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BEHAVIORAL ECOLOGY – ORIGINAL RESEARCH

No evidence for an association between *Clock* gene allelic variation and migration timing in a long-distance migratory shorebird (*Limosa lapponica baueri*)

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Abstract

The gene *Clock* is a key part of the Core Circadian Oscillator, and the length of the polyglutamine (poly-Q) repeat sequence in *Clock* (*Clk*polyQcds) has been proposed to be associated with the timing of annual cycle events in birds. We tested whether variation in *Clk*polyQcds corresponds to variation in migration timing in the bar-tailed godwit (*Limosa lapponica baueri*), a species in which individuals show strong annual consistency in their migration timing despite the New Zealand population migrating across a 5-week period. We describe allelic variation of the *Clk*polyQcds in 135 godwits over-wintering in New Zealand (N.Z.) and investigate whether polymorphism in this region is associated with northward migration timing (chronophenotype) from N.Z. or (for 32 birds tracked by geolocator) after the primary stopover in Asia. Six *Clock* alleles were detected (Q_7–Q_{12}) and there was substantial variation between individuals (heterozygosity of 0.79). There was no association between *Clk*polyQcds polymorphism and migration timing from N.Z. The length of the shorter *Clock* allele was related to migration timing from Asia, though this relationship arose largely from just a few northern-breeding birds with longer alleles. Other studies show no consistent associations between *Clk*polyQcds and migration timing in birds, although *Clock* may be associated with breeding latitude in some species (as an adaptation to photoperiodic regime). Apparent relationships with migration timing could reflect latitude-related variation in migration timing, rather than *Clock* directly affecting migration timing. On current evidence, *Clk*polyQcds is not a strong candidate for driving migration timing in migratory birds generally.

Keywords *Clock* · Polyglutamine · Circannual · Phenology · Migration · Bar-tailed godwit

Introduction

Long-distance migratory birds are presented with the annually repeating challenge of ensuring that a crucial life-history event, migratory departure from their over-wintering sites, is timed so that subsequent reproduction-related life-history events (e.g., courtship, mating and nesting) occur at appropriate times at far-distant locations (Both et al. 2006; Nussey et al. 2007). Because initiation of migration occurs at sites that may be thousands of kilometers away from the breeding sites (Jenni and Kery 2003; Both et al. 2006, 2010), consistent migration timing (e.g., Conklin et al. 2013) depends strongly on a genetically influenced timing mechanism (Berthold and Querner 1981).

Avian migration timing is thought to be determined by inputs from an internal/intrinsic circannual clock interacting with responses to extrinsic, seasonally varying *Zeitgebers*, which at temperate latitudes is principally the seasonally changing photoperiod (Helm et al. 2009; Both et al. 2010;

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Helm et al. 2013; Kölzsch et al. 2015; Majumdar et al. 2015). The genetic and physiological mechanisms generating internal circannual clocks remain largely unknown (Rani and Kumar 2013; Evans et al. 2015; Majumdar et al. 2015). In contrast, some genetic and physiological elements of the avian internal circadian clock used to measure seasonally changing photoperiod can be deduced from their evolutionary and functional relationships with similar (i.e., homologous) elements in more experimentally accessible model organisms (Panda et al. 2002; Hardin 2005; Helfer et al. 2006). Although behaviors are typically complex traits with continuous distributions of phenotypic values and presumably polygenic control (Tschirren and Bensch 2010; Liedvogel and Lundberg 2014), genetic polymorphisms can be directly linked with behavioral variation in natural populations (Easton et al. 2003; Fidler et al. 2007; Korsten et al. 2010; Tschirren and Bensch 2010; Caprioli et al. 2012; Mueller et al. 2013; Wetzel et al. 2015).

The core circadian oscillator (CCO) in birds and mammals is generated by a number of genes/proteins that work together to form an oscillatory transcription/translation feedback loop (Hastings 2000; Bell-Pedersen et al. 2005; Albrecht 2012; Cassone 2014; Hurley et al. 2016). One critical element of the CCO is the gene *Clock* (*Circadian Locomotor Output Cycles Kaput*) encoding one half of a heterodimeric transcription factor CLOCK/BMAL1, a transcription-activating complex that regulates the expression of several CCO genes (e.g., Period, Cryptochrome) (Zhang and Kay 2010; Cassone 2014) in addition to some 'output' genes (Chong et al. 2000; Ripperger et al. 2000; Yoshitane and Fukada 2009; Reischl and Kramer 2011; Rey et al. 2011).

An evolutionarily conserved feature of CLOCK protein orthologs is a polyglutamine (poly-Q) repeat sequence located toward the C-terminus, which may affect the binding affinity and thus its functionality as a transcription factor (Gemayel et al. 2015). Variation in the length of *Clock*polyQ has consequences that can be observed at the behavioral level. Specifically, experimental studies of *Drosophila melanogaster* and mice showed that variation in the length of *Clock*polyQ resulted in altered circadian behavior (King et al. 1997; Darlington et al. 1998). Although the exact mechanism by which *Clock*polyQ variation links mechanistically with timing-related traits is unclear, variation in the glutamine repeat length could be a source of quantitative variation of a phenotype (i.e., behavior) within a population (Darlington et al. 1998; Saleem et al. 2001; Resuehr et al. 2007; Hands et al. 2008).

Variation in *Clock*polyQ in birds has been mostly studied in relation to latitudinal clines across populations or breeding phenology traits (e.g., laying date) (Johnsen et al. 2007; Liedvogel et al. 2009; Bourret and Garant 2015; Zhang et al. 2017). Analogous studies in fish and flies support the idea that *Clock* allelic variation is associated with latitudinal adaptations (Costa et al. 1992; Leder et al. 2006; O'Malley and Banks 2008). It has been suggested that the effect of *Clock* on breeding timing may be via its effect on the hypothalamus-pituitary-gonad (HPG) endocrine axis (Zhang et al. 2017). *Clock* has also been suggested to relate to migration timing or distance in birds, but results have been variable. Some studies support an association (Johnsen et al. 2007; Bourret and Garant 2015; Saino et al. 2015), others do not (Chakarov et al. 2013; Kuhn et al. 2013; Peterson et al. 2015; Bazzi et al. 2017) and some found a lack of *Clock* gene poly-Q (*Clk*polyQcds) polymorphism in the species studied (Bazzi et al. 2015, 2016b). In this study, we investigated whether variation in *Clk*polyQcds genotype is associated with variation in timing of individual bar-tailed godwits (*Limosa lapponica baueri*, hereafter 'godwits') on northward migration from New Zealand to Alaska.

Godwits that over-winter in Australasia are long-distance migratory shorebirds that present an extreme example of the importance of integrating the timing of migration with the timing of breeding far away (Conklin et al. 2010). The annual migration schedule of godwits that over-winter in N.Z. consists of three non-stop flights over open ocean: departing N.Z. in austral late summer/early autumn godwits fly ~ 10,000 km to feeding grounds in coastal east Asia, followed by a stopover of 4–7 weeks and then a flight of ~ 7000 km to western and northern Alaska to breed, and then, departing in the boreal autumn, a > 11,500 km flight directly across the Pacific Ocean to N.Z. (Gill et al. 2009; Battley et al. 2012). Given the short temporal window for successful breeding in Alaska (Meltofte et al. 2007), it is expected that initiation of all three migratory flights is under strong selection pressure with severe penalties for inappropriate timing (Drent et al. 2003; Conklin et al. 2013; Visser et al. 2015). Godwits depart N.Z. over an approximately 5-week period in austral late summer/early autumn (late February–early April) but individual godwits typically depart within the same week each year (Battley 2006; Conklin and Battley 2012; Conklin et al. 2013). Similar inter-individual variation in departure times from the Asian stopover sites grounds has also been reported albeit from a smaller dataset (Conklin et al. 2013). Correlations between departure times from N.Z. or Asia and ultimate breeding latitude indicate that the inter-individual variation in migration dates is correlated with variation in the spring thaw across the Alaskan breeding range, with northern-breeding godwits migrating and breeding later than southern-breeding birds (Conklin et al. 2010). Thus, godwits appear to offer an excellent opportunity to test the association between genetic variation and migration timing across an entire migration.

Materials and methods

Collection and storage of godwit blood samples

Godwits were captured by cannon-net or mist-net at two sites on the North Island (Firth of Thames, 37.17°S 175.32°E, $n=13$; Manawatu River estuary, 40.47°S, 175.22°E, $n=68$) and at one site on the South Island (Catlins Coast, 46.48°S, 169.70°E, $n=54$) of N.Z. Birds were banded, measured, aged (based on plumage characteristics) and given a unique combination of color bands or an engraved 3-letter leg flag to allow individual recognition in the field. Juveniles (age 1) and adults (age 3+) can generally be identified; immatures (age 2 or 3) are more difficult and often impossible to distinguish, in which case they are recorded as immatures. Most birds in our sample were adults ($n=123$): one was banded as a juvenile, 10 as immatures and one was of uncertain age. Potentially 12 birds in the sample included their first northward migration (which generally occurs at age 3 or 4; P.F.B. and J.R.C, unpubl. data). Blood samples (ca. 50.0 µl) were collected from the metatarsal or brachial vein using micro-haematocrit capillary glass tubes and preserved in either 96% (v/v) ethanol or Queen's Lysis buffer (QLB) (10 mM Tris, 10 mM NaCl,10 mM EDTA, 1.0% (w/v) *n*-lauroylsarcosine, pH 8.0) (Seutin et al. 1991) before long-term storage at ambient temperature. All sampling was carried out under both N.Z. Department of Conservation (DOC) permits and Animal Ethics Committee approval from Massey University (#07/163, # 12/90) and the University of Otago (#66/03).

Determination of individual chronophenotypes

Departure dates of godwits leaving N.Z. were recorded based on daily observations of individually marked (color-banded or engraved-flagged) godwits at the study sites between 2004 and 2016 (Online resource 1). Fieldwork at the Firth of Thames was conducted in 2004–2006 and 2014–2016, at the Manawatu Estuary from 2008–2016 and at the Catlins Coast in 2013–2016. At the Manawatu Estuary where the population is small (ca. 200–280 birds per year), virtually exact departure information (i.e., 0–1 days of uncertainty) could be determined from direct observations of departing flocks, daily records of marked birds and detailed flock counts (Conklin and Battley 2011; Battley and Conklin 2017). At the Catlins Coast (population ca. 400 birds), daily color-band readings combined with flock counts were used to infer migration dates of individuals, while at the Firth of Thames (population > 3000 birds), the last observation of repeatedly observed individuals was taken to represent the migration timing (with some direct observations of departing birds). In total, we have data for 135 birds with 426 migration dates across the study period. A subset of those birds was also tracked via geolocator through their Asian stopover in 2008–2009 (using MK14 geolocators, British Antarctic Survey, UK) and 2013–2014 (MK4093, Biotrack, UK and Intigeo-C65 K, Migrate Technology, UK) (32 birds with 41 observations, Online resource 2). We used conductivity data (wet-dry transitions) to determine departure date from the stopover (Battley and Conklin 2017). We determined general breeding region from the light data using BAStrack software for British Antarctic Survey loggers (Fox 2010), Geolight (Lisovski and Hahn 2012) for Biotrack loggers and the R package *PolarGeolocation* (Lisovski 2018) for Migrate Technology loggers. As there is considerable uncertainty around estimates of high-latitude positions, especially for geolocators that do not record continuous light levels (MK14 and MK4093), we grouped breeding regions into 'north' (Seward Peninsula and north) and 'south' (Yukon-Kuskokwim Delta), i.e., a cutoff at Latitude 64°N). This division corresponds to regions that differ in their light regimes (northern birds have no discernible darkness at night; Conklin 2011).

DNA-based sex determination

Godwits were molecularly sexed using methodology of Fridolfsson and Ellegren (1999) which relies on an intron within the Chromo Helicase DNA-binding (CHD) gene differing in length between the CHD alleles carried on the Z (CHD-Z, ~0.6 kb) and the W (CHD-W, ~0.5 kb) chromosomes. Thus, male bird gDNA (ZZ) amplifies a single band and female gDNA (ZW) either two bands or, in some cases as ours, a single band shorter than that of males (Fridolfsson and Ellegren 1999). Godwit gDNA was used as the template for the PCR using the primer pair: 2550F (5′–GTTACTGATTCGTCTACGAGA–3′) and 2718R (5′–ATTGAAATGATCCAGTGCTTG–3′) (Fridolfsson and Ellegren 1999). The PCR mix consisted of: 1×MyTaq™ HS Mix (Cat. No. BIO25045, Bioline, London, UK), forward primer (2550F) (0.2 µM), reverse primer (2718R) (0.2 µM), 0.5 µg/µl non-acetylated bovine serum albumin (BSA; Cat. No. B8667, Sigma-Aldrich, St. Louis, USA) with a final volume of 10.0 µl. Thermocycling parameters consisted of: 94 °C/2 min; 94 °C/2 min; 60 °C/40 s, ramping +1 °C/s to 72 °C, 72 °C/40 s, 10 cycles; 94 °C/30 s, 50 °C/30 s, 72 °C/35 s, 30 cycles; 72 °C/5 min.; hold at 4 °C. Amplification products were separated by agarose gel (2.5% (w/v)) electrophoresis and visualized by staining in ethidium bromide before photographing on a UV transilluminator (ChemiDoc™ MP, Imaging System, BioRad, Hercules, USA). Samples of gDNA generating a single

Fig. 1 Alignment of predicted protein sequences corresponding to the ▸ five godwit *Clk*polyQcds alleles sequenced in this study. An exon of the godwit *Clock* gene ortholog containing a poly-glutamine (poly-Q) coding region was amplified and sequenced. Allele names denote the number of glutamine (Q) residues predicted to be encoded in a variable length region (shaded). Glutamine residues encoded by CAA are single underlined. All Qs depict glutamine residues encoded by the triplet CAG except for one encoded by CAA (single-underlined) found in the *Clk*polyQ$_8$ (KR653306). Positions of predicted protein identity are indicated by dots (•) with gaps introduced to the poly-Q region to achieve alignment indicated by (-). GenBank accession numbers for the five allelic sequences are: *Clk*polyQ$_8$ (KR653306), *Clk*polyQ$_9$ (JN676984), *Clk*polyQ$_{10}$ (KU051417), *Clk*polyQ$_{11}$ (JN676983) and *Clk*polyQ$_{12}$ (KU051418)

***Clock*polyQcds alleles**		**GenBank acc.no.**
*Clk*polyQ12	TKLVTAPVACGAVMVVPSTMFMGQVVTAYPTFAAQQQPQTLSVTQQQQQQQQQQQQGQQDQQQPQPQLTTVQQPAQPQLT	KU051418
*Clk*polyQ11	…QQQQQQQQQQQ-…	JN676983
*Clk*polyQ10	…QQQQQQQQQQ--…	KU051417
*Clk*polyQ9	…QQQQQQQQQ---…	JN676984
*Clk*polyQ8	…QQQQQQQQ----…	KR653306

amplicon ~ 0.5 kb were interpreted as male and a larger amplicon ~ 0.6 kb as female. Our N.Z. dataset comprises 70 females and 65 males, and our Asian dataset comprises 17 females and 15 males.

Genotyping of the *Clk*polyQcds locus

The godwit *Clk*polyQcds genotyping procedure followed Johnsen et al. (2007) in first amplifying, cloning into plasmids and sequencing a range of *Clk*polyQcds alleles that then provided templates to generate size standards for subsequent precise determination of *Clk*polyQcds allele lengths/genotypes. Godwit *Clk*polyQcds sequences were amplified using the generic avian *Clk*polyQcds primers: forward Gen*Clk*For: 5′–TTTTCTCAAGGTCAGCAGCTTGT–3′ and Gen*Clk*Rev reverse: 5′–CTGTAGGAACTGTTGYGG KTGCTG–3′ (Johnsen et al. 2007) with reaction conditions 1x BioMix (BioLine, London, UK), 0.8 µM both forward and reverse primers, 1.0 µL gDNA, double-distilled water to a final volume of 50.0 µL with thermocycling conditions: 94 °C/2 min; 94 °C/30 s, 60 °C/30 s, 72 °C/60 s, 10 cycles; 94 °C/30 s, 64 °C/30 s, 72 °C/60 s, 30 cycles; 72 °C/7 min; hold at 15 °C. The resulting PCR products were visualized on an ethidium bromide-stained agarose gel [2.0% (w/v)], extracted using a commercial kit (Zymoclean™ Gel DNA Recovery Kit, Zymo Research, Irvine, USA), ligated into the T-tailed cloning vector pGEM-Teasy (Promega, Madison, USA) and transformed into competent DH5α *E. coli* cells. Plasmids were purified (High Pure Plasmid Isolation Kit, Roche Diagnostics, Penzberg, Germany) and their inserts sequenced by an external contractor (Massey Genome Service, Massey University, N.Z.). From six godwit genomic DNA samples, two distinct *Clk*polyQcds allele sequences were found and denoted *Clk*polyQ$_9$ and *Clk*polyQ$_{11}$ based on variation in the length of a poly-glutamine (poly-Q) coding region (Fig. 1). Using the *Clk*polyQ$_9$ and *Clk*polyQ$_{11}$ allele sequences godwit-specific *Clk*polyQcds, PCR primers were designed for further genotyping, forward primer *LimClk*For: 5′–TGTAAAACGACGGCCAGT TGGGACAGGTGGTGACGGCTTAC–3′; reverse primer

*Lim*ClkRev: 5′–GTTTCTGCTGAACGGTGGTGAG–3′. The godwit *Clk*polyQcds sequences correspond to coordinates 63–85 and 217–200 of *Clk*polyQ$_{11}$ (GenBank acc. no.: JN676983). An 18 bp generic M13 'tag' sequence (5′–TGT AAAACGACGGCCAGT–3′) was included on the 5′ end of *LimClk*For to allow florescent labeling of amplicons using the three primer PCR strategy of Schuelke (2000). A 5′ 'PIG tail' sequence (5′–GTTT–3′) was included on *LimClk*Rev in an effort to enhance consistency in amplicon size by promoting the addition of 3′ A's by the *Taq* DNA polymerase (Brownstein et al. 1996). Godwit *Clk*polyQcds genotyping PCR mixes consisted of: 1x MyTaq™ HS Mix (cat. no. BIO25045, Bioline, London, UK), *LimClk*For (0.2 µM), *LimClk*Rev (0.6 µM), and a florescent dye-labeled generic M13-tag primer (5′–TGTAAAACGACGGCCAGT–3′) (0.6 µM) Schuelke (2000), with thermocycling conditions: 94 °C/2 min; 94 °C/30 s, 59 °C/30 s, 72 °C/30 s, 15 cycles; 94 °C/30 s, 54 °C (ramping +0.2 °C/s)/30 s, 72 °C/30 s, 25 cycles; 72 °C/5 min; 60 °C/30 min; 15 °C/hold. Among 20 godwit gDNA samples screened, six were identified that collectively displayed five different *Clk*polyQcds length variant alleles, either in heterozygous or homozygous conditions. Using the generic *Clk*polyQcds primer pair of Johnsen et al. (2007) (i.e., Gen*Clk*For and Gen*Clk*Rev), the five different *Clk*polyQcds allelic sequences were amplified and sequenced (Fig. 1). Five plasmids corresponding to the five *Clk*polyQcds alleles *Clk*polyQ$_{8\text{-}12}$ were then used as templates for the PCR with primers *LimClk*For and *LimClk*Rev in combination with 6-FAM™ labeled M13 tag primer. The resulting amplicons were diluted and pooled to generate 6-FAM™ labeled standards for each of the five *Clk*polyQcds alleles. To genotype the full set of godwits ($n = 135$), their corresponding gDNA samples were used as templates with the generic M13 tag primer labeled with either VIC® (green) or NED™ (yellow). The samples sent to an external contractor (ABI3730 Genetic Analyzer, GeneScan™-500 LIZ™ size standard, Massey Genome Service, Massey University, Palmerston North, N.Z.) for amplicon length estimations consisted of pools of: (1) the 6-FAM™ labeled standards (2) amplification products from one godwit gDNA labeled with VIC® and (3) amplification products from one bird gDNA labeled with NED™. Peak Scanner™ v2.0 (Life Technologies, Carlsbad, USA) was used to view and analyze the electropherogram data.

In preliminary experiments, we found, as reported by Sutton et al. (2011), that the dye PET™ adds an apparent 3 bp to the estimated length of a DNA fragment when compared with FAM™, VIC® and NED™ labeling (data not shown). Therefore, only the dyes FAM™ (for the standards) and VIC® and NED™ (for genotyping) were used in this study. Repeat genotyping of three gDNA samples with either VIC® or NED™ labeled generic M13 tag primers returned the same genotype with examples of the genotyping electropherogram shown in Online Resource 3. Therefore, it was concluded that genotypes generated using FAM™, VIC® and NED™ labeled generic M13 tag primers could be combined and compared.

Statistical analyses

General parameters (He, Ho, Na, allele frequencies) were calculated for the pooled dataset using GENEAIEX v.6.501 (Peakall and Smouse 2012). We tested for departure from Hardy–Weinberg equilibrium (HWE) using ARLEQUIN v.3.5 (Excoffier et al. 2005) with 1,000,000 steps in Markov chain and 1,000,000 dememorization steps. We checked for normality of poly-Q unit frequency distributions for each sex with a Shapiro test. Differences between sexes in allele length for either locus were assessed with a *t* test. *Clk*polyQcds genotype frequencies between sexes were compared with a Kruskal–Wallis test (non-parametric). Since we do not have a priori knowledge about the genotype–phenotype relationship (i.e., dominance, co-dominance, partial dominance), we ran replicated analyses for the length of the shorter allele, length of the longer allele and mean length of the two alleles. To test for relationships between *Clock* and migration timing, we ran linear mixed models (using the R package 'lme4'; Bates et al. 2015) of departure date in relation to combinations of fixed (*Clock* alleles, sex, and for the Asia analysis, breeding region) and random (site in N.Z., year and individual) factors using all migration dates for each individual bird. We evaluated models by comparison of AIC values (generated via the AIC function), treating models differing by AIC ≤ 2 as having similar support. We also tested for differences in support between models by comparing them using the anova function. All statistical analyses were performed in R v.3.5.1 (R Core Team 2018). Finally, we performed population genetic analysis and compared AMOVA-*Fst* values from the *Clk*polyQcds locus to those from a set of microsatellites. Details of these analyses and results are presented in Online Resource 4.

There is no a priori reason to think that the putative associations tested here would appear just in one of the sexes since the *Clock* gene is autosomal. However, natural and sexual selection can affect allele/genotype frequencies differently in males and females (Kissner et al. 2003; Ellegren and Parsch 2007; Spottiswoode and Saino 2010; Saino et al. 2013; Bazzi et al. 2017); therefore, we also tested the potential association between *Clk*polyQcds and migration departure time with an interaction between *Clock* and sex; results (not shown) were no different from those without this interaction.

Results

*Clock*polyQ genotyping and general parameters

We successfully amplified the *Clk*polyQcds region from 135 individuals. In the course of this genotyping, a sixth *Clk*polyQcds length allele was identified and denoted Q_7 based on its estimated length, although its sequence data were not obtained. Predicted protein sequences corresponding to the five sequenced *Clk*polyQcds alleles (denoted Q_8–Q_{12}) were identical except for the poly-Q repeat length (Fig. 1). Average observed heterozygosity was 0.79 (females = 0.77, males = 0.80). The most common alleles were Q_9 (36%), Q_{11} (24%) and Q_{10} (22%) (Table 1). Genotype frequencies did not deviate from Hardy–Weinberg equilibrium for sexes combined or considered separately (all $P = 0.8$). *Clk*polyQcds allele and genotype frequencies were not different between the sexes (Table 1).

*Clk*polyQcds polymorphism and migratory departure dates

There were no clear patterns of association between *Clk*polyQcds genotype and migration timing (Fig. 2). Nonetheless for the N.Z. departure dates, the best generalized linear mixed models indicated that there was a slight sex difference in migration departure timing (males being ca. 2 days later than females) and a suggestion of an effect of *Clk*polyQcds genotype (for the longer allele and the mean allele length; $P = 0.074$–0.089; Table 2). This apparent effect of *Clk*polyQcds genotype was, however, due entirely to a single individual with the longest allele lengths (mean 11.5). When this individual was removed from the analysis, no effect of *Clk*polyQcds genotype was detected from the remaining 134 birds.

For departures from Asia, three models with similar AIC scores indicated that (compared to northern-breeding females) males in the sample migrated 4.6–4.7 days later and southern breeders migrated 11.3–11.5 d earlier (Table 2). Two models found some support for a relationship between *Clk*polyQcds genotype and migration timing (significant for the shorter allele; marginal for mean allele length together; Table 2). While the effect of the shorter *Clock* allele was statistically significant, a plot of modeled departure date in relation to *Clock* (Fig. 3) suggests that the relationship was largely due to one or two northern-breeding birds. Again, as with the New Zealand departures, this relationship disappeared when the latest bird was removed from the analysis.

Discussion

New Zealand-wintering bar-tailed godwits provide an excellent system in which to test for genetic influences on migration timing, as the measure of phenology is clear and unequivocal (i.e., the date that major trans-oceanic migratory flights are embarked upon), and is variable between individuals yet strongly consistent within individuals. We found that godwits were highly polymorphic at the *Clk*polyQcds locus (heterozygosity 0.79) with a wide range of genotypes so that relationships are unlikely to be strongly influenced by outliers. Despite this, we found no convincing evidence for a relationship between *Clk*polyQcds genotype and migration departure timing in godwits.

*Clk*polyQcds polymorphism in the bar-tailed godwit

We identified six alleles within the 135 bar-tailed godwits genotyped, which is similar to what previous studies reported from other species (Johnsen et al. 2007; Liedvogel et al. 2009; Liedvogel and Sheldon 2010; Dor et al. 2011a, b; Caprioli et al. 2012; Chakarov et al. 2013; Kuhn et al. 2013; Bazzi et al. 2015; Saino et al. 2015; Stuber et al. 2016) (Table 3). The number of poly-Q repeats in godwit *Clock* alleles ranged from Q_7 to Q_{12}. Previous studies found *Clock* poly-Q allelic variation from Q_5 to Q_{16} (Johnsen et al. 2007; Dor et al. 2011a); therefore, godwit variation is within the range already reported. We found no significant differences in poly-Q allele content or genotype frequencies between sexes, as expected of an autosomal locus, but there was higher diversity of poly-Q genotypes in females than in males. Heterozygosity in godwits (0.79) was higher than any reported in previous studies (Table 3) ranging from monomorphic in sedge warbler (*Acrocephalus schoenobaenus*), reed warbler (*Acrocephalus scirpaceus*) and European bee-eater (*Merops apiaster*) to 0.64 in blue tit (*Cyanistes caeruleus*) (Dor et al. 2011b; Saino et al. 2015; Bazzi et al. 2016b), indicating high diversity in poly-Q alleles in godwits. Bazzi et al. (2016a) found

Table 1 Summary statistics of *Clk*polyQcds allele frequencies among godwits over-wintering in New Zealand

Dataset	*n*	*k*	Ho	Q7	Q8	Q9	Q10	Q11	Q12
Total New Zealand	135	6	0.79	0.07	0.08	0.36	0.22	0.24	0.03
Females	70	6	0.77	0.06	0.08	0.36	0.21	0.26	0.04
Males	65	6	0.80	0.08	0.07	0.37	0.24	0.22	0.02

n number of individuals, *k* number of alleles, *Ho* observed heterozygosity

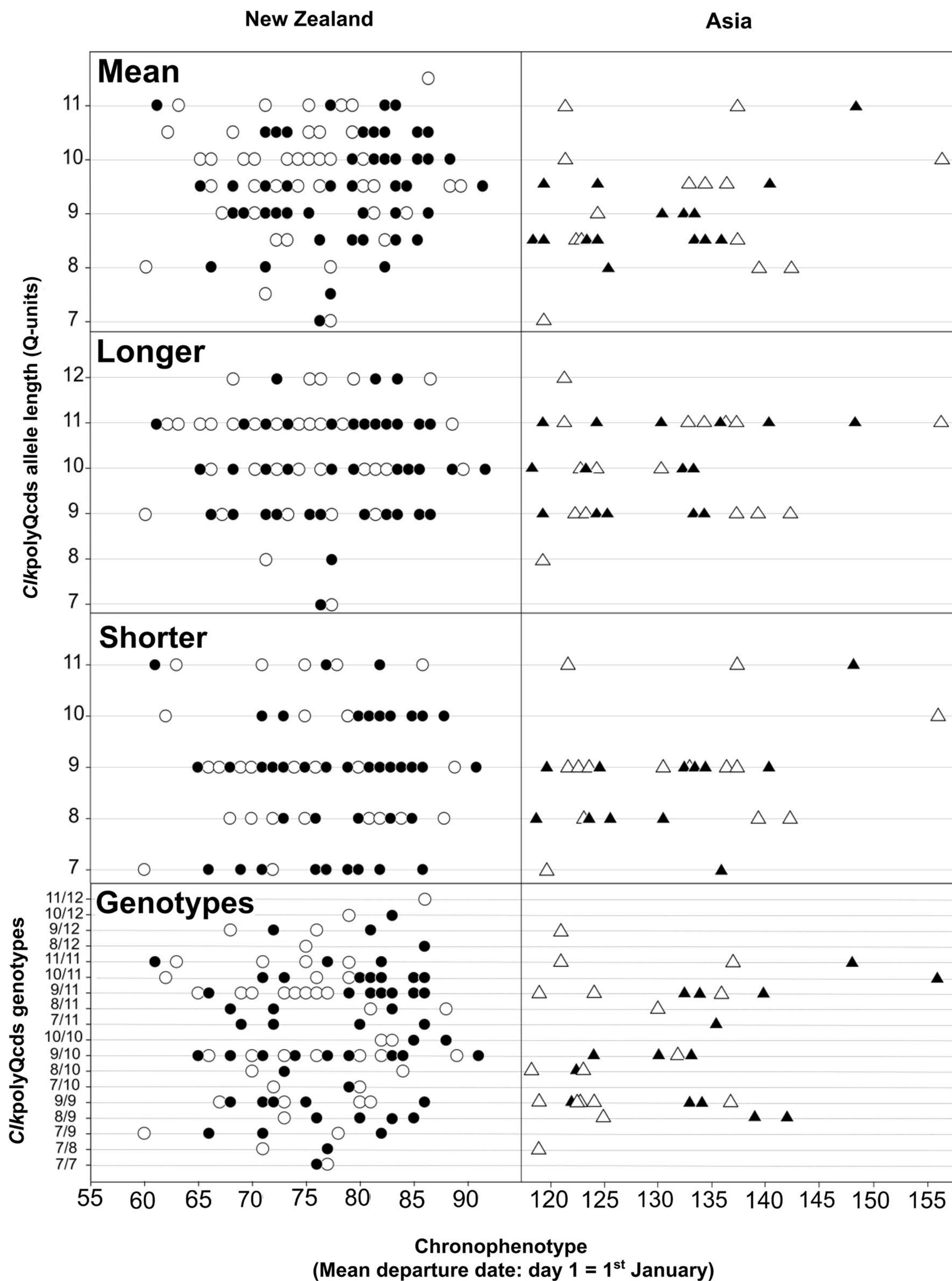

Fig. 2 Relationships between godwit mean migratory departure dates and *Clk*polyQcds genotype. The *Clk*polyQcds genotypes of individual female and male godwits are summarized, as mean allele length, length of longer allele, length of shorter allele and actual genotype (allele combination). Mean migratory departure dates of individual godwits from N.Z. and Asia are given as Gregorian calendar date (day 1 = 1 January). See "Materials and methods" for details of how migratory departure dates were obtained and calculated. N.Z. departure dataset consists of 135 godwits (70 female (○), 65 male (●)); Asia departure dataset consists of 32 godwits (17 female (Δ), 15 male (▲)). Note that while mean departure dates are shown for clarity, statistical tests were done on all available dates for each bird

Table 2 Results from generalized linear mixed model analyses of migration date from New Zealand and from Asia

From	AIC	ΔAIC	Parameter	Estimate	SE	*df*	*t*	*P* value
New Zealand								
Model 1	2393.04	0.00	Intercept	65.06	5.99	21.16	10.39	< 0.001
			Longer allele	0.87	0.48	132.08	1.80	0.074
			Sex (male)	2.04	0.96	130.42	2.13	0.035
Model 2	2393.06	0.02	Intercept	64.88	6.29	25.03	10.31	< 0.001
			Mean allele	0.95	0.56	132.61	1.72	0.089
			Sex (male)	2.01	0.96	130.37	2.10	0.038
Model 3	2394.34	1.29	Intercept	64.34	6.35	26.02	10.13	< 0.001
			Shorter allele	0.19	0.54	130.13	0.35	0.730
			Longer allele	0.78	0.55	130.33	1.41	0.160
			Sex (male)	2.04	0.96	129.48	2.12	0.036
Asia								
Model 1	239.84	0.00	Intercept	112.11	11.74	26.21	9.55	< 0.001
			Shorter allele	1.94	1.09	26.17	1.78	0.086
			Longer allele	0.46	1.02	24.91	0.45	0.658
			Sex (male)	4.68	2.08	26.40	2.25	0.033
			Breed south	− 11.27	2.15	27.61	− 5.25	< 0.001
Model 2	239.86	0.025	Intercept	115.21	9.34	27.75	12.34	< 0.001
			Shorter allele	2.12	1.00	27.48	2.12	0.043
			Sex (male)	4.66	2.05	27.42	2.28	0.031
			Breed south	− 11.40	2.10	28.54	− 5.43	< 0.001
Model 3	240.11	0.27	Intercept	112.87	1164	27.16	9.61	< 0.001
			Mean allele	2.30	1.18	26.73	1.97	0.059
			Sex (male)	4.65	2.06	27.42	2.26	0.032
			Breed south	− 11.17	2.14	28.90	− 5.22	< 0.001

Parameter contrasts are against females (sex) and northern Alaska (breeding region). Shown are parameter estimates for fixed factors from models within two AIC units of the lowest AIC model that were not significantly different in each set of analyses. All models had year and bird ID as random factors; New Zealand models also had site as a random factor

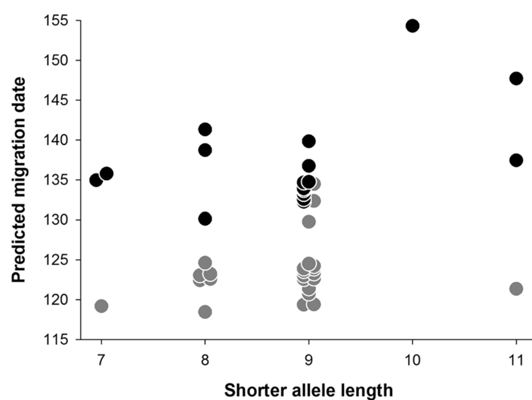

Fig. 3 Predicted migration date from Asia in relation to the shorter *Clk*polyQ allele for northern Alaska-breeding godwits (black dots) and southern Alaska-breeding godwits (gray dots). Predicted values are from a mixed model regression of migration date versus *Clock*, sex, breeding region, year and bird ID. Points have been randomly offset on the *x*-axis for clarity

in trans-Saharan migrants (mainly passerine) that long-distance migration was associated with low gene diversity, which they interpreted as reflecting tighter migratory timing constraints. Specifically, they showed that genetic diversity [i.e., following the methodology used in Bazzi et al. (2016a): genetic diversity was measured as $\hat{h}$: see Nei and Roychoudhury (1974) and formula 12.33 in Nei and Kumar (2000)] of long-distance trans-Saharan migratory passerines was between 0 and 0.78, with the icterine warbler (*Hippolais icterina*), which migrates a distance of ~ 7700 km, having one of the lowest genetic diversities ($\hat{h}$ ~ 0.08), and the subalpine warbler (*Sylvia cantillans*), which migrates a distance of ~ 3000 km, having the largest genetic diversity ($\hat{h}$ ~ 0.78). Bar-tailed godwits do not match this pattern as they are an extreme long-distance migrant (> 11,000 km) yet with high *Clock* diversity using the methodology of Bazzi et al. (2016a: $\hat{h}$ = 0.75), suggesting that their observation of low *Clock* allelic diversity in long-distance passerine migrants is not generalizable.

Table 3 Summary of previous studies on the *Clk*polyQcds polymorphism and diverse phenology-related traits in birds. Studies are listed by year of publication. Observed heterozygosity (Ho) is indicated when the publication reported it. * indicates number of alleles when alleles were not reported (genotyped)

References	Species	Range of polymorphism	Ho	Feature tested	Reported findings
Johnsen et al. (2007)	*Luscinia svecica* *Cyanister caeruleus*	Q10–Q16 Q9–Q17	0.21 0.49	Breeding latitude	Longer alleles more common in higher latitude at the breeding site (only in *Cyanister caeruleus*)
Liedvogel et al. (2009)	*Cyanistes caeruleus*	Q10–Q16 (Q15 not detected)	0.56	Breeding time Incubation duration Breeding success and survival	Breeding time weakly associated with *Clk*polyQcds (only in females). Larger polyQ-alleles found in females with more breeding success
Liedvogel and Sheldon 2010)	*Parus major*	Q10–Q16 (Q14 accounted for 96% of the overall allelic diversity)	0.08	Breeding time	Very low polymorphism. No evidence for association of *Clk*polyQcds with breeding time
Dor et al. 2011b)	*Tachycineta bicolour* *T. thalassina* *T. albilinea* *T. leucorrhoa* *T. meyeni*	Q6–Q9 Q6–Q9 Q7–Q9 Q7–Q9 Q6–Q8	0.47 0.35 0.34 0.05 0.44	Breeding latitude Clutch size Clutch initiation Incubation duration	No evidence for association of *Clk*polyQcds with latitude, clutch initiation or incubation duration
Dor et al. (2011a)	*Hirundo rustica*	Q6–Q8	0.03	*Clk*polyQcds locus against microsatellite loci and mitochondrial DNA.	Very low polymorphism (> 96% homozygotes). Conclusion: High diversity at *Clk*polyQ is not general across all avian species
Caprioli et al. (2012)	*Hirundo rustica*	Q5–Q8 (Q7 accounted for 96.7% of the overall allelic diversity)	0.07	Breeding time	Very low polymorphism. Evidences of association between breeding date and two rare *Clk*polyQ genotypes (Q7Q8 vs. Q7Q7)
Kuhn et al. (2013)	*Ficedula hypoleuca*	Q10–Q14	0.50	Genetic differences over time	No significant genetic variation over time
Chakarov et al. (2013)	*Buteo buteo* *Permis apivorus* *Accipiter gentilis* *Milvus milvus* *Milvus migrans* *Falco tinnunculus* *Falco peregrinus* *Falco cherrug*	Q8 Q9 Q9–Q11 (Q10 not detected) Q6–Q8 (Q7 not detected) Q8 Q7 Q7 Q7	– – – – – – – –	Breeding time	*Clk*polyQcds is monomorphic in a large clade of birds of prey. Conclusion: *Clk*polyQcds polymorphism seems not to be necessary for adaptive variation in phenology
Saino et al. (2013)	*Hirundo rustica*	Q6–Q8	–	Timing of moult	Q7/Q8 barn swallows at the *Clk*polyQcds delayed moult compared to the other individuals with different genotypes. However, sample size of this study is quite small and very low polymorphism (~93% homozygotes Q7Q7).

Table 3 (continued)

References	Species	Range of polymorphism	Ho	Feature tested	Reported findings
Peterson et al. (2015)	*Junco hyemalis hyemalis* *J. h. carolinensis* *J. h. aikeni* *J. h. oreganus* *J.h. thurberi* *J. h. caniceps* *J. h. mearnsi* *J. h. insularis* *J. phaeonotus phaeonotus* *J. p. alticola* *J. p. bairdi*	4* 3 3 3 4 3 4 1 4 3 4	0.27 0.36 0.49 0.19 0.31 0.14 0.32 0.00 0.37 0.56 0.16	Migratory status Migratory distance Migratory restlessness	No evidence for a predictable relationship between migratory behavior (sedentary vs. migratory) and lengths of *Clk*polyQcds
Bourret and Garant (2015)	*Tachycineta bicolour*	Q6–Q9	0.51	Laying date Incubation duration	Association between laying date and *Clk*polyQcds only in females. Association between incubation duration and *Clk*polyQcds only in females
Saino et al. (2015)	*Luscinia megarhynchos* *Ficedula hypoleuca* *Anthus trivialis* *Saxicola rubetra*	Q9–Q13 Q10–Q15 (Q14 not detected) Q6–Q10 Q9–Q16 (Q10 not detected)	0.55 0.48 0.25 0.13	Spring migration dates in a stopover	Longer alleles are more common in later migratory birds in *Anthus trivialis* using the *Clk*polyQcds mean allele length. Longer alleles are more common in later migratory females in *Anthus trivialis* using the *Clk*polyQcds longer allele length. Negative and significant relationship between mean *Clk*polyQcds allele length and wing length
Bazzi et al. (2015)	*Hirundo rustica*	Q6–Q8	0.07 Caprioli et al. (2012) 0.03 Dor et al. (2011a)	Departure from breeding grounds Arrival to the wintering area Departure from the wintering area Arrival to the breeding ground	Two males with genotype Q6Q7 departed earlier compared to those with genotype Q7Q7. A single female Q7Q8 departed later compared to those genotyped Q7Q7. Three Q6Q7 individuals had similar phenology between them compared to individuals with genotype Q7Q7
Bazzi et al. (2016b)	*Cardellina pusilla*	–	0.02	Timing of spring migration in a stopover site Inferred breeding latitude	98% of the individuals were homozygous at *Clock* (almost no variability at the *Clock* gene). Rare heterozygotes did not deviate from homozygous migration phenology

Table 3 (continued)

References	Species	Range of polymorphism	Ho	Feature tested	Reported findings
Bazzi et al. (2017)	*Phylloscopus trochilus*	–	–	Timing of spring migration across the Mediterranean Sea (i.e., in route) Moult speed	*Clk*polyQcds did not predict time of spring migration or moult speed. Significant predictability of *Npas2* allele size and spring migration date were obtained only in females and of *Creb1* allele size and moult speed only in males
Bazzi et al. (2016a)	*Acrocephalus arundinaceus* *Acrocephalus schoeobaenus* *Acrocephalus scirpaceus* *Anthus trivialis* *Caprimulgus europaeus* *Ficedula hypoleuca* *Hippolais icterina* *Jynx torquilla* *Lanius senator* *Luscinia megarhynchos* *Merops apiaster* *Muscicapa striata* *Oenanthe oenanthe* *Oriolus oriolus* *Phoenicurus phoenicurus* *Phylloscopus sibilatrix* *Phylloscopus trochilus* *Saxicola rubetra* *Streptopelia turtur* *Sylvia borin* *Sylvia cantillans* *Sylvia communis* *Upupa epops*	– –	– –	Between species Timing of spring migration across the Mediterranean Sea (i.e., in route) Migration distance Breeding latitude	Note: This is a between-species study (species level). Species breeding at northern latitudes had significantly longer *Clk*pol-yQcds alleles. There was no association between migratory date and *Clock* allele size. *Clock* gene diversity (see Nei and Roychoudhury 1974, formula 12.33 in Nei and Kumar 2000) was found to be related to migratory behavior: Specifically long-distance migratory species were found to be associated with lower *Clock* diversity
Zhang et al. (2017)	*Calandrella cheleensis*	Q7–Q13 (Q8 not detected)	0.58	Activation of Hypothalamus-Pineal-Gonadal endocrine axis Breeding time	Mean *Clk*polyQcds allele length is positively correlated with the standardized laying date of the first egg
Contina et al. (2018)	*Passerina ciris*	8	0.60	Between populations Intra-population: Fall departure date, arrival date, migration duration, body mass, wing chord length	No association between *Clk*polyQcds polymorphism and fall departure date, migration duration, moult site arrival date, spring arrival date, body mass and wing chord

Table 3 (continued)

References	Species	Range of polymorphism	Ho	Feature tested	Reported findings
Sokolovskis et al. (2019)	*P. t. trochilus*	Q9–Q12	0.30	*Clk*polyQcds polymorphism used as genetic marker to compare genetic diversity between the three subspecies	Size ranges and frequencies of different *Clk*polyQcds were almost identical across all three subspecies
	P. t. acredula	Q9–Q12	0.33		
	P. t. yukatensis	Q9–Q12	0.19		
Present study	*Limosa lapponica baueri*	Q7–Q12	0.79	Departure date for migration to the breeding grounds Departure date for migration from a stopover to the breeding grounds	No association between *Clk*polyQcds polymorphism and inter-individual migration departure time from the over-wintering sites (N.Z.) or from the stopover (Eastern Asia)

Relationships with migration timing

We found no unequivocal support for an association between *Clk*polyQcds genotype and migration timing for godwits departing either N.Z. or Asia. In N.Z., the only suggestion of a marginal relationship was shown to be an effect of a single individual, and *Clk*polyQcds genotype showed no relationship with departure timing for the general population. In Asia, while there was a statistical relationship between the presence of the shorter *Clk*polyQcds allele and migration timing, the relationship was very weak and its statistical significance was again driven by a single late-departing northern-breeding bird. These results strongly suggest that variation in *Clk*polyQcds does not directly influence migration timing in the population of bar-tailed godwits examined in this study, contrasting with claims about *Clk*polyQcds genotype associations in other species. In barn swallows (*Hirundo rustica*), it has been suggested that the *Clk*polyQcds polymorphism may influence phenological variation (Bazzi et al. 2015), despite > 90% of the swallows being monomorphic for *Clk*polyQcds (Dor et al. 2011b; Caprioli et al. 2012; Bazzi et al. 2015) so *Clk*polyQcds variation cannot influence the majority of the population. Similarly, while the title of a paper studying the timing of passage of passerines through the Mediterranean implies strong predictive power (Saino et al. 2015, "Polymorphism at the Clock gene predicts phenology of long-distance migration in birds"), their results were variable between species and between sexes (and effects absent in most comparisons). A potential confounding influence in studies of birds on migration is that the destinations and breeding latitudes are usually unknown, and relationships between body size and latitude may also be unknown (which is relevant if for example wing length is used to "correct" migration date, e.g., Saino et al. 2015). In New Zealand-wintering godwits, geographical variation and migration timing are comparatively well resolved, and we know that late migrants are largely northern breeders (Conklin et al. 2010) and that northern breeders are smaller than southern breeders (Conklin et al. 2011). The strength of the relationship between migration time and eventual breeding latitude is much stronger for godwits leaving Asia than New Zealand (Conklin et al. 2010), yet the annual consistency in individual departure dates is similar for both migration stages (Conklin et al. 2013). These findings indicate that godwits have strong individual migration schedules both at departure and after their stopover. If *Clk*polyQcds genotype did strongly influence migration timing, this ought to be detectable at both points of the migration (though admittedly the sample size for birds leaving Asia is limited).

The changing view of *Clk*polyQcds and avian time-related traits

The length of the *Clk*polyQ region has been described as a modifier of the functionality (i.e., as transcription-activating factor) of the protein CLOCK, thereby potentially influencing annual timing-related phenotypes (Gekakis et al. 1998). The discovery of a poly-Q region (i.e., region rich in glutamine) in the avian *Clock* gene and its polymorphism gave rise to studies trying to understand the meaning of the existence of this diversity at the *Clk*polyQ region in the different aspects of the phenology (i.e., breeding time). The paper that essentially started the interest in *Clock* (Johnsen et al. 2007) proposed that variation in *Clock* may relate to microevolutionary responses to photoperiod related to latitude. Subsequent studies have investigated potential influences of *Clock* on phenological events in a wide range of birds (mostly passerines; see Table 3 for a summary of these studies). Subsequent studies suggested that *Clk*polyQcds genotype may influence migration timing (O'Malley and Banks 2008; Saino et al. 2015; Bazzi et al. 2017), but an increasing number of studies have failed to find any association (Bazzi et al. 2016a, b, 2017; Contina et al. 2018). In combination with the results of the study reported here, we conclude that there is little support for variation in *Clk*polyQ genotype being a significant determinant of migration timing in birds.

The functional significance of variation in *Clk*polyQ length is thought to lie in responses to photoperiod, with a tendency toward longer alleles in more northern populations reflecting selection pressures from longer daylengths at higher latitudes (Johnsen et al. 2007; Bazzi et al. 2016a). There is some evidence for *Clk*polyQ length of individual birds relating to laying date in passerines (Liedvogel et al. 2009; Caprioli et al. 2012; Bourret and Garant 2015; Zhang et al. 2017). Zhang et al. (2017) proposed, based on rat (*Rattus rattus*) studies by Resuehr et al. (2007), that CLOCK/BMAL1 binds to E-box elements in the gonadotropin releasing hormone receptor (GnRH-R) gene promoter region. GnRH plays a role in the upper stream of the hypothalamic–pituitary–gonadal axis (HPG), triggering physiological changes that prepare the organism for reproduction (Tsutsui and Ubuka 2018). Thus, it is possible that through a mechanism—which is still not clear—the length of *Clk*polyQ affects this binding and consequently produces the observed inter-individual variation in breeding/laying time (Zhang et al. 2017). In short, the most recent studies consider that the role of *Clock* is more likely an adaptation to photoperiodic regimes at the breeding grounds with possible implications for breeding time rather than a direct determinant of migratory timing. However, given that individual and population-level patterns of migration can exist (e.g., Tøttrup et al. 2012; Briedis et al. 2016; Pedersen et al. 2018), it is possible that variation in breeding latitude and associated migration timing could be correlated with differences in *Clk*polyQ composition and give rise to apparent *Clock*–migration relationships. If underlying mechanisms that drive bird phenological characteristics differ between species (Liedvogel and Sheldon 2010) or even between distinct populations (Saino et al. 2015; Bazzi et al. 2016b), it is still possible that the *Clk*polyQ polymorphism plays some role in generating migratory timing phenotypes in other species.

Other factors and limitations

Migration timing is influenced not only by the internal "clock" but also by environmental conditions (Marra et al. 2005; Bauer et al. 2008; Bourret and Garant 2015). Over a decade of observations at our main godwit study site in N.Z. during the migration departure period have confirmed that conditions are, in general, favorable with unsuitable winds (e.g., northerlies to westerlies) typically lasting for only a few days in sequence (e.g., Conklin and Battley 2011). Environmental variation probably influences an individual's decision on when to depart on a scale of days, rather than weeks. There are some suggestions that individuals may fine-tune their migrations (Sergio et al. 2014; Evens et al. 2017), with first-time northward migrants having lower repeatability in migration departure date than adults (Battley 2006). This suggests that the experience gained by an individual after years of completed migratory cycles could blur the strength in which timing-associated genes are detected (Berthold 2001). A significant improvement in our study would be using immature individuals' first departure dates for migration, as these may be more genetically driven than subsequent migrations in which experience may play a role. We expect, however, that the genetic influence on an individual's migratory behavior remains determinant in the adult's average departure date; i.e., an immature early chronophenotype would become an adult early chronophenotype. Godwits are also social migrants that behaviorally advertise their 'intention' to migrate as a way of recruiting flockmates, and such social advertising could potentially 'overrule' innate timing preferences. However, godwits frequently ignore social cues from migrants on a given day only to migrate a day or two later (Conklin and Battley 2011), implying that individual preferences can and frequently do overrule social cues from other birds. First-time migrants could possibly be more influenced by social cues from recruiting flocks than experienced birds, but evidence from the Firth of Thames (Battley 2006, Fig. 1) indicates that even in the presence of large numbers of migrating adults, first-time migrants still departed over virtually the whole migration period. This suggests that first-time migrants already have internally set programs that establish the stage of the season within which to migrate. Social cues may simply aid the joining of a flock within this receptive period.

Conclusions

Our results indicate that the *Clk*polyQcds polymorphism is not associated with the timing of northward migration in bar-tailed godwits. It adds to a growing list of studies that have failed to establish a link between *Clock*, calling into question the generality of any claimed *Clock*-timing associations. However, we limited our analysis to a single trait; other traits under circannual control (i.e., fat storage, moult, reproduction) might be good candidates for future studies. One possibility for the lack of concordance across *Clock*polyQ studies is that migratory timing is indeed a complex trait governed by numerous genes (polygenic) in which *Clock* variability seems not to contribute substantially or consistently to the observed chronophenotype variability (Pulido and Berthold 2003; Bazzi et al. 2016b; Hess et al. 2016). Phenotype–genotype studies are complex since an individual phenotype can be influenced by many factors (i.e., environment and social context) and it could potentially change according to a particular environment (i.e., phenotypic plasticity). Another alternative explanation is that *Clock*polyQ plays no role in departure time decisions for migration at all. Finally, yet importantly, genetic components are not the only molecular-related sources of behavioral variation: epigenetic–phenotype associations are still in their early stages of being investigated, with the potential to play an important role in future discovery (Crews 2011; Powledge 2011; Liedvogel and Lundberg 2014; Baerwald et al. 2016). Indeed, a recent study found evidence of methylation level at the *Clk*polyQcds playing an important role regulating individual variation in migration timing (Saino et al. 2017). To what extent epigenetics is involved in determining variation in migratory timing of individuals and populations across taxa is not yet sufficiently explored, but is a promising avenue for future work.

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Author contribution statement Fieldwork was done principally by PFB (bird capture efforts at all sites and monitoring in the Firth of Thames from 2004–06) and JRC (captures and departure monitoring at the Manawatu River estuary). APM was responsible for microsatellite data generation in the lab, subsequent analyses and writing the manuscript. AEF guided and supervised the laboratory work and AEF and PFB gave input to data analyses. All authors contributed to drafting of the manuscript.

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Data accessibility Sequences were deposited in GenBank Accession nos KR653306 (*Clk*polyQ$_8$), JN676984 (*Clk*polyQ$_9$), KU051417 (*Clk*polyQ$_{10}$), JN676983 (*Clk*polyQ$_{11}$) and KU051418 (*Clk*polyQ$_{12}$).

References

Albrecht U (2012) Timing to perfection: the biology of central and peripheral circadian clocks. Neuron 74:246–260. https://doi.org/10.1016/j.neuron.2012.04.006

Baerwald MR et al (2016) Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. Mol Ecol 25:1785–1800. https://doi.org/10.1111/mec.13231

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67:48. https://doi.org/10.18637/jss.v067.i01

Battley PF (2006) Consistent annual schedules in a migratory shorebird. Biol Lett 2:517–520. https://doi.org/10.1098/rsbl.2006.0535

Battley PF, Conklin JR (2017) Geolocator wetness data accurately detect periods of migratory flight in two species of shorebird. Wader Study 124:112–119. https://doi.org/10.18194/ws.00068

Battley PF et al (2012) Contrasting extreme long-distance migration patterns in bar-tailed godwits *Limosa lapponica*. J Avian Biol 43:21–32. https://doi.org/10.1111/j.1600-048x.2011.05473.x

Bauer S, Gienapp P, Madsen J (2008) The relevance of environmental conditions for departure decision changes en route in migrating geese. Ecology 89:1953–1960. https://doi.org/10.1890/07-1101.1

Bazzi G et al (2015) *Clock* gene polymorphism and scheduling of migration: a geolocator study of the barn swallow *Hirundo rustica*. Sci Rep 5:12443. https://doi.org/10.1038/srep12443

Bazzi G et al (2016a) *Clock* gene polymorphism, migratory behaviour and geographic distribution: a comparative study of trans-Saharan migratory birds. Mol Ecol 25:6077–6091. https://doi.org/10.1111/mec.13913

Bazzi G et al (2016b) *Adcyap1* polymorphism covaries with breeding latitude in a Nearctic migratory songbird, the wilson's warbler (*Cardellina pusilla*). Ecol Evol 6:3226–3239. https://doi.org/10.1002/ece3.2053

Bazzi G et al (2017) Candidate genes have sex-specific effects on timing of spring migration and moult speed in a long-distance migratory bird. Curr Zool 63:479–486. https://doi.org/10.1093/cz/zow103

Bell-Pedersen D et al (2005) Circadian rhythms from multiple oscillators: lessons from diverse organisms. Nat Rev Genet 6:544–556. https://doi.org/10.1038/nrg1633

Berthold P (2001) Bird migration: a general survey, 2nd edn. Oxford University Press, c2001, Oxford

Berthold P, Querner U (1981) Genetic basis of migratory behavior in European warblers. Science 212:77–79. https://doi.org/10.1126/science.212.4490.77

Both C, Bouwhuis S, Lessells CM, Visser ME (2006) Climate change and population declines in a long-distance migratory bird. Nature 441:81–83. https://doi.org/10.1038/nature04539

Both C, Van Turnhout CAM, Bijlsma RG, Siepel H, Van Strien AJ, Foppen RPB (2010) Avian population consequences of climate change are most severe for long-distance migrants in seasonal habitats. Proc Biol Sci 277:1259–1266. https://doi.org/10.1098/rspb.2009.1525

Bourret A, Garant D (2015) Candidate gene–environment interactions and their relationships with timing of breeding in a wild bird

population. Ecol Evol 5:3628–3641. https://doi.org/10.1002/ece3.1630

Briedis M et al (2016) Breeding latitude leads to different temporal but not spatial organization of the annual cycle in a long-distance migrant. J Avian Biol 47:743–748. https://doi.org/10.1111/jav.01002

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 20(1004–1006):1008–1010

Caprioli M et al (2012) *Clock* gene variation is associated with breeding phenology and maybe under directional selection in the migratory barn swallow. PLoS One 7:e35140. https://doi.org/10.1371/journal.pone.0035140

Cassone VM (2014) Avian circadian organization: a chorus of clocks. Front Neuroendocrinol 35:76–88. https://doi.org/10.1016/j.yfrne.2013.10.002

Chakarov N, Jonker R, Boerner M, Hoffman J, Kruger O (2013) Variation at phenological candidate genes correlates with timing of dispersal and plumage morph in a sedentary bird of prey. Mol Ecol 22:5430–5440. https://doi.org/10.1111/mec.12493

Chong NW, Bernard M, Klein DC (2000) Characterization of the chicken serotonin N-acetyltransferase gene—activation via clock gene heterodimer/e box interaction. J Biol Chem 275:32991–32998. https://doi.org/10.1074/jbc.m005671200

Conklin JR (2011) Extreme migration and the annual cycle: individual strategies in New Zealand bar-tailed godwits. PhD thesis, Massey University, Palmerston North

Conklin JR, Battley PF (2011) Impacts of wind on individual migration schedules of New Zealand bar-tailed godwits. Behav Ecol 22:854–861. https://doi.org/10.1093/beheco/arr054

Conklin JR, Battley PF (2012) Carry-over effects and compensation: late arrival on non-breeding grounds affects wing moult but not plumage or schedules of departing bar-tailed godwits *Limosa lapponica baueri*. J Avian Biol 43:252–263. https://doi.org/10.1111/j.1600-048x.2012.05606.x

Conklin JR, Battley PF, Potter MA, Fox JW (2010) Breeding latitude drives individual schedules in a trans-hemispheric migrant bird. Nat Commun 1:67. https://doi.org/10.1038/ncomms1072

Conklin JR, Battley PF, Potter MA, Ruthrauff DR (2011) Geographic variation in morphology of Alaskan-breeding bar-tailed godwits (*Limosa lapponica*) is not maintained on their nonbreeding grounds in New Zealand. Auk 128:363–373. https://doi.org/10.1525/auk.2011.10231

Conklin JR, Battley PF, Potter MA (2013) Absolute consistency: individual versus population variation in annual-cycle schedules of a long-distance migrant bird. PLoS One 8:e54535. https://doi.org/10.1371/journal.pone.0054535

Contina A, Bridge ES, Ross JD, Shipley JR, Kelly JF (2018) Examination of *Clock* and *Adcyap1* gene variation in a neotropical migratory passerine. PLoS One 13:e0190859. https://doi.org/10.1371/journal.pone.0190859

Costa R, Peixoto AA, Barbujani G, Kyriacou CP (1992) A latitudinal cline in a Drosophila *Clock* gene. Proc Biol Sci 250:43–49. https://doi.org/10.1098/rspb.1992.0128

Crews D (2011) Epigenetic modifications of brain and behavior: theory and practice. Horm Behav 59:393–398. https://doi.org/10.1016/j.yhbeh.2010.07.001

Darlington TK et al (1998) Closing the circadian loop: CLOCK-induced transcription of its own inhibitors per and tim. Science 280:1599–1603. https://doi.org/10.1126/science.280.5369.1599

Dor R et al (2011a) *Clock* gene variation in *Tachycineta* swallows. Ecol Evol 2:95–105. https://doi.org/10.1002/ece3.73

Dor R et al (2011b) Low variation in the polymorphic *Clock* gene poly-Q region despite population genetic structure across barn swallow (*Hirundo rustica*) populations. PLoS One 6:e28843. https://doi.org/10.1371/journal.pone.0028843

Drent R, Both C, Green M, Madsen J, Piersma T (2003) Pay-offs and penalties of competing migratory schedules. Oikos 103:274–292. https://doi.org/10.1034/j.1600-0706.2003.12274.x

Easton A, Arbuzova J, Turek FW (2003) The circadian *Clock* mutation increases exploratory activity and escape-seeking behavior. Genes Brain Behav 2:11–19. https://doi.org/10.1034/j.1601-183x.2003.00002.x

Ellegren H, Parsch J (2007) The evolution of sex-biased genes and sex-biased gene expression. Nat Rev Genet 8:689–698. https://doi.org/10.1038/nrg2167

Evans JA, Leise TL, Castanon-Cervantes O, Davidson AJ (2015) Neural correlates of individual differences in circadian behaviour. Proc Biol Sci 282:20150769

Evens R, Beenaerts N, Witters N, Artois T (2017) Repeated migration of a juvenile European nightjar *Caprimulgus europaeus*. J Ornithol 158:881–886. https://doi.org/10.1007/s10336-017-1459-2

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

Fidler AE, van Oers K, Drent PJ, Kuhn S, Mueller JC, Kempenaers B (2007) *Drd4* gene polymorphisms are associated with personality variation in a passerine bird. Proc Biol Sci 274:1685–1691. https://doi.org/10.1098/rspb.2007.0337

Fox JW (2010) Geolocator manual. Version 8. British Antartic Survey. United Kingdom

Fridolfsson AK, Ellegren H (1999) A simple and universal method for molecular sexing of non-ratite birds. J Avian Biol 30:116–121. https://doi.org/10.2307/3677252

Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP (1998) Role of the CLOCK protein in the mammalian circadian mechanism. Science 280:1564–1569

Gemayel R et al (2015) Variable glutamine-rich repeats modulate transcription factor activity. Mol Cell 59:615–627. https://doi.org/10.1016/j.molcel.2015.07.003

Gill RE Jr et al (2009) Extreme endurance flights by landbirds crossing the Pacific Ocean: ecological corridor rather than barrier? Proc Biol Sci 276:447–458. https://doi.org/10.1098/rspb.2008.1142

Hands S, Sinadinos C, Wyttenbach A (2008) Polyglutamine gene function and dysfunction in the ageing brain. Biochim Biophys Acta 1779:507–521. https://doi.org/10.1016/j.bbagrm.2008.05.008

Hardin PE (2005) The circadian timekeeping system of *Drosophila*. Curr Biol 15:R714–R722. https://doi.org/10.1016/j.cub.2005.08.019

Hastings MH (2000) Circadian clockwork: two loops are better than one. Nat Rev Neurosci 1:143–146. https://doi.org/10.1038/35039080

Helfer G, Fidler AE, Vallone D, Foulkes NS, Brandstaetter R (2006) Molecular analysis of clock gene expression in the avian brain. Chronobiol Int 23:113–127. https://doi.org/10.1080/07420520500521871

Helm B, Schwabl I, Gwinner E (2009) Circannual basis of geographically distinct bird schedules. J Exp Biol 212:1259–1269. https://doi.org/10.1242/jeb.025411

Helm B et al (2013) Annual rhythms that underlie phenology: biological time-keeping meets environmental change. Proc Biol Sci 280:20130016. https://doi.org/10.1098/rspb.2013.0016

Hess JE, Zendt JS, Matala AR, Narum SR (2016) Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. Proc Biol Sci 283:20153064

Hurley JM, Loros JJ, Dunlap JC (2016) Circadian oscillators: around the transcription–translation feedback loop and on to output. Trends Biochem Sci 41:834–846. https://doi.org/10.1016/j.tibs.2016.07.009

Jenni L, Kery M (2003) Timing of autumn bird migration under climate change: advances in long-distance migrants, delays in short-distance migrants. Proc Biol Sci 270:1467–1471. https://doi.org/10.1098/rspb.2003.2394

Johnsen A et al (2007) Avian *Clock* gene polymorphism: evidence for a latitudinal cline in allele frequencies. Mol Ecol 16:4867–4880. https://doi.org/10.1111/j.1365-294x.2007.03552.x

King DP et al (1997) Positional cloning of the mouse circadian clock gene. Cell 89:641–653

Kissner KJ, Weatherhead PJ, Francis CM (2003) Sexual size dimorphism and timing of spring migration in birds. J Evol Biol 16:154–162. https://doi.org/10.1046/j.1420-9101.2003.00479.x

Kölzsch A et al (2015) Forecasting spring from afar? Timing of migration and predictability of phenology along different migration routes of an avian herbivore. J Anim Ecol 84:272–283. https://doi.org/10.1111/1365-2656.12281

Korsten P et al (2010) Association between DRD4 gene polymorphism and personality variation in great tits: a test across four wild populations. Mol Ecol 19:832–843. https://doi.org/10.1111/j.1365-294x.2009.04518.x

Kuhn K et al (2013) Differentiation in neutral genes and a candidate gene in the pied flycatcher: using biological archives to track global climate change. Ecol and Evol 3:4799–4814. https://doi.org/10.1002/ece3.855

Leder EH, Danzmann RG, Ferguson MM (2006) The candidate gene, *Clock*, localizes to a strong spawning time quantitative trait locus region in rainbow trout. J Hered 97:74–80. https://doi.org/10.1093/jhered/esj004

Liedvogel M, Lundberg M (2014) The genetics of migration. In: Hansson LA, Akesson S (eds) Animal movements across scales. Oxford University Press, Oxford, pp 219–231

Liedvogel M, Sheldon BC (2010) Low variability and absence of phenotypic correlates of *Clock* gene variation in a great tit *Parus major* population. J Avian Biol 41:543–550. https://doi.org/10.1111/j.1600-048x.2010.05055.x

Liedvogel M, Szulkin M, Knowles SCL, Wood MJ, Sheldon BC (2009) Phenotypic correlates of *Clock* gene variation in a wild blue tit population: evidence for a role in seasonal timing of reproduction. Mol Ecol 18:2444–2456. https://doi.org/10.1111/j.1365-294x.2009.04204.x

Lisovski S (2018) Light-level geolocation in polar regions with 24-hour daylight. Wader Study 125:129–134. https://doi.org/10.18194/ws.00109

Lisovski S, Hahn S (2012) GeoLight – processing and analysing light-based geolocator data in R. Methods Ecol Evol 3:1055–1059. https://doi.org/10.1111/j.2041-210x.2012.00248.x

Majumdar G, Trivedi AK, Gupta NJ, Kumar V (2015) Circadian synchronization determines critical day length for seasonal responses. Physiol Behav 147:282–290. https://doi.org/10.1016/j.physbeh.2015.05.005

Marra PP, Francis CM, Mulvihill RS, Moore FR (2005) The influence of climate on the timing and rate of spring bird migration. Oecologia 142:307–315. https://doi.org/10.1007/s00442-004-1725-x

Meltofte H, Piersma T, Boyd H, McCaffery BJ, Tulp IYM (2007) Effects of climate variation on the breeding ecology of Arctic shorebirds. Meddelelser om Groenland, Bioscience 59:1–48

Mueller JC, Partecke J, Hatchwell BJ, Gaston KJ, Evans KL (2013) Candidate gene polymorphisms for behavioural adaptations during urbanization in blackbirds. Mol Ecol 22:3629–3637. https://doi.org/10.1111/mec.12288

Nei M, Kumar S (2000) Molecular evolution and phylogenetics. Oxford University Press, Oxford

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76:379–390

Nussey DH, Wilson AJ, Brommer JE (2007) The evolutionary ecology of individual phenotypic plasticity in wild populations. J Evol Biol 20:831–844. https://doi.org/10.1111/j.1420-9101.2007.01300.x

O'Malley KG, Banks MA (2008) A latitudinal cline in the Chinook salmon (*Oncorhynchus tshawytscha*) Clock gene: evidence for selection on PolyQ length variants. Proc Biol Sci 275:2813–2821. https://doi.org/10.1098/rspb.2008.0524

Panda S, Hogenesch JB, Kay SA (2002) Circadian rhythms from flies to human. Nature 417:329–335. https://doi.org/10.1038/417329a

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pedersen L, Jackson K, Thorup K, Tøttrup AP (2018) Full-year tracking suggests endogenous control of migration timing in a long-distance migratory songbird. Behav Ecol Sociobiol 72:139. https://doi.org/10.1007/s00265-018-2553-z

Peterson MP, Abolins-Abols M, Atwell JW, Rice RJ, Mila B, Ketterson ED (2015) Variation in candidate genes CLOCK and ADCYAP1 does not consistently predict differences in migratory behavior in the songbird genus *Junco*. F1000Res 2:2–115

Powledge TM (2011) Behavioral epigenetics: how nurture shapes nature. Bioscience 61:588–592. https://doi.org/10.1525/bio.2011.61.8.4

Pulido F, Berthold P (2003) Quantitative genetic analysis of migratory behaviour. In: Berthold P, Gwinner E, Sonnenschein E (eds) Avian Migration. Springer, Berlin, pp 53–77

Rani S, Kumar V (2013) Avian circannual systems: persistence and sex differences. Gen Comp Endocrinol 190:61–67. https://doi.org/10.1016/j.ygcen.2013.04.013

Reischl S, Kramer A (2011) Kinases and phosphatases in the mammalian circadian clock. FEBS Lett 585:1393–1399. https://doi.org/10.1016/j.febslet.2011.02.038

Resuehr D, Wildemann U, Sikes H, Olcese J (2007) E-box regulation of gonadotropin-releasing hormone (GnRH) receptor expression in immortalized gonadotrope cells. Mol Cell Endocrinol 278:36–43. https://doi.org/10.1016/j.mce.2007.08.008

Rey G, Cesbron F, Rougemont J, Reinke H, Brunner M, Naef F (2011) Genome-wide and phase-specific DNA-binding rhythms of BMAL1 control circadian output functions in mouse liver. PLoS Biol 9:e1000595. https://doi.org/10.1371/journal.pbio.1000595

Ripperger JA, Shearman LP, Reppert SM, Schibler U (2000) CLOCK, an essential pacemaker component, controls expression of the circadian transcription factor DBP. Genes Dev 14:679–689

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Saino N et al (2013) Timing of molt of barn swallows is delayed in a rare Clock genotype. Peerj 1:e17. https://doi.org/10.7717/peerj.17

Saino N et al (2015) Polymorphism at the Clock gene predicts phenology of long-distance migration in birds. Mol Ecol 24:1758–1773. https://doi.org/10.1111/mec.13159

Saino N et al (2017) Migration phenology and breeding success are predicted by methylation of a photoperiodic gene in the barn swallow. Sci Rep 7:45412. https://doi.org/10.1038/srep45412

Saleem Q, Anand A, Jain S, Brahmachari SK (2001) The polyglutamine motif is highly conserved at the Clock locus in various organisms and is not polymorphic in humans. Hum Genet 109:136–142. https://doi.org/10.1007/s004390100550

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. Nat Biotechnol 18:233–234. https://doi.org/10.1038/72708

Sergio F et al (2014) Individual improvements and selective mortality shape lifelong migratory performance. Nature 515:410. https://doi.org/10.1038/nature13696

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Can J Zool 69:82–90. https://doi.org/10.1139/z91-013

Sokolovskis K et al (2019) Phenotypic and genetic characterization of the East Siberian Willow Warbler (*Phylloscopus trochilus yakutensis* Ticehurst, 1935) in relation to the European subspecies. J Ornithol 160:721–731. https://doi.org/10.1007/s10336-019-01653-y

Spottiswoode C, Saino N (2010) Sexual selection and climate change. In: Møller AP, Fiedler W, Berthold P (eds) Oxford University Press. OUP Oxford, pp 169-189

Stuber EF, Baumgartner C, Dingemanse NJ, Kempenaers B, Mueller JC (2016) Genetic correlates of individual differences in sleep behavior of free-living great tits (*Parus major*). G3 Genes Genomes Genet 6:599–607. https://doi.org/10.1534/g3.115.024216

Sutton JT, Robertson BC, Jamieson IG (2011) Dye shift: a neglected source of genotyping error in molecular ecology. Mol Ecol Resour 11:514–520. https://doi.org/10.1111/j.1755-0998.2011.02981.x

Tøttrup AP et al (2012) The annual cycle of a trans-equatorial Eurasian-African passerine migrant: different spatio-temporal strategies for autumn and spring migration. Proc Biol Sci 279:1008–1016. https://doi.org/10.1098/rspb.2011.1323

Tschirren B, Bensch S (2010) Genetics of personalities: no simple answers for complex traits. Mol Ecol 19:624–626. https://doi.org/10.1111/j.1365-294X.2009.04519.x

Tsutsui K, Ubuka T (2018) How to contribute to the progress of neuroendocrinology: discovery of GnIH and progress of GnIH research. Front Endocrinol. https://doi.org/10.3389/fendo.2018.00662

Visser ME et al (2015) Effects of spring temperatures on the strength of selection on timing of reproduction in a long-distance migratory bird. PLoS Biol 13:e1002120–e1002120. https://doi.org/10.1371/journal.pbio.1002120

Wetzel DP, Hatch MI, Westneat DF (2015) Genetic sources of individual variation in parental care behavior. Behav Ecol Sociobiol 69:1933–1943. https://doi.org/10.1007/s00265-015-2006-x

Yoshitane H, Fukada Y (2009) CIPC-dependent phosphorylation of CLOCK and NPAS2 in circadian clockwork. Sleep Biol Rhythms 7:226–234. https://doi.org/10.1111/j.1479-8425.2009.00411.x

Zhang EE, Kay SA (2010) Clocks not winding down: unravelling circadian networks. Nat Rev Mol Cell Biol 11:764–776. https://doi.org/10.1038/nrm2995

Zhang S, Xu X, Wang W, Yang W, Liang W (2017) *Clock* gene is associated with individual variation in the activation of reproductive endocrine and behavior of Asian short toed lark. Sci Rep 7:15002. https://doi.org/10.1038/s41598-017-15064-7