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Epigenetic Buffering in Introduced House Sparrows

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EPIGENETIC BUFFERING IN INTRODUCED HOUSE SPARROWS

by

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(Under the Direction of Aaron W. Schrey)

ABSTRACT

Epigenetic buffering, as an environmentally induced increase in variance of epigenetic states that increases phenotypic variation to buffer populations against decreased fitness, may be a factor that resolves the genetic paradox of introduced species. DNA methylation is a molecular mechanism that could facilitate epigenetic buffering by changing in response to environmental stress. Therefore, epigenetic buffering can be detected through increased variance in DNA methylation in novel or heterogeneous environments. Introduced house sparrows (*Passer domesticus)* have well-documented phenotypic changes with low genetic diversity, high epigenetic diversity, and high variance in DNA methylation that provide a characteristic signature of epigenetic buffering. Here, I screened DNA methylation among introduced and native house sparrows from multiple widely separated geographic locations to test for evidence of epigenetic buffering. I used epiRADseq to detect differentially methylated regions and estimate total DNA methylation. I found that introduction history explained the patterns of DNA methylation among introduced and native house sparrows in a manner that supports epigenetic buffering. Recently introduced house sparrows had the highest variance in DNA methylation, and the most significantly different methylated sites. Established introduced house sparrows also had higher variance than native house sparrows. House sparrows from British Columbia and Brazil were more similar to recently introduced sparrows in methylation patterns, while the older established introduced sparrows, South Africa and Florida USA, were more similar to native house sparrows. I show that variance in DNA methylation is highest in the novel environments for the house sparrow. As environmental novelty encompasses intense stress and potentially heterogeneous environments, I suggest that epigenetic buffering is likely an important phenomenon for response to such conditions.

INDEX WORDS: DNA methylation, EpiRADseq, Rapid adaptation, Genetic paradox

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CHAPTER 1

INTRODUCTION

A population facing novel stressors can use heritable bet-hedging strategies to explore adaptive responses through increased phenotypic variation (Jablonka and Lamb 1989; O'Dea et al. 2016). Epigenetic buffering is a method of heritable bet-hedging, which precipitates an increase in epigenetic variation resulting in increased phenotypic variation in response to changes in environmental conditions (O'Dea et al. 2016). If a population uses epigenetic buffering, variance in epigenetic states will increase in more heterogenous, or stressful, environments to buffer the population against decreased fitness (O'Dea et al. 2016). Epigenetic buffering occurs on rapid timescale compared to genetic changes, acting within an individual's lifetime and across several generations (Hawes et al. 2018). Because epigenetic marks can be induced by environmental changes and in multiple individuals in response to the same cues, epigenetic buffering can rapidly manifest within a population and persist until the need for buffering is alleviated (Jablonka and Lamb 1989; Jablonka et al. 1995; Jablonka and Lamb 1998; Pál and Miklós 1999).

Molecular epigenetic mechanisms are particularly powerful for a population that is responding to novel stressors because they can change rapidly and contemporaneously (Hu et al. 2019). The most well-studied epigenetic mechanism is DNA methylation (Schrey et al. 2013). DNA methylation can change phenotypes without variation in DNA sequence (Cubas et al. 1999; Sepers et al. 2019; Husby 2020) and can generate phenotypic variation even for populations with low genetic diversity (Massicotte et al. 2011; Liebl et al. 2013; Carneiro and Lyko 2020). DNA methylation regulates gene expression, albeit with complicated, site-specific, effects (Nätt et al. 2012). DNA methylation can change in response to novel environmental stressors and can facilitate appropriate regulation of gene expression for that stressor (Foust et al. 2015; Fu et al. 2021).

Three case-studies provide evidence of the potential impact of epigenetic buffering: a hostage nectivorous yeast (*Metschnikowia reukaufii*), an epigenetic switching brewer's yeast (*Saccharomyces cerevisiae*), and an asexually reproducing fish (*Chrosomus eos-neogaeus*). In *M. reukaufii*, DNA

methylation facilitates the response to highly variable sugar environments among flowers by epigenetic buffering among the heterogeneous habitats (Herrera et al. 2011). Floral nectar composition is related to high inter-individual phenotypic variation (Pozo et al. 2015), suggesting that changes in DNA methylation occur in response to floral nectar composition, increasing phenotypic variability in *M. reukaufii*. The epigenetic switching brewer's yeast thrive in fluctuating environments using epigenetic variation to facilitate their persistence until DNA sequence mutations consolidate advantageous phenotypes (Stajic et al. 2022). Yeast with the epigenetic switcher phenotype can persist in stable environments, but not as well as non-switchers, showing that epigenetic switching is not maladaptive and implying that maintaining the potential for epigenetic variation might be necessary in all environments (Stajic et al. 2022). In the asexual reproducing *C. eon-neogaeus* among natural populations spanning a broad geographic range, there was high epigenetic variation among individuals with the same genotype, showing that epigenetic variation responds to environmental conditions (Massicotte et al. 2011; Massicotte and Angers 2012). Individuals had differentiated epigenetic profiles among geographic locations and environmental conditions, further supporting environmentally induced epigenetic changes (Massicotte et al. 2011; Angers et al. 2012; Leung et al. 2016). Further, common garden experiments show *C. eon-neogaeus* spawned in natural environments displayed high variation in DNA methylation that decreased after individuals were transplanted to controlled common conditions (Leung et al. 2016).

Epigenetic buffering could help resolve the genetic paradox (O'Dea et al. 2016; Hawes et al. 2018). Introduction forces organisms to face novel and heterogeneous environments, while also confronting the genetic paradox. The genetic paradox refers to the phenomenon of species with no prior genetic adaptations to a particular environment becoming successfully introduced (Frankham 2005; Pérez et al. 2006). As introduction events can result in decreased genetic diversity compared to native populations, additional mechanisms increase phenotypic variation before genetic assimilation can occur. Introduced populations could, therefore, use epigenetic buffering to test multiple different phenotypes until adaptive phenotypes are consolidated and the introduced population is buffered against decreased fitness from the introduction (O'Dea et al. 2016; Hawes et al. 2018). Avian species display DNA

methylation patterns corresponding to environmental stress, environmental change, novelty, and for changes in behavior, particularly in novel environments. For example, any increase or decrease in brood size resulted in a change of DNA methylation in nestling zebra finch (*Taeniopygia guttata*; Sheldon et al. 2018). The zebra finch also displays different patterns of DNA methylation when reared at warmer temperatures (Sheldon et al. 2020). Great tits (*Parus major*) from urban environments show more variation in DNA methylation than those from rural environments (Watson et al. 2021), suggesting that urban environments might also induce epigenetic buffering. Further, throughout development house wrens (*Troglodytes aeon*) had more change in DNA methylation when the nest condition (urban or rural) of the hatchlings matched the nest condition of the parents (vonHoldt et al. 2021). Domesticated and wild-type chickens (*Gallus gallus*) had differences in DNA methylation that are related to differences in aggression (Nätt et al. 2012).

The house sparrow (*Passer domesticus*) has been introduced or expanded its native range on all continents, except Antarctica (Anderson 2006; Liebl et al. 2015). This human-commensal songbird displays phenotypic differences between native and introduced populations (Anderson 2006; Lima et al. 2012; Hanson et al. 2020b), among introductions (Johnston and Selander 1973; Martin and Fitzgerald 2005; Lima et al. 2012) and among individuals across introduced ranges (Liebl and Martin 2012; Liebl and Martin 2013; Liebl and Martin 2014; Martin et al. 2017). Traditional genetic mechanisms likely are not sufficient to account for the success of the house sparrow in introduced areas (Liebl et al. 2015). Introduced populations have lower genetic diversity and less genetic differentiation than native populations (Schrey et al. 2011; Liebl et al. 2015; Andrew et al. 2018). Also, the genetic characteristics of introduced house sparrows are shaped by admixture among locations and long-distance, human-mediated dispersal (Schrey et al. 2011; Schrey et al. 2014; Sheldon et al. 2018). Therefore, additional mechanisms, such as phenotypic differences, epigenetic differences, and combinations of these, have been investigated to understand introduction success.

Phenotypic differences directly associated with introduction success have been studied in introduced house sparrow populations. Introduced individuals approached and ate novel foods with less

latency (Martin and Fitzgerald 2005; Kelly et al. 2020), were more exploratory (Liebl and Martin 2012), had greater stress responses (Liebl and Martin 2012; Liebl and Martin 2013; Martin et al. 2017), and increased expression of the immunologically important genes Toll-like receptor (TLR) 2 and 4 towards introduction range edge (Martin et al. 2014; Martin et al. 2015; Martin et al. 2017). As phenotypic changes in these populations occur despite low genetic diversity (as in the Kenyan system) and/or have rapidly manifested as different from native populations, epigenetic mechanisms may be facilitating aspects of the observed phenotypic differences (Schrey et al. 2011; Liebl et al. 2015; Kilvitis et al. 2019).

Across house sparrow introductions, DNA methylation characteristics indicate that epigenetic buffering is likely an important mechanism facilitating their success. In Kenya, where house sparrows reached some locations as recently as 2005, variation in DNA methylation was highest among individuals captured in the most recently introduced location (Hanson et al. 2022). Also in Kenyan house sparrows, diversity in DNA methylation among individuals compensated for decreased genetic diversity, and range-edge populations had higher diversity in DNA methylation compared to range-core populations (Liebl et al. 2013). Kenyan house sparrows and those from Florida, U.S.A, widely separated introduced populations with different times since introduction, had significantly different DNA methylation (Schrey et al. 2012). Introduced Australian sparrows had high diversity in DNA methylation and low genetic diversity, but with weaker trends than Kenyan house sparrows, likely because the Australian introduction was nearly 100 years earlier (Sheldon et al. 2018). Importantly, in house sparrows, DNA methylation in the promoter of Toll-like receptor 4 has been correlated to variation in gene expression (Kilvitis et al. 2019). In Senegal, introduced house sparrows from the range-edge had a positive relationship between DNA methylatransferase-1 expression levels and corticosterone expression levels, while individuals at the range-core had a negative relationship (Kilvitis et al. 2018), indicating that expression of DNA methylating enzymes changes with introduction.

My objective was to screen DNA methylation in house sparrows from multiple native and introduced areas to determine if the pattern of variation supported epigenetic buffering. I used epiRADseq, a next-generation sequencing approach that allows genome-wide patterns in methylation to

be quantified as a continuous variable without a reference genome (Schield et al. 2016). The epiRADseq protocol is well-suited to assess differences in DNA methylation for species without a reference genome using a reduced representation method. I identified differentially methylated regions (DMR), or locations where methylation differences occur between comparisons, and estimated levels of total methylation. I hypothesized the variance in DNA methylation would be highest among house sparrows from the most recently introduced locations and that DNA methylation would differ among recently introduced, established introduced, and native populations of house sparrow. This study is the broadest geographic scope of any epigenetic study on introduced house sparrows to date.

CHAPTER 2

METHODS

Data collection

I screened house sparrows (n = 45; Table 1, Figure 1) across their global distribution targeting native - Turkey (n = 8) and France (n = 4) – and introduced locations. I used the documented year of initial introduction to divide introduced locations into recently introduced, introduced for less than 70 y: Kenya (n = 14; 1950-2005, Liebl et al. 2013; Coon and Martin 2014) and Panama (n = 4; 1980, Hanson et al. 2020a) and established introduced, introduced for more than 70 y: Florida USA (n = 4; 1886, Peña-Peniche et al. 2021), South Africa (n = 4; 1900, Liebl et al. 2015), Brazil (n = 3; 1905, Lima et al. 2012), and British Columbia (n = 4; 1915, Hanson et al. 2020a). I extracted DNA from blood samples stored in 100% ethanol or dried on Whatman paper using the Dneasy Kit (Qiagen, Valencia CA USA).

Next-Generation Sequencing

I screened variation in DNA methylation among individuals using epiRADseq (Schield et al. 2016) on the Ion Torrent PGM platform (Thermo Fisher Scientific, Waltham, MA). Briefly, epiRADseq is a ddRADseq protocol that uses a DNA methylation sensitive restriction enzyme (*HpaII*), which fails to cut when the CCGG restriction site is modified by DNA methylation. Thus, variation in DNA methylation is assayed as read count variation. I followed a 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, I ligated Ion Torrent IonXpress barcoded adaptors and y-adaptors. I ran emulsion PCR following manufacturers protocols of the Ion PGM-Hi-Q-View OT2-200 kit on the Ion Express OneTouch2 platform. I sequenced resultant fragments following manufacturers protocols of the Ion PGM-Hi-Q-View Sequencing 200 Kit using an Ion 316v2 BC Chips.

Data analysis

I demultiplexed runs and conducted quality control with Torrent Suite version 4.4.3. I trimmed sequences to 150 bp, the number of sequences generated per individual ranged from 5,838 to 579,265. I

performed a de novo assembly and constructed a pseudo-reference of 11,371,350 bases using Geneious Prime v. 2021.2.2. I mapped individual sequences with BWA Galaxy Version 0.7.17.4 (Li and Durbin 2009; Li and Durbin 2010). I used featureCounts Galaxy Version 1.6.4+galaxy1 (Liao et al. 2013) to determine read counts of fragments within 150 bp bins spanning the pseudo-reference. I targeted the 20,000 bins with the most coverage for analysis.

I used edgeR, Galaxy Version 3.24.1+galaxy1 (Robinson et al. 2010; Liu et al. 2015) to detect DMR present in house sparrows using an FDR of 0.05. First, I detected DMR pairwise among sampling locations. Second, I detected DMR among introduction categories (recent, established, native). I sorted DMR among introduction categories with Venn diagrams.

I calculated an index of methylation (IOM) as an estimate of total methylation, across bins, standardized by sequencing depth. For every individual, I divided the total number of counted reads (across bins) by the total number of sequences observed for that individual and subtracted this ratio from 1. I compared IOM among introduction categories, estimating mean and variance for introduction categories using t-tests and f-tests. I also correlated the IOM for each sample location with the year of initial introduction using Pearson's correlations to determine how DNA methylation patterns changed over time (using year zero for native populations). All statistical tests of IOM used alpha = 0.05 and were corrected for multiple tests when appropriate (Rice 1989).

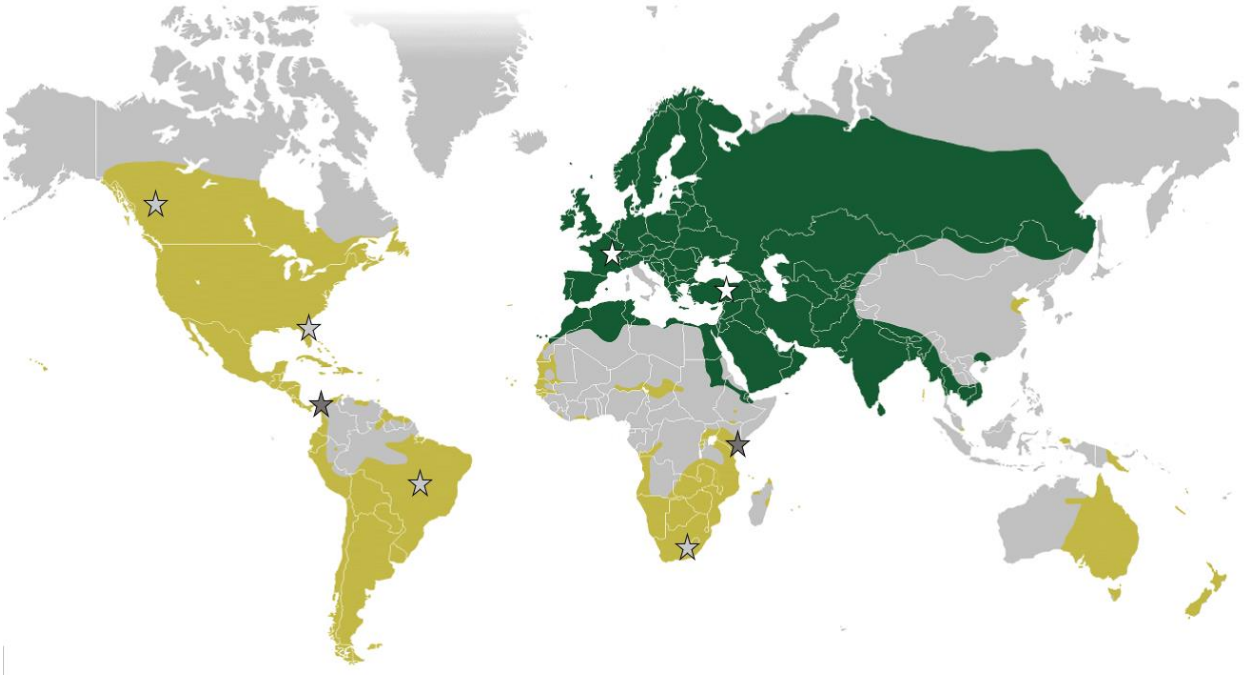

Figure 1. Sampling locations of house sparrows indicated by stars on a map of the global distribution (modified from Hanson et al. 2020a). Yellow shading indicates the introduced range and green shading indicates the native range. Dark gray stars represent locations with recently introduced individuals, light gray represent locations with established introduced individuals, and white stars represent locations with native individuals.

CHAPTER 3

RESULTS

Differences in DNA methylation among house sparrows supported epigenetic buffering, both among introduction categories and among geographic locations. I detected 37,501 DMR in house sparrows across all locations. Recently introduced house sparrows had the greatest number of DMR, and these were predominantly between recently introduced and native house sparrows. Among introduction categories, I detected more than twice as many DMR between recently introduced and native house sparrows (11,538 DMR; Figure 2) compared to the number detected between recently introduced and established introduced (4,226 DMR) and established introduced compared to native (4,110 DMR) house sparrows (Figure 2). Only 45 DMR (out of 19,874) were shared among all introduction categories (Figure 2).

Significant DMR were detected in 15 of the 28 pairwise comparisons among geographic locations (Table 2). Pairwise comparisons including house sparrows from the recently introduced location Kenya had the highest number of DMR, and DMR were detected between Kenyan house sparrows and all other locations. Pairwise comparisons including house sparrows from the native location Turkey had the second highest number of DMR. Interestingly, DMR were not detected between individuals from the two native locations Turkey and France, or between individuals from Turkey and two established introduced locations, Florida USA and South Africa. Pairwise comparisons including individuals from the established introduced location Brazil had the fewest DMR, with DMR only detected between Brazilian house sparrows and Kenyan house sparrows and Brazilian house sparrows and individuals from Turkey.

My estimates of total methylation, IOM, also supported epigenetic buffering among introduced house sparrows (Figure 3). Individual IOM scores were significantly correlated to year of initial introduction, with year of introduction included as a continuous variable and year 0 used to represent native house sparrows ($r = -0.505$, $P = 0.0002$). Recently introduced house sparrows had the highest variance in DNA methylation ($\sigma^2 = 0.006$; Table 1). Established introduced house sparrows had the second highest variance in DNA methylation ($\sigma^2 = 0.002$). Native house sparrows had the lowest variance

(σ^2 = 0.0004). Both recently introduced (*f-test P* = 0.00003) and established introduced (*f-test P* = 0.005) house sparrows had significantly higher variance in DNA methylation compared to native house sparrows. Total DNA methylation was significantly lower in recently introduced house sparrows (IOM = 0.864; Table 1) compared to both established introduced (IOM = 0.913; *t-test* recent vs. established *P* = 0.020) and native (IOM = 0.9625; *t-test* recent vs. native *P* = 0.0001) house sparrows. Also, total DNA methylation in established introduced house sparrows was significantly lower than in native house sparrows (*t-test P* = 0.001).

I found a transition in total DNA methylation within the established introduced house sparrows between years 1900 and 1905. The more recently introduced of the established introduced category, years 1905 and after, had higher variance (σ^2 = 0.004; *f-test P* < 0.0001) and lower mean (0.865: *t-test P* < 0.0001; Figure 4) compared to the longer established individuals, years 1900 and before, variance (σ^2 = 0.0003) and mean (0.959).

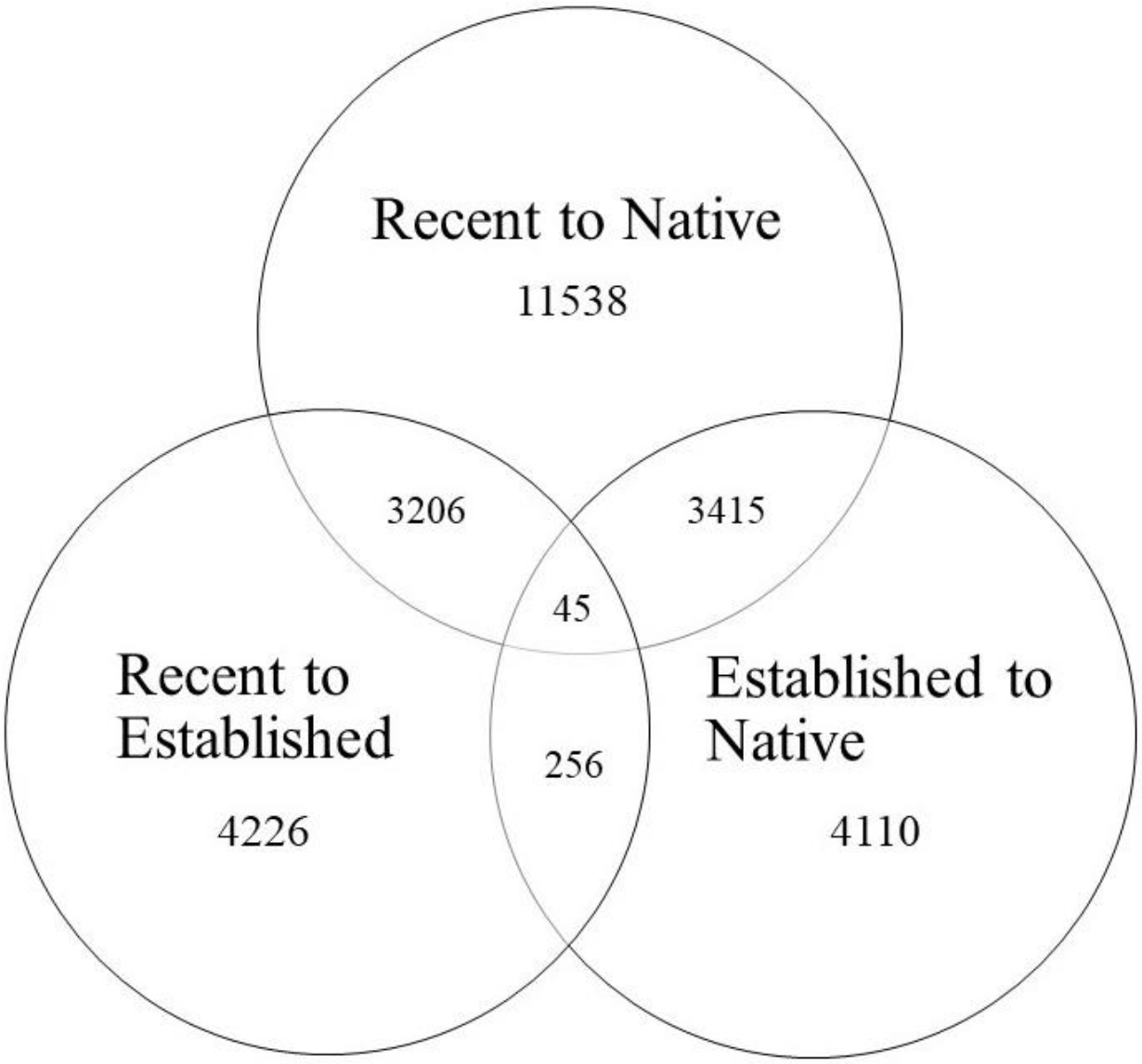

Figure 2. Venn diagram showing the number of differentially methylated regions (DMR) and pattern of sharing among house sparrows from three categories of introduction. The highest DMR counts occurred in comparisons including recently introduced house sparrows. The smallest number was shared among all comparisons.

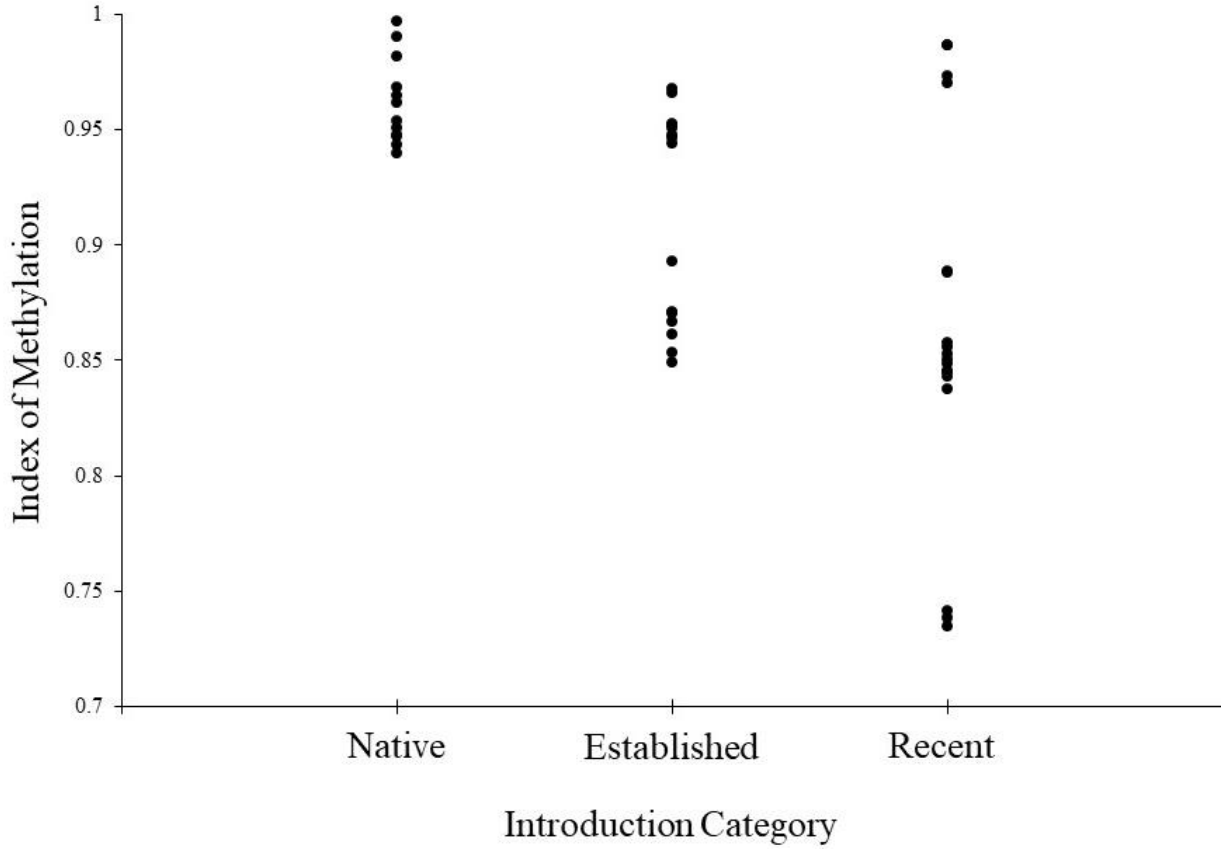

Figure 3. DNA methylation level among house sparrows estimated by an index of methylation (IOM) for each location grouped by introduction categories. Recently introduced house sparrows had the highest variance in DNA methylation (σ^2 = 0.006; *f-test* recent vs. native P = 0.00003, established P = 0.052) and the lowest mean IOM score (IOM = 0.864; native P = 0.0001, established P = 0.02). Established introduced individuals had higher variance in DNA methylation than the native house sparrows (σ^2 = 0.002; *f-test* established vs. native P = 0.005). House sparrows from native locations had the lowest variance in DNA methylation (σ^2 = 0.0004) and the highest mean IOM scores (IOM = 0.963).

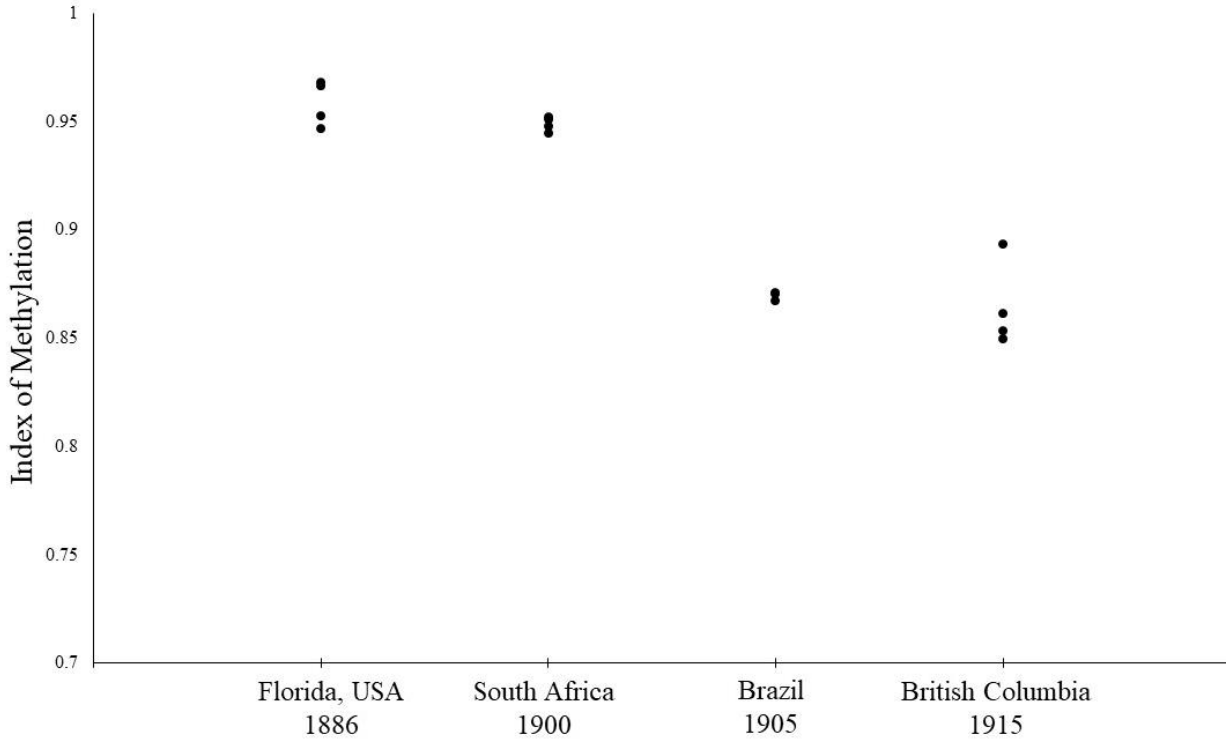

Figure 4. DNA methylation levels among house sparrows estimated with an index of methylation (IOM) from each location in the established introduced category. Results show a split in mean IOM between those introduced before 1900 and those introduced after 1905 (1900 and before IOM = 0.959; 1905 and after IOM = 0.865; *t-test* $P < 0.0001$).

Table 1. House sparrow collection locations with sample size (n), separated by introduction category based on year of initial introduction (Year) and those from the native range. DNA methylation was estimated for each location by an index of methylation (IOM) score. Mean IOM with variance are provided.

Country	Year	n	IOM Mean (Variance)
Recent			0.864 (0.006)
Panama	1980	4	0.862
Kenya	2005	14	0.864
Mombasa	1950	2	*0.848*
Voi	1960	3	*0.846*
Nairobi	1990	4	*0.797*
Nakuru	2000	1	*0.845*
Garissa	2000	1	*0.987*
Kakamega	2005	3	*0.950*
Established			0.913 (0.002)
British Columbia	1915	4	0.864
Brazil	1905	3	0.870
South Africa	1900	4	0.949
Tampa, FL, USA	1886	4	0.958
Native			0.963 (0.0004)
France	-	4	0.960
Turkey	-	8	0.964

Table 2. Pairwise comparisons of differentially methylated regions among house sparrows for all sampling locations. The number presented is the total significant DMR detected for that comparison at FDR = 0.05.

	Kenya	Panama	British Columbia	Brazil	South Africa	Tampa, FL, USA	France	Turkey
Kenya	-							
Panama	6521	-						
British Columbia	6542	0	-					
Brazil	1423	0	0	-				
South Africa	299	1145	1771	0	-			
Tampa, FL, USA	188	721	1437	0	0	-		
France	169	0	278	0	0	0	-	
Turkey	6181	4863	5565	398	0	0	0	-

CHAPTER 4

DISCUSSION

Introduction history explained the differences in DNA methylation among introduced and native house sparrows. Also, DNA methylation among introduced house sparrows showed evidence of epigenetic buffering and supported epigenetic buffering as an important mechanism in overcoming the genetic paradox. As expected, epigenetic buffering was evident in the most recently introduced areas. The most recently introduced sparrows had the highest variance in DNA methylation, followed by the established introduced, then the native sparrows (Table 1; Figure 2). Previous work in introduced house sparrows has shown that the most recently introduced populations in Kenya have higher variance in DNA methylation (Hanson et al. 2022) and a compensatory relationship occurred between epigenetic and genetic diversity (Liebl et al. 2013), but these studies have been limited in scope to a single introduction location. Here, we found that the increase in variance of DNA methylation was common among the introduced locations. However, the oldest of the established introduced category, Florida USA and South Africa, more closely resembled the native category sparrows in variance of DNA methylation and did not display DMR compared to these sampling locations. These results further the findings of Sheldon et al. (2018), in that the Australian house sparrow introduction shows epigenetic diversity still high compared to genetic diversity, but that DNA methylation differences between introduction clusters are likely responses to specific environmental conditions. The oldest of the introduced house sparrows display patterns of DNA methylation differences dependent on environmental responses, as native house sparrows do, instead of random increases in DNA methylation variation used to buffer the population against decreased fitness and lower genetic diversity risks associated with early introduction. As such, we can speculate that the lack of DMR among the oldest of the introduced house sparrows and native house sparrows and more similar variance in DNA methylation could be a result of a decrease or dissipation in epigenetic buffering.

Introduction history also affected the number of DMR, with locations with the maximum observed epigenetic buffering having the most differences. Recently introduced house sparrows had the

most DMR across all comparisons (Table 2; Figure 1). As the introduced sites showed DMR among locations, we can expect that epigenetic buffering is occurring as the increase in DMR is not idiosyncratically manifested among locations. The most DMRs occurred in comparisons between recent and native house sparrows, followed by comparisons between recent and established house sparrows, and the least DMR occurred in comparisons between established and native house sparrows (Figure 1). The introduction condition seems to contribute to the greatest differences in methylation patterns, as the comparison between the recently introduced to native and the established introduced to native resulted in the most shared DMR (3,415). A temporal component to changes in DNA methylation patterns, namely the "recently introduced" state, contributed 3,206 shared DMR between the recently introduced to native and the recently introduced to established introduced comparisons. 256 DMR were shared between the recently introduced to established introduced and the established introduced to native comparisons and 45 DMR were shared among all comparisons. I interpreted these results as meaning that the introduction status changes methylation patterns in a complex manner and introduction event-specific conditions and cues drive changes.

My results found that variance in DNA methylation increased in response to introduction and remained high for at least 70 years, after which variance decreased to levels similar to native populations. House sparrows in the established introduced category had a significant transition between introduction occurring before and after 1905. House sparrows that were introduced after 1905 closely resembled the recently introduced group in both variance and total methylation, while those introduced before 1905 more closely resembled individuals from the native group (Figure 3). Additionally, the older of the established introduced house sparrows (Florida USA and South Africa) did not display DMR from the native individuals (Table 2), suggesting that the distribution of DNA methylation is being influenced by epigenetic buffering in the most recent introductions, as it broadens the methylation landscape.

Epigenetic buffering provides an alternative route to traditional genetic mechanisms for increasing phenotypic variation in introduced populations facing novel environments and rapid change. Epigenetic buffering would also be beneficial in other contexts, especially any changes that a species

must respond to over ecological timescales. Further studies are needed to understand the effect epigenetic buffering has on gene expression and if epigenetic buffering manifests idiosyncratic changes by location or a general response among introductions. Future studies would benefit from a whole genome approach for the detection of site-specific changes or increases in variance at particular targets in the genome. Conservation efforts could consolidate screening for epigenetic buffering with other population genetics, as these measures will be more useful in predicting population persistence in the face of rapid change. Epigenetic buffering should therefore be considered in future studies as it might be a good indicator of environmental change tolerance/flexibility to novelty in the face of a dynamic world.

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