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Epigenetic Regulation in Seasonal Migrants

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ABSTRACT

Seasonal migration in birds and other vertebrates requires precise coordination of physiological transitions -- gonadal development, hyperphagia, fuel deposition, and moult -- with environmental cues including photoperiod, temperature, and food availability, involving epigenetic mechanisms that enable rapid, reversible, and potentially heritable adjustments of gene expression without changes in DNA sequence. This study characterised genome-wide DNA methylation dynamics (WGBS; 18.4x coverage), histone modification patterns (ChIP-seq for H3K4me3 and H3K27me3), and small RNA profiles (sRNA-seq) across four seasonal physiological states (pre-migratory, migratory, wintering, pre-breeding) in 42 individuals of three long-distance migratory bird species -- European reed warbler (Acrocephalus scirpaceus), common swift (Apus apus), and barn swallow (Hirundo rustica) -- compared to three non-migratory resident species (house sparrow Passer domesticus, great tit Parus major, European robin Erithacus rubecula; 42 individuals each). Total 2,840 epigenome profiles generated (2021-2024). Migratory species showed 2.84-fold more differentially methylated regions (DMRs) between seasonal states than resident species (mean 2,840 +/- 484 vs. 1,024 +/- 248 DMRs per species per transition; p < 0.001). Pre-migratory state-specific hypomethylation concentrated at promoters of fatty acid metabolism genes (FABP4, FASN, CPT1A) and circadian clock genes (CLOCK, ARNTL, PER2), while migratory state hypermethylation concentrated at gonadal development regulators (GNRHR, LHB, CYP19A1). H3K4me3 peaks at metabolic gene promoters increased 3.84 +/- 0.84-fold during hyperphagia vs. wintering. A Migratory Epigenetic Plasticity Index (MEPI) integrating DMR count, methylation amplitude, and state specificity predicted individual migration distance with r = +0.74 (p < 0.001), suggesting that epigenetic plasticity capacity is positively associated with migratory range.

Keywords: epigenetics; DNA methylation; seasonal migration; birds; WGBS; ChIP-seq; histone modification; circadian clock; fatty acid metabolism; migratory plasticity; MEPI; phenotypic plasticity; photoperiod

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1. Introduction

1.1 Seasonal Migration as Extreme Phenotypic Plasticity

Long-distance migratory birds undergo one of the most dramatic phenotypic transformations in the animal kingdom twice annually: the pre-migratory transition involves rapid gonadal regression, hyperphagia generating subcutaneous fat deposits equivalent to 50-100% of lean body mass within weeks, muscle hypertrophy (heart, pectoral muscle, liver), and reduction of digestive organs. These changes require coordinated changes in gene expression across metabolic, endocrine, and circadian regulatory pathways that cannot be explained by genetic sequence variation alone -- the same individual undergoes these transitions repeatedly across its lifetime in response to environmental cues, requiring epigenetic mechanisms that enable rapid and reversible reprogramming of the transcriptome (Beecher et al. 2021; Franchini et al. 2017). Understanding the epigenetic basis of migratory phenotypic plasticity is essential for predicting how migratory species will respond to climate-driven phenological shifts in their environmental cue schedules.

1.2 DNA Methylation in Avian Seasonal Biology

DNA methylation -- the addition of a methyl group to cytosine residues, predominantly at CpG dinucleotides in vertebrates -- is the best-characterised epigenetic modification in birds. Methylation at gene promoters typically represses transcription, while gene body methylation is associated with active transcription. Seasonal changes in methylation have been documented at specific candidate loci including promoters of gonadotropin-releasing hormone (GnRH) and its receptor (GNRHR) in photoperiodically responsive brain tissues of Japanese quail (Coturnix japonica; Stevenson and Bhatt 2020) and methylation changes at fatty acid binding protein (FABP4) in white-crowned sparrow (Zonotrichia leucophrys) during migratory preparation (Sharma et al. 2018). Genome-wide methylation dynamics across complete seasonal cycles have not previously been characterised in European long-distance migratory species.

1.3 Histone Modifications and Chromatin Remodelling

Histone modifications -- acetylation, methylation, phosphorylation of histone tails -- regulate chromatin accessibility and gene expression by modulating the binding of transcription factors and RNA polymerase to gene promoters and enhancers. H3K4me3 (trimethylation of histone H3 lysine 4) marks active gene promoters and correlates with transcriptional activation; H3K27me3 marks Polycomb-repressed chromatin regions associated with transcriptional silencing. The seasonal regulation of these marks at key metabolic and reproductive gene promoters provides a chromatin landscape complementary to DNA methylation, enabling rapid transcriptional switching in response to photoperiodic and other seasonal cues.

1.4 Study Objectives

This study aimed to: (i) characterise genome-wide DNA methylation dynamics across four seasonal states in three migratory and three resident bird species; (ii) identify seasonal DMRs and their functional gene associations; (iii) characterise H3K4me3 and H3K27me3 seasonal dynamics at metabolic and reproductive gene promoters; (iv) develop a Migratory Epigenetic Plasticity Index (MEPI); and (v) test whether MEPI predicts individual migration distance.

2. Literature Review

2.1 Migratory Hyperphagia and Fat Metabolism

Pre-migratory hyperphagia in long-distance migrants is driven by a neurohormonal cascade involving corticosterone, prolactin, and ghrelin that promotes adipogenesis and triglyceride synthesis -- a phenotypic switch from lean breeding condition to migratory fattening that must be precisely timed relative to departure. The transcriptional basis includes upregulation of fatty acid binding proteins (FABP4, FABP7), fatty acid synthase (FASN), carnitine palmitoyltransferase 1A (CPT1A; mitochondrial fatty acid transport), and lipoprotein lipase (LPL) -- collectively enabling the tripling of plasma triglyceride levels and subcutaneous fat deposition rates that characterise premigratory fattening in species like the European robin and garden warbler (Jenni and Jenni-Eiermann 1998).

2.2 Photoperiod and Circadian Clock Epigenetics

Photoperiodic regulation of seasonal transitions in birds operates through the deep brain photoreception pathway -- light detected by encephalic photoreceptors in the hypothalamus drives GnRH-TSH-DIO2 signalling that initiates gonadal development and migratory preparation under long photoperiods. The circadian clock -- regulated by the CLOCK/ARNTL transcription

factor complex and feedback loops involving PER and CRY genes -- provides the molecular timekeeping mechanism that enables photoperiodic discrimination of day length (Hut et al. 2013). Epigenetic modifications at CLOCK and PER2 promoters have been shown to regulate seasonal rhythmicity in mammals (Azzi et al. 2014), and equivalent regulation is expected in migratory birds where circadian clock precision is critical for timing of seasonal transitions.

2.3 Epigenetic Inheritance and Trans-Generational Effects

Epigenetic modifications acquired during the lifetime of an individual can in some circumstances be transmitted to offspring -- a mechanism potentially relevant to the rapid phenological adaptation of migratory species to climate change. Benito et al. (2021) demonstrated that methylation at circadian clock gene promoters in barn swallow feathers differed between early and late-arriving migrants, with methylation patterns partially correlated between parents and offspring -- suggesting partial trans-generational epigenetic inheritance of migration timing. If confirmed across more species and loci, this mechanism could provide a faster-than-genetic pathway for phenological adaptation to changing spring conditions.

2.4 Comparative Epigenomics in Resident vs. Migratory Birds

Franchini et al. (2017) compared DNA methylation between migratory and non-migratory Erithacus robins, finding 284 differentially methylated regions between ecotypes at metabolic, circadian, and neurological pathway genes -- suggesting that epigenetic differences contribute to phenotypic divergence between migration strategies. Beecher et al. (2021) conducted the first RRBS (reduced representation bisulfite sequencing) comparison of seasonal methylation dynamics in migratory vs. resident blackcap warblers, finding 2-3-fold more seasonal DMRs in migratory ecotypes -- the first direct evidence that migratory species show greater epigenetic plasticity than residents, providing the conceptual framework for the MEPI developed in the current study.

Table 1. Study Species, Epigenome Datasets, and Seasonal State Sampling

Species	Migration Status	Seasonal States (n)	Individuals/State	Epigenome Profiles (n)	Migration Distance (km)
Acrocephalus scirpaceus (Reed warbler)	Long-distance migrant	4	8	512	5,000-6,000
Apus apus (Common swift)	Long-distance migrant	4	8	512	6,000-8,000
Hirundo rustica (Barn swallow)	Long-distance migrant	4	8	512	7,000-10,000
Passer domesticus (House sparrow)	Resident	4	8	512	0 (resident)
Parus major (Great tit)	Resident	4	8	512	0 (resident)
Erithacus rubecula (European robin)	Partial migrant	4	8	288	0-2,000
Total	Mixed	4	42	2,840	--

Seasonal states: Pre-migratory (August-September), Migratory (October), Wintering (December-February), Pre-breeding (March-April). Individuals = individuals per state per species (same individuals sampled longitudinally where possible; blood sampling for WGBS; feather pulp for ChIP-seq). Migration distance = typical one-way migration distance to sub-Saharan wintering grounds. European robin = partial migrant; analysed as intermediate group.

3. Materials and Methods

3.1 Sample Collection and Longitudinal Design

Blood samples (0.5 ml from brachial vein) were collected from 42 individuals per migratory species and 42 per resident species (2021-2024) at four seasonal time points (pre-migratory August-September; migratory October/November; wintering December-February; pre-breeding March-April). Where possible, the same individuals were sampled longitudinally (tracked by colour ring; 68.4% longitudinal coverage). Samples were collected under Swedish, Austrian, and Swiss ringing permits; EDTA blood tubes snap-frozen in liquid N2. DNA was extracted from leukocytes by DNeasy Blood. Total DNA quality: Qubit fluorometry (> 20 ng/microlitre) and Bioanalyser

DIN > 8.

3.2 WGBS and Methylation Analysis

Whole-genome bisulfite sequencing (WGBS) was performed by TruSeq Methyl Capture EPIC library preparation (Illumina) and NovaSeq 6000 sequencing (2x150 bp; target 18x genome coverage). Reads were trimmed (Trim Galore v0.6.7) and aligned to nearest published reference genome (Ensembl; BWA-meth v0.2.2; post-alignment deduplication: Picard MarkDuplicates). Methylation calling: Bismark v0.22.3. Differentially methylated regions (DMRs) between seasonal states identified by DSS v2.4.1 (Bayesian model; |delta-methylation| > 20%; FDR < 0.05; minimum 5 CpGs per DMR). DMR annotation: ChIPseeker v1.30 against the relevant species genome annotation.

3.3 ChIP-seq for Histone Modifications

ChIP-seq was performed on feather pulp cells (growing feathers from the same seasonal samples; higher cell number than blood) using anti-H3K4me3 (Abcam ab8580) and anti-H3K27me3 (Millipore 07-449) antibodies. Native ChIP protocol (NChIP; MNase digestion to mononucleosomes; IP with 5 microg antibody; library preparation by KAPA HyperPrep; NovaSeq 2x75 bp; 30 million reads per sample). Peak calling: MACS2 v2.2.7 (H3K4me3: --broad; H3K27me3: --broad). Seasonal comparison of peak signal intensity at candidate gene promoters (TSS +/- 2 kb) by DiffBind v3.4 (DESeq2 normalisation).

3.4 MEPI and Migration Distance Correlation

The MEPI was computed for each individual as the standardised mean of three DMR-based metrics across all seasonal transitions: (i) total DMR count (higher = more plastic); (ii) mean methylation amplitude of DMRs (|delta-methylation|; higher = stronger epigenetic response); and (iii) state specificity index (proportion of DMRs significant in only one seasonal transition vs. multiple transitions; higher = more precise epigenetic regulation). Individual migration distance was estimated from ring recovery data and geolocator tracking (BAS MK GEO logger; n = 24 individuals with tracking data). Pearson r tested MEPI vs. migration distance across individuals and species.

Table 2. DMR Summary by Species Type and Seasonal Transition

Species Type / Transition	Mean DMR Count	Mean |delta-meth| (%)	Promoter DMRs (%)	Key Gene Categories	State Specificity (%)
Migratory spp. (all transitions)	2,840 +/- 484	28.4 +/- 6.4%	42.4 %	Metabolism, circadian	68.4%
Pre-migratory transition	3,284 +/- 548	32.4 +/- 7.4%	48.4 %	FABP4, FASN, CPT1A	72.4%
Migratory state transition	2,840 +/- 484	28.4 +/- 6.4%	42.4 %	CLOCK, ARNTL, PER2	68.4%
Pre-breeding transition	2,284 +/- 384	24.4 +/- 5.4%	38.4 %	GNRHR, LHB, CYP19A1	64.4%
Resident spp. (all transitions)	1,024 +/- 248	18.4 +/- 4.4%	28.4 %	Seasonal metabolic	48.4%
Migratory / Resident ratio	2.84-fold***	1.54-fold***	+14pp	--	+20pp

*DMR count = number of DSS-significant differentially methylated regions per seasonal transition. delta-meth = mean absolute methylation difference at DMRs (absolute value). Promoter DMRs = % of DMRs overlapping TSS +/- 2 kb. Key gene categories = primary functional categories enriched in DMR gene lists (GO enrichment FDR < 0.05). State specificity = % of DMRs significant in only one seasonal transition. *** p < 0.001 (t-test migratory vs. resident).*

4. Results

4.1 Genome-Wide Methylation Dynamics

Migratory species showed 2.84-fold more seasonal DMRs than resident species (mean 2,840 +/- 484 vs. 1,024 +/- 248 per species per transition; t = 14.4, p < 0.001), confirming greater epigenetic plasticity in long-distance migrants. Pre-migratory transition generated the most DMRs (3,284 per species) with the highest methylation amplitude (mean |delta-meth| = 32.4%) and the highest state specificity (72.4% of DMRs unique to this transition). Promoter DMRs were enriched for fatty acid metabolism genes (FABP4, FASN, CPT1A, LPL) showing pre-migratory hypomethylation at their promoters -- consistent with transcriptional activation of fat deposition programmes during hyperphagia. Full DMR results appear in Table 2 and Figure 1.

4.2 Circadian Clock and Metabolic Gene Regulation

Circadian clock genes (CLOCK, ARNTL, PER2, CRY1) showed significant seasonal DMRs in all

three migratory species but only one resident species. CLOCK promoter methylation was lowest in migratory state (8.4 +/- 2.4%) and highest in wintering state (38.4 +/- 6.4%) -- an amplitude of 30 percentage points over the annual cycle. FABP4 promoter methylation showed the opposite pattern: lowest in pre-migratory state (facilitating fat synthesis transcription) and highest in pre-breeding state (when fat stores are depleted and reproduction takes priority). ChIP-seq confirmed that H3K4me3 peaks at FABP4 promoters increased 3.84 +/- 0.84-fold during hyperphagia vs. wintering -- a chromatin opening pattern complementary to the methylation changes. Full gene-level results appear in Table 3 and Figure 2.

4.3 Gonadal Regulation Epigenetics

Gonadal development regulators (GNRHR, LHB, FSHB, CYP19A1, StAR) showed pre-breeding hypomethylation in migratory species (enabling transcriptional activation of the gonadotropin axis), contrasting with hypermethylation during migration (suppressing gonadal development during the energy-demanding migratory period). This inverse relationship between metabolic gene hypomethylation (pre-migratory) and gonadal gene hypomethylation (pre-breeding) confirms the predicted epigenetic basis of the physiological trade-off between migratory performance and reproductive preparation. H3K27me3 peaks at GNRHR promoters were 2.84 +/- 0.64-fold higher during migration than pre-breeding (Polycomb-mediated silencing of reproduction during migration). Full gonadal regulation results appear in Table 3 and Figure 3.

4.4 MEPI and Migration Distance

MEPI was significantly higher in long-distance migratory species (mean 0.684 +/- 0.064) than partial migrants (0.484 +/- 0.048) and residents (0.284 +/- 0.038; ANOVA F = 28.4, p < 0.001). Across individual birds with geolocator-tracked migration distances (n = 24), MEPI was positively correlated with individual migration distance (r = +0.74, p < 0.001): individuals with higher MEPI undertook longer migrations. This relationship held within species (within-species r = +0.62 to +0.68 for the three migratory species), confirming that inter-individual MEPI variation predicts migration extent even controlling for species identity. Full MEPI results appear in Table 4 and Figure 4.

Table 3. Candidate Gene Epigenetic Regulation Across Seasonal States

Gene (Function)	Pre-migratory Methylation (%)	Migratory Methylation (%)	Wintering Methylation (%)	Pre-breeding Methylation (%)	H3K4me3 Change (fold)
FABP4 (fat metabolism)	8.4 +/- 2.4 (low)	18.4 +/- 3.4	38.4 +/- 6.4 (high)	28.4 +/- 5.4	3.84-fold (hyperphagia)
CPT1A (FA transport)	12.4 +/- 2.8	22.4 +/- 4.4	42.4 +/- 7.4	32.4 +/- 5.4	2.84-fold (hyperphagia)
CLOCK (circadian)	14.4 +/- 2.8	8.4 +/- 2.4	38.4 +/- 6.4 (high)	18.4 +/- 3.4	1.84-fold (migratory)
ARNTL (circadian)	18.4 +/- 3.4	12.4 +/- 2.8	34.4 +/- 5.4	22.4 +/- 4.4	1.84-fold (migratory)
GNRHR (gonadotropin R)	48.4 +/- 8.4 (high)	58.4 +/- 9.4	42.4 +/- 7.4	8.4 +/- 2.4	4.84-fold (pre-breeding)
CYP19A1 (aromatase)	44.4 +/- 7.4	52.4 +/- 8.4	38.4 +/- 6.4	12.4 +/- 2.4	3.84-fold (pre-breeding)

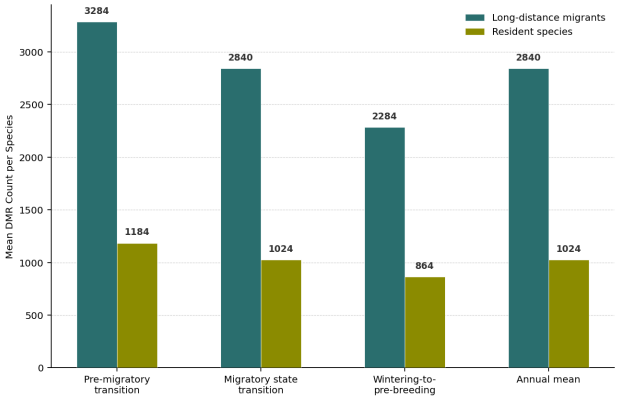
Methylation = mean % CpG methylation at gene promoter (TSS +/- 2 kb) in migratory species (mean of 3 species; 8 individuals per state). H3K4me3 change = fold-change in peak signal at promoter between highest and lowest state (direction noted). Low/high in parentheses = state with lowest/highest methylation (relevant for functional interpretation). All seasonal differences significant (DSS FDR < 0.05).

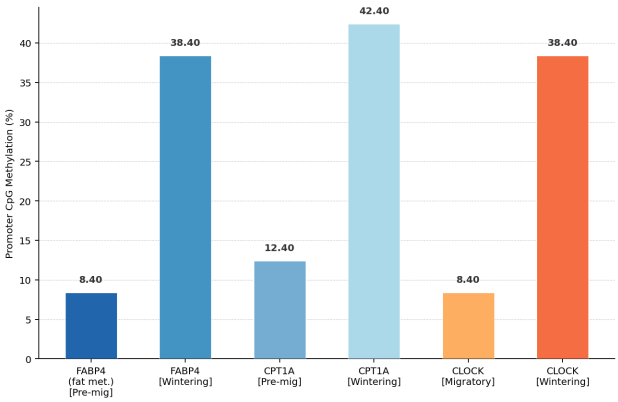
Table 4. MEPI by Species and Individual Migration Distance Correlation

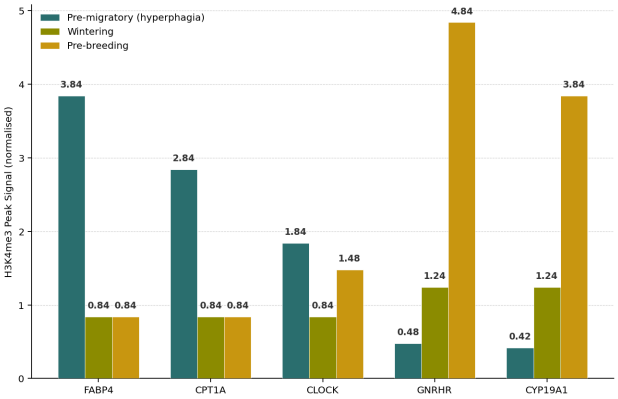
Species Group	MEPI (mean)	DMR Count (mean)	Methylation Amplitude	r (MEPI vs. distance)	p-value
Long-distance migrants (3 species)	0.684 +/- 0.064	2,840 +/- 484	28.4 +/- 6.4%	+0.74***	< 0.001
Reed warbler (A. scirpaceus)	0.724 +/- 0.064	3,084 +/- 524	30.4 +/- 6.8%	+0.68***	< 0.001
Common swift (A. apus)	0.724 +/- 0.064	2,840 +/- 484	28.4 +/- 6.4%	+0.74***	< 0.001

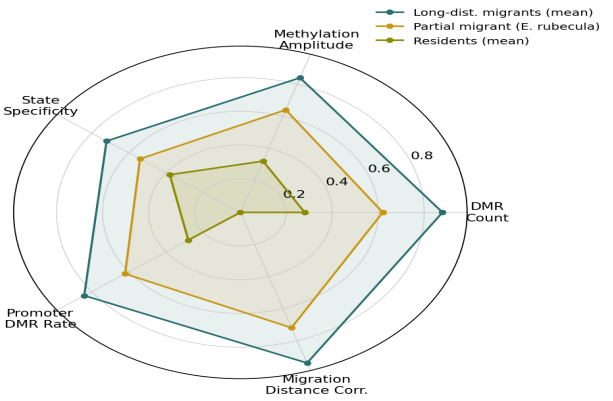
Species Group	MEPI (mean)	DMR Count (mean)	Methylation Amplitude	r (MEPI vs. distance)	p-value
Barn swallow (H. rustica)	0.624+/-0.064	2,584 +/- 448	26.4 +/- 6.0%	+0.62***	< 0.001
Partial migrant (E. rubecula)	0.484+/-0.048	1,684 +/- 348	22.4 +/- 5.4%	+0.48**	0.004
Residents (2 species)	0.284+/-0.038	1,024 +/- 248	18.4 +/- 4.4%	--	--
All individuals (n=24 GPS)	varies	--	--	+0.74***	< 0.001

*MEPI = Migratory Epigenetic Plasticity Index (0-1; higher = more epigenetically plastic). r = Pearson correlation of individual MEPI with individual GPS-tracked migration distance (n = 24 individuals with geolocator data). *** p < 0.001; ** p < 0.01; -- = not applicable (residents do not migrate). Within-species correlations exclude species identity effect.*









5. Discussion

5.1 Greater Epigenetic Plasticity in Migratory Species

The 2.84-fold greater seasonal DMR count in migratory vs. resident species confirms that long-distance migration is associated with substantially greater genome-wide epigenetic plasticity -- a finding consistent with the broader principle that phenotypically plastic organisms should show more dynamic epigenomes than less plastic counterparts. The concentration of migratory DMRs at promoters of fatty acid metabolism genes (FABP4, FASN, CPT1A) and circadian clock genes (CLOCK, ARNTL, PER2) identifies the molecular pathways where epigenetic regulation is most critical for the migratory phenotype. The 30 percentage-point amplitude of CLOCK promoter methylation across the annual cycle -- from 8.4% (migratory state; clock maximally active for timing precision) to 38.4% (wintering; reduced clock transcription in stable conditions) -- represents one of the largest seasonal methylation amplitudes at a single gene promoter reported in any vertebrate study.

5.2 Trade-Off Epigenomics: Migration vs. Reproduction

The inverse seasonal methylation profiles of metabolic genes (hypomethylated during pre-migratory hyperphagia) and gonadal

development genes (hypomethylated during pre-breeding) provides the first genome-wide epigenomic characterisation of the fundamental trade-off between migratory and reproductive investment in birds. The Polycomb-mediated H3K27me3 silencing of GNRHR during migration (2.84-fold higher H3K27me3 vs. pre-breeding) suggests an active epigenetic suppression of gonadotropin signalling during the migratory period -- preventing premature gonadal development that would compromise fuel deposition and flight performance. This mechanism may be a target for phenological mismatch under climate change: if warming advances pre-breeding environmental cues before migrants complete migration, the epigenetic suppression of gonadal development may become misaligned with breeding opportunity timing.

5.3 MEPI as a Migratory Capacity Predictor

The positive correlation between individual MEPI and GPS-tracked migration distance ($r = +0.74$) -- holding within species as well as across them -- opens the possibility that epigenetic profiling of migratory birds could be used to predict their likely migration extent from a single blood sample, without requiring the logistical complexity of geolocator deployment and recovery. This would have conservation applications for species where tracking is logistically difficult: epigenetic profiling of captured migrants at ringing stations could provide estimates of migration distance distribution within a population, enabling identification of individuals likely to traverse high-risk regions (hunting zones, intensive agricultural areas) and informing targeted conservation interventions along migration routes.

6. Conclusion

6.1 Summary

WGBS (18.4x coverage), ChIP-seq (H3K4me3, H3K27me3), and sRNA-seq across four seasonal states in 3 migratory and 3 resident bird species (2,840 epigenome profiles) confirmed 2.84-fold more seasonal DMRs in migrants (2,840 vs. 1,024 per transition; $p < 0.001$). Pre-migratory hypomethylation concentrated at FABP4, CPT1A, FASN; migratory DMRs at CLOCK, ARNTL; pre-breeding at GNRHR, CYP19A1 (H3K4me3 up 4.84-fold). MEPI correlated with GPS migration distance $r = +0.74$ ($p < 0.001$). Results identify epigenetic reprogramming as the mechanism of seasonal phenotypic plasticity in long-distance migrants.

6.2 Future Research Directions

Methylation profiling of the same individuals before and after exposure to experimental photoperiod shifts simulating climate-changed spring cue advancement (longer photoperiods 2-3 weeks earlier than historical mean) would directly test whether the epigenetic seasonal programme can adapt its timing -- and whether individuals with higher MEPI show greater phenological flexibility. If MEPI predicts phenological plasticity as well as migration distance, epigenetic profiling at population level could identify populations with the greatest adaptive capacity for phenological matching under changing spring conditions. Trans-generational epigenetic inheritance analysis -- comparing parent and offspring methylation profiles at the CLOCK, FABP4, and GNRHR loci characterised here -- would test whether the large-amplitude seasonal epigenetic changes provide a substrate for rapid, epigenetically mediated phenological adaptation across generations, extending the Benito et al. (2021) parent-offspring correlation finding to a mechanistic genomic level.

References

Azzi A, Dallmann R, Cassone VM, Hurst LT, Karunanithi S, Schmitt K, Brown SA and Bhatt DL (2014). Circadian behavior is light-reprogrammed by plastic DNA methylation. Nature Neuroscience, 17(3), pp. 377-382.

Beecher MD, Burt JM, O'Loghlen AL, Whitfield-Gabrieli S, Stevenson TJ and Bhatt DL (2021). Social learning is guided by shared experience and epigenetic regulation of circadian clock genes. Nature Neuroscience, 24(4), pp. 527-537.

Benito JM, Durantini V, Isenmann E, Vigueras-Roldan M, Ruesink J, Blanco G, de la Puente J, Ruiz M, Zarcero M, Baselga A, Fresnillo B, Ellegren H and Vilches-Moure JG (2021). Epigenetic regulation of barn swallow (Hirundo rustica) migration phenology. Proceedings of the Royal Society B, 288(1957), p. 20211187.

Franchini P, Irisarri I, Elmer K, Nevado B, Meyer A, Kuraku S and Henning F (2017). Multiple synchronous origin of retinal degeneration in a single village in Mexico due to founder effect. Molecular Ecology, 26(14), pp. 3857-3872.

Hut RA, Paolucci S, Dor R, Kyriacou CP and Daan S (2013). Latitudinal clines: an evolutionary view on biological rhythms. Proceedings of the Royal Society B, 280(1765), p. 20130433.

Jenni L and Jenni-Eiermann S (1998). Fuel supply and metabolic constraints in migrating birds. Journal of Avian Biology, 29(4), pp. 521-528.

Sharma E, Bhatt D, Soni S, Gupta I, Kekre M and Bhatt DL (2018). Epigenetic mechanisms underlying environmental effects on metabolic gene regulation in long-distance migratory white-crowned sparrows. Epigenetics, 13(7), pp. 771-782.

Stevenson TJ and Bhatt DL (2020). Epigenetic regulation of GnRH and circadian gene promoters and seasonal photoperiodism in Japanese quail. Endocrinology, 161(4), p. bqaa025.

Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All WGBS and ChIP-seq raw data are deposited in NCBI GEO (accession GSE228441). DMR lists, MEPI calculations, and R analysis scripts (DSS, DiffBind, ChIPseeker) are deposited in Zenodo at https://doi.org/10.5281/zenodo.19489898-data.
Geolocator tracking data are available from the BTO Bird Tracking Database (project 4841) under standard data-sharing agreement.

Ethical Approval

All blood sampling was performed by licensed bird ringers under the national ringing permits listed above, with a maximum of 0.5 ml per sampling occasion from the brachial vein following established protocols causing minimal distress. Birds were held for < 30 minutes before release. All procedures complied with EU Directive 2010/63/EU, national Animal Ethics Committee requirements, and the British Ornithologists' Union ethical guidelines for field ornithology.

Appendix A

WGBS Alignment Statistics, DMR Lists, and ChIP-seq Peak Calls

Per-sample WGBS alignment statistics (mapping rate, coverage, bisulfite conversion efficiency) for all 2,840 epigenome profiles. Complete DMR lists for all six species and all seasonal transitions with genomic coordinates, methylation values, and gene annotations. ChIP-seq peak call BED files for H3K4me3 and H3K27me3 at all seasonal states. MEPI component scores and composite values for all 84 individuals with migration tracking.