



Time trees and Clock genes: a Systematic Review and Comparative Analysis of contemporary Avian Migration Genetics

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1 Time trees and Clock genes: a Systematic Review and Comparative 2 Analysis of contemporary Avian Migration Genetics

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27
28 **Short title:** Time trees and Clock genes.

29 **Abstract**

30 Timing is a crucial aspect for survival and reproduction in seasonal environments
31 leading to carefully scheduled annual programs of migration in many species. The
32 exact mechanisms through which birds (class: Aves [Linnaeus, 1758]) can keep track
33 of time, anticipate seasonal changes, and adapt their behaviour, has become a subject
34 of inquiry. One proposed mechanism regulating annual behaviour is the circadian
35 clock, controlled by a highly conserved set of genes, collectively called “clock genes”
36 which are well established in controlling the daily rhythmicity of physiology and
37 behaviour. Due to diverse migration patterns observed within and between species, in
38 a seemingly endogenously programmed manner, the field of migration genetics has
39 sought and tested several candidate genes within the clock circuitry that may portend
40 the observed differences in breeding and migration behaviour. Among others, length
41 polymorphisms within genes such as the *Clock* and *Adcyap1* have been hypothesised
42 to play a putative role, though association and fitness studies in various species have
43 yielded mixed results. To contextualize the existing body of data, here we conducted
44 a systematic review of all published studies relating polymorphisms in clock genes to
45 seasonality in a phylogenetically and taxonomically informed manner. This was
46 complemented by a standardised comparative re-analysis of candidate gene
47 polymorphisms of 76 bird species, of which 58 are migrants and 18 are resident.
48 Genetic diversity estimates were assessed, Mantel tests performed, and Phylogenetic
49 generalised least square models fitted to evaluate relationships between candidate
50 gene allele length and population averages for geographic range, migration distance,
51 timing of migration, taxonomic relationships, and divergence times. The combined
52 meta-analysis provided evidence (i) of an association between *Clock* gene variation
53 and Autumn migration as well as an association between *Adcyap1* gene variation and
54 Spring migration in migratory species, (ii) that these candidate genes are not
55 diagnostic markers to distinguish migratory from sedentary birds, and (iii) of correlated
56 variability in both genes to divergence time, potentially reflecting ancestrally inherited
57 genotypes rather than contemporary changes driven by selection. These finding
58 highlight a tentative association between candidate genes and migration attributes as
59 well as genetic constraints to evolutionary adaptation.

60 **Keywords:** Migration, Birds, Circadian, *Clock*, *Adcyap1*, Candidate genes,
61 Phylogenetic, Time trees, Divergence times, Ornithology.

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104 I. INTRODUCTION

105 Each year, billions of birds take to the sky to make their annual journey from non-breeding
106 to breeding grounds often migrating at night with several key stop-over sites to refuel and
107 rest along well-established flyways. This event occurs like clockwork, carefully timed to
108 ensure an optimal flight to reach their destination. These annual flight plans are
109 orchestrated to balance day length (Sockman & Hurlbert, 2020), night time visibility
110 (Brown & Mewaldt, 1968; Pyle *et al.*, 1993; Norevik *et al.*, 2019), and time spent at staging
111 sites (Roques *et al.*, 2022). The repeated occurrence of this characteristic behaviour on
112 an annual basis with seemingly little temporal deviation has sparked interest in the field
113 of chronobiology, with emphasis on both intrinsic as well as environmental cues
114 contributing to timekeeping in birds (Åkesson *et al.*, 2017).

115 One proposed mechanism of intrinsic annual timekeeping is the circadian clock,
116 which regulates daily activity in almost every organism from bacteria to mammals
117 (Aguilar-Roblero, 2015). The circadian clock comprises several genes which can be
118 defined as: "... genes that interact with each other to make up an auto-regulatory
119 feedback loop, in which its activation and repression cycle takes about one day" (Albrecht
120 & Ripperger, 2009). Circadian clock genes therefore have a central axis with a positive
121 feedback loop, which promotes transcription, and a negative feedback loop, which
122 prevents transcription (detailed in **Figure 1**), and the expression levels of these key
123 elements fluctuate throughout the day in different tissues (Albrecht & Ripperger, 2009;
124 Aguilar-Roblero, 2015).

125 The circadian clock is tied to many extrinsic stimulating factors (*Zeitgebers*) that
126 oscillates the expression levels within the brain, such as photoperiod (length of
127 sunlight; Leclerc *et al.*, 2010), changing temperatures (Jenni & Kéry, 2003; Pancerasa
128 *et al.*, 2018), and availability of food sources (Hau & Gwinner, 1996; Stephan, 2002;
129 Scheuerlein & Gwinner, 2002). For example, as the photoperiod changes due to the
130 orbit of the earth and seasonality, with days becoming shorter and nights becoming
131 longer (see **Figure 2**), the internal phase of the circadian clock adjusts in order to
132 maintain appropriate sleep-wake cycles in a process known as entrainment (Albrecht
133 & Ripperger, 2009; Robart, McGuire, & Watts, 2018). As photoperiod is tied to latitude,
134 this partially explains how environmental changes modulate the circadian clock on a
135 circannual basis possibly driving the timing of migration (see **Figure 3**).

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2
3 136 For many migratory bird species it has been demonstrated that they persistently
4
5 137 exhibit seasonally appropriate migration related behaviours, such as migratory
6
7 138 restlessness (*Zugunruhe*) and moulting phenology, even when kept under constant
8
9 139 conditions in captivity (Newton, 2007; Aguilar-Roblero, Díaz-Muñoz, & Fanjul-Moles,
10
11 140 2015). Therefore, the intrinsic clock can function in isolation of environmental factors
12
13 141 and may be under genetic control, although the possibility of an epigenetic effect has
14
15 142 also been proposed (Saino *et al.*, 2017; Merlin & Liedvogel, 2019).

15 143 One confounding observation for the argument of migration being a genetically
16
17 144 programmed behaviour is the occurrence of differential migration patterns within a
18
19 145 single species. Differential migration is the observation that specific populations or
20
21 146 groups within a single species follow divergent migratory strategies. These patterns of
22
23 147 differential migration include differences in the timing, direction, duration, or distance
24
25 148 of migration as well as the occurrence of migration behaviour; as many bird species
26
27 149 do not exhibit migratory behaviour but instead form resident populations within their
28
29 150 range. The current assumption surrounding resident populations of bird species is that
30
31 151 the ancestors of current species were all migratory and that species adapted resident
32
33 152 behaviour on an individual basis (de Zoeten & Pulido, 2020).

32 153 For example, species such as the Streaky-breasted flufftail, *Sarothrura boehmi*
34
35 154 [Reichenow, 1900] (Taylor & Kirwan, 2020) and European bee-eater, *Merops apiaster*
36
37 155 [Linnaeus, 1758] (Fry & Boesman, 2020), show distinct populations of resident and
38
39 156 migratory birds despite being classified as a single species. While migration studies
40
41 157 on other species, such as congeneric spotted eagle hybrids between *Clanga clanga*
42
43 158 [Pallas, 1811] and *Clanga pomarine* [Brehm, 1831], have shown that hybridization
44
45 159 between species with divergent movement patterns results in a new population with
46
47 160 migration strategies that differ from their progenitors (Väli *et al.*, 2018). Furthermore,
48
49 161 birds like the Common chaffinch, *Fringilla coelebs* [Linnaeus, 1758], from their
50
51 162 Scandinavian range, were first described by Linnaeus as a “bachelor bird” due to the
52
53 163 earlier timing and longer migration of females while males from the same population
54
55 164 appeared nearly resident year-round. In contrast, in most species the males are often
56
57 165 the first to depart or migrate farther (Dierschke, Mendel, & Schmaljohann, 2005;
58
59 166 Briedis *et al.*, 2019). This raises the pertinent question as to how differential responses
60
167 to environmental stimuli, and resulting variable migration patterns, are established and

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2
3 168 maintained between individuals within a species or between closely related sub-
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5 169 species—if migration behaviour is a genetically programmed trait.

6
7 170 Due to the potential for genetic studies to complement behavioural studies,
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9 171 several approaches have been evaluated for the identification of putative polymorphic
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11 172 repeats within clock regulating genes that could be used in behaviour association
12
13 173 studies of birds (Steinmeyer, Mueller, & Kempenaers, 2009). The first included the
14
15 174 identification of polymorphic genes in the Blue tit (recently separated into the Eurasian
16
17 175 blue tit, *Cyanistes caeruleus* [Linnaeus, 1758], and African blue tit, *Cyanistes*
18
19 176 *teneriffae* [Lesson, 1831]). This study also developed the methods to successfully
20
21 177 assay the identified polymorphisms in Eurasian blackcap (*Sylvia atricapilla* [Linnaeus,
22
23 178 1758]) populations from which most other studies emanated. Several candidate genes
24
25 179 have since been proposed and tested including Circadian Locomotor Output Cycles
26
27 180 Protein Kaput (*Clock*), Adenylate Cyclase Activating Polypeptide 1 (*Adcyap1*), CAMP
28
29 181 responsive element binding protein 1 (*CREB1*), Neuronal PAS domain protein 2
30
31 182 (*NPAS2*), and Dopamine Receptor D4 (*DRD4*). Of these genes, the first two, *Clock*
32
33 183 and *Adcyap1*, have been studied extensively.

34
35 184 The *Clock* gene (detailed in **Figure 4**) is located on the 4th chromosome at the
36
37 185 12th band of the q-arm in humans and on chromosome 4 in chickens (*Gallus*
38
39 186 *domesticus* [Linnaeus, 1758]). Towards the 3'-end there is a polyglutamine repeat
40
41 187 (Poly-Q) region that tends to vary in length between and within species resulting in
42
43 188 shorter and longer alleles. The second gene, *Adcyap1* (detailed in **Figure 5**), encodes
44
45 189 the Pituitary-Adenylate Cyclase-Activating Polypeptide (PACAP) protein that
46
47 190 stimulates the production of melatonin in the pineal gland, thereby conveying light
48
49 191 information from the retina to the brain, regulating the circadian rhythm (Simonneaux,
50
51 192 Ouichou, & Pévet, 1993; Hannibal *et al.*, 1998). The *Adcyap1* gene is located on the
52
53 193 18th chromosome of the human genome, at band 11 and sub-band 32 of the p-arm
54
55 194 and on chromosome 2 in chickens. The 3'-untranslated region (UTR) contains a
56
57 195 homopolymer run of adenines (A) and guanines (G) which vary in length within and
58
59 196 between species resulting in shorter and longer alleles.

60
61 197 Studies have aimed to answer questions surrounding differential migratory
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63 198 behaviour by comparing migratory attributes (or related breeding phenology) of
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65 199 individuals or populations to putative variation within candidate clock genes (Caprioli
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67 200 *et al.*, 2012; Saino *et al.*, 2015; Delmore *et al.*, 2016). The central hypothesis of these

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3 201 candidate gene studies can be summarised as follows: variation, in the form of length
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5 202 polymorphisms, within genes associated with the circadian clock may result in
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7 203 differential responses to environmental cues due to delayed or enhanced entrainment,
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9 204 resulting in differences in migration behaviour which may drive early speciation
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11 205 through a migratory divide.

12 206 A significant association between candidate gene variability and factors
13
14 207 contributing to migration patterns, annual synchronicity in life events, or geographical
15
16 208 processes has been illustrated in multiple lineages within the order Passeriformes,
17
18 209 including Palearctic and Nearctic warblers (Bazzi *et al.*, 2017; Ralston *et al.*, 2019),
19
20 210 swallows (Caprioli *et al.*, 2012; Bazzi *et al.*, 2015), tits (Liedvogel *et al.*, 2009), chats
21
22 211 (Justen *et al.*, 2022), and flycatchers (Kuhn *et al.*, 2013); whilst associations were not
23
24 212 clear or absent for other lineages (Contina *et al.*, 2018; Parody-Merino *et al.*, 2019).
25
26 213 Additionally, although some heritable pattern was observed in a study comparing
27
28 214 migratory species from several lineages that share the same trans-Saharan migratory
29
30 215 flyway (Bazzi *et al.*, 2016a), a cross-species comparative study on a subset of
31
32 216 candidate genes failed to detect a clear, generalised relationship between clock gene
33
34 217 diversity and divergent migration behaviour in terms of migratory versus resident bird
35
36 218 species (Lugo Ramos, Delmore, & Liedvogel, 2017).

37 219 Thus, the current literature on the putative role of these polymorphisms in
38
39 220 shaping differential migration among bird species presents conflicting evidence that
40
41 221 needs to be clarified to reframe our understanding of migration genetics. Furthermore,
42
43 222 the context within which the initial data was interpreted need reappraisal due to
44
45 223 the frequent taxonomic revisions that occur in bird classification and the disparities
46
47 224 that exist between phylogeny and taxonomy for species concepts (Sangster, 2014).
48
49 225 This is of particular importance as understanding how a biological clock is able to
50
51 226 anticipate environmental cues and adapt to them, along with any potential genetic
52
53 227 constraints, is informative to conservation given the potential effect of climate change
54
55 228 and habitat erosion on migratory behaviour (Carey, 2009).

56 229 The aim of this study is to (1) systematically synthesise and review the existing
57
58 230 literature on polymorphisms in clock genes, primarily within the context of migration,
59
60 231 breeding, and annual life events, to probe for existing patterns, (2) perform a
232 comparative analysis of the existing data to test for an association between clock
233 genes and attributes of migration such as latitude, distance, and timing, and (3)

234 contextualise the existing evidence from the review and the comparative analysis in a
235 taxonomic, phylogenetic, and paleogeographic informed manner to compare effects
236 within and between lineages with shared evolutionary histories.

237

238 II. SUMMARY OF METHODS

239 (1) Literature search and systematic review

240 A systematic approach was used to search for and synthesise the available literature.
241 Literature was searched on the Scopus (www.scopus.com) and Dimensions
242 (www.dimensions.ai) databases using the following Boolean search string: ("Clock
243 genes" OR "Clock" OR "Adcyap1") AND ("Birds" OR "Avian") AND ("Migration" OR
244 "Flying"). Search results were exported in the comma separated value format and the
245 literature was subsequently summarised, guided by citation networks visualised using
246 CitNetExplorer 1.0.0 and VOSviewer 1.6.16 (van Eck & Waltman, 2017). The results
247 retrieved from Scopus was converted to the appropriate format with the R package
248 'Scopus2CitNet 0.1.0.0' in RStudio 1.4.1106 (RStudio Team, 2021), running R version
249 4.0.5 (R Core Team, 2020). Due to the diverse array of species in which these studies
250 have been conducted, the literature was first organised by year of publication followed
251 by species, with taxonomic grouping based firstly on order, as either passerine or non-
252 passerine, followed by families. Families were grouped, based on higher taxonomic
253 classifications, in superfamilies and parvorders for the sake of a concise and cohesive
254 comparison.

255 (2) Species

256 Species for comparative analysis were selected based on existing literature available
257 on either the *Clock* or *Adcyap1* gene in relation to migration phenology and/or for which
258 genomic or transcriptomic studies have been conducted, including unpublished data
259 from eight species. As most studies thus far have focused on Palearctic and Nearctic
260 birds, species were further complemented with migrant and resident bird species from
261 other locations to have a globally distributed dataset. This included the addition of
262 several species of Manakins, resident birds found in the equatorial parts of the
263 Neotropics, the endangered island restrained Elfin woods warbler, *Setophaga angelae*
264 [Kepler & Parkes, 1972], and the Australasian Superb fairy-wren, *Malurus cyaneus*
265 [Ellis, 1782]. Our final dataset included 76 species, 76 for *Clock* and 71 for *Adcyap1*,

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2
3 266 of which 58 were classified as migrants and 18 were classified as residents. Migrants
4 were species with complete or partial migratory behaviour (used to describe species
5 267 that have both resident and migrating populations, in this context species with a
6 268 singular resident population which was not sampled were treated as generally
7 269 migratory), while the term resident refers to species that do not follow an annual cycle
8 270 of migration (although some do follow a pattern of altitudinal migration within their
9 271 resident range, a small subset included species that have a single migratory
10 272 population that were not sampled and were treated as generally resident).
11 273

17 274 **(3) Genetic data**

19 275 Data was summarized from the literature based on species, number of alleles, most
20 276 common allele, and observed heterozygosity when available. Additional information
21 277 such as the number of extant and presently recognised subspecies was retrieved from
22 278 Birds of the World (Billerman *et al.*, 2020). *Clock* and *Adcyap1* data for additional
23 279 species, from a wider geographic distribution and including additional resident birds,
24 280 was retrieved from the National Centre for Biotechnology Information (NCBI) website
25 281 using Basic Local Alignment and Search Tool (BLAST) searches (Altschul *et al.*, 1990)
26 282 against reference genomes and available databases, including PopSet and Nucleotide
27 283 (Agarwala *et al.*, 2018); where no sequence data was available a further BLAST
28 284 search was done against the Sequence Read Archive (SRA) for specific species.
29 285 Population level allele data was retrieved for 40 species in total, 39 for *Clock* and 37
30 286 for *Adcyap1*, from either the supplementary material of the article or the online data
31 287 repositories Dryad (www.datadryad.org), Figshare (www.figshare.com), or additional
32 288 data received directly from authors (see acknowledgements). *Clock* data was
33 289 transformed to only represent the actual number of poly-Q repeats, as different studies
34 290 used different primers resulting in variable lengths in the raw data. *Adcyap1* was
35 291 consistently amplified and sequenced with the same primer set or region facilitating
36 292 between study comparisons. Summary statistics for the data is indicated in **Table 4**
37 293 and **Table 5**.

52 294 **(4) Migration and range data estimates**

53 295 Migration data was computed using QGIS 3.16.15 (QGIS Development Team, 2022)
54 296 from shapefiles extracted from the geodatabase of distribution maps compiled by
55 297 BirdLife International, version 2021.1 (BirdLife International & Handbook of the Birds
56
57
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1
2
3 298 of the World, 2021), supplemented with shapefile data from eBird (Fink *et al.*, 2021).
4
5 299 Centroids were computed for the non-breeding and breeding ranges to determine the
6
7 300 average coordinates by latitude and longitude (in degrees) for each. Furthermore, the
8
9 301 average migration distance between centroids was calculated (in meters). Geographic
10
11 302 distance matrixes for the breeding and non-breeding coordinates of each species were
12
13 303 generated with the java application Geographic Distance Matrix Generator 1.2.3
14
15 304 (Ersts, 2012) in degrees. Species classified as partial migrants, for which breeding
16
17 305 and non-breeding range data was available, were treated as migrant, while data
18
19 306 deficient partial migrants were treated as resident birds. For resident species, the data
20
21 307 was computed only once based on the full range. Summary statistics for the data is
22
23 308 indicated in **Table 4** and **Table 5**.

24 309 Population and species specific migration dates, not stated in publications, were
25
26 310 retrieved from eBird (Fink *et al.*, 2021) and, in the case of species sampled in Italy,
27
28 311 supplemented with data from the Italian bird migration atlas (Spina & Volponi, 2008,
29
30 312 2009). A similar approach was used for data on buntings and larks from Asia (Ali,
31
32 313 Ripley, & Roberts, 1999). As seasons and migration dates vary by hemisphere, dates
33
34 314 were normalised to a standard reference point (Sockman & Hurlbert, 2020) that
35
36 315 roughly corresponds to the photoperiod and temperature (**Figure 2** and **3**). The
37
38 316 difference in days was calculated between the start, middle and end dates for Spring
39
40 317 migration and the Summer solstice (approximately 15h light and 9h dark) and Spring
41
42 318 equinox (12h light and 12h dark), while Autumn migration dates were normalised in
43
44 319 reference to the Winter solstice (approximately 9h light and 15h dark) and Autumn
45
46 320 equinox (12h light and 12h dark), of each respective hemisphere depending on the
47
48 321 species range.

49 322 **(5) Population genetics**

50 323 POPGENE 1.32 (Yeh *et al.*, 1997) was used to test for Hardy-Weinberg (Hardy, 1908;
51
52 324 Weinberg, 1908) equilibrium using Chi-squared (χ^2) tests (with significance measured
53
54 325 at $\alpha = 0.02$), to calculate the observed (H_o) and expected (H_e) heterozygosity, and to
55
56 326 create a genetic distance matrix using F_{ST} values. Python for Population Genetics
57
58 327 (PyPop) version 0.7.0 (Lancaster *et al.*, 2007) was used to test for selection and
59
60 328 neutrality as well as linkage disequilibrium. Neutrality was assessed using Slatkin's
329
330 329 implementation (Slatkin, 1994) of the Ewens-Watterson (Ewens, 1972; Watterson,
1977) test, with the probability values expressed as the relative degree at which the

1
2
3 331 observed F -value occurs in a sample distribution with simulation run with 10,000
4
5 332 repeats. Linkage disequilibrium was assessed using two measures: the overall
6
7 333 Linkage Disequilibrium, D' (Hedrick, 1987), and Cramer's V Statistic, W_n (Cramer,
8
9 334 1946). A P -value < 0.05 is indicative of overall significant linkage disequilibrium.

10 335 **(6) Mantel tests**

11
12
13 336 Mantel tests (Mantel, 1967) were conducted using the Mantel 2.1.0 (Carr, 2021)
14
15 337 package in Python 3.9 (Python Team, 2021). Test were done to compare genetic
16
17 338 distance within two candidate genes and attributes of migration including the distance
18
19 339 between latitude of both breeding and non-breeding ranges among species, as well
20
21 340 as the relationship between the genetic distance and taxonomic distance and
22
23 341 divergence times as measures of evolutionary distance, to assess the strength of
24
25 342 heritability of genotypes within lineages.

26
27 343 Test were run between the genetic distance matrixes, generated with CONVERT
28
29 344 1.31, the geographic distance matrixes, generated with Geographic Distance Matrix
30
31 345 Generator 1.2.3 (for both breeding and non-breeding coordinates), the taxonomic
32
33 346 distance matrixes, generated using the R package vegan 2.5-7 (Oksanen *et al.*, 2020),
34
35 347 and the divergence times matrix. Divergence times between pairs of species, in
36
37 348 millions of years ago (MYA), were retrieved from the Time Tree resource (Kumar *et al.*,
38
39 349 2017) website (www.timetree.org) using a custom Python script called Python
40
41 350 Automated Retrieval of Time Trees (PAReTT version 1.0.1) and exported as a
42
43 351 vectorised matrix. A P -value < 0.02 and Z -value > 1.96 (or < -1.96) is considered
44
45 352 significant.

46 353 **(7) Phylogenetic Generalised Least Squares analysis**

47
48 354 Phylogenetic generalised least square (PGLS) models were fitted individually using
49
50 355 the R package caper 1.0.1 (Orme *et al.*, 2018) to avoid error from repeat sampling.
51
52 356 PGLS was used to relate both *Clock* and *Adcyap1* length to breeding and non-
53
54 357 breeding latitude as the distance from the equator in degrees, as well as to total
55
56 358 migration distance between regions and to the normalised dates for the beginning,
57
58 359 middle, and end of Spring and Autumn migration, assuming Brownian motion and a
59
60 360 lambda (λ) = 1.0. Phylogenetic signal for each gene was measured using the R
61
62 361 package phytools 0.7-90 (Revell, 2012) to compute both lambda and kappa for the
63
64 362 gene and tree data and verify the presence of Brownian motion. The tree used for the

1
2
3 363 phylogeny was generated from the 'Ericson' phylogeny (Jetz *et al.*, 2012) by sampling
4 364 5000 trees from the Bird Tree website (www.birdtree.org). The trees were summarised
5 365 to a 60% consensus tree by maximum clade credibility using TreeAnnotator 2.6.3, part
6 366 of BEAST 2.6.3 (Bouckaert *et al.*, 2014), with a ten percent burn-in. The final tree was
7 367 edited in BioRender.com.

12 368 **(8) Time trees and paleogeography**

14 369 Time trees were computed from calibrated divergence time estimates using the Time
15 370 Tree resource (Kumar *et al.*, 2017) to visualise the evolutionary history and
16 371 relatedness of study species in terms of shared common ancestry and the length of
17 372 time individual lineages have been evolving independently. Trees were downloaded
18 373 from the website (www.timetree.org) with a custom Python script called Python
19 374 Automated Retrieval of Time Trees (PARETT version 1.0.1). The relevant topography
20 375 of Earth for each time period was also reconstructed in GPlates 2.3.0 (Müller *et al.*,
21 376 2018) with the PALEOMAP paleoAtlas (Scotese, 2016) to visualise relevant barriers
22 377 to gene flow, and potential differences in selective forces between modern and
23 378 historical geography, for each time period, that may have contributed to selection and
24 379 speciation across the genomes of study species.

33 380

35 381 **III. SYSTEMATIC REVIEW OF PUBLISHED STUDIES**

37 382 The results of studies assaying candidate gene polymorphisms and comparing them
38 383 to relevant attributes of seasonal phenology are summarised in **Tables 1** and **2**.
39 384 Several non-candidate-gene studies, conducted at the genome, epigenome, or
40 385 transcriptome level, are summarised in **Table 3** as such studies have frequently
41 386 complemented candidate gene studies in terms of species covered. Because many
42 387 biological traits among birds are highly heritable within lineages (Silva *et al.*, 2017;
43 388 Lamichhaney *et al.*, 2018; Cava, Perlut, & Travis, 2019), study species are
44 389 summarised and discussed according to their taxonomic and phylogenetic relatedness
45 390 illustrated in **Figure 6**, and the time tree of estimated divergence times of lineages
46 391 illustrated in **Figure 7**.

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2
3 392 **(1) *Passerine birds***
4

5
6 393 **(a) *Tits (Family: Paridae, 10 MYA)***
7

8 394 Johnsen *et al.* (2007) were the first to investigate if polymorphic differences in the
9
10 395 *Clock* gene exist within avian populations based on evolutionary and functional studies
11
12 396 of allelic variants of fruit flies (*D. melanogaster*) circadian clock genes (Tauber &
13
14 397 Kyriacou, 2005), and their selective differences between Northern and Southern
15
16 398 regions. They characterised *Clock* gene variation in the Poly-Q regions of two bird
17
18 399 species focally in the context of breeding phenology. The focal species investigated in
19
20 400 this study were fourteen populations of the Blue tit, including subspecies, across a
21
22 401 latitudinal gradient. Their primary findings found evidence of significant relationships
23
24 402 between latitudinal clines in habitat and *Clock* gene lengths between thirteen of the
25
26 403 studied Blue tit populations, as well as significant levels of allelic differentiation in a
27
28 404 between-subspecies analysis. The most common allele was the Q₁₂ allele which
29
30 405 represented 44% or more of the genotypes, accounting for all individuals in the Italian
31
32 406 subpopulation. From this they concluded that, at least in the Blue tit population, the
33
34 407 longer alleles correspond to greater clines in habitat location, partially distinguishes
35
36 408 subspecies, and reveals genetic geographic processes not associated with other
37
38 409 microsatellite markers (Johnsen *et al.*, 2007). Studies isolated to a single Blue tit
39
40 410 population of the Oxfordshire woods in the United Kingdom and timing of breeding
41
42 411 (Liedvogel *et al.*, 2009) and clutch size (Liedvogel, Cornwallis, & Sheldon, 2012)
43
44 412 across a two year period found that shorter alleles corresponded to earlier breeding,
45
46 413 shortened incubation, and higher fledgeling rates among females whilst allele
47
48 414 frequencies were homogenously distributed. This served as further supporting
49
50 415 evidence that *Clock* gene polymorphisms can be linked to adaptations to local
51
52 416 environments and may explain population variation in Blue tits. Similar phenotypic
53
54 417 correlates were, however, found to be absent from the sympatric Great tit, *Parus major*
55
56 418 [Linnaeus, 1758], which indicates that correlates between behaviour and *Clock*
57
58 419 polymorphisms may not be universal to all passerine birds (Liedvogel & Sheldon,
59
60 420 2010), although studies on period length within this species have found there to be
421 distinct heritable differences in their migratory behaviour (Helm & Visser, 2010).

1
2
3 422 (b) *Warblers* (Families: *Acrocephalidae*, *Parulidae*, *Phylloscopidae*, *Sylviidae*, 30
4 423 MYA)

5
6
7 424 *Clock*, *Adcyap1*, *NPAS2*, and *CREB1* genes were studied in several populations of
8
9 425 Eurasian blackcap (Mueller, Pulido, & Kempenaers, 2011) for relatedness to migratory
10
11 426 behaviour by comparing length polymorphisms to individual calculated migratory
12
13 427 distance; including samples from populations that together represent most recognised
14
15 428 subspecies. A fifth gene, *DRD4*, which has been linked to “exploratory behaviour” in
16
17 429 Great tit populations (Korsten *et al.*, 2010; Mueller *et al.*, 2013a) was also analysed in
18
19 430 parallel. Only the *Adcyap1* gene rendered significant results and longer alleles were
20
21 431 positively associated with a higher latitude of breeding while shorter alleles
22
23 432 corresponded to higher degrees of migratory restlessness. The absence of a role for
24
25 433 *DRD4* in warblers has also been illustrated in the Seychelles warbler, *Acrocephalus*
26
27 434 *sechellensis* [Oustalet, 1877] in later studies (Edwards *et al.*, 2015). Both *Clock* and
28
29 435 *Adcyap1* were later assayed in several warbler species across four families including
30
31 436 *Acrocephalidae*, *Parulidae*, *Phylloscopidae*, and *Sylviidae*.

32
33 437 In the family *Acrocephalidae*, Bazzi *et al.* (2016a) studied four species:
34
35 438 Eurasian reed warbler, *Acrocephalus scirpaceus* [Hermann, 1804]; Great reed
36
37 439 warbler, *Acrocephalus arundinaceus* [Linnaeus, 1758]; Icterine warbler, *Hippolais*
38
39 440 *icterina* [Vieillot, 1817]; and Sedge warbler, *Acrocephalus schoenobaenus* [Linnaeus,
40
41 441 1758]. Low diversity was observed within the *Clock* gene in this family with nearly all
42
43 442 individuals homozygous for the same allele, while heterozygosity for the *Adcyap1*
44
45 443 allele varied from 0.18 to 0.81. Overall, the greatest diversity was observed for Great
46
47 444 reed warbler which has two known subspecies. No indication was given as to whether
48
49 445 sampling included the known subspecies but given the single sampling site on a
50
51 446 Mediterranean island it is possible that the full scope of the subspecies was not
52
53 447 included.

54
55 448 Of the *Phylloscopidae* family, Bazzi *et al.* (2016a) studied two species, the
56
57 449 Willow warbler, *Phylloscopus trochilus* [Linnaeus, 1758], and Wood warbler,
58
59 450 *Phylloscopus sibilatrix* [Bechstein, 1793], and found greater *Clock* allele diversity as
60
451 compared to the family *Acrocephalidae* with heterozygosity scores of 0.63 and 0.43
452 respectively and a similar trend for *Adcyap1*. Later publications with a focus on the
453 Willow warbler (Bazzi *et al.*, 2017) illustrated a high degree of homozygosity for the Q₉

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2
3 454 allele, accounting for more than 98% of all individuals, but no significant relationship
4
5 455 was found in relation to migration phenology.

6 456 Among the *Sylviidae* warblers, which includes the Eurasian blackcap (Mueller
7
8 457 *et al.*, 2011), Bazzi *et al.* (2016a) assessed these genes for the Common whitethroat,
9
10 458 *Curruca (Sylvia) communis* [Latham, 1787], Eastern subalpine warbler, *Curruca*
11
12 459 (*Sylvia) cantillans* [Pallas, 1764], and Garden warbler, *Sylvia borin* [Boddaert, 1783].
13
14 460 The *Sylviidae* warblers followed the same general trend as seen in *Phylloscopidae*
15
16 461 warblers with slightly higher heterozygosity for both *Clock* (0.68-0.76) and *Adcyap1*
17
18 462 (0.66-0.88).

19 463 Two family members of the *Parulidae* warblers have been studied as well; the
20
21 464 Wilson's warbler, *Cardellina pusilla* [Wilson, 1811] (Bazzi *et al.*, 2016b); and Blackpoll
22
23 465 warbler, *Setophaga striata* [Forster, 1772] (Ralston *et al.*, 2019). Wilson's warbler was
24
25 466 similarly homozygous for the Q₉ allele to Willow warbler and no effect was observed
26
27 467 on migration phenology. To the contrary, Ralston *et al.* (2019) did find a relationship
28
29 468 between Spring- and Autumn migration and allele length diversity for both *Clock* and
30
31 469 *Adcyap1*. In the Willow warbler, *NPAS2* and *CREB1* were also assayed; *NPAS2*
32
33 470 positively predicted the date of migration while *CREB1* positively predicted moult
34
35 471 speed, however, both effects were sex-specific and only present in males (Bazzi *et al.*,
36
37 472 *et al.*, 2017).

38 473 This illustrates that within the broader group that is the warblers, the effects for
39
40 474 clock gene polymorphisms and directional selection of polymorphisms may be isolated
41
42 475 to specific families, and in particular, ones with higher levels of speciation. Several
43
44 476 non-gene linkage studies have been conducted in warblers as well. The first used a
45
46 477 panel of single nucleotide polymorphisms in Wilson's warbler to assess potential
47
48 478 genomic regions driving differences in geographic distribution (Ruegg *et al.*, 2014b).
49
50 479 Other approaches measured mRNA expression levels in Willow warbler using
51
52 480 transcriptomics approaches to elucidate potential gene candidates (Lundberg *et al.*,
53
54 481 2013; Boss *et al.*, 2015). This "shotgun" approach was later used in the same species
55
56 482 though whole genome sequencing (Lundberg *et al.*, 2017) and found distinct haplotype
57
58 483 distributions between migratory phenotypes despite low allele variability; with only a
59
60 484 fraction of the tested regions showing measurable and potentially useful variations.

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3 485 (c) Swallows (Family: Hirundinidae, 18 MYA)
4

5 486 Dor *et al.* (2011) analysed the Poly-Q region of five populations, representing three
6
7 487 subspecies of Barn swallows, *Hirundo rustica* [Linnaeus, 1758], and found low levels
8
9 488 of variability despite the evident population structure. Additionally, similar to the high
10
11 489 prevalence of the Q₁₃ allele in Blue tits (Johnsen *et al.*, 2007), nearly 98% of all allelic
12
13 490 diversity was accounted for by the Q₇ allele (Dor *et al.*, 2011). From this it was
14
15 491 suggested that perhaps social cues take preference over other environmental cues
16
17 492 which may result in the negative selection for *Clock* polymorphisms. Later studies of
18
19 493 the same species in European populations revealed a similar trend for the Q₇ allele,
20
21 494 accounting for 96% of the observed genotypes in Italy (Caprioli *et al.*, 2012) and 91%
22
23 495 in Switzerland (Bazzi *et al.*, 2015). They did, however, succeed in elucidating an
24
25 496 association between *Clock* alleles and breeding phenology in females while more rare
26
27 497 genotypes occurred in males (Caprioli *et al.*, 2012) as well as correlations to diverse
28
29 498 departure and arrival times (Bazzi *et al.*, 2015). Differences between genotypes and
30
31 499 timing of moulting have also been identified (Saino *et al.*, 2013), and moulting is known
32
33 500 to co-vary with migratory phenology (Saino *et al.*, 2015b). The apparent lack of genetic
34
35 501 variability may be due to these genes already being under directional selection given
36
37 502 the observed changes in migratory phenology in response to climate change (Altwegg
38
39 503 *et al.*, 2012) in these species.

40
41 504 Dor *et al.* (2012) also studied *Clock* length polymorphisms in five species of
42
43 505 *Tachycineta* swallows: Tree swallow, *T. bicolor* [Vieillot, 1808]; Violet-green swallow,
44
45 506 *T. thalassina* [Swainson, 1827]; Mangrove swallow, *T. albilinea* [Lawrence, 1863];
46
47 507 White-rumped swallow, *T. leucorrhoa* [Vieillot, 1817]; and Chilean swallow, *T. meyeni*
48
49 508 [Cabanis, 1850]. With the exception of the Violet-green swallow, these species are
50
51 509 often classed together in genus *Iridoprocne* (Whittingham *et al.*, 2002). The first three
52
53 510 species displayed a characteristic higher proportion of the Q₈ allele, whilst White-
54
55 511 rumped swallows had higher prevalence of the Q₇ allele. By contrast, Chilean swallow
56
57 512 had a near equal distribution of both the Q₇ and Q₈ alleles (Dor *et al.*, 2012). Although
58
59 513 study specimens derived from a wide range of breeding latitudes there was no
60
514 significant correlation found when comparing breeding latitude and *Clock* mean allele
515 size and a small effect in some species on breeding/laying time in females (Dor *et al.*,
516 2012). A major limiting factor of this study was the sampling of each species from only
517 one location, and therefore only one population, which may impact the ability to

1
2
3 518 adequately resolve allele diversity and evidence for selection within a singular species.
4
5 519 Another study evaluated the polymorphisms of four genes, identified by Steinmeyer *et*
6
7 520 *al.* (2009), in Tree swallow in relation to two phenological traits related to migration:
8
9 521 laying date and incubation duration. For the *Clock* gene, the Q₈ allele was the most
10
11 522 abundant (61.6% versus the 64% observed by Dor *et al.* 2012). *Adcyap1* was found
12
13 523 to be highly polymorphic with thirteen alleles (like observations in Eurasian blackcaps,
14
15 524 see Mueller *et al.* 2011). A significant correlation was observed in all genes measured,
16
17 525 with most relationships dependent upon environmental variables such as latitude,
18
19 526 temperature, and breeding density—in particular, longer alleles were associated with
20
21 527 more Eastern latitudes (Bourret & Garant, 2015). Expression level studies have also
22
23 528 been applied to Tree swallows, however, while several regions were identified that
24
25 529 putatively co-varied with migration phenology, none corresponded to clock genes
26
27 530 previously identified as candidate genes (Brown, 2019).

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29 531 *(d) Larks (Family: Alaudidae, 40 MYA)*

30
31 532 Asian short-toed lark (*Alaudala cheleensis* [Swinhoe, 1871], published as *Calandrella*
32
33 533 *cheleensis*) was found to have a near equal distribution of the Q₉ (slightly higher) and
34
35 534 Q₁₁ alleles. A significant relationship was found with both the timing of egg laying as
36
37 535 well as a correlation with the seasonal endocrine response to initiate breeding (Zhang
38
39 536 *et al.*, 2017). This species is, however, currently under taxonomic review, and not
40
41 537 universally recognised (de Juana & Suárez, 2020; BirdLife International, 2021; HBW
42
43 538 and BirdLife International, 2021).

44
45 539 *(e) Sparrows, Juncos, and Buntings (Families: Passerellidae, Emberizidae, 34 MYA)*

46
47 540 The finding that hybrid speciation among congeneric spotted eagle leads to divergent
48
49 541 migration strategies (Väli *et al.*, 2018) has been assessed in other instances of
50
51 542 hybridization; notably the Italian sparrow, *Passer italiae* [Vieillot, 1817] (Guldvog,
52
53 543 2015), a hybrid of the House- (*Passer domesticus* [Linnaeus, 1758]) and Spanish
54
55 544 sparrow (*Passer hispaniolensis* [Temminck, 1820]). Despite the evident differences in
56
57 545 migration behaviour, as closely related sympatric sparrow species have been
58
59 546 observed to follow different migratory phenology (Borowske, Gjerdrum, & Elphick,
60
61 547 2017), all three species were found to be homozygous for the Q₁₁ *Clock* allele.
62
63 548 Furthermore, higher diversity without evident patterning, able to partition migrating and
64
65 549 sedentary populations, was observed for *Adcyap1*. These results were consistent with

1
2
3 550 previous findings in White-crowned sparrow, *Zonotrichia leucophrys* [Forster, 1772],
4 551 using transcriptomic approaches (Jones *et al.*, 2008b). The latter study did find a
5
6 552 significant variation in the expression of one clock related gene, *Copine 4 (CPNE4)*,
7
8 553 which has also been observed in other bird species (Ruegg *et al.*, 2014b; Delmore *et*
9
10 554 *al.*, 2015; Bossu *et al.*, 2022), while another study in Song sparrow, *Melospiza melodia*
11
12 555 [Wilson, 1810], found a significant link between exploratory behaviour and migration
13
14 556 distance respectively when comparing SNP's in the *DRD4* gene among migrants
15
16 557 (Posliff, 2020).

17
18 558 A study of fifteen populations of the highly divergent junco species complex,
19
20 559 including eight subspecies of the Dark-eyed junco, *Junco hyemalis* [Linnaeus, 1758],
21
22 560 and two subspecies of the Yellow-eyed junco, *Junco phaeonotus* [Wagler, 1831],
23
24 561 found no consistent relationship across all congeneric species for *Clock* or *Adcyap1*
25
26 562 length polymorphisms and the measured phenological traits. They did, however,
27
28 563 observe longer *Clock* alleles in two subspecies known to migrate longer distances as
29
30 564 well as a relationship between *Adcyap1* length and migratory restlessness, similar to
31
32 565 that observed by Mueller *et al.* (2011), in one of two captive species (Peterson *et al.*,
33
34 566 2013).

35
36 567 Several transcriptomic studies have been done on the migratory songbirds of the
37
38 568 family *Emberizidae*. The first measured expression levels in captive Red-headed
39
40 569 bunting, *Emberiza bruniceps* [Brandt, 1841], under varied photoperiods, for several
41
42 570 genes including *Bmal1*, *Clock*, *Cry1/2*, and *Per2* (Singh, Rani, & Kumar, 2013). A
43
44 571 strong correlation between light-dark phases and differential gene expression was
45
46 572 observed in all the assayed tissue types, providing further evidence of light attuned
47
48 573 oscillations withing the circadian circuitry of migratory birds (Singh *et al.*, 2013) similar
49
50 574 to previous observations in model organisms (Leclerc *et al.*, 2010). When comparing
51
52 575 expression levels under photoperiods consistent with triggers for Spring or Autumn
53
54 576 migration, including *Adcyap1*, the functional consequences thereof were clearly
55
56 577 illustrated; Autumnal conditions lead to considerably higher expression levels whereas
57
58 578 Spring conditions lead to many metabolic and endocrine changes (Sharma *et al.*,
59
60 579 2018b). Interestingly there was also differential expression of *DNA methyltransferase*
61
62 580 *3 (DNMT3)* and *Tet methylcytosine dioxygenase 2 (TET2)*, two enzymes involved in
63
64 581 methylation, indicating a possible role of epigenetic control over migratory and non-
65
66 582 migratory states in line with previous findings in Barn swallows (Saino *et al.*, 2017).

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2
3 583 Similar transcriptomic studies in the congeneric Black-headed bunting, *E.*
4 584 *melanocephala* [Scopoli, 1769], revealed a similar pattern of differential expression in
5 585 neural and peripheral tissues, oscillating with seasonal life history events such as
6 586 photoperiod (Singh *et al.*, 2015). Studies on *Emberizidae* buntings did, however, not
7 587 assess potential inter-individual variation in the measured response—which may be
8 588 due to both species of bunting under consideration being classified as monotypic.

13
14
15 589 *(f) Cardinals (Family: Cardinalidae, 17 MYA)*

16 590 Unlike the Red- and Black-headed bunting, the Painted bunting, *Passerina ciris*
17 591 [Linnaeus, 1758], along with other buntings such as the Lazuli bunting, *Passerina*
18 592 *amoena* [Say, 1823], is classified in the family *Cardinalidae* (Lowther *et al.*, 2020).
19 593 Three Painted bunting populations, representing both subspecies, were compared for
20 594 allelic diversity in both the *Clock* and *Adcyap1* gene; migration phenology was only
21 595 analysed in the Western population for which geolocator data was available (Contina
22 596 *et al.*, 2018). Greater allelic diversity was observed for both genes in the Western
23 597 population, where the most common allele was Q₁₁, as compared to the Eastern
24 598 population, where the Q₁₂ was more abundant. No significant cline was detected
25 599 relative to the initiation of, nor the duration of, Autumn migration. and it was concluded
26 600 that individual allele studies may have limited resolution for this species. The same
27 601 authors later assessed the migratory divide between subspecies using a panel of
28 602 single nucleotide polymorphisms (SNP's), similar to those previously used in thrushes
29 603 (Ruegg *et al.*, 2014a) and warblers (Ruegg *et al.*, 2014b) and were able to detect at
30 604 least three distinct breeding populations across the continental United States that may
31 605 form pertinent conservation units (Contina *et al.*, 2019).

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36 606 *(g) Flycatchers and Chats (Family: Muscicapidae, 16 MYA)*

37 607 Johnsen *et al.* (2007) also investigated *Clock* gene variation in the Poly-Q regions of
38 608 twelve populations of the Bluethroat (*Cyanecula svecica* [Linnaeus, 1758], cited as
39 609 *Luscinia svecica*), formerly classified with the Thrushes in the family *Turdidae*
40 610 (reclassified as an Old-World Flycatcher). No significant relationship between the
41 611 evident allelic diversity and latitudinal clines were found, with 85.4% or more of the
42 612 genotypes being represented by the Q₁₃ allele and all individuals from the Italian
43 613 subpopulation were homozygous for this allele (Johnsen *et al.*, 2007). A study which
44 614 included another Old-World Flycatcher, the Common nightingale (*Luscinia*

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3 615 *megarhynchos* [Brehm, 1831]), found a near equal prevalence of the Q₁₁ and Q₁₂
4 616 alleles with a significant correlation between longer alleles and later migration date
5 617 (Saino *et al.*, 2015a). Another member, the Northern wheatear (*Oenanthe Oenanthe*
6 618 [Linnaeus, 1758]), also display consistently longer alleles (Bazzi *et al.*, 2016a).
7
8 619 Transcriptomic studies on this species also detected seasonal differences in the
9 620 expression of key clock elements in birds with different migration phenology (Frias-
10 621 Soler *et al.*, 2020, 2021).

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15 622 In the European pied flycatcher, *Ficedula hypoleuca* [Pallas, 1764], studies
16 623 found five Poly-Q alleles in contemporary (Saino *et al.*, 2015a) and historical samples
17 624 (Kuhn *et al.*, 2013), with the Q₁₂ allele accounting for 70% of individuals (Saino *et al.*,
18 625 2015a). Neither study was able to find a significant association between migration
19 626 timing and polymorphisms in the *Clock* (Kuhn *et al.*, 2013; Saino *et al.*, 2015a) or
20 627 *Adcyap1* (Saino *et al.*, 2015a) genes, however, Saino *et al.* (2015a) did detect a slight
21 628 relationship between timing and sex. A similar pattern of *Clock* and *Adcyap1* lengths
22 629 was also found in the related Spotted flycatcher, *Muscicapa striata* [Pallas, 1764]
23 630 (Bazzi *et al.*, 2016a), and Whinchat, *Saxicola rubetra* [Linnaeus, 1758] (Saino *et al.*,
24 631 2015a). A more recent study in flycatchers evaluated polymorphisms in the four genes
25 632 identified by Steinmeyer *et al.* (2009) in the Collared flycatcher (*Ficedula albicollis*
26 633 [Temminck, 1815]) but found no evidence of a relationship between genetic diversity
27 634 of the clock genes and migration phenology over a four year period (Krist *et al.*, 2021).

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36 635 In addition, a study conducted on the stonechat species complex (Justen *et al.*,
37 636 2022) found no clear correlates between phenological attributes such as breeding
38 637 latitude and the observed *Clock* genotypes but did detect a relationship between
39 638 timing of Autumn migration for the migratory species. This study included the African
40 639 stonechat (*Saxicola torquatus* [Linnaeus, 1766]) subspecies resident in central Africa
41 640 (but not those resident in South Africa), Canary island stonechat (*Saxicola dacotiae*
42 641 [Meade-Waldo, 1889]), European stonechat (*Saxicola rubicola* [Linnaeus, 1766]), and
43 642 Siberian stonechat (*Saxicola maurus* [Pallas, 1773]), and found *Clock* alleles that
44 643 ranged from Q₈ to Q₁₅—with the most common alleles being Q₁₃ and Q₁₄, similar to
45 644 the common Q₁₄ allele observed in the congeneric Whinchat species (Saino *et al.*,
46 645 2015a). This illustrates that, at least within the family *Muscicapidae*, migration
47 646 phenology may vary independent of genetic polymorphisms but there is evidence for
48 647 differential expression of the circadian clock genes (Frias-Soler *et al.*, 2020).
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3 648 (h) *Pipits* (Family: Motacillidae, 22 MYA)
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5 649 As compared to the flycatchers and chats, Tree pipits, *Anthus trivialis* [Linnaeus,
6 650 1758], were found to have shorter *Clock* alleles (allele range Q₆₋₁₀), with the most
7 651 prevalent Q₉ allele accounting for 85% of the observed genotypes (Saino *et al.*,
8 652 2015a). Saino *et al.* (2015a) were able to detect a significant relationship between
9 653 migration date and longer alleles in female, but not male, Tree pipits but had no
10 654 unequivocal explanation for sex dependence of migration phenology as such a
11 655 dependence in *Clock* photoperiodic responses remains to be documented for this
12 656 species. It is possible that the sex dependence is related to time of egg laying and
13 657 hatching, which would be more strongly related to timing of migration in females as
14 658 seen in Blue tits (Liedvogel *et al.*, 2009).

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24 659 (i) *Thrushes* (Family: Turdidae, 18 MYA)
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26 660 High resolution genetic markers have also been used in the Swainson's thrush,
27 661 *Catharus ustulatus* [Nuttall, 1840], to detect genomic regions of divergence related to
28 662 migration. Several genes were detected to have higher levels of differentiation than
29 663 expected, including genes previously associated with the circadian clock and
30 664 migratory behaviour such as *Adcyap1*, *CREB1*, *NPAS2*, and *Per3*, between the two
31 665 subspecies (Ruegg *et al.*, 2014a). The authors concluded that future work should
32 666 include more data from hybrid zones to assess the potential effect of this differentiation
33 667 on maintaining barriers to gene flow between subspecies (Ruegg *et al.*, 2014a). This
34 668 was addressed in subsequent studies (Delmore *et al.*, 2015) in a hybrid zone between
35 669 coastal and inland populations of Swainson's thrush. Delmore *et al.* (2015) used Next
36 670 Generation Sequencing (NGS) of the whole genome and targeted their analyses to
37 671 gene regions previously associated with migratory phenology; including *Clock*,
38 672 *Adcyap1*, *NPAS2* and *DRD4*. Between group analyses revealed high levels of
39 673 heterogeneity in diversity estimates while within group diversity was lower in areas of
40 674 higher speciation. This study was complimented with further analyses (Delmore *et al.*,
41 675 2016) aimed at comparing known differences in migration phenology with gene
42 676 diversity from the same dataset and three genes were found to co-vary with
43 677 phenology, one of which was the *Clock* gene.

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57 678 A genetic analysis of clock genes in Eurasian blackbirds, *Turdus merula*
58 679 [Linnaeus, 1758], in relation to urbanization assayed six established candidate genes
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3 680 including *Clock*, *Adcyap1*, *NPAS2*, *CREB1* and *DRD4* (Mueller *et al.*, 2013b). The
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5 681 authors found two *Clock* alleles, with the most common genotype being homozygous
6
7 682 Q_7/Q_7 , while the *Adcyap1* gene was considerably more diverse with more than twenty
8
9 683 detected alleles. Transcriptomic analyses of twelve Eurasian blackbirds displaying
10
11 684 differential resident versus migratory behaviour found several clusters of differentially
12
13 685 expressed genes, however, none were within elements of the circadian clock genes;
14
15 686 one was associated with moult rate, which may influence timing of migration (Franchini
16
17 687 *et al.*, 2017). Although this study was able to detect differential gene expression
18
19 688 between two morphs within a sympatric population the absence of relevant
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21 689 phenological function for the identified genes is limiting, highlighting the importance of
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23 690 “shotgun” approaches remaining grounded in genes of likely phenological
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25 691 consequence.

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27 692 A candidate gene approach has been applied to studying two congenic species of
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29 693 bluebird: Mountain bluebird, *Sialia currucoides* [Bechstein, 1798]; and Western
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31 694 bluebird, *Sialia Mexicana* [Swainson, 1832] (Sauve *et al.*, 2021). Both the *Adcyap1*
32
33 695 and *DRD4* genes were assayed and a comparison to migration phenology done since
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35 696 Western bluebirds are partial migrants, often switching between strategies, while
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37 697 Mountain bluebirds are obligate migrants. Their analyses revealed a potential role for
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39 698 *DRD4*, a gene previously linked to exploratory behaviour in tits (Korsten *et al.*, 2010;
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41 699 Mueller *et al.*, 2013a) and swans (Van Dongen *et al.*, 2015), but absent in warblers
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43 700 (Mueller *et al.*, 2011; Edwards *et al.*, 2015). To the contrary no role was evident for the
44
45 701 *Adcyap1* gene (Sauve *et al.*, 2021) although the allelic diversity was comparatively
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47 702 lower than that observed in studies which did find such a correlation in e.g. Eurasian
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49 703 blackcaps (Mueller *et al.*, 2011).

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51 704 (j) *Shrikes and Orioles (Families: Laniidae, Oriolidae, 30 MYA)*

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53 705 The Eurasian golden oriole, *Oriolus oriolus* [Linnaeus, 1758], and Woodchat shrike,
54
55 706 *Lanius senator* [Linnaeus, 1758], were included as part of a larger study evaluating
56
57 707 inter-species differences in clock length polymorphisms (Bazzi *et al.*, 2016a) and three
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59 708 elements of migration phenology; migration date, migration distance, and latitude of
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709 breeding. This analysis largely consisted of phylogeny corrected generalised least-
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711 squares (PGLS) regression models (Freckleton, Harvey, & Pagel, 2002). Both orioles
and shrikes had significantly shorter Poly-Q repeats with the average allele equivalent

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2
3 712 to the Q₉ allele observed in pipits (Saino *et al.*, 2015a). The allele diversity of these
4
5 713 species does, however, remain to be tested for intra-species variation in relation to
6
7 714 diverse migratory strategies.

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9 715 **(2) Non-passerine birds**

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12 716 *(a) Buzzards, Hawks, and Kites (Order: Accipitriformes, Family: Accipitridae, 67 MYA)*

13
14 717 Contrary to the results observed in passerine species, most buzzards such as the
15
16 718 Eurasian buzzard, *Buteo buteo* [Linnaeus, 1758], is monoallelic for the *Clock* allele,
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18 719 with all genotyped individuals homozygous for the Q₈ allele. The same study also
19
20 720 assayed the *Adcyap1*, *NPAS2*, and *CREB1* genes and found a significant
21
22 721 phenological correlates with the observed polymorphisms in juveniles when
23
24 722 comparing timing of dispersal as well as dispersal distance (Chakarov *et al.*, 2013).
25
26 723 Interestingly no major differences were detected between the German (*N* = 976) and
27
28 724 Bulgarian (*N* = 23) populations, indicating that general population structure for gene
29
30 725 polymorphisms is already evident from smaller samples and higher sampling may not
31
32 726 significantly improve study resolution. This study also included other family members,
33
34 727 such as the Red kite, *Milvus milvus* [Linnaeus, 1758], and Northern goshawk, *Accipiter*
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36 728 *gentilis* [Linnaeus, 1758], but found no significant relationship among individuals of
37
38 729 these species.

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41 730 *(b) Hornbills and Hoopoes (Order: Bucerotiformes, Family: Upupidae, 65 MYA)*

42
43 731 In Eurasian hoopoe, *Upupa epops* [Linnaeus, 1758], the average allele size for *Clock*
44
45 732 corresponded to the Q₈ allele with three alleles present with near equal distribution for
46
47 733 Q₇ and Q₈; the calculated heterozygosity was 0.50. The *Adcyap1* gene had
48
49 734 comparable diversity with three alleles ranging in size from 157 to 159 bp and a higher
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51 735 calculated heterozygosity of 0.56 (Bazzi *et al.*, 2016a).

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53
54 736 *(c) Woodpeckers and Wrynecks (Order: Piciformes, Family: Picidae, 62 MYA)*

55
56 737 The Eurasian wryneck, *Jynx torquilla* [Linnaeus, 1758], had an average allele size for
57
58 738 *Clock* corresponding to the Q₈ allele with four alleles present ranging in size from Q₆
59
60 739 to Q₁₀; the calculated heterozygosity was 0.44. Consistent with the findings in the
61
62 740 Eurasian hoopoe, the *Adcyap1* gene had a near equal number of alleles to the clock
63
64 741 genes with five alleles ranging in sizes from 131 to 137 bp; considerably shorter than

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2
3 742 that observed in other non-passerines. Additionally, the calculated heterozygosity was
4
5 743 considerably higher at an estimated 0.68 (Bazzi *et al.*, 2016a).
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8 744 *(d) Kingfishers and Bee-eaters (Order: Coraciiformes, Family: Meropidae, 62 MYA)*

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10 745 European bee-eater was mono-allelic for *Clock* and corresponded to the Q₄ allele,
11
12 746 which is considerably shorter in comparison to other non-passerines. *Adcyap1* had
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14 747 several more alleles with a total of six, ranging in sizes from the shortest at 155 bp to
15
16 748 the longest at 169 bp. There was, however, considerable heterozygosity for the most
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18 749 common allele, 163 bp, and an estimated diversity of only 0.19 (Bazzi *et al.*, 2016a).
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20 750 This cohort only included individuals from the migratory group of European bee-eaters
21
22 751 in Italy and did not include samples from the resident population found in South Africa.

23 752 *(e) Nightjars (Order: Caprimulgiformes, Family: Caprimulgidae, 70 MYA)*

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25 753 The European nightjar, *Caprimulgus europaeus* [Linnaeus, 1758], had an average
26
27 754 allele size for *Clock* corresponding to the Q₈ allele with two alleles; the calculated
28
29 755 heterozygosity was 0.35 as most individuals were homozygous. The *Adcyap1* allele
30
31 756 was highly divergent in comparison to other non-passerine species as well as the
32
33 757 *Clock* allele with nine alleles and a calculated heterozygosity of approximately 0.81
34
35 758 (Bazzi *et al.*, 2016a). Once more this illustrated that a sample of approximately thirty
36
37 759 individuals is still adequate to resolve most of the allelic diversity.

38 760 *(f) Pigeons and Doves (Order: Columbiformes, Family: Columbidae, 74 MYA)*

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40 761 European turtle dove, *Streptopelia turtur* [Linnaeus, 1758], had an average allele size
41
42 762 for *Clock* corresponding to the Q₇ allele with a minimum of two alleles, but with a
43
44 763 heterozygosity of 0.03, were nearly monoallelic. The *Adcyap1* gene was more
45
46 764 polymorphic with five detected alleles ranging in size from 148 to 152 bp; an
47
48 765 intermediate length as compared to the Eurasian hoopoe and Eurasian wryneck,
49
50 766 closer in range to the European nightjar. The overall diversity was, however, lower
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52 767 with a calculated heterozygosity of 0.30 and many individuals homozygous for the
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54 768 most common 150 bp allele (Bazzi *et al.*, 2016a).
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3 769 (g) Shorebirds (Order: Charadriiformes, Families: Laridae, Scolopacidae,
4 770 Charadriidae, 78 MYA)

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7 771 The females of a large breeding colony of the largely resident species of
8 772 Yellow-legged gull, *Larus michahellis* [Naumann, 1840], was studied for length
9 773 polymorphisms in two regions of the *Clock* gene, *Adcyap1* and *NPAS2* (Romano *et al.*
10 774 *et al.*, 2018). The observed polymorphisms were compared to timing of breeding
11 775 (measured as laying date) but found similar distributions of all assayed alleles between
12 776 early and late laying birds. From this the authors concluded that the influence of
13 777 photoperiod selection on clock gene polymorphisms may not be universal to all bird
14 778 species (Romano *et al.*, 2018). It should be noted that this population represented only
15 779 one of the two recognised subspecies, *L. m. michahellis* and not *L. m. atlantis*, which
16 780 could affect the results as differences are likely to be more evident in early speciation
17 781 (Rolland *et al.*, 2014), between ecological niches (Gómez *et al.*, 2016), or along lateral
18 782 gradients (Linck, Freeman, & Dumbacher, 2019).

19 783 Among Bar-tailed godwit subspecies *Limosa lapponica baueri* [Linnaeus, 1758],
20 784 which overwinter in New Zealand, a high degree of *Clock* variability was observed with
21 785 three nearly equally distributed alleles Q₉₋₁₁ (Parody-Merino *et al.*, 2019). Statistical
22 786 analyses revealed no clear relationship between allele size and timing of migration,
23 787 but a slight latitudinal cline was observed with longer alleles corresponding to
24 788 individuals travelling further North. The authors made the rather bold conclusion that
25 789 clock gene polymorphisms are “not a strong candidate for driving migration timing in
26 790 migratory birds generally.”, however, the fact that this species—known to make one of
27 791 the most arduous annual journeys—displayed significantly more heterozygosity than
28 792 other bird species (e.g., tits or warblers) should not be overlooked. Furthermore, this
29 793 study only tested one distinct subspecies and may therefore lack the ability to detect
30 794 diversification of this locus if the diversification is part of the speciation process.

31 795 A more recent study included an assessment of diversity within the *Adcyap1* gene
32 796 in several species of shorebirds: the Collared plover (*Charadrius collaris* [Vieillot,
33 797 1818]), Semipalmated sandpiper (*Calidris pusilla* [Linnaeus, 1766]), Semipalmated
34 798 plover (*Charadrius semipalmatus* [Bonaparte, 1825]), and Spotted sandpiper (*Actitis*
35 799 *macularius* [Linnaeus, 1766]). Their study found several alleles within each species
36 800 but was limited in its resolution by small sample sizes (de Almeida Miranda *et al.*,
37 801 2022).

802 IV. CROSS-SPECIES COMPARATIVE ANALYSIS

803 (1) Population Genetics

804 The results for the population genetics analyses are summarised in **Table 6**, while
805 more detailed results are available in the supplementary tables. The total number of
806 Poly-Q alleles were thirteen and ranged in size from four repeats, observed in the
807 European bee-eater, to sixteen repeats observed in the Blue tit, Common redstart,
808 and Whinchat. The total number of *Adcyap1* alleles were far more numerous with fifty-
809 one alleles ranging in size from 131 to 189 bp. Sequence data was not available for
810 all species and as such the total reported allele length of *Adcyap1* was used rather
811 than just the length of the “AG” repeat region, still staying true to the central hypothesis
812 that any length difference in this region is responsible for altered gene regulation
813 resulting in different entrainment. Both the *Clock* and *Adcyap1* alleles passed the
814 Hardy-Weinberg equilibrium test for nearly all species, with only five species failing
815 equilibrium assumptions in each case. For the *Clock* gene, the species that failed
816 assumptions were the Common chiffchaff, *Phylloscopus collybita* [Vieillot, 1817], the
817 Magnolia warbler, *Setophaga magnolia* [Wilson, 1811], the Common nightingale, the
818 Common whitethroat, and the Willow warbler. Species which failed equilibrium
819 assumptions for *Adcyap1* were the Blackpoll warbler, the Common redstart, the
820 European nightjar, the Willow warbler, and the Wilson’s warbler. The overall test for
821 equilibrium between species failed with a P -value < 0.02 , as anticipated. Three
822 species, the European turtle dove, the Great reed warbler, and the Wilson’s warbler,
823 had equal observed and expected heterozygosity for the *Clock* allele while four
824 species, the Eurasian reed warbler, the European bee-eater, the Sedge warbler, and
825 the White-throated sparrow (*Zonotrichia albicollis* [Gmelin, 1789]) were mono-allelic.
826 For both alleles the observed heterozygosity (H_o) was slightly higher than the
827 calculated expected (H_e) in nearly half of the tested species, 17/39 for *Clock* and 19/37
828 for *Adcyap1* respectively, however, the overall test revealed a mean observed
829 heterozygosity of 0.325 for *Clock* and 0.678 for *Adcyap1*, which was lower than the
830 calculated expected heterozygosity. The neutrality tests detected significant evidence
831 for deviation from neutrality in three of the thirty-nine species, the Blackpoll warbler,
832 the Great reed warbler, and the Whinchat, for the *Clock* allele and five species, the
833 Dark-eyed junco, the European bee-eater, the Magnolia warbler, the Tree swallow,
834 the Willow warbler, the Wilson’s warbler, and the Woodchat shrike, for the *Adcyap1*

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3 835 allele. Overall tests for selection between species detected no evidence for deviation
4 836 from neutrality in *Clock* but did detect deviation from neutrality for *Adcyap1*. For the
5 837 thirty-six species for which data of both alleles was available, linkage disequilibrium
6 838 was detected in three species, the Common whitethroat, the Eurasian golden oriole,
7 839 and the Eurasian wryneck—while the remainder appeared to be in equilibrium.

12 840 **(2) Mantel tests**

14 841 A significant relationship was detected between the genetic distance of the *Clock*
15 842 alleles, expressed as F_{ST} , and both the breeding and non-breeding latitude distances,
16 843 with a P -value of 0.099 ($P < 0.10$) and 0.018 ($P < 0.02$) respectively, as well as with
17 844 regards to the taxonomic distance and divergence times, with a P -value of 0.013 ($P <$
18 845 0.02) and 0.051 ($P < 0.10$) respectively. Apart from divergence times, in all cases the
19 846 Z -value and R -value were indicative that the values are positively correlated. In
20 847 contrast, no significant relationship was detected between the genetic distance of
21 848 *Adcyap1* alleles and geographic attributes of migration phenology or taxonomic
22 849 distance, but a significant relationship was found in relation to the divergence times
23 850 with a P -value of 0.010 ($P < 0.02$). The negative values of the Z -value and R -value is
24 851 indicative of a negative correlation between the observed genetic distances and
25 852 divergence time. The results for the Mantel tests are summarised in **Table 7**.

35 853 **(3) Phylogenetic generalised least square analyses**

37 854 Phylogenetic generalised least square (PGLS) analyses revealed no significant
38 855 relationship between the average latitudes of breeding and non-breeding ranges and
39 856 the allele length of the most common *Clock* or *Adcyap1* allele of the more than seventy
40 857 species under consideration; the only detected geo-spatial relationship being between
41 858 *Clock* allele length and total migration distance. A correlation was, however, detected
42 859 between allele length for *Clock* and the start and middle dates of Autumn migration
43 860 with at a 90% confidence interval, as related to the Summer solstice and Autumn
44 861 equinox. A similar correlation was detected between *Adcyap1* and the middle and end
45 862 dates of Spring migration in relation to the date of the Winter solstice. The strength of
46 863 the overall phylogenetic signal, as assessed by estimating the lambda and kappa
47 864 parameters, was strongly correlated to the observed most common alleles in both
48 865 genes. The results of the PGLS are summarised with the results of the Mantel tests in
49 866 **Table 7**. A phylogenetic ANOVA between migratory and resident species revealed no

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2
3 867 significant partitioning based on *Clock* or *Adcyap1* allele length (data not shown) while
4
5 868 the largely sedentary species complex of manakins had clock alleles ranging in size
6
7 869 from Q₈ to Q₁₁, overlapping with those observed in migratory species.

8 9 870 **(4) Time tree and paleogeography**

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11 871 Divergence times for the main lineages for studied species are represented in the
12
13 872 reconstructed time tree (**Figure 7**) indicating the relative divergence times for
14
15 873 speciation, in millions of years ago (MYA). Each panel indicates a paleogeographic
16
17 874 epoch, colour coded to the corresponding period. The time scale starts at the primary
18
19 875 speciation event dividing the species of the order *Passeriformes* from the non-
20
21 876 passerine birds circa 85 million years ago (MYA) during the Upper Cretaceous period.
22
23 877 The average divergence times for the study species was approximately 35 MYA with
24
25 878 more recent divisions ranging from as recent as 10 to 20 MYA during the Paleogene
26
27 879 and early Neogene. Most recent speciation events within lineages occurred in the
28
29 880 Miocene and Pliocene and continued to modern times with the most recent speciation
30
31 881 for the dataset being 1.85 MYA between the different flycatcher species. Some basal
32
33 882 differences were observed between the phylogenetic tree and the time tree, most
34
35 883 notably the closer partitioning of Bluethroat species with the thrushes and Painted-
36
37 884 and Lazuli buntings with Eurasian buntings according to their former taxonomic
38
39 885 classification.

37
38 886 Paleogeographic reconstructions (**Figure 7** and **Video 1**) show that the primary
39
40 887 division between most lineages occurred prior to the formation of most continents, and
41
42 888 would likely have required several range shifts, which would only have occurred
43
44 889 approximately 50 MYA with a substantial amount of continental drift continuing until
45
46 890 about 21 MYA when most continents started to assume their contemporary positions.
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48 891 There was still, however, a continuation of tectonic plate movement resulting in
49
50 892 significant geographic remodelling on most continents, shaping the landscape within
51
52 893 which range selection and speciation occurred.

51 52 894 **V. DISCUSSION**

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54 895 Our systematic review of the existing body of scientific evidence for a potential role of
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56 896 diversity within genes, associated with the circadian clock machinery, regulating, or
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58 897 shaping diverse migration phenology clearly presents the conflicting evidence. On the
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60 898 one hand, several studies were successful at illustrating such a relationship (Liedvogel

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3 899 *et al.*, 2009; Caprioli *et al.*, 2012; Kuhn *et al.*, 2013) whilst on the other hand, several
4
5 900 studies found no evidence of an association (Peterson *et al.*, 2013; Contina *et al.*,
6
7 901 2018; Parody-Merino *et al.*, 2019). This is further confounded by the fact that several
8
9 902 transcriptomic studies in related species detected no marked difference within the
10
11 903 expression levels of key clock genes (Jones *et al.*, 2008a; Franchini *et al.*, 2017;
12
13 904 Brown, 2019) often used in candidate gene association studies.

14 905 It should, however, be noted that several studies may have had pitfalls within
15
16 906 the study design or data analyses that could confound results. For example, most of
17
18 907 the initial studies relied solely on the use of the Mantel test (Mantel, 1967) to find a
19
20 908 correlation between genetic distance and geographic distance, as has been a common
21
22 909 element of spatial genetics and ecology for some time. More recently, however,
23
24 910 several authors have critiqued the suitability of the Mantel test in these instances
25
26 911 (Guillot & Rousset, 2013; Legendre, Fortin, & Borcard, 2015) as the test classically
27
28 912 assumes that one is comparing two distance matrices of which both are a measure of
29
30 913 differentness—rather than a physical distance—and that geographic distance
31
32 914 expressed as kilometres may exaggerate the relationship. Other potential statistical
33
34 915 confounders include the post-hoc grouping of study individuals based on observed
35
36 916 outlier status (Bazzi *et al.*, 2015) or sex (Dor *et al.*, 2011), rather than predetermined
37
38 917 grouping, small sample sizes (de Almeida Miranda *et al.*, 2022), and the averaging of
39
40 918 alleles in heterozygotes to analyse mean allele length (Zhang *et al.*, 2017).

41 919 Analysing individuals by mean allele length makes several assumptions about
42
43 920 the underlying biology of the system that have yet to be proven. Firstly, it assumes
44
45 921 equal bi-allelic expression from a single locus, an absence of parental imprinting (Jang
46
47 922 *et al.*, 2013), and identical regulation of both alleles. This contradicts the central
48
49 923 hypothesis of altered entrainment cycles in the presence of a length polymorphism as
50
51 924 well as some existing evidence for heritable patterns of methylation in birds (Romano
52
53 925 *et al.*, 2017; Saino *et al.*, 2019). Secondly it assumes knowledge of the copy number
54
55 926 variation for the studied genes (Skinner *et al.*, 2014), which may be particularly
56
57 927 complex given that birds have different karyotypes with chromosomes ranging
58
59 928 between 78 and 82 in most species (Degrandi *et al.*, 2020). Initial studies grouped
60
929 individuals based on homozygosity, carrying the most common allele, and
930 heterozygosity with a longer or shorter allele to model likely gene effects without
931 assuming equal bi-allelic expression from a single locus (Johnsen *et al.*, 2007).

1
2
3 932 Some studies may also have failed due to deviation from the central hypothesis
4
5 933 by analysing total fragment length and including alleles that differed by only one base-
6
7 934 pair in the dataset (Contina *et al.*, 2018), thereby not implicitly measuring only the
8
9 935 identified length polymorphism, as well as including an excessively narrow cohort in
10
11 936 the analysis by focusing on a singular subspecies (Parody-Merino *et al.*, 2019); the
12
13 937 central hypothesis implies that the genetic differences establish a migratory divide that
14
15 938 leads to speciation, the context within which these polymorphism are studied is unclear
16
17 939 when a singular subspecies is studied as speciation is unlikely to be documented
18
19 940 below the subspecies level. Additionally, in many of the studied species for which
20
21 941 known subspecies exist, no clear effort was made to assign individuals to specific
22
23 942 subspecies, but rather all individuals assumed to belong to the same subspecies
24
25 943 based on study site, failing to control for varied population assignment confounding
26
27 944 results. The importance of accounting for subspecies has been highlighted by the
28
29 945 increasing number of documented cases of hybrid speciation among birds, including
30
31 946 eagles (Väli *et al.*, 2018), finches (Lamichhaney *et al.*, 2018), manakins (Barrera-
32
33 947 Guzmán *et al.*, 2017), sparrows (Hermansen *et al.*, 2011), warblers (Brelford, Milá,
34
35 948 & Irwin, 2011; Ralston, Ermacor, & Kirchman, 2015) and tits (Janas *et al.*, 2021).

36
37 949 Most studies published to date have focused on European species of birds
38
39 950 within the order *Passeriformes*. This may be due to a slight mis-nomenclature in early
40
41 951 works in ornithology as the term “passer” in Latin refers to sparrows while the French
42
43 952 “passer” may refer to movement (Vieillot, 1816). This may have been misleading in
44
45 953 the sense that passerines were considered migratory while non-passerines by large
46
47 954 sedentary in earlier ornithological works like the *Histoire Naturelle* (Leclerc, 1770).
48
49 955 Other reasons may be the suggested higher diversification, and possibly speciation,
50
51 956 rate among passerines (Jetz *et al.*, 2012; Prum *et al.*, 2015), differences in trapping
52
53 957 method, or an interest in more complex migration strategies. This interpretation is,
54
55 958 however, subjective as mist net trapping has been effectively used in many non-
56
57 959 passerine species including kingfishers (Dalton *et al.*, 2022), cuckoos (Chaisi *et al.*,
58
59 960 2019), flufftails (Dalton *et al.*, 2016), and hornbills (Theron *et al.*, 2013), and some of
60
961 the most exceptional long distance migrations have been observed in non-passerine
962 species (Parody-Merino *et al.*, 2019).

963 The full dataset for *Clock* revealed a limited number of genotypes with a small
964 number of alleles and widespread homozygosity while *Adcyap1* was considerably

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2
3 965 more diverse with many more genotypes per species and more heterozygotes;
4 966 although, a general trend was found for higher observed than expected heterozygosity
5 967 within species while between species analyses showed less heterozygosity. Our
6 968 diversity estimates were generally well correlated to those of published studies.
7 969 Population genetics analyses found most tested species to be in Hardy-Weinberg
8 970 equilibrium, with only a few species failing the equilibrium assumptions. In the case of
9 971 the Willow warbler and Common chiffchaff, this was anticipated as the dataset
10 972 included individuals of known subspecies. For the remainder, it is possible that within
11 973 these species this was due to similar substructure or heterozygote deficiencies or
12 974 excess, attributed to a variety of factors such as inbreeding, hybridization or recent
13 975 population bottlenecks (Hedrick, 1987; Lade *et al.*, 1996; Luikart & Cornuet, 1998).

14 976 Our comparative analysis of available data found a significant correlation
15 977 between *Clock* alleles and both breeding and non-breeding latitudes using Mantel
16 978 tests (Mantel, 1967), but not PGLS (Freckleton *et al.*, 2002), indicative that concerns
17 979 regarding false positives using the former method in spatial genetics may be
18 980 vindicated (Guillot & Rousset, 2013; Legendre *et al.*, 2015). No comparable
19 981 relationship was found for *Adcyap1*, although this may be due to difficulty in comparing
20 982 this gene across taxa in the absence of sequence data as the use of the full length for
21 983 the allele does not solely account for length variation due to a specific length
22 984 polymorphism (Bazzi *et al.*, 2016a). Additionally, no evident trend was found for
23 985 partitioning migratory and sedentary species based on either candidate gene,
24 986 consistent with previous findings using phylogenetic approaches (Lugo Ramos *et al.*,
25 987 2017). When specifically analysing the relationship between alleles and the timing of
26 988 migration among migratory species, results supported a correlation between *Clock*
27 989 alleles and the timing of Autumn migration (Ralston *et al.*, 2019; Justen *et al.*, 2022)
28 990 while the opposite was found for *Adcyap1*, where some evidence supports of a
29 991 relationship with the timing and staging of Spring migration (Bazzi *et al.*, 2016a;
30 992 Ralston *et al.*, 2019).

31 993 Interestingly, an analysis of genetic distance in relation to divergence times
32 994 found a significant correlation for both *Clock* and *Adcyap1* alleles, as well as a
33 995 relationship between taxonomic distance and *Clock* alleles, indicating that
34 996 contemporary genotypes may still resemble the ancestral genotypes inherited millions
35 997 of years ago. This was further supported by the strong phylogenetic signal for both

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3 998 genes, as measured by both lambda and kappa parameters, indicating that closely
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5 999 related species have markedly similar allele lengths and variation is best explained
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7 1000 within lineages, similar to previous findings (Bazzi *et al.*, 2016a). This also supports
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9 1001 findings from European pied flycatcher studies that illustrated similar alleles in
10 1002 contemporary (Saino *et al.*, 2015a) and historical samples (Kuhn *et al.*, 2013).
11 1003 Considering the evident paleogeographic remodelling which coincided with the
12 1004 divergence of these lineages over that past 85 million years, along with recent climatic
13 1005 changes, recent selective sweeps would have abrogated this relationship if these
14 1006 genes were under strong selection in terms of adapting migration strategies in
15 1007 response to geographic or environmental changes. In the comparative analysis, the
16 1008 tests for deviation from assumptions of neutrality and evidence of selection failed to
17 1009 detect any evident selection in either gene for most species.

24 1010 A substantial amount of genetic research in ornithology from the 1990's (Vos *et al.*
25 1011 *et al.*, 1995) to shortly after the turn of the century (Bensch & Åkesson, 2005) focused on
26 1012 identifying genes with length polymorphisms that co-vary with latitude (Bensch,
27 1013 Åkesson, & Irwin, 2002) and could be used as molecular markers in population
28 1014 assignment or barcoding (Ottvall *et al.*, 2005). This included the identification of
29 1015 several markers which have been successfully used for population assignment in
30 1016 several bird species such as Willow warbler (Bensch *et al.*, 2002), House wren,
31 1017 *Troglodytes aedon* [Vieillot, 1809] (Arguedas & Parker, 2000), Superb fairy-wren
32 1018 (Double *et al.*, 1997), and Long-tailed manakin, *Chiroxiphia linearis* [Bonaparte, 1838]
33 1019 (McDonald & Potts, 1994). Therefore, although some evidence exists that
34 1020 polymorphisms within the tested candidate genes *Clock* and *Adcyap1* co-vary with
35 1021 elements of migration behaviour, considering the relationship was also illustrated in
36 1022 several sedentary species (Johnsen *et al.*, 2007; Liedvogel *et al.*, 2012), caution
37 1023 should be applied when translating these findings into a causal relationship.

49 1024 Future studies are currently needed in the field of migration genetics to address
50 1025 current gaps in our understanding of the systematics regulating circadian machinery.
51 1026 Such studies should ideally expand the breadth of species for which data is available,
52 1027 include transcriptional studies comparing expression levels and dominance of alleles
53 1028 in heterozygous individuals, studies comparing the expression levels between species
54 1029 with known polymorphic length variation in candidate genes, studies on the copy
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3 1030 number variation of circadian genes in avian species, and epigenetic studies to
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5 1031 address the potential ancillary role thereof in regulating these genes.

6 7 1032 **VI. CONCLUSIONS**

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9 1033 1. Some evidence exists that polymorphisms within the poly-Q region of the *Clock*
10 1034 gene are related to geo-spatial differences in the range of migrating birds, as well
11 1035 as the timing of Autumn migration.
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13 1036 2. Little evidence exists that polymorphism expressed as total allele length of
14 1037 *Adcyap1* has a geo-spatial pattern, however some evidence suggests a
15 1038 relationship with the timing of Spring migration.
16
17 1039 3. Both *Clock* and *Adcyap1* have a strong phylogenetic signal indicative that alleles
18 1040 and genotypes are highly heritable within lineages.
19
20 1041 4. For both *Clock* and *Adcyap1* the patterning is well correlated to divergence times
21 1042 and may therefore still reflect the ancestrally inherited genotypes rather than
22 1043 recently acquired changes.
23
24 1044 5. No clear evidence exists that either candidate gene can be used to distinguish
25 1045 migratory from sedentary birds across all taxa.
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27 1046

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30
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49 1056 **VIII. DATA AVAILABILITY**

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51 1057 Data used in this study are available for download from the Zenodo repository and is
52 1058 available at the following link: <https://doi.org/10.5281/zenodo.6637839>. The custom
53 1059 python script PARETT version 1.0.1 is available for download for installation from
54 1060 source code on GitHub (<https://github.com/LSLeClercq/PARETT>).
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3 1061 **IX. REFERENCES**
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1601 **Tables**

1602 **Table 1:** Summary of candidate gene studies relating polymorphisms in the *Clock*
 1603 gene to migration related behaviour in class *Aves*.

Species	Sub-species	Reference	Total alleles (Poly-Q)	Study
Bluethroat (<i>Cyanecula svecica</i>) *	Y(12)	(Johnsen <i>et al.</i> , 2007)	7 (Q ₁₃)	a
Blue tit (<i>Cyanistes caeruleus</i>) †	Y (9)	(Johnsen <i>et al.</i> , 2007)	9 (Q ₁₂)	a
		(Liedvogel <i>et al.</i> , 2009; 2012)	6 (Q ₁₂)	a, c, d
		(Steinmeyer <i>et al.</i> , 2009)	5 (Q ₁₂)	-
Great tit (<i>Parus major</i>) †	Y(15)	(Liedvogel & Sheldon, 2010)	5 (Q ₁₄)	c
Barn swallow (<i>Hirundo rustica</i>) *	Y (7)	(Dor <i>et al.</i> , 2011)	3 (Q ₇)	a, c
		(Caprioli <i>et al.</i> , 2012)	3 (Q ₇)	c
		(Bazzi <i>et al.</i> , 2015)	3 (Q ₇)	e
Tree swallow (<i>Tachycineta bicolor</i>) *	N	(Dor <i>et al.</i> , 2012)	4 (Q ₈)	a, c, d
		(Bourret & Garant, 2015)	4 (Q ₈)	a, c
Violet-green swallow (<i>T. thalassina</i>) *	Y (2)	(Dor <i>et al.</i> , 2012)	4 (Q ₈)	a, c, d
Mangrove swallow (<i>Tachycineta albilinea</i>) ‡	N	(Dor <i>et al.</i> , 2012)	2 (Q ₈)	a, c, d
White-rumped swallow (<i>T. leucorrhoa</i>) *	N	(Dor <i>et al.</i> , 2012)	3 (Q ₇)	a, c, d
Chilean swallow (<i>Tachycineta meyeni</i>) *	N	(Dor <i>et al.</i> , 2012)	3 (Q ₈)	a, c, d
Tree swallow (<i>Tachycineta bicolor</i>) *	N	(Dor <i>et al.</i> , 2012)	4 (Q ₈)	a, c, d
		(Bourret & Garant, 2015)	4 (Q ₈)	a, c
Eurasian buzzard (<i>Buteo buteo</i>) ‡	Y (6)	(Chakarov <i>et al.</i> , 2013)	1 (Q ₈)	a, c, e
Red kite (<i>Milvus milvus</i>) ‡	Y (2)	(Chakarov <i>et al.</i> , 2013)	2 (Q ₈)	a, c, e
Northern goshawk (<i>Accipiter gentilis</i>) ‡	Y(10)	(Chakarov <i>et al.</i> , 2013)	2 (Q ₁₁)	a, c, e
European pied flycatcher (<i>Ficedula hypoleuca</i>) *	Y (3)	(Kuhn <i>et al.</i> , 2013)	5 (Q ₁₂)	a, c
		(Saino <i>et al.</i> , 2015a)	5 (Q ₁₂)	e
		(Bazzi <i>et al.</i> , 2016a)	5 (Q ₁₂)	a, e
Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Mueller <i>et al.</i> , 2013b)	2 (Q ₇)	g
Dark-eyed junco (<i>Junco hyemalis</i>) †	Y(14)	(Peterson <i>et al.</i> , 2013)	7 (Q ₁₁)	a, b
Yellow-eyed junco (<i>Junco phaeonotus</i>) ‡	Y (4)	(Peterson <i>et al.</i> , 2013)	5 (Q ₁₁)	a, b
Common nightingale (<i>L. megarhynchos</i>) *	Y (3)	(Saino <i>et al.</i> , 2015a)	5 (Q ₁₂)	e
		(Bazzi <i>et al.</i> , 2016a)	5 (Q ₁₂)	a, e
Tree pipit (<i>Anthus trivialis</i>) *	Y (2)	(Saino <i>et al.</i> , 2015a)	5 (Q ₉)	e
		(Bazzi <i>et al.</i> , 2016a)	5 (Q ₉)	a, e
Whinchat (<i>Saxicola rubetra</i>) *	N	(Saino <i>et al.</i> , 2015a)	7 (Q ₁₄)	e
		(Bazzi <i>et al.</i> , 2016a)	7 (Q ₁₄)	a, e
Common redstart (<i>Phoenicurus phoenicurus</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	4 (Q ₁₄)	a, e
Common whitethroat (<i>Curruca communis</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	9 (Q ₁₀)	a, e
Eastern subalpine warbler (<i>Curruca cantillans</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	4 (Q ₉)	a, e
Eurasian golden oriole (<i>Oriolus oriolus</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₆)	a, e
Eurasian hoopoe (<i>Upupa epops</i>) *	Y (7)	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₈)	a, e
Eurasian reed warbler (<i>Acrocephalus scirpaceus</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	1 (Q ₁₁)	a, e
Eurasian wryneck (<i>Jynx torquilla</i>) *	Y (6)	(Bazzi <i>et al.</i> , 2016a)	4 (Q ₈)	a, e

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3	European bee-eater (<i>Merops apiaster</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	1 (Q ₄)	a, e
4	European nightjar (<i>Caprimulgus europaeus</i>) *	Y (6)	(Bazzi <i>et al.</i> , 2016a)	3 (Q ₈)	a, e
5	European turtle dove (<i>Streptopelia turtur</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₇)	a, e
6	Garden warbler (<i>Sylvia borin</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	6 (Q ₁₁)	a, e
7	Great reed warbler (<i>Acrocephalus arundinaceus</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₁₂)	a, e
8	Icterine warbler (<i>Hippolais icterina</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₈)	a, e
9	Northern wheatear (<i>Oenanthe oenanthe</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	5 (Q ₁₄)	a, e
10	Sedge warbler (<i>Acrocephalus schoenobaenus</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	1 (Q ₁₁)	a, e
11	Spotted flycatcher (<i>Muscicapa striata</i>) *	Y (7)	(Bazzi <i>et al.</i> , 2016a)	2 (Q ₉)	a, e
12	Willow warbler (<i>Phylloscopus trochilus</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016a)	5 (Q ₉)	a, e
13			(Bazzi <i>et al.</i> , 2017)	5 (Q ₉)	e, f
14	Wood warbler (<i>Phylloscopus sibilatrix</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	3 (Q ₁₁)	a, e
15	Woodchat shrike (<i>Lanius senator</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016a)	3 (Q ₆)	a, e
16	Wilson's warbler (<i>Cardellina pusilla</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016b)	2 (Q ₉)	a, e
17	Asian short-toed lark (<i>Alaudala cheleensis</i>) †	Y (4)	(Zhang <i>et al.</i> , 2017)	6 (Q ₉)	c
18	Painted bunting (<i>Passerina ciris</i>) *	Y (2)	(Contina <i>et al.</i> , 2018)	6 (Q ₁₁)	e
19	Yellow-legged gull (<i>Larus michahellis</i>) †	Y (2)	(Romano <i>et al.</i> , 2018)	2 (Q ₅)	c
20	Bar-tailed godwit (<i>Limosa lapponica baueri</i>) *	Y (3)	(Parody-Merino <i>et al.</i> , 2019)	6 (Q ₉)	a, e
21	Blackpoll warbler (<i>Setophaga striata</i>) *	N	(Ralston <i>et al.</i> , 2019)	1 (Q ₆)	a, e
22	Collared flycatcher (<i>Ficedula albicollis</i>) *	N	(Krist <i>et al.</i> , 2021)	4 (Q ₁₂)	e
23	African stonechat (<i>Saxicola torquatus</i>) ‡	Y(16)	(Justen <i>et al.</i> , 2022)	6 (Q ₁₃)	a, e
24	Canary island stonechat (<i>Saxicola dacotiae</i>) ‡	N	(Justen <i>et al.</i> , 2022)	3 (Q ₁₄)	a, e
25	European stonechat (<i>Saxicola rubicola</i>) *	Y (2)	(Justen <i>et al.</i> , 2022)	7 (Q ₁₄)	a, e
26	Siberian stonechat (<i>Saxicola maurus</i>) *	Y (5)	(Justen <i>et al.</i> , 2022)	5 (Q ₁₃)	a, e

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1605 Note: Species are listed in chronological order of publication. The presence of
 1606 subspecies is indicated (Y = Yes, N = No) along with the total alleles and most frequent
 1607 Poly-Q allele. Studies used latitude/longitude/spatial analyses (a), migratory
 1608 restlessness (b), timing of egg laying/breeding (c), clutch size (d), timing of migration
 1609 (e), moult rate (f), or urbanization (g). (Migration status: * Migratory † Partial Migratory
 1610 ‡ Sedentary)

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1612 **Table 2:** Summary of candidate gene studies relating polymorphisms in genes to
 1613 elements of migration related behaviour in class Aves.

Species	Sub-species	Reference	Total alleles	Study
Adcyap1 gene: (NCBI Gene ID: 116)				
Blue tit (<i>Cyanistes caeruleus</i>) †	Y (9)	(Steinmeyer <i>et al.</i> , 2009)	7 (162)	-
Eurasian blackcap (<i>Sylvia atricapilla</i>) †	Y (5)	(Mueller <i>et al.</i> , 2011)	13 (161)	a, b
		(Mettler <i>et al.</i> , 2015)	11 (161)	e
Eurasian buzzard (<i>Buteo buteo</i>) ‡	Y (6)	(Chakarov <i>et al.</i> , 2013)	3 (152)	a, c, e
Red kite (<i>Milvus milvus</i>) ‡	Y (2)	(Chakarov <i>et al.</i> , 2013)	2 (139)	a, c, e
Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Mueller <i>et al.</i> , 2013b)	19 (165)	g
Dark-eyed junco (<i>Junco hyemalis</i>) †	Y(14)	(Peterson <i>et al.</i> , 2013)	16 (161)	a, b
Yellow-eyed junco (<i>Junco phaeonotus</i>) ‡	Y (4)	(Peterson <i>et al.</i> , 2013)	11 (161)	a, b
Tree swallow (<i>Tachycineta bicolor</i>) *	N	(Bourret & Garant, 2015)	13 (173)	a, c
European pied flycatchers (<i>Ficedula hypoleuca</i>) *	Y (3)	(Saino <i>et al.</i> , 2015a)	11 (180)	e
		(Bazzi <i>et al.</i> , 2016a)	11 (180)	a, e
Common nightingale (<i>L. megarhynchos</i>) *	Y (3)	(Saino <i>et al.</i> , 2015a)	7 (151)	e
		(Bazzi <i>et al.</i> , 2016a)	7 (151)	a, e
Tree pipit (<i>Anthus trivialis</i>) *	Y (2)	(Saino <i>et al.</i> , 2015a)	12 (170)	e
		(Bazzi <i>et al.</i> , 2016a)	12 (170)	a, e
Whinchat (<i>Saxicola rubetra</i>) *	N	(Saino <i>et al.</i> , 2015a)	13 (169)	e
		(Bazzi <i>et al.</i> , 2016a)	13 (169)	a, e
Common redstart (<i>Phoenicurus phoenicurus</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	13 (169)	a, e
Common whitethroat (<i>Curruca communis</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	13 (172)	a, e
Eastern subalpine warbler (<i>Curruca cantillans</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	7 (168)	a, e
Eurasian golden oriole (<i>Oriolus oriolus</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	7 (163)	a, e
Eurasian hoopoe (<i>Upupa epops</i>) *	Y (7)	(Bazzi <i>et al.</i> , 2016a)	3 (157)	a, e
Eurasian reed warbler (<i>Acrocephalus scirpaceus</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	10 (169)	a, e
Eurasian wryneck (<i>Jynx torquilla</i>) *	Y (6)	(Bazzi <i>et al.</i> , 2016a)	5 (135)	a, e
European bee-eater (<i>Merops apiaster</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	3 (163)	a, e
European nightjar (<i>Caprimulgus europaeus</i>) *	Y (6)	(Bazzi <i>et al.</i> , 2016a)	7 (154)	a, e
European turtle dove (<i>Streptopelia turtur</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	3 (150)	a, e
Garden warbler (<i>Sylvia borin</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	6 (169)	a, e
Great reed warbler (<i>Acrocephalus arundinaceus</i>) *	Y (2)	(Bazzi <i>et al.</i> , 2016a)	2 (163)	a, e
Icterine warbler (<i>Hippolais icterina</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	7 (169)	a, e
Northern wheatear (<i>Oenanthe oenanthe</i>) *	Y (4)	(Bazzi <i>et al.</i> , 2016a)	6 (167)	a, e
Sedge warbler (<i>Acrocephalus schoenobaenus</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	5 (163)	a, e
Spotted flycatcher (<i>Muscicapa striata</i>) *	Y (7)	(Bazzi <i>et al.</i> , 2016a)	5 (162)	a, e
Willow warbler (<i>Phylloscopus trochilus</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016a)	10 (174)	a, e
		(Bazzi <i>et al.</i> , 2017)	10 (174)	e, f
Wood warbler (<i>Phylloscopus sibilatrix</i>) *	N	(Bazzi <i>et al.</i> , 2016a)	5 (162)	a, e
Woodchat shrike (<i>Lanius senator</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016a)	8 (176)	a, e
Wilson's warbler (<i>Cardellina pusilla</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2016b)	9 (158)	a, e

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3	Painted bunting (<i>Passerina ciris</i>) *	Y (2)	(Contina <i>et al.</i> , 2018)	4 (169)	e
4	Yellow-legged gull (<i>Larus michahellis</i>) †	Y (2)	(Romano <i>et al.</i> , 2018)	4 (162)	b
5	Blackpoll warbler (<i>Setophaga striata</i>) *	N	(Ralston <i>et al.</i> , 2019)	16 (189)	a, e
6	Collared flycatcher (<i>Ficedula albicollis</i>) *	N	(Krist <i>et al.</i> , 2021)	6 (182)	e
7	Mountain bluebird (<i>Sialia currucoides</i>) *	N	(Sauve <i>et al.</i> , 2021)	7 (169)	a
8	Western bluebird (<i>Sialia mexicana</i>) †	Y (6)	(Sauve <i>et al.</i> , 2021)	7 (170)	a
9	Collared plover (<i>Charadrius collaris</i>) ‡	N	(de Almeida Miranda <i>et al.</i> , 2022)	6 (172)	a
10	Semipalmated sandpiper (<i>Calidris pusilla</i>) *	N	(de Almeida Miranda <i>et al.</i> , 2022)	6 (188)	a
11	Semipalmated plover (<i>Charadrius semipalmatus</i>) *	N	(de Almeida Miranda <i>et al.</i> , 2022)	5 (178)	a
12	Spotted sandpiper (<i>Actitis macularius</i>) *	N	(de Almeida Miranda <i>et al.</i> , 2022)	4 (196)	a

CREB1 gene: (NCBI Gene ID: 1385)

13	Blue tit (<i>Cyanistes caeruleus</i>) †	Y (9)	(Steinmeyer <i>et al.</i> , 2009)	7 (548)	-
14	Eurasian blackcap (<i>Sylvia atricapilla</i>) †	Y (5)	(Mueller <i>et al.</i> , 2011)	10 (532)	a, b
15	Eurasian buzzard (<i>Buteo buteo</i>) ‡	Y (6)	(Chakarov <i>et al.</i> , 2013)	3 (533)	a, c, e
16	Red kite (<i>Milvus milvus</i>) ‡	Y (2)	(Chakarov <i>et al.</i> , 2013)	2 (534)	a, c, e
17	Northern goshawk (<i>Accipiter gentilis</i>) ‡	Y(10)	(Chakarov <i>et al.</i> , 2013)	1 (534)	a, c, e
18	Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Mueller <i>et al.</i> , 2013b)	2 (532)	g
19	Tree swallow (<i>Tachycineta bicolor</i>) *	N	(Bourret & Garant, 2015)	3 (518)	a, c
20	Willow warbler (<i>Phylloscopus trochilus</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2017)	4 (529)	e, f
21	Collared flycatcher (<i>Ficedula albicollis</i>) *	N	(Krist <i>et al.</i> , 2021)	9 (534)	e

NPAS2 gene: (NCBI Gene ID: 4862)

22	Blue tit (<i>Cyanistes caeruleus</i>) †	Y (9)	(Steinmeyer <i>et al.</i> , 2009)	5 (Q ₁₂)	-
23	Eurasian blackcap (<i>Sylvia atricapilla</i>) †	Y (5)	(Mueller <i>et al.</i> , 2011)	2 (Q ₈)	a, b
24	Eurasian buzzard (<i>Buteo buteo</i>) ‡	Y (6)	(Chakarov <i>et al.</i> , 2013)	2 (Q ₉)	a, c, e
25	Red kite (<i>Milvus milvus</i>) ‡	Y (2)	(Chakarov <i>et al.</i> , 2013)	2 (Q ₈)	a, c, e
26	Northern goshawk (<i>Accipiter gentilis</i>) ‡	Y(10)	(Chakarov <i>et al.</i> , 2013)	2 (Q ₁₁)	a, c, e
27	Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Mueller <i>et al.</i> , 2013b)	3 (Q ₁₀)	g
28	Tree swallow (<i>Tachycineta bicolor</i>) *	N	(Bourret & Garant, 2015)	7 (Q ₁₁)	a, c
29	Willow warbler (<i>Phylloscopus trochilus</i>) *	Y (3)	(Bazzi <i>et al.</i> , 2017)	5 (Q ₁₀)	e, f
30	Yellow-legged gull (<i>Larus michahellis</i>) †	Y (2)	(Romano <i>et al.</i> , 2018)	1 (Q ₇)	b
31	Collared flycatcher (<i>Ficedula albicollis</i>) *	N	(Krist <i>et al.</i> , 2021)	4 (Q ₁₁)	e

DRD4 gene: (NCBI Gene ID: 1815)

32	Great tit (<i>Parus major</i>) †	Y(15)	(Korsten <i>et al.</i> , 2010)	SNP	h
33			(Mueller <i>et al.</i> , 2013a)	SNP	h
34	Eurasian blackcap (<i>Sylvia atricapilla</i>) †	Y (5)	(Mueller <i>et al.</i> , 2011)	SNP	h
35	Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Mueller <i>et al.</i> , 2013b)	SNP	g, h
36	Seychelles warbler (<i>Acrocephalus sechellensis</i>) †	N	(Edwards <i>et al.</i> , 2015)	SNP	h
37	Black swan (<i>Cygnus atratus</i>) ‡	N	(Van Dongen <i>et al.</i> , 2015)	SNP	g, h
38	Song sparrow (<i>Melospiza melodia</i>) *	Y(25)	(Posliff, 2020)	SNP	a, h
39	Mountain bluebird (<i>Sialia currucoides</i>) *	N	(Sauve <i>et al.</i> , 2021)	SNP	a

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Western bluebird (*Sialia mexicana*) †

Y (6)

(Sauve *et al.*, 2021)

SNP

a

1614 Note: Species are listed in chronological order of publication. The presence of
 1615 subspecies is indicated (Y = Yes, N = No) along with the total alleles and most frequent
 1616 Poly-Q allele. Studies used latitude/longitude/spatial analyses (a), migratory
 1617 restlessness (b), timing of egg laying/breeding (c), clutch size (d), timing of migration
 1618 (e), moult rate (f), urbanization (g) or exploratory behaviour (h). (Migration status: *
 1619 Migratory † Partial Migratory ‡ Sedentary)

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1621 **Table 3:** Summary of studies that used non-candidate gene approaches to identify
 1622 genetic regions that either co-vary with migration phenology or are expressed
 1623 differentially on a circannual basis in class *Aves*.

Species	Sub-species	Reference	Type of study
White-crowned sparrow (<i>Zonotrichia leucophrys</i>) †	Y (5)	(Jones <i>et al.</i> , 2008a, 2008b)	a
Willow warbler (<i>Phylloscopus trochilus</i>) *	Y (3)	(Lundberg <i>et al.</i> , 2013)	a
		(Boss <i>et al.</i> , 2015)	a
		(Lundberg <i>et al.</i> , 2017)	b
Red-headed bunting (<i>Emberiza bruniceps</i>) *	N	(Singh <i>et al.</i> , 2013)	a
		(Sharma <i>et al.</i> , 2018b)	a, c
		(Trivedi <i>et al.</i> , 2019)	a
Swainson's thrush (<i>Catharus ustulatus</i>) *	Y (6)	(Ruegg <i>et al.</i> , 2014a)	b
		(Delmore <i>et al.</i> , 2015, 2016)	b
		(Johnston <i>et al.</i> , 2016)	b
Wilson's warbler (<i>Cardellina pusilla</i>) *	Y (3)	(Ruegg <i>et al.</i> , 2014b)	b
House sparrow (<i>Passer domesticus</i>) †	Y(14)	(Guldvog, 2015)	a
Italian sparrow (<i>Passer italiae</i>) ‡	N	(Guldvog, 2015)	a
Spanish sparrow (<i>Passer hispaniolensis</i>) †	Y (2)	(Guldvog, 2015)	a
Black-headed bunting (<i>Emberiza melanocephala</i>) *	N	(Singh <i>et al.</i> , 2015)	a
		(Mishra, Singh, & Kumar, 2017)	a
		(Sharma <i>et al.</i> , 2018a)	a
Eurasian blackbird (<i>Turdus merula</i>) †	Y (7)	(Franchini <i>et al.</i> , 2017)	a
Barn swallow (<i>Hirundo rustica</i>) *	Y (7)	(Saino <i>et al.</i> , 2017)	c
Tree swallows (<i>Tachycineta bicolor</i>) *	N	(Brown, 2019)	a
Painted bunting (<i>Passerina ciris</i>) *	Y (2)	(Contina <i>et al.</i> , 2019)	b
Gray catbird (<i>Dumetella carolinensis</i>) *	N	(DeMoranville <i>et al.</i> , 2019)	a
Great tit (<i>Parus major</i>) †	Y(15)	(Laine <i>et al.</i> , 2019)	a
		(Mäkinen <i>et al.</i> , 2019)	c
		(Viitaniemi <i>et al.</i> , 2019)	c
Eurasian blackcap (<i>Sylvia atricapilla</i>) †	Y (5)	(Delmore <i>et al.</i> , 2020a, 2020b)	b
Northern wheatear (<i>Oenanthe oenanthe</i>) *	Y (4)	(Frias-Soler <i>et al.</i> , 2020, 2021)	a
Chestnut-crowned babblers (<i>Pomatostomus ruficeps</i>) ‡	N	(Liebl <i>et al.</i> , 2021)	c
American kestrel (<i>Falco sparverius</i>) †	Y(17)	(Bossu <i>et al.</i> , 2022)	b

1624 Note: Species are listed in chronological order of publication with subspecies number
 1625 indicated (Y = Yes, N = No) along with the method used. Study method used
 1626 transcriptomics (a), genomics (b), or epigenetics (c); studies that included clock genes
 1627 are discussed in the text. (Migration status: * Migratory † Partial Migratory ‡

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1629 **Table 4:** Summary statistics for data used in the cross-species comparative analyses of the *Clock* gene.

Variable (x)	N	Mean (\bar{x})	Std deviation (σ)	Minimum (x_{\min})	Maximum (x_{\max})
Mantel tests:					
Genetic distance (F_{ST})	39	1.54	1.98	0.00	9.42
Geographic distance (°):					
Breeding latitude	39	58.129	48.351	0.000	156.573
Non-breeding latitude	39	71.046	63.941	0.000	178.575
Taxonomic distance (weighed)	39	81.12	21.69	0.00	100.00
Evolutionary distance:					
Mean divergence times (MYA)	39	39.86	19.19	0.00	75.00
PGLS models:					
<i>Migrants</i>					
Allele size (Poly-Q's)	58	9	2.22	4	14
Observed Heterozygosity (H_o)	48	0.325	0.249	0.000	0.839
Breeding latitude (°)	58	17.987	13.039	0.837	56.049
Non-breeding latitude (°)	58	48.429	9.7407	4.669	66.762
Migration distance (m)	58	4,573,174.68	2,232,252.07	875,528.03	12,388,216.66
Migration distance (°)	58	41.018	19.934	8.250	111.290
<i>Residents</i>					
Allele size	18	10	1.94	8	14
Observed Heterozygosity (H_o)	8	0.131	0.154	0.000	0.343
Latitude (°)	18	23.876	17.396	2.419	55.384
Range (m)	18	691,024.34	727,105.36	10,530.99	2,657,514.74

1630 **Table 5:** Summary statistics for data used in the cross-species comparative analyses of the *Adcyap1* gene.

Variable (x)	N	Mean (\bar{x})	Std deviation (σ)	Minimum (x_{\min})	Maximum (x_{\max})
Mantel tests:					
Genetic distance (F_{ST})	37	1.96	2.23	0.00	9.46
Geographic distance (°):					
Breeding latitude	37	57.436	48.853	0.000	156.573
Non-breeding latitude	37	71.872	64.245	0.000	178.575
Taxonomic distance (weighed)	37	80.51	22.09	0.00	100
Evolutionary distance:					
Mean divergence times (MYA)	37	39.63	19.71	0.00	75.00
PGLS models:					
<i>Migrants</i>					
Allele size (base pairs, bp)	54	166	9.60	135	195
Observed Heterozygosity (H_o)	39	0.670	0.179	0.1905	0.914
Breeding latitude (°)	54	17.964	13.486	0.838	56.050
Non-breeding latitude (°)	54	47.549	14.537	4.669	66.762
Migration distance (m)	54	4,669,913.60	2,263,790.40	875,528.03	12,388,216.66
Migration distance (°)	54	41.873	20.240	8.250	111.290
<i>Residents</i>					
Allele size	17	170	13.55	139	198
Observed Heterozygosity (H_o)	2	0.538	0.319	0.312	0.764
Latitude (°)	17	24.457	17.751	2.419	55.384
Range (m)	17	715,937.68	741,521.71	10,530.99	2,657,514.74

Table 6: Summary of results for population genetics comparative analysis testing for Hardy-Weinberg equilibrium, heterozygosity, neutrality, and linkage disequilibrium.

	<i>Clock</i>			<i>Adcyap1</i>		
Hardy-Weinberg	<i>N</i>	Total	<i>P</i>-value	<i>N</i>	Total	<i>P</i>-value
In equilibrium	34	39	< 0.02	32	37	< 0.02
Not in equilibrium	5			5		
Heterozygosity	<i>N</i>	Total	H_o/H_e	<i>N</i>	Total	H_o/H_e
Observed (H_o) > Expected (H_e)	17	39	0.325/0.33	19	37	0.678/0.69
Expected (H_e) > Observed (H_o)	15			18		
Observed (H_o) = Expected (H_e)	3			-		
Single allele	4			-		
Neutrality (Ewes-Watterson)	<i>N</i>	Total	<i>P</i>-value	<i>N</i>	Total	<i>P</i>-value
Neutral	32	39	> 0.05	32	37	< 0.05
Selection	3			5		
Single allele	4			-		
Linkage Disequilibrium	<i>N</i>	Total				
<i>Clock</i> vs <i>Adcyap1</i>						
In equilibrium	33	36				
Not in equilibrium	3					

This table summarises the main findings for the population genetics analysis of individual species for which allele data was available. For both tested alleles most species passed the Hardy-Weinberg equilibrium test, with only five species failing equilibrium assumptions in each case. The overall test for equilibrium between species failed, as anticipated, with a P -value < 0.02. For both alleles the observed heterozygosity (H_o) was slightly higher than the expected (H_e) in nearly half of the tested species but the overall test for the total cohort revealed an observed value that was lower than the expected value. Three species had perfectly matched observed and expected heterozygosity. The *Clock* allele was mono-allelic in four of the thirty-nine species. The neutrality tests only detected significant (P -value < 0.05) evidence for selection in three species for the *Clock* allele and five species for the *Adcyap1* allele; overall tests for selection detected no evidence thereof for *Clock* but did detect deviation from neutrality for *Adcyap1*. For the thirty-six species for which data of both alleles was available, linkage disequilibrium was detected in three species while the remainder appeared to be in equilibrium.

1649 **Table 7:** Results for statistical comparative analysis using Mantel tests and
 1650 Phylogenetic Generalised Least Squares (PGLS) regression.

Mantel tests	<i>Clock</i>			<i>Adcyap1</i>			
	Z-value	P-value	R-value	Z-value	P-value	R-value	
Geography:							
Breeding latitudes	0.086	0.099*	1.638	0.036	0.499	0.657	
Non-Breeding latitudes	0.124	0.018***	2.426	0.033	0.520	0.631	
Taxonomy:							
Taxonomic distance	0.116	0.013***	2.479	0.058	0.239	1.185	
Evolutionary history:							
Mean divergence time	-0.139	0.051*	-1.901	-0.190	0.010***	-2.573	
PGLS		P-value	R ²	DF	P-value	R ²	DF
Geography:							
Breeding latitudes		0.275	0.02	74	0.300	0.02	69
Non-Breeding latitudes		0.220	0.03	56	0.472	0.01	52
Migration distance		0.093*	0.04	74	0.342	0.01	69
Timing:							
Spring migration vs Winter solstice (9:15 LD)	Start	0.343	0.02	56	0.273	0.02	52
	Mid	0.325	0.02	56	0.096*	0.05	52
	End	0.406	0.01	56	0.070*	0.06	52
Spring migration vs Spring equinox (12:12 LD)	Start	0.638	0.00	56	0.450	0.01	52
	Mid	0.454	0.01	56	0.480	0.01	52
	End	0.389	0.01	56	0.532	0.01	52
Autumn migration vs Summer solstice (15:9 LD)	Start	0.077*	0.05	56	0.905	0.00	52
	Mid	0.058*	0.06	56	0.643	0.00	52
	End	0.221	0.03	56	0.443	0.01	52
Autumn migration vs Autumn equinox (12:12 LD)	Start	0.097*	0.05	56	0.950	0.00	52
	Mid	0.070*	0.06	56	0.602	0.01	52
	End	0.236	0.03	56	0.420	0.01	52
Phylogenetic signal (Evolutionary history):							
Lambda (λ)		0.000***	0.92	74	0.000***	0.75	69
Kappa (κ)		0.001***	0.73	74	0.001***	0.61	69

1651 Summary table of both Mantel and PGLS regression analysis results for both the *Clock*
 1652 and *Adcyap1* genes respectively. For Mantel tests, the Z-value, P-value, and R-value
 1653 results are reported for tests comparing the genetic distance between species to the
 1654 geographic distance between latitudes, the taxonomic distance between species, as
 1655 well as the temporal distance given by divergence times. A significant relationship was

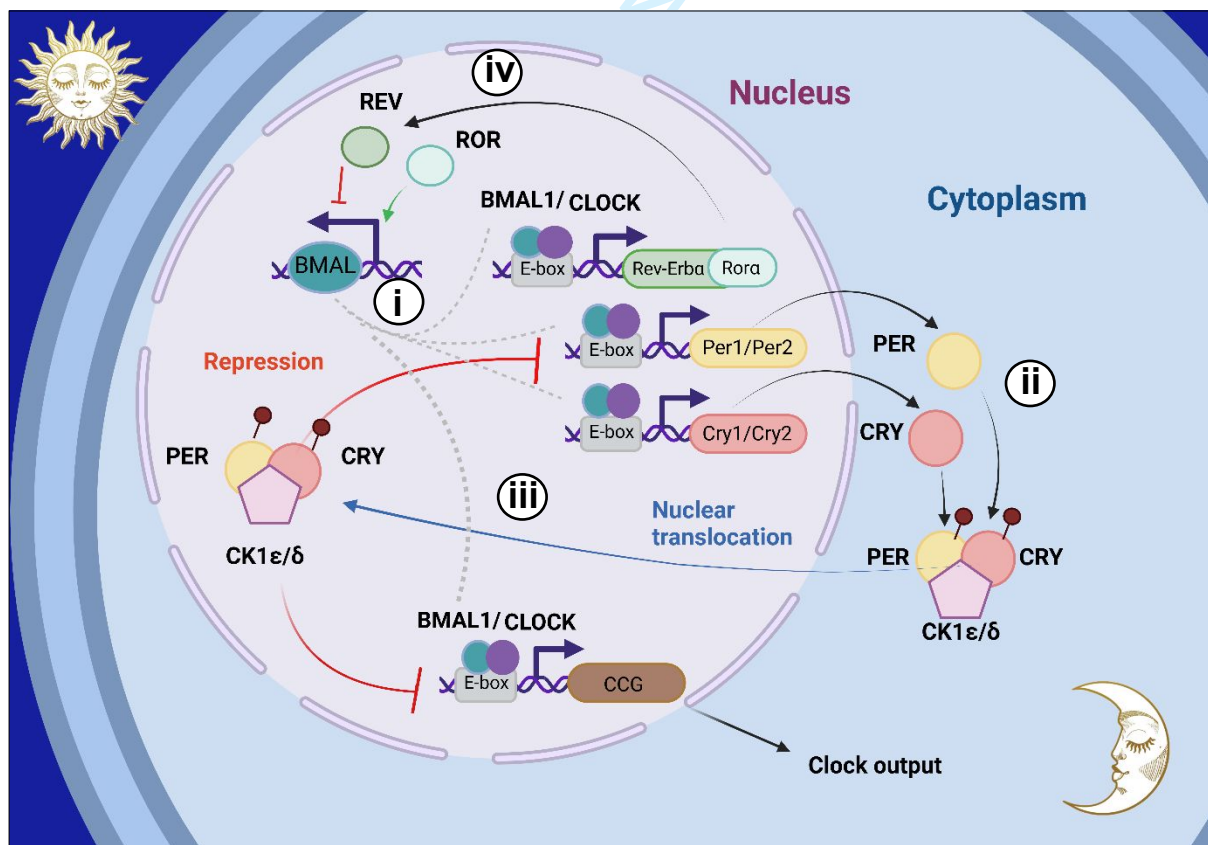
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3 1656 detected between the genetic distance and both breeding and non-breeding latitude
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5 1657 distances ($P < 0.10$ and $P < 0.02$) as well as taxonomic distance ($P < 0.10$) and
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7 1658 divergence time ($P < 0.10$) for the *Clock* alleles, however, a significant relationship
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9 1659 was only found between the genetic distances of *Adcyap1* alleles and the divergence
10 1660 time ($P < 0.02$). For PGLS, the P -value, correlation coefficient (R^2), and degrees of
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12 1661 freedom (DF) are reported for tests comparing the most common allele for each
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14 1662 species to their breeding and non-breeding range latitudes, migration distance
15 1663 between ranges, and migration dates as compared to annual dates with known relative
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17 1664 photoperiods. No significant relationship was found between latitudes and allele
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19 1665 length; however, a correlation was observed between allele length for *Clock* and the
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21 1666 timing of Autumn migration as well as between *Adcyap1* and the timing of Spring
22 1667 migration. A significant relationship was also detected between *Clock* allele length and
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24 1668 total migration distance between breeding and non-breeding latitudes. The strength of
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26 1669 the phylogenetic signal for each gene was also assessed by estimating the lambda
27 1670 and kappa parameters and in both genes a strong phylogenetic signal was detected
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29 1671 for the observed most common alleles.

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31 1672 (Significance: * P -value < 0.10 , ** P -value < 0.05 , *** P -value < 0.02)
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3 1673 **Figures**

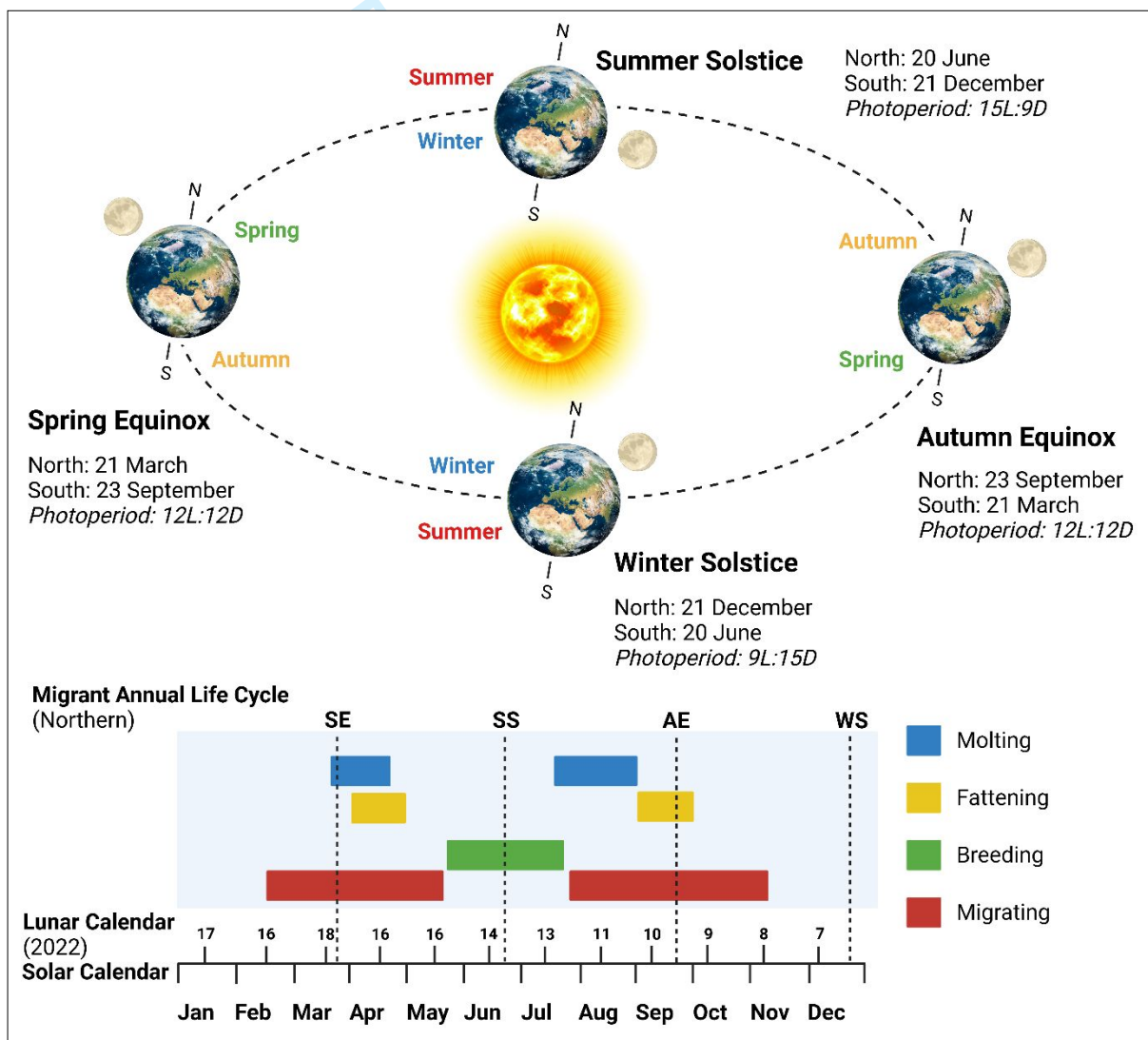
4 1674 **Figure 1:** Diagrammatic representation of the regulation of the circadian clock. **i)** ,
5 1675 Brain and muscle ARNT-like protein 1 (*Bmal1*, blue circles) forms a dimer with
6 1676 Circadian Locomotor Output Cycles Protein Kaput (*Clock*, purple circles) in the
7 1677 nucleus, which binds to the enhancer box (E-box, in grey) region of nuclear receptors
8 1678 REV-ERB (Rev-Erba, green) and retinoic acid-related orphan nuclear receptors
9 1679 (*Rora*), as well as the *Period 1* or *2* (*Per1/2*, yellow), *Cryptochrome 1* or *2* (*Cry1/2*,
10 1680 orange), and other Circadian Clock Genes (CCG's, brown). **ii)** When the dimer binds
11 1681 to the *Per1/Per2* and *Cry1/Cry2* E-box, PER and CRY proteins are expressed and,
12 1682 following phosphorylation, form a complex with Casein kinase 1 isoform epsilon or
13 1683 delta (CK1 ϵ/δ) in the cytoplasm. **iii)** The PER/CRY complex then undergoes nuclear
14 1684 translocation (blue arrow) where it downregulates *Per1/Per2*, *Cry1/Cry2*, and other
15 1685 CCG transcription by inhibiting the binding of *Bmal1/Clock* complexes to E-box regions
16 1686 (showed by the red lines). **iv)** Concurrently, the binding of *Bmal1/Clock* complexes to
17 1687 the enhancer elements of *Rev-Erba* and *Rora* results in the expression of the REV
18 1688 and ROR proteins which act on the transcription elements of *Bmal1*, where REV acts
19 1689 as an inhibitor while ROR acts as an inducer.

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3 1692 **Figure 2:** Diagrammatic illustration of the seasonal cycles of the year indicating the
4 1693 four key dates for changing photoperiods of the year: the Summer solstice, the Winter
5 1694 solstice, the Spring equinox, and the Autumn equinox. The equinoxes correspond to
6 1695 near equal day and night (12h Light: 12h Dark) while the Summer solstice is the
7 1696 longest day (15h Light: 9h Dark) and the Winter solstice is the shortest day (9h Dark:
8 1697 15h Light). The dates of the solstices and equinoxes fall on the exact opposite days in
9 1698 the Northern and Southern hemispheres each year. The bottom panel indicates the
10 1699 annual timing of major life events during the year for migrants of the northern
11 1700 hemisphere, which encompasses most migratory species in this review. Dates are
12 1701 adapted from the literature and relative to the solar and lunar calendars.

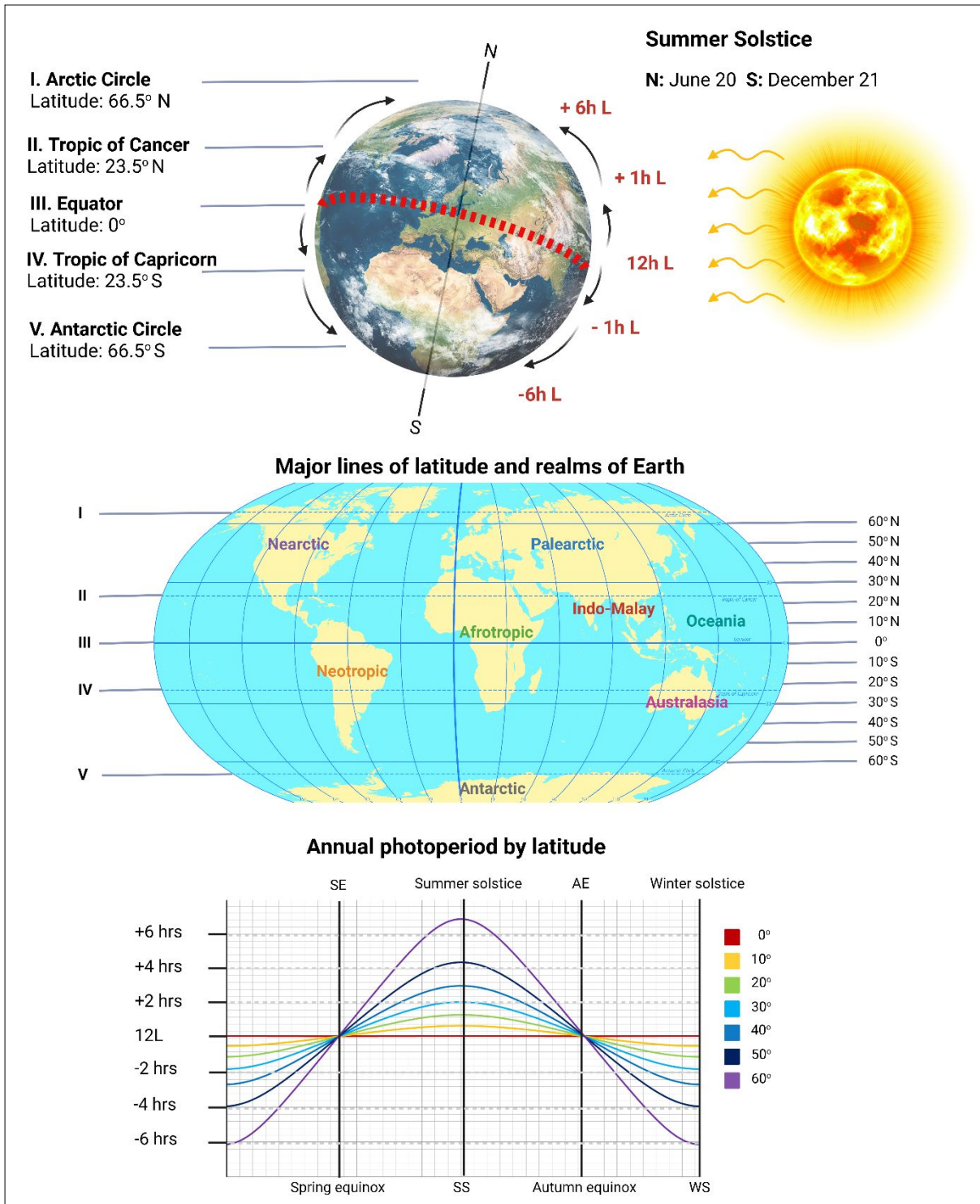


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3 1705 **Figure 3:** Diagrammatic representation of the major lines of latitude of Earth and the
4 relative photoperiod at each latitude between the four key dates for changing
5 1706 photoperiods. First, the orientation of the Earth on the Summer solstice is indicated
6 with the difference in daylength between the equator (III) and the two major tropics
7 1707 and the arctic circles. In the centre, these lines are indicated on a map, along with the
8 1708 intermediate lines of latitude. Below this, the annual variation in photoperiod is charted
9 for each ten-degree increment in latitude between the two solstices and equinoxes.
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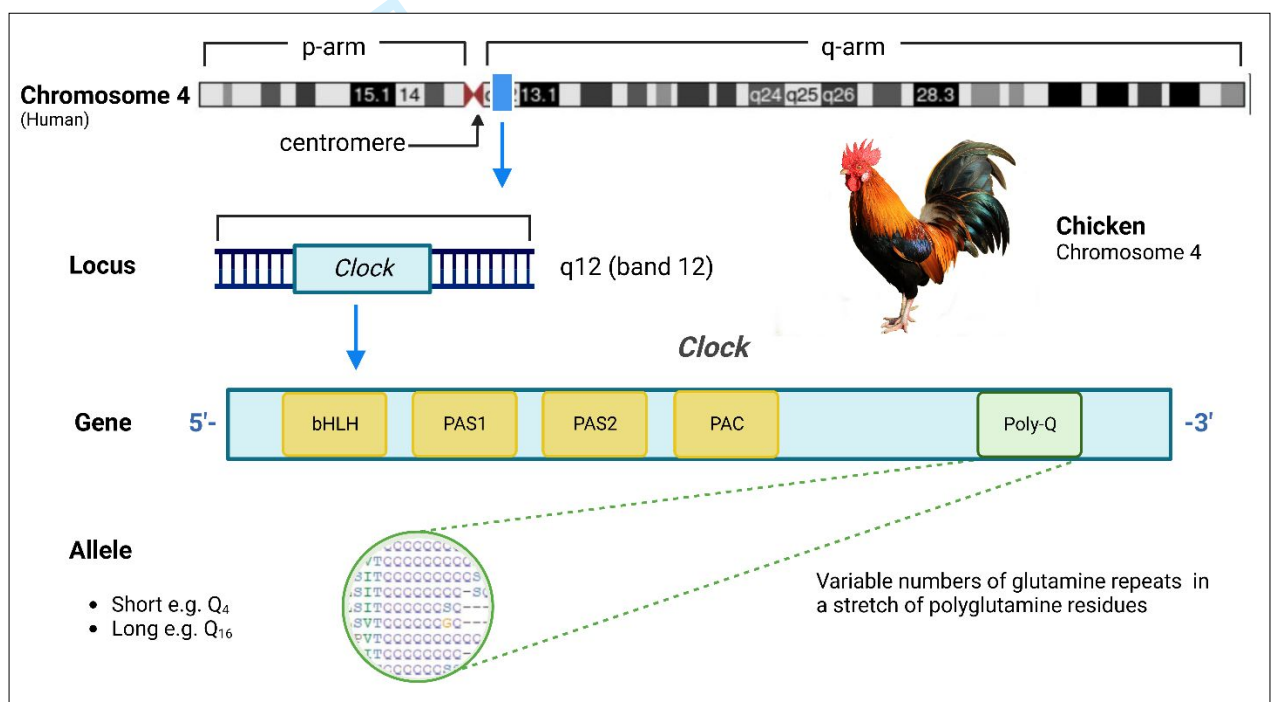
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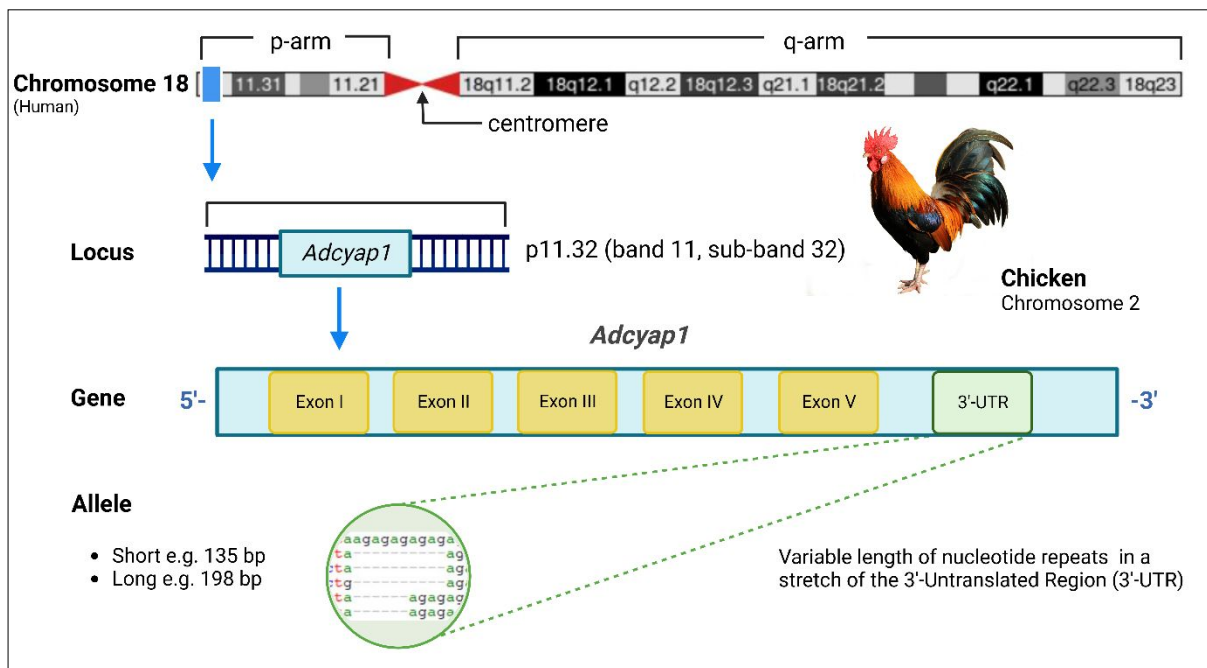
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3 1713 **Figure 4:** Depiction of the *Clock* gene (NCBI Gene ID: 9575) and the variable poly
4 1714 glutamine (Poly-Q) repeat region associated with migration phenology. The top panel
5 1715 indicates the location of the *Clock* gene in the human genome on chromosome four at
6 1716 position 12.0 on the q-arm of the chromosome. The second panel indicates the gene
7 1717 transcript with its four primary domains basic helix-loop-helix (bHLH), Period-Ah
8 1718 receptor nuclear translocator (ARNT)-Single minded protein (PAS1/PAS2), and PAS-
9 1719 associated C-terminal (PAC) in yellow. The Poly-Q region is indicated in green, which
10 1720 is a stretch of amino acid residues of glutamine (Q) of varied length within and between
11 1721 species.
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1725 **Figure 5:** Depiction of the *Adcyap1* gene (NCBI Gene ID: 116) and the variable 3'-
 1726 UTR region associated with migration phenology. The top panel indicates the location
 1727 of the *Adcyap1* gene in the human genome on chromosome eighteen at position 11.32
 1728 on the p-arm of the chromosome. The second panel indicates the organization of the
 1729 gene starting from the 5'-end, with the five exons indicated in orange. The three prime
 1730 untranslated region (3'-UTR) is indicated in green, which contains a stretch of nucleic
 1731 acid repeats of adenine and guanine that vary within and between species.
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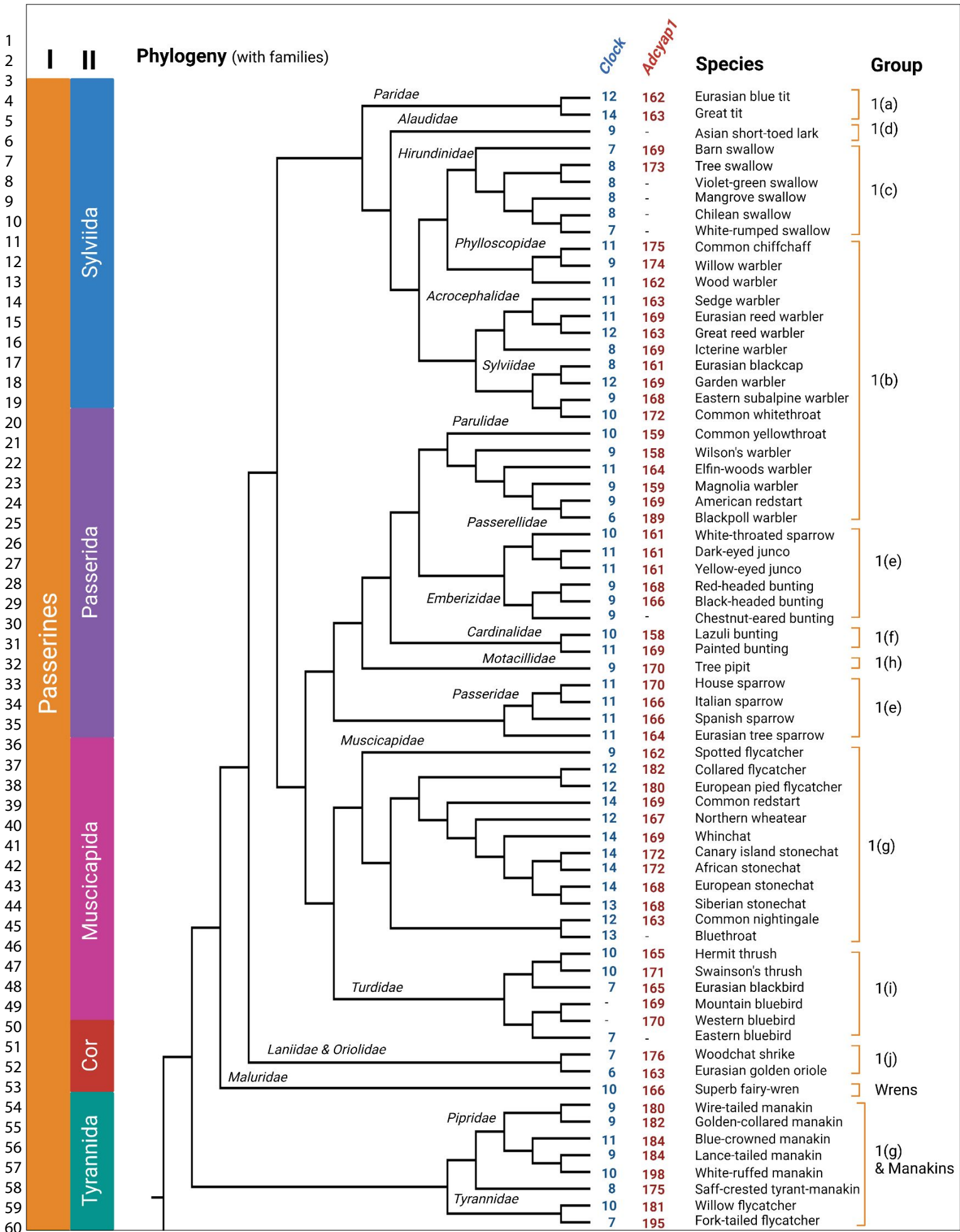
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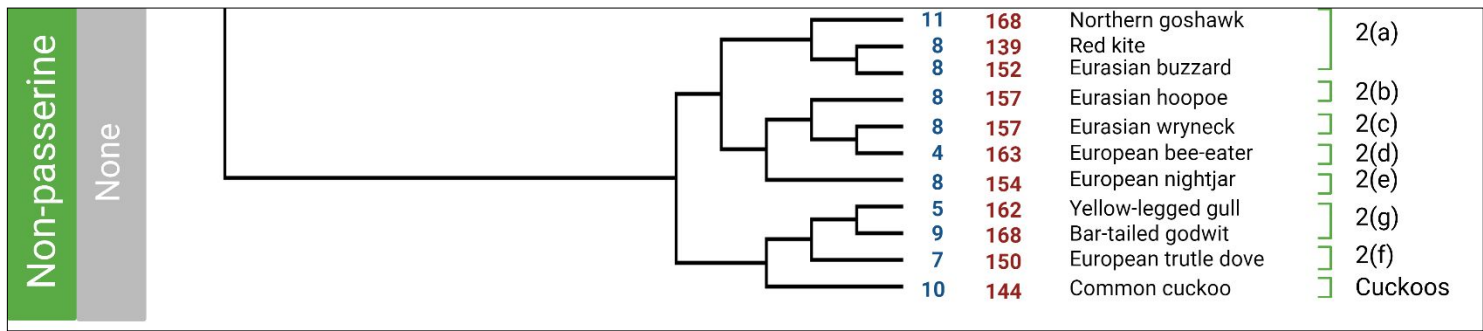
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3 1735 **Figure 6:** Phylogenetic tree displaying the relatedness and taxonomy of species for
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5 1736 which clock genes have been assayed in class *Aves* from previous studies, as well as
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7 1737 additional species included in the present analysis, used for both the phylogenetic
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9 1738 hypothesis for comparing studies as well as the PGLS analysis. The final tree shows
10 1739 the major taxonomic divisions between species used in the comparative analyses by
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12 1740 order (I), passerine and non-passerine, as well as parvorders (II), including Sylviida,
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14 1741 Passerida, Muscicapida, Corvida (Cor), and Tyrannida. Families are indicated at the
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16 1742 relevant clades of the tree while the main grouping, used in discussing and comparing
17 1743 studies in the text, are indicated to the right. The most common *Clock* allele, as number
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19 1744 of Poly-Q repeats, is indicated for each species along with the length, in base pairs,
20 1745 for the most common *Adcyap1* allele.

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3 **1776** **Figure 7:** Time tree of study species indicating the relative divergence times for
4 speciation, in millions of years ago (MYA), of the major lineages for the study species.
5 **1777** Each panel indicates a paleogeographic epoch, colour coded to the corresponding
6 **1778** period. The time scale starts at the primary division of passerine and non-passerine
7 **1779** birds more than 75 MYA during the Upper Cretaceous period, with more recent
8 **1780** divisions within lineages starting around 70 MYA and continuing to as recent as 20
9 **1781** MYA during the Paleogene and early Neogene. Most recent speciation events within
10 **1782** lineages occurred in the Miocene and Pliocene (Plio) and continued to modern times.
11 **1783** The left panel shows the corresponding paleogeography from before the continents
12 **1784** were fully assembled (75 MYA) to when most continents had assumed their
13 **1785** contemporary shape, approximately 30 MYA, and finally assumed their modern-day
14 **1786** positions in terms of latitude and longitude (today).
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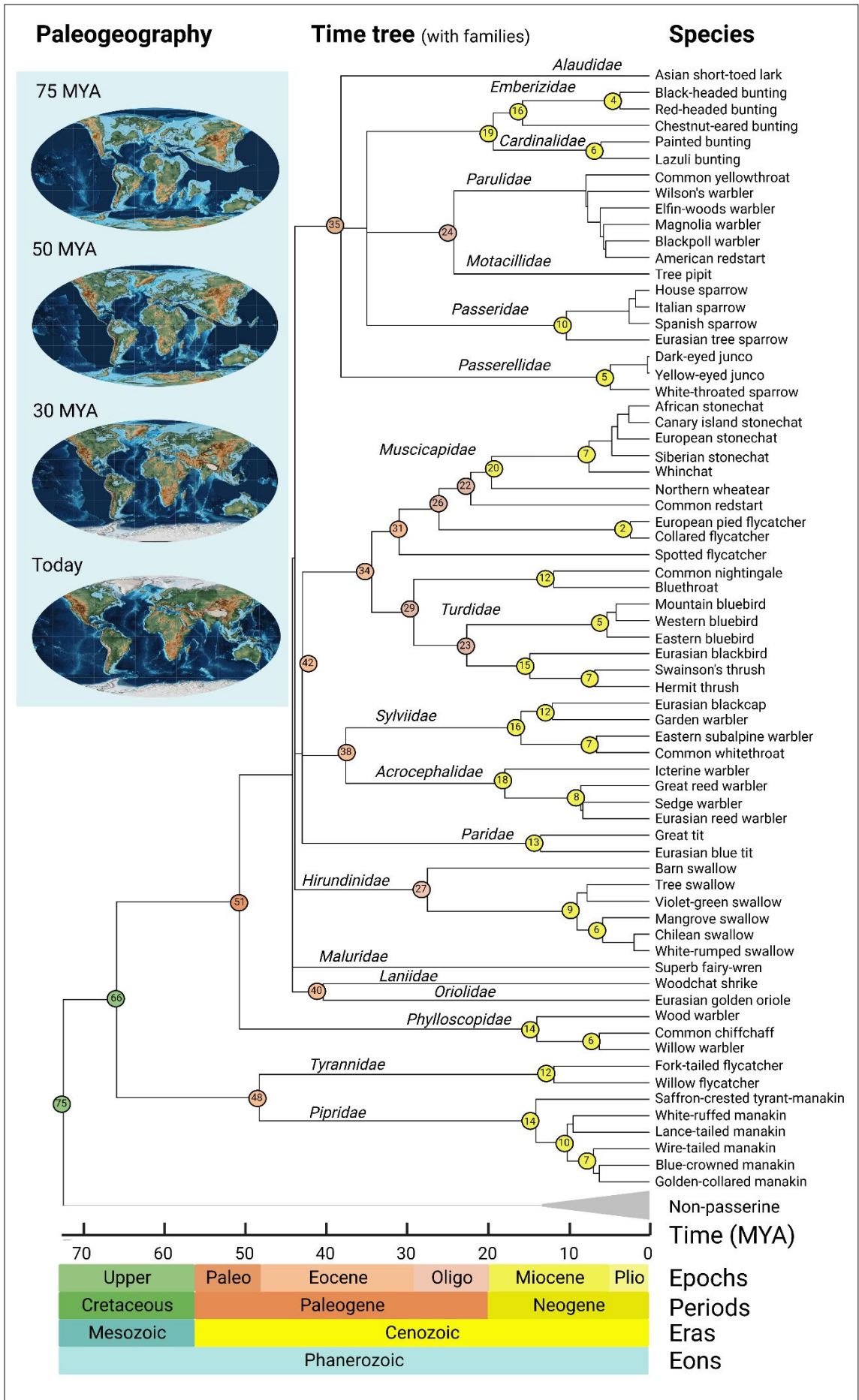


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I. DETAILED METHODS

(1) Literature search and systematic review

A systematic approach was used to search for and synthesise the available literature. Both white literature and grey literature was searched on the Scopus (www.scopus.com) and Dimensions (www.dimensions.ai) databases using the following Boolean search string: ("Clock genes" OR "Clock" OR "Adcyap1") AND ("Birds" OR "Avian") AND ("Migration" OR "Flying"). White literature included the primary scientific literature inclusive of research articles, short communications, review articles, and conference abstracts. Grey literature included ancillary sources of scientific information including dissertations, theses, and internet sources such as websites. Search results were exported in the comma separated value format and the literature was subsequently summarised, guided by citation networks visualised using CitNetExplorer 1.0.0 and VOSviewer 1.6.16 (van Eck & Waltman, 2017). The results retrieved from Scopus was converted to the appropriate format with the R package 'Scopus2CitNet 0.1.0.0' in RStudio 1.4.1106 (RStudio Team, 2021), running R version 4.0.5 (R Core Team, 2020). Due to the diverse array of species in which these studies have been conducted, the literature was first organised by candidate gene and year of publication followed by species, with taxonomic grouping in the text based firstly on

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3 order, as either passerine or non-passerine, followed by families. Families were
4 grouped, based on higher taxonomic classifications, in superfamilies and parvorders
5 for the sake of a concise and cohesive comparison. The proper taxonomic
6 classification was validated against the Handbooks of the Birds of the World (del Hoyo
7 *et al.*, 2013) and BirdLife International checklist, version 6 (HBW and BirdLife
8 International, 2021). Instances where the taxonomic classification has changed since
9 the publication of a study, or is currently under review, are highlighted throughout the
10 text. The inclusion criteria of a study were confined to studies that primarily measure
11 *Clock* or *Adcyap1* gene polymorphisms, as well as other candidate genes studied in
12 parallel, within a population of birds with views on comparing putative variation to
13 elements of the annual synchronicity in life events and differential migration. These
14 included latitude/longitude/spatial analyses, timing of migration, migratory
15 restlessness, timing of egg laying/breeding, clutch size, moult rate, urbanization and
16 exploratory behaviour. This was complimented with a free term search for studies
17 overlapping in the range of species covered with a focus on genomic, transcriptomic,
18 or epigenetic methods of identifying genetic variation that relates to any of the
19 aforementioned attributes.

32 (2) Species

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35 Species for comparative analysis were selected based on existing literature available
36 on either the *Clock* or *Adcyap1* gene in relation to migration phenology and/or for which
37 genomic or transcriptomic studies have been conducted. This included unpublished
38 data from eight species: American redstart, *Setophaga ruticilla* [Linnaeus, 1758];
39 Common chiffchaff, *Phylloscopus collybita* [Vieillot, 1817]; Common yellowthroat,
40 *Geothlypis trichas* [Linnaeus, 1766]; Hermit thrush, *Catharus guttatus* [Pallas, 1811],
41 Magnolia warbler, *Setophaga magnolia* [Wilson, 1811]; Swainson's thrush, *Catharus*
42 *ustulatus* [Nuttall, 1840]; and White-throated sparrow, *Zonotrichia albicollis* [Gmelin,
43 1789]. As most studies thus far have focused on Palearctic and Nearctic birds, species
44 were further complemented with migrant and resident bird species from other locations
45 to have a globally distributed dataset. This included the addition of several species of
46 Manakins, resident birds found in the equatorial parts of the Neotropics, the
47 endangered island restrained Elfin woods warbler, *Setophaga angelae* [Kepler &
48 Parkes, 1972], and the Australasian Superb fairy-wren, *Malurus cyaneus* [Ellis, 1782].
49 Our final dataset included 76 species, 76 for *Clock* and 71 for *Adcyap1*, of which 58
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3 were classified as migrants and 18 were classified as residents. Migrants were species
4 with complete or partial migratory behaviour (used to describe species that have both
5 resident and migrating populations, in this context species with a singular resident
6 population which was not sampled were treated as generally migratory), while the term
7 resident refers to species that do not follow an annual cycle of migration (although
8 some do follow a pattern of altitudinal migration within their resident range, a small
9 subset included species that have a single migratory population that were not sampled
10 and were treated as generally resident).

17 **(3) Genetic data**

19 Data was summarized from the literature based on species, number of alleles, most
20 common allele, and observed/expected heterozygosity when available. Additional
21 information such as the number of extant and presently recognised subspecies was
22 retrieved from Birds of the World (Billerman *et al.*, 2020). *Clock* and *Adcyap1* data for
23 additional species, from a wider geographic distribution and including additional
24 resident birds, was retrieved from the National Centre for Biotechnology Information
25 (NCBI) website using Basic Local Alignment and Search Tool (BLAST) searches
26 (Altschul *et al.*, 1990) against reference genomes and available databases, including
27 PopSet and Nucleotide (Agarwala *et al.*, 2018); where no sequence data was available
28 a further BLAST search was done against the Sequence Read Archive (SRA) for
29 specific species. Population level allele data was retrieved for 40 species in total, 39
30 for *Clock* and 37 for *Adcyap1*, from either the supplementary material of the article or
31 the online data repositories Dryad (Liedvogel *et al.*, 2010; Liedvogel, Cornwallis, &
32 Sheldon, 2012; Chakarov *et al.*, 2013; Bourret & Garant, 2015; Saino *et al.*, 2015;
33 Ralston *et al.*, 2019), Figshare (Mettler, Segelbacher, & Schaefer, 2015), or additional
34 data received directly from authors (see acknowledgements). *Clock* data was
35 transformed to only represent the actual number of poly-Q repeats, as different studies
36 used different primers resulting in variable lengths in the raw data while the gene itself
37 is conserved among lineages with the exception of the Poly-Q repeat. *Adcyap1* was
38 consistently amplified and sequenced with the same primer set or region facilitating
39 between study comparisons, however, the variable length in the 3'-UTR was not solely
40 attributable to the "AG" repeat as several species had a secondary source of sequence
41 length variation several base pairs upstream of the "AG" repeat region, based on
42 sequences retrieved from NCBI. Because any variation in length of *Adcyap1* is
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3 theorised to delay entrainment, the total variation, including the “AG” repeat region
4 and secondary sources of length variation, was taken into consideration for the
5 analyses.
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8 9 **(4) Migration and range data estimates**

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11 In order to compare candidate gene diversity to the latitude of their breeding and non-
12 breeding ranges, as well as the average migratory distance, estimates were computed
13 using QGIS 3.16.15 (QGIS Development Team, 2022) from shapefiles extracted from
14 the geodatabase of distribution maps compiled by BirdLife International, version
15 2021.1 (BirdLife International & Handbook of the Birds of the World, 2021),
16 supplemented with shapefile data from eBird (Fink *et al.*, 2021). Centroids were
17 computed for the non-breeding and breeding ranges to determine the average
18 coordinates by latitude and longitude (in degrees) for each. Furthermore, the average
19 migration distance between centroids was calculated (in meters), using a distance
20 matrix. Geographic distance matrixes for the breeding and non-breeding coordinates
21 of each species were generated with the java application Geographic Distance Matrix
22 Generator 1.2.3 (Ersts, 2012) in degrees. Species classified as partial migrants, for
23 which breeding and non-breeding range data was available, were treated as migrants
24 due to the resident portion belonging to a singular cryptic population that was not
25 within the sample range of published studies. Conversely, data deficient partial
26 migrants, often having a singular small population that remains migratory, were treated
27 as largely resident birds. For resident species, the data was computed only once
28 based on the full range.
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42 To compare candidate gene diversity to the timing of migration, population and
43 species specific migration dates, not stated in publications, were retrieved from eBird
44 (Fink *et al.*, 2021) and, in the case of species sampled in Italy, supplemented with data
45 from the Italian bird migration atlas (Spina & Volponi, 2008, 2009). A similar approach
46 was used for data on buntings and larks from Asia (Ali, Ripley, & Roberts, 1999). As
47 seasons and migration dates vary by hemisphere, dates were normalised to a
48 standard reference point that roughly corresponds to the photoperiod and
49 temperature. The difference in days was calculated between the start, middle and end
50 dates for Spring migration and the Summer solstice and Spring equinox, while Autumn
51 migration dates were normalised in reference to the Winter solstice and Autumn
52 equinox, of each respective hemisphere depending on the range of a specific species.
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3 The Summer solstice corresponds to the longest day and shortest night
4 (approximately 15h light: 9h dark) and the Winter solstice to the shortest day and
5 longest night (approximately 9h light: 15h dark), while the equinoxes correspond to
6 near equal photoperiods (12h light: 12h dark). The basic premise of these seasonal
7 date conversions and photoperiod are depicted in **Figure 3**.
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10 11 12 **(5) Population genetics**

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14 Allele data derived from population based studies was converted to the POPGENE
15 (.dat) format, and allele frequencies calculated, using CONVERT 1.31 (Glaubitz,
16 2004). POPGENE 1.32 (Yeh *et al.*, 1997) was used to test for Hardy-Weinberg (Hardy,
17 1908; Weinberg, 1908) equilibrium using Chi-squared (χ^2) tests (with significance
18 measured at $\alpha = 0.02$), to calculate the observed (H_o) and expected (H_e)
19 heterozygosity, and to create a genetic distance matrix using F_{ST} values. Python for
20 Population Genetics (PyPop) version 0.7.0 (Lancaster *et al.*, 2007) was used to test
21 for selection and neutrality as well as linkage disequilibrium. Neutrality was assessed
22 using Slatkin's implementation (Slatkin, 1994) of the Ewens-Watterson (Ewens, 1972;
23 Watterson, 1977) test, with the probability values expressed as the relative degree at
24 which the observed F -value occurs in a sample distribution with simulation run with
25 10,000 repeats. F -values which correspond to the upper or lower five percent are
26 considered significant (Manly, 1985). Linkage disequilibrium was assessed using two
27 measures: the overall Linkage Disequilibrium, D' (Hedrick, 1987), and Cramer's V
28 Statistic, W_n (Cramer, 1946). For each locus pair the log-likelihood of obtaining the
29 observed data given the inferred genotype frequencies, and the likelihood of the data
30 under the null hypothesis of linkage equilibrium is used to calculate the S statistic,
31 which is defined as twice the difference between the likelihoods. An empirical
32 distribution of S is created by shuffling genotypes among individuals, thus creating
33 linkage equilibrium, and the P -value is given as the fraction of permutations (1000)
34 that results in values of S greater/equal to that observed. A P -value < 0.05 is indicative
35 of overall significant linkage disequilibrium.
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52 53 **(6) Mantel tests**

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55 To assess geographic processes related to candidate gene diversity, spatial analyses
56 were done through Mantel tests (Mantel, 1967) using the Mantel 2.1.0 (Carr, 2021)
57 package in the Spyder 5 IDE, running under Python 3.9 (Python Team, 2021). Test
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were done to compare genetic distance within two candidate genes and attributes of migration including the distance between latitude of both breeding and non-breeding ranges among species, as well as the relationship between the genetic distance and taxonomic distance and divergence times as measures of evolutionary distance, to access the strength of heritability of genotypes within lineages.

Tests were run between the genetic distance matrixes, generated with CONVERT 1.31, the geographic distance matrixes, generated with Geographic Distance Matrix Generator 1.2.3 (for both breeding and non-breeding coordinates), the taxonomic distance matrixes, generated using the 'tax2dist()' function of the R package vegan 2.5-7 (Oksanen *et al.*, 2020), and the divergence times matrix. For the taxonomic distance matrix the following taxonomic weights were applied to each rank: base = 2.09, genus = 16.34, species = 7.73, family = 20.42, superfamily = 9.08, parvorder = 32.67, and order = 11.67; missing values for non-passerine species were sampled from the gamma distribution using the R package gam 1.2 (Hastie, 2020). Divergence times between pairs of species, in millions of years ago (MYA), were retrieved from the Time Tree resource (Kumar *et al.*, 2017) website (www.timetree.org) using a custom Python script called Python Automated Retrieval of Time Trees (PAReTT version 1.0.1) and exported as a vectorised matrix. A P -value < 0.02 and Z -value > 1.96 (or < -1.96) is considered significant.

(7) Phylogenetic Generalised Least Squares analysis

Phylogenetic generalised least square (PGLS) models were fitted individually using the R package caper 1.0.1 (Orme *et al.*, 2018) to avoid error from repeat sampling. PGLS was used to relate both *Clock* and *Adcyap1* length to breeding and non-breeding latitude as the distance from the equator in degrees, as well as to total migration distance between regions and to the normalised dates for the beginning, middle, and end of Spring and Autumn migration, assuming Brownian motion and a lambda (λ) = 1.0. Phylogenetic signal for each gene was measured using the R package phytools 0.7-90 (Revell, 2012) to compute both lambda and kappa for the gene and tree data and verify the presence of Brownian motion. The tree used for the phylogeny was generated from the 'Ericson' phylogeny (Jetz *et al.*, 2012) by sampling 5000 trees from the Bird Tree website (www.birdtree.org). The trees were summarised to a 60% consensus tree by maximum clade credibility using TreeAnnotator 2.6.3, part of BEAST 2.6.3 (Bouckaert *et al.*, 2014), with a ten percent burn-in. The tree was

viewed and checked for taxonomic correctness in FigTree 1.4.3 (Rambaut, 2017) and tip labels were renamed to match the data set using the R package phylotools 0.2.4 (Zhang, 2021) prior to analysis. The final tree was edited in BioRender.com.

(8) Time trees and paleogeography

Time trees were computed from calibrated divergence time estimates using the Time Tree resource (Kumar *et al.*, 2017) to visualise the evolutionary history and relatedness of study species in terms of shared common ancestry and the length of time individual lineages have been evolving independently. Trees were downloaded from the website (www.timetree.org) with a custom Python script called Python Automated Retrieval of Time Trees (PARETT version 1.0.1). The tree was exported as a newick file and tip labels were renamed to match the common names of the data set using the R package phylotools 0.2.4 (Zhang, 2021) and the final tree was edited in BioRender.com. The relevant topography of Earth for each time period was also reconstructed in GPlates 2.3.0 (Müller *et al.*, 2018) with the PALEOMAP paleoAtlas (Scotese, 2016) to visualise relevant barriers to gene flow, and potential differences in selective forces between modern and historical geography, for each time period, that may have contributed to selection and speciation across the genomes of study species.

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For Review Only

Supplementary Table 1: Population genetics results (heterozygosity and Hardy-Weinberg) from comparative analysis of allele data for forty species from class Aves.

Species	Study	Clock						Adcyap1				
		N	Alleles	Freq	H _o	H _e	HW	Alleles	Freq	H _o	H _e	HW
American redstart	(unpublished)	26	2	Q ₉	↓0.154	↑0.208	0.149	7	161	↑0.778	↓0.759	0.545
Barn swallow	(Bazzi <i>et al.</i> , 2015)	64	3	Q ₇	↑0.094	↓0.091	0.988	-	-	-	-	-
Blackpoll warbler	(Ralston <i>et al.</i> , 2019)	72	4	Q ₆	↑0.722	↓0.714	0.968	13	189	↓0.722	↑0.786	0.044**
Blue tit	(Johnsen <i>et al.</i> , 2007)	950	6	Q ₁₂	↑0.559	↓0.550	0.812	-	-	-	-	-
Collared flycatcher	(Krist <i>et al.</i> , 2021)	406	4	Q ₁₂	↑0.328	↓0.325	0.306	6	182	↑0.595	↓0.581	0.558
Common chiffchaff	(unpublished)	55	4	Q ₁₁	↓0.291	↑0.426	0.007***	-	-	-	-	-
Common nightingale	(Bazzi <i>et al.</i> , 2016a)	150	5	Q ₁₂	↓0.527	↑0.564	0.006***	7	163	↓0.440	↑0.474	0.637
Common redstart	(Bazzi <i>et al.</i> , 2016a)	43	6	Q ₁₄	↓0.512	↑0.524	0.907	13	169	↓0.771	↑0.804	0.000***
Common whitethroat	(Bazzi <i>et al.</i> , 2016a)	25	5	Q ₁₀	↓0.520	↑0.680	0.000***	13	172	↓0.769	↑0.879	0.000
Common yellowthroat	(unpublished)	31	4	Q ₁₀	↑0.484	↓0.445	0.870	9	159	↓0.742	↑0.768	0.940
Dark-eyed junco	(Peterson <i>et al.</i> , 2013)	36	4	Q ₁₁	↑0.417	↓0.349	0.890	7	161	↑0.800	↓0.797	0.210
Eastern subalpine warbler	(Bazzi <i>et al.</i> , 2016a)	31	6	Q ₉	↑0.774	↓0.758	0.490	7	168	↑0.773	↓0.664	0.987
Eurasian blackbird	(Mueller <i>et al.</i> , 2013b)	792	2	Q ₇	↓0.028	↑0.030	0.045	25	165	↓0.782	↑0.820	0.846
Eurasian blackcap	(Mueller <i>et al.</i> , 2011)	936	-	-	-	-	-	11	161	↑0.700	↓0.699	0.336
Eurasian golden oriole	(Bazzi <i>et al.</i> , 2016a)	30	2	Q ₆	↑0.067	↓0.066	0.895	7	163	↓0.633	↑0.636	0.815
Eurasian hoopoe	(Bazzi <i>et al.</i> , 2016a)	25	3	Q ₈	↑0.560	↓0.496	0.687	3	157	↓0.500	↑0.559	0.771
Eurasian reed warbler	(Bazzi <i>et al.</i> , 2016a)	24	1	Q ₁₁	0.000	0.000	NA	10	169	↑0.875	↓0.813	0.996
Eurasian wryneck	(Bazzi <i>et al.</i> , 2016a)	30	4	Q ₈	↓0.433	↑0.438	0.976	5	135	↑0.710	↓0.684	0.856
European bee-eater	(Bazzi <i>et al.</i> , 2016a)	35	1	Q ₄	0.000	0.000	NA	6	163	↑0.194	↓0.185	1.000
European nightjar	(Bazzi <i>et al.</i> , 2016a)	39	2	Q ₈	↓0.333	↑0.345	0.824	9	154	↓0.744	↑0.806	0.000***
European pied flycatcher	(Bazzi <i>et al.</i> , 2016a)	226	5	Q ₁₂	↑0.478	↓0.464	0.716	11	180	↓0.695	↑0.709	1.000
European turtle dove	(Bazzi <i>et al.</i> , 2016a)	29	2	Q ₇	0.035	0.035	1.000	5	150	↓0.241	↑0.256	0.577
Garden warbler	(Bazzi <i>et al.</i> , 2016a)	31	6	Q ₁₂	↑0.839	↓0.718	0.904	6	169	↓0.710	↑0.721	0.189

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3	Great reed warbler	(Bazzi <i>et al.</i> , 2016a)	20	2	Q ₁₂	0.050	0.050	1.000	2	163	↑0.191	↓0.177	0.679
4	Hermit thrush	(unpublished)	30	3	Q ₁₀	↓0.233	↑0.244	0.455	7	165	↑0.833	↓0.794	0.871
5	Icterine warbler	(Bazzi <i>et al.</i> , 2016a)	29	2	Q ₈	↑0.069	↓0.068	0.893	7	169	↓0.517	↑0.662	0.497
6	Magnolia warbler	(unpublished)	33	5	Q ₉	↓0.273	↑0.326	0.001***	8	159	↑0.914	↓0.835	0.751
7	Northern wheatear	(Bazzi <i>et al.</i> , 2016a)	30	4	Q ₁₂	↓0.500	↑0.540	0.982	6	167	↓0.567	↑0.577	0.863
8	Painted bunting	(Contina <i>et al.</i> , 2018)	60	6	Q ₁₁	↓0.583	↑0.601	0.986	7	169	↑0.817	↓0.709	0.801
9	Sedge warbler	(Bazzi <i>et al.</i> , 2016a)	30	1	Q ₁₁	0.000	0.000	NA	4	163	↑0.433	↓0.397	0.991
10	Spotted flycatcher	(Bazzi <i>et al.</i> , 2016a)	29	2	Q ₉	↑0.103	↓0.100	0.812	5	162	↑0.690	↓0.683	0.660
11	Swainson's thrush	(unpublished)	29	2	Q ₁₀	↑0.276	↓0.242	0.424	11	171	↓0.828	↑0.846	0.324
12	Tree pipit	(Bazzi <i>et al.</i> , 2016a)	153	5	Q ₉	↓0.242	↑0.256	0.101	12	170	↓0.763	↑0.802	0.137
13	Tree swallow	(Bourret & Garant, 2015)	921	4	Q ₈	↓0.503	↑0.507	0.680	25	173	↓0.902	↑0.907	0.954
14	Whinchat	(Bazzi <i>et al.</i> , 2016a)	208	7	Q ₁₄	↑0.125	↑0.120	1.000	13	169	↑0.787	↓0.769	0.947
15	White-throated sparrow	(unpublished)	32	1	Q ₁₀	0.000	0.000	NA	10	161	↑0.781	↓0.775	0.853
16	Willow warbler	(Bazzi <i>et al.</i> , 2016a)	495	5	Q ₉	↓0.366	↑0.397	0.025**	10	174	↑0.839	↓0.825	0.000***
17	Wilson's warbler	(Bazzi <i>et al.</i> , 2016b)	102	2	Q ₉	0.020	0.020	0.944	9	158	↓0.775	↑0.814	0.002***
18	Wood warbler	(Bazzi <i>et al.</i> , 2016a)	30	5	Q ₁₁	↑0.633	↓0.628	0.970	5	162	↑0.552	↓0.531	0.734
19	Woodchat shrike	(Bazzi <i>et al.</i> , 2016a)	20	4	Q ₇	↑0.550	↓0.537	0.949	8	176	↑0.722	↓0.649	0.998

Summary table indicating the common name of the forty species for which allele data was available along with the study from which the data was derived. The sample size is given (N) along with the total number of alleles detected and the most abundant (Freq) allele for *Clock* and *Adcyap1* respectively. Both the observed (H_o) and expected (H_e) heterozygosity was determined and the relative relationship between them indicated with arrows. In many cases the observed value was higher than the expected value. The Chi-square test statistic for departure from Hardy-Weinberg (HW) equilibrium is also indicated along with its level of significance. (Significance: * P -value < 0.10, ** P -value < 0.05, *** P -value < 0.02)

Supplementary Table 2: Population genetics results (Fixation index and Ewens-Watterson) from comparative analysis of allele data for forty species from class *Aves*.

Species	Study	Clock					Adcyap1				
		F_{IS}	F -value	Lower 95% CI	Upper 95% CI	P -value	F_{IS}	F -value	Lower 95% CI	Upper 95% CI	P -value
American redstart	(unpublished)	0.246	0.796	0.501	0.962	0.426	-0.044	0.255	0.191	0.596	0.264
Barn swallow	(Bazzi <i>et al.</i> , 2015)	-0.042	0.910	0.373	0.969	0.855	NA	NA	NA	NA	NA
Blackpoll warbler	(Ralston <i>et al.</i> , 2019)	-0.018	0.291	0.319	0.932	0.009***	0.075	0.219	0.128	0.443	0.562
Blue tit	(Johnsen <i>et al.</i> , 2007)	-0.017	0.450	0.281	0.955	0.307	NA	NA	NA	NA	NA
Collared flycatcher	(Krist <i>et al.</i> , 2021)	-0.010	0.676	0.338	0.980	0.518	-0.025	0.420	0.269	0.926	0.301
Common chiffchaff	(unpublished)	0.311	0.578	0.310	0.912	0.561	NA	NA	NA	NA	NA
Common nightingale	(Bazzi <i>et al.</i> , 2016a)	0.063	0.438	0.285	0.922	0.307	0.068	0.528	0.219	0.770	0.791
Common redstart	(Bazzi <i>et al.</i> , 2016a)	0.013	0.482	0.228	0.748	0.729	0.026	0.208	0.114	0.338	0.737
Common whitethroat	(Bazzi <i>et al.</i> , 2016a)	0.220	0.334	0.252	0.778	0.341	0.108	0.138	0.109	0.297	0.291
Common yellowthroat	(unpublished)	-0.106	0.562	0.302	0.877	0.614	0.019	0.244	0.156	0.499	0.479
Dark-eyed junco	(Peterson <i>et al.</i> , 2013)	-0.209	0.656	0.307	0.893	0.729	-0.018	0.214	0.198	0.650	0.060*
Eastern subalpine warbler	(Bazzi <i>et al.</i> , 2016a)	-0.038	0.254	0.222	0.710	0.093*	-0.191	0.351	0.188	0.576	0.712
Eurasian blackbird	(Mueller <i>et al.</i> , 2013b)	0.069	0.970	0.505	0.999	0.525	0.046	0.181	0.102	0.396	0.544
Eurasian blackcap	(Mueller <i>et al.</i> , 2011)	NA	NA	NA	NA	NA	-0.001	0.301	0.188	0.772	0.320
Eurasian golden oriole	(Bazzi <i>et al.</i> , 2016a)	-0.035	0.936	0.502	0.967	0.784	-0.013	0.375	0.194	0.629	0.695
Eurasian hoopoe	(Bazzi <i>et al.</i> , 2016a)	-0.151	0.514	0.360	0.922	0.319	0.088	0.452	0.359	0.925	0.153
Eurasian reed warbler	(Bazzi <i>et al.</i> , 2016a)	NA	1.000	NA	NA	NA	-0.099	0.204	0.138	0.409	0.478
Eurasian wryneck	(Bazzi <i>et al.</i> , 2016a)	-0.007	0.569	0.299	0.873	0.627	-0.055	0.327	0.255	0.791	0.190
European bee-eater	(Bazzi <i>et al.</i> , 2016a)	NA	1.000	NA	NA	NA	-0.063	0.817	0.225	0.726	0.996***
European nightjar	(Bazzi <i>et al.</i> , 2016a)	0.022	0.659	0.501	0.975	0.252	0.066	0.204	0.163	0.528	0.191
European pied flycatcher	(Bazzi <i>et al.</i> , 2016a)	-0.033	0.537	0.290	0.939	0.502	0.015	0.295	0.157	0.560	0.637
European turtle dove	(Bazzi <i>et al.</i> , 2016a)	-0.018	0.966	0.502	0.966	0.783	0.040	0.749	0.252	0.779	0.946*
Garden warbler	(Bazzi <i>et al.</i> , 2016a)	-0.187	0.293	0.221	0.711	0.236	-0.001	0.291	0.222	0.710	0.225
Great reed warbler	(Bazzi <i>et al.</i> , 2016a)	-0.026	0.951	0.501	0.951	1.000***	-0.105	0.828	0.501	0.954	0.490
Hermit thrush	(unpublished)	0.026	0.761	0.361	0.935	0.685	-0.067	0.219	0.195	0.633	0.087*

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3	Icterine warbler	(Bazzi <i>et al.</i> , 2016a)	-0.036	0.933	0.502	0.966	0.783	0.206	0.349	0.194	0.615	0.629
4	Magnolia warbler	(unpublished)	0.150	0.679	0.257	0.803	0.887	-0.111	0.177	0.177	0.585	0.023**
5	Northern wheatear	(Bazzi <i>et al.</i> , 2016a)	0.058	0.469	0.299	0.873	0.420	0.002	0.432	0.221	0.703	0.701
6	Painted bunting	(Contina <i>et al.</i> , 2018)	0.021	0.404	0.233	0.798	0.490	-0.162	0.297	0.209	0.728	0.285
7	Sedge warbler	(Bazzi <i>et al.</i> , 2016a)	NA	1.000	NA	NA	NA	-0.111	0.610	0.301	0.873	0.684
8	Spotted flycatcher	(Bazzi <i>et al.</i> , 2016a)	-0.055	0.902	0.502	0.966	0.591	-0.028	0.329	0.251	0.778	0.204
9	Swainson's thrush	(unpublished)	-0.160	0.762	0.502	0.966	0.375	0.005	0.168	0.129	0.386	0.273
10	Tree pipit	(Bazzi <i>et al.</i> , 2016a)	0.051	0.745	0.287	0.924	0.809	0.044	0.202	0.143	0.526	0.294
11	Tree swallow	(Bourret & Garant, 2015)	0.008	0.493	0.351	0.989	0.186	0.005	0.094	0.103	0.390	0.008***
12	Whinchat	(Bazzi <i>et al.</i> , 2016a)	-0.041	0.880	0.230	0.846	0.985***	-0.026	0.233	0.144	0.544	0.428
13	White-throated sparrow	(unpublished)	NA	1.000	NA	NA	NA	-0.024	0.237	0.144	0.448	0.573
14	Willow warbler	(Bazzi <i>et al.</i> , 2016a)	0.078	0.604	0.302	0.964	0.543	-0.021	0.178	0.168	0.626	0.045**
15	Wilson's warbler	(Bazzi <i>et al.</i> , 2016b)	-0.010	0.981	0.503	0.990	0.831	0.044	0.190	0.182	0.666	0.040**
16	Wood warbler	(Bazzi <i>et al.</i> , 2016a)	-0.026	0.383	0.254	0.786	0.372	-0.058	0.479	0.253	0.778	0.657
17	Woodchat shrike	(Bazzi <i>et al.</i> , 2016a)	-0.050	0.476	0.295	0.814	0.521	-0.144	0.369	0.162	0.463	0.902*

Summary table indicating the common name of the forty species for which allele data was available along with the study from which the data was derived. For each species the Fixation index (F_{IS}) is reported along with the F -value results from the Ewens-Watterson test for neutrality or selection for *Clock* and *Adcyap1* respectively. Both the observed F -value and upper and lower bounds of the 95% confidence interval (CI), estimated from 10 000 random samplings, was determined. The probability, based on Slatkin's implementation of the EW test, test statistic for departure from neutrality is also indicated along with its level of significance. (Significance: * P -value < 0.10, ** P -value < 0.05, *** P -value < 0.02)

Supplementary Table 3: Population genetics results (Linkage Disequilibrium) from comparative analysis of allele data for thirty-six species from class Aves.

Species	Study	D'	W_n	S	P-value
American redstart	(unpublished)	0.288	0.223	2.320	0.935
Blackpoll warbler	(Ralston et al., 2019)	0.221	0.316	26.350	0.927
Collared flycatcher	(unpublished)	0.143	0.100	12.780	0.517
Common nightingale	(Bazzi et al., 2016a)	0.259	0.218	17.560	0.403
Common whitethroat	(Bazzi et al., 2016a)	0.617	0.741	47.060	0.026*
Common yellowthroat	(unpublished)	0.394	0.391	18.990	0.487
Common redstart	(Bazzi et al. 2016a)	0.458	0.417	34.740	0.059
Dark-eyed junco	(Peterson et al., 2013)	0.476	0.501	16.500	0.279
Eastern subalpine warbler	(Bazzi et al., 2016a)	0.616	0.453	18.490	0.722
Eurasian blackbird	(Mueller et al., 2013b)	0.291	0.179	12.870	0.625
Eurasian golden oriole	(Bazzi et al., 2016a)	0.964	0.782	11.650	0.004*
Eurasian hoopoe	(Bazzi et al., 2016a)	0.560	0.290	6.130	0.120
Eurasian reed warbler	(Bazzi et al., 2016a)	0.000	NA	0.000	0.000
Eurasian wryneck	(Bazzi et al., 2016a)	0.437	0.384	19.020	0.025*
European bee-eater	(Bazzi et al., 2016a)	0.000	NA	0.000	0.000
European nightjar	(Bazzi et al., 2016a)	0.427	0.486	9.600	0.396
European pied flycatcher	(Bazzi et al., 2016a)	0.309	0.258	27.040	0.444
European turtle dove	(Bazzi et al., 2016a)	1.000	0.053	0.300	0.237
Garden warbler	(Bazzi et al., 2016a)	0.381	0.455	23.430	0.497
Great reed warbler	(Bazzi et al., 2016a)	1.000	0.053	0.210	0.192
Hermit thrush	(unpublished)	0.550	0.543	16.240	0.074
Icterine warbler	(Bazzi et al., 2016a)	0.945	0.556	6.990	0.153
Magnolia warbler	(unpublished)	0.436	0.479	24.060	0.269
Northern wheatear	(Bazzi et al., 2016a)	0.258	0.381	11.510	0.506
Painted bunting	(Contina et al., 2018)	0.345	0.281	24.550	0.364
Sedge warbler	(Bazzi et al., 2016a)	0.000	NA	0.000	0.000
Spotted flycatcher	(Bazzi et al., 2016a)	1.000	0.242	3.160	0.334
Swainson's thrush	(unpublished)	0.555	0.415	5.040	0.969
Tree pipit	(Bazzi et al., 2016a)	0.384	0.280	20.410	0.691
Tree swallow	(Bourret & Garant, 2015)	0.116	0.159	64.420	0.580
Whinchat	(Bazzi et al., 2016a)	0.391	0.228	36.950	0.538
White-throated sparrow	(unpublished)	0.000	NA	0.000	0.000
Willow warbler	(Bazzi et al., 2016a)	0.290	0.319	37.790	0.308
Wilson's warbler	(Bazzi et al., 2016b)	1.000	0.176	4.260	0.370
Wood warbler	(Bazzi et al., 2016a)	0.403	0.280	12.960	0.514
Woodchat shrike	(Bazzi et al., 2016a)	0.466	0.556	16.480	0.686

Summary table indicating the common name of the thirty-six species for which allele data was available for both *Clock* and *Adcyap1* along with the study from which the data was derived. Two measures of linkage disequilibrium are reported, D' and W_n , along with the calculated S-statistic and P-value. (Significance: *P-value < 0.05)

Supplementary files: Videos

Video 1: Paleogeographic reconstruction of the continents and their historical positions and movements during the major speciation periods of the studied avian lineages. The reconstruction ranges from 85 million years ago (Ma) when many of the main divisions between avian taxa occurred, prior to the formation of most continents while East Africa was still separated from the main African continent, North and South America were still separated and before South-East Asia merged with the main Asian continent. Continents started to assume contemporary shapes approximately 50 Ma in the early Eocene with a substantial amount of continental drift continuing until about 21 Ma in the early Miocene, when most continents started to assume their contemporary positions. There was still, however, a continuation of tectonic plate movement resulting in significant geographic remodelling alongside widespread climate changes on most continents.

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