, FKBP5 expression in house sparrows (*Passer domesticus*)is correlated with HPA flexibility and exploratory behavior (Zimmer et al., 2021). Thus, FKBP5 appears to be a key regulator of the HPA axis across many vertebrates (Zimmer et al., 2020). In our study, methylation of FKBP5 decreased over the course of the study period. Decreased methylation of FKBP5 is expected to upregulate this gene and inhibit glucocorticoid receptor signaling (Zannas et al., 2016). Correspondingly, the change that we observed in our birds could be related to an effort to downregulate the stress response during breeding, as HPA axis activation can inhibit reproductive success (Bókony et al., 2009; Wingfield & Sapolsky, 2003). FKBP5 was also notable because individual bird identity explained a substantial proportion (48%) of the variation in methylation in this gene (Table S3). The consistency in methylation of this gene across individuals suggests that perhaps some epigenetic programming of this gene occurs early in life and/or is transgenerationally inherited. Indeed, another study found that methylation in FKBP5 is a heritable epigenetic marker of trauma in humans (Yehuda et al., 2016).

A growing number of studies investigate the plasticity of DNA methylation in response to environmental conditions in free-living animals. However, most of these studies have focused on early life epigenetic programming (McNew et al., 2021; Sheldon et al., 2020; Watson et al., 2019) and studies that sample the same individuals repeatedly are particularly rare (e.g. Anderson et al., 2021; Rubenstein et al., 2016). Our results highlight that DNA methylation patterns can change within an individual in response to specific experimental manipulation, and moreover, may be naturally labile over short periods. Although we did not see changes across all the genes we studied in response to plumage dulling, the change in methylation in CRHR1 suggests that epigenetic mechanisms may mediate the effects of social environment on the physiology of free-living social birds.

**DATA AVAILABILITY**

Data and scripts used in the analysis are available at <https://github.com/smcnew/tres_pryo> and will be archived permanently on zenodo upon acceptance.

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**REFERENCES**

Alwin, D. F., & Wray, L. A. (2005). A life-span developmental perspective on social status and health. *The Journals of Gerontology: Series B*, *60*(Special\_Issue\_2), S7–S14. https://doi.org/10.1093/geronb/60.Special\_Issue\_2.S7

Anderson, J. A., Johnston, R. A., Lea, A. J., Campos, F. A., Voyles, T. N., Akinyi, M. Y., Alberts, S. C., Archie, E. A., & Tung, J. (2021). High social status males experience accelerated epigenetic aging in wild baboons. *ELife*, *10*, e66128. https://doi.org/10.7554/eLife.66128

Beck, M. L., Hopkins, W. A., & Hawley, D. M. (2015). Relationships among plumage coloration, blood selenium concentrations and immune responses of adult and nestling tree swallows. *The Journal of Experimental Biology*, *218*(Pt 21), 3415–3424. https://doi.org/10.1242/jeb.123794

Bentz, A. B., George, E. M., Wolf, S. E., Rusch, D. B., Podicheti, R., Buechlein, A., Nephew, K. P., & Rosvall, K. A. (2021). Experimental competition induces immediate and lasting effects on the neurogenome in free-living female birds. *Proceedings of the National Academy of Sciences*, *118*(13), e2016154118. https://doi.org/10.1073/pnas.2016154118

Bókony, V., Lendvai, Á. Z., Liker, A., Angelier, F., Wingfield, J. C., & Chastel, O. (2009). Stress Response and the Value of Reproduction: Are Birds Prudent Parents? *The American Naturalist*, *173*(5), 589–598. https://doi.org/10.1086/597610

Clinchy, M., Zanette, L., Boonstra, R., Wingfield, J. C., & Smith, J. N. M. (2004). Balancing food and predator pressure induces chronic stress in songbirds. *Proceedings. Biological Sciences / The Royal Society*, *271*(1556), 2473–2479. https://doi.org/10.1098/rspb.2004.2913

Creel, S., Dantzer, B., Goymann, W., & Rubenstein, D. R. (2013). The ecology of stress: Effects of the social environment. *Functional Ecology*, *27*(1), 66–80. https://doi.org/10.1111/j.1365-2435.2012.02029.x

Deviche, P., Beouche-Helias, B., Davies, S., Gao, S., Lane, S., & Valle, S. (2014). Regulation of plasma testosterone, corticosterone, and metabolites in response to stress, reproductive stage, and social challenges in a desert male songbird. *General and Comparative Endocrinology*, *203*, 120–131. https://doi.org/10.1016/j.ygcen.2014.01.010

Engh, A. L., Beehner, J. C., Bergman, T. J., Whitten, P. L., Hoffmeier, R. R., Seyfarth, R. M., & Cheney, D. L. (2006). Behavioural and hormonal responses to predation in female chacma baboons (Papio hamadryas ursinus). *Proceedings of the Royal Society B: Biological Sciences*, *273*(1587), 707–712. https://doi.org/10.1098/rspb.2005.3378

Guidotti, G., Calabrese, F., Anacker, C., Racagni, G., Pariante, C. M., & Riva, M. A. (2013). Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: Modulation by antidepressant treatment. *Neuropsychopharmacology*, *38*(4), Article 4. https://doi.org/10.1038/npp.2012.225

Korte, S. M., Koolhaas, J. M., Wingfield, J. C., & McEwen, B. S. (2005). The Darwinian concept of stress: Benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. *Neuroscience & Biobehavioral Reviews*, *29*(1), 3–38. https://doi.org/10.1016/j.neubiorev.2004.08.009

Landys, M. M., Goymann, W., Schwabl, I., Trapschuh, M., & Slagsvold, T. (2010). Impact of season and social challenge on testosterone and corticosterone levels in a year-round territorial bird. *Hormones and Behavior*, *58*(2), 317–325. https://doi.org/10.1016/j.yhbeh.2010.02.013

Lee, R. S., & Sawa, A. (2014). Environmental Stressors and Epigenetic Control of the Hypothalamic-Pituitary-Adrenal Axis. *Neuroendocrinology*, *100*(4), 278–287. https://doi.org/10.1159/000369585

Mason, N. A., & Bowie, R. C. K. (2020). Plumage patterns: Ecological functions, evolutionary origins, and advances in quantification. *The Auk*, *137*(4), ukaa060. https://doi.org/10.1093/auk/ukaa060

McNew, S. M., Boquete, M. T., Espinoza‐Ulloa, S., Andres, J. A., Wagemaker, N. C. A. M., Knutie, S. A., Richards, C. L., & Clayton, D. H. (2021). Epigenetic effects of parasites and pesticides on captive and wild nestling birds. *Ecology and Evolution*, *0*(0), 1:17. https://doi.org/10.1002/ece3.7606

Menke, A., Klengel, T., Rubel, J., Brückl, T., Pfister, H., Lucae, S., Uhr, M., Holsboer, F., & Binder, E. B. (2013). Genetic variation in FKBP5 associated with the extent of stress hormone dysregulation in major depression. *Genes, Brain and Behavior*, *12*(3), 289–296. https://doi.org/10.1111/gbb.12026

Monaghan, P. (2014). Organismal stress, telomeres and life histories. *Journal of Experimental Biology*, *217*(1), 57–66. https://doi.org/10.1242/jeb.090043

Mougeot, F., Martínez-Padilla, J., Bortolotti, G. R., Webster, L. M. I., & Piertney, S. B. (2010). Physiological stress links parasites to carotenoid-based colour signals. *Journal of Evolutionary Biology*, *23*(3), 643–650. https://doi.org/10.1111/j.1420-9101.2009.01926.x

Plank, A.-C., Frey, S., Basedow, L. A., Solati, J., Canneva, F., von Hörsten, S., Kratz, O., Moll, G. H., & Golub, Y. (2021). Prenatally traumatized mice reveal hippocampal methylation and expression changes of the stress-related genes Crhr1 and Fkbp5. *Translational Psychiatry*, *11*(1), 183. https://doi.org/10.1038/s41398-021-01293-y

Potticary, A. L., & Duckworth, R. A. (2020). Multiple environmental stressors induce an adaptive maternal effect. *The American Naturalist*, *196*(4), 487–500. https://doi.org/10.1086/710210

Razzoli, M., Nyuyki-Dufe, K., Gurney, A., Erickson, C., McCallum, J., Spielman, N., Marzullo, M., Patricelli, J., Kurata, M., Pope, E. A., Touma, C., Palme, R., Largaespada, D. A., Allison, D. B., & Bartolomucci, A. (2018). Social stress shortens lifespan in mice. *Aging Cell*, *17*(4), e12778. https://doi.org/10.1111/acel.12778

Rubenstein, D. R., Skolnik, H., Berrio, A., Champagne, F. A., Phelps, S., & 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*(8), 1714–1728. https://doi.org/10.1111/mec.13483

Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). *How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions*. *21*(1), 35.

Schartner, C., Ziegler, C., Schiele, M. A., Kollert, L., Weber, H., Zwanzger, P., Arolt, V., Pauli, P., Deckert, J., Reif, A., & Domschke, K. (2017). CRHR1 promoter hypomethylation: An epigenetic readout of panic disorder? *European Neuropsychopharmacology*, *27*(4), 360–371. https://doi.org/10.1016/j.euroneuro.2017.01.005

Sheldon, E. L., Schrey, Aaron. W., Hurley, L. L., & Griffith, S. C. (2020). Dynamic changes in DNA methylation during postnatal development in zebra finches Taeniopygia guttata exposed to different temperatures. *Journal of Avian Biology*, *51*(5). https://doi.org/10.1111/jav.02294

Singh-Manoux, A., Adler, N. E., & Marmot, M. G. (2003). Subjective social status: Its determinants and its association with measures of ill-health in the Whitehall II study. *Social Science & Medicine*, *56*(6), 1321–1333. https://doi.org/10.1016/S0277-9536(02)00131-4

Snyder-Mackler, N., Sanz, J., Kohn, J. N., Voyles, T., Pique-Regi, R., Wilson, M. E., Barreiro, L. B., & Tung, J. (2019). Social status alters chromatin accessibility and the gene regulatory response to glucocorticoid stimulation in rhesus macaques. *Proceedings of the National Academy of Sciences*, *116*(4), 1219–1228. https://doi.org/10.1073/pnas.1811758115

Sotnikov, S. V., & Markt, P. O. (2014). Epigenetic regulation of corticotropin-releasing hormone receptor 1: Implication for anxiety-related disorders. *Receptors & Clinical Investigation*. https://doi.org/10.14800/rci.175

Stevens, S., Valderas, J. M., Doran, T., Perera, R., & Kontopantelis, E. (2016). Analysing indicators of performance, satisfaction, or safety using empirical logit transformation. *BMJ*, *352*, i1114. https://doi.org/10.1136/bmj.i1114

Stoffel, M. A., Nakagawa, S., & Schielzeth, H. (2017). rptR: Repeatability estimation and variance decomposition by generalized linear mixed‐effects models. *Methods in Ecology and Evolution*, *8*(11), 1639–1644. https://doi.org/10.1111/2041-210X.12797

Taff, C. C., Campagna, L., & 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*(16), 3722–3737. https://doi.org/10.1111/mec.15186

Taff, C. C., Zimmer, C., Scheck, D., Ryan, T. A., Houtz, J. L., Smee, M. R., Hendry, T. A., & Vitousek, M. N. (2021). Plumage manipulation alters associations between behaviour, physiology, the internal microbiome and fitness. *Animal Behaviour*, *178*, 11–36. https://doi.org/10.1016/j.anbehav.2021.05.012

Taff, C. C., Zimmer, C., & Vitousek, M. N. (2019). Achromatic plumage brightness predicts stress resilience and social interactions in tree swallows (Tachycineta bicolor). *Behavioral Ecology*, *30*(3), 733–745. https://doi.org/10.1093/beheco/arz010

Turecki, G., & Meaney, M. J. (2016). Effects of the social environment and stress on glucocorticoid receptor gene methylation: A systematic review. *Biological Psychiatry*, *79*(2), 87–96. https://doi.org/10.1016/j.biopsych.2014.11.022

Warton, D. I., & Hui, F. K. C. (2011). The arcsine is asinine: The analysis of proportions in ecology. *Ecology*, *92*(1), 3–10. https://doi.org/10.1890/10-0340.1

Watson, H., Salmón, P., & Isaksson, C. (2019). Dynamic changes in DNA methylation during embryonic and postnatal development of an altricial wild bird. *Ecology and Evolution*, *9*(17), 9580–9585. https://doi.org/10.1002/ece3.5480

Wingfield, J. C., Maney, D. L., Breuner, C. W., Jacobs, J. D., Lynn, S., Ramenofsky, M., & Richardson, R. D. (1998). Ecological bases of hormone—behavior interactions: The “emergency life history stage.” *American Zoologist*, *38*(1), 191–206. https://doi.org/10.1093/icb/38.1.191

Wingfield, J. C., & Sapolsky, R. M. (2003). Reproduction and Resistance to Stress: When and How. *Journal of Neuroendocrinology*, *15*(8), 711–724. https://doi.org/10.1046/j.1365-2826.2003.01033.x

Yehuda, R., Daskalakis, N. P., Bierer, L. M., Bader, H. N., Klengel, T., Holsboer, F., & Binder, E. B. (2016). Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. *Biological Psychiatry*, *80*(5), 372–380. https://doi.org/10.1016/j.biopsych.2015.08.005

Young, C., Majolo, B., Heistermann, M., Schülke, O., & Ostner, J. (2014). Responses to social and environmental stress are attenuated by strong male bonds in wild macaques. *Proceedings of the National Academy of Sciences*, *111*(51), 18195–18200. https://doi.org/10.1073/pnas.1411450111

Zannas, A. S., & Binder, E. B. (2014). Gene–environment interactions at the FKBP5 locus: Sensitive periods, mechanisms and pleiotropism. *Genes, Brain and Behavior*, *13*(1), 25–37. https://doi.org/10.1111/gbb.12104

Zannas, A. S., Wiechmann, T., Gassen, N. C., & Binder, E. B. (2016). Gene–stress–epigenetic regulation of FKBP5: Clinical and translational implications. *Neuropsychopharmacology*, *41*(1), Article 1. https://doi.org/10.1038/npp.2015.235

Zimmer, C., Hanson, H. E., & Martin, L. B. (2021). FKBP5 expression is related to HPA flexibility and the capacity to cope with stressors in female and male house sparrows. *Hormones and Behavior*, *135*, 105038. https://doi.org/10.1016/j.yhbeh.2021.105038

Zimmer, C., Hanson, H. E., Wildman, D. E., Uddin, M., & Martin, L. B. (2020). FKBP5: A key mediator of how vertebrates flexibly cope with adversity. *BioScience*, *70*(12), 1127–1138. https://doi.org/10.1093/biosci/biaa114