

Revealing the Demographic History of the European nightjar (*Caprimulgus europaeus*).

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Abstract

A species' demographic history provides important context to contemporary population genetics and a possible insight into past responses to climate change. An individual's genome provides a window into the evolutionary history of contemporary populations. Pairwise Sequentially Markovian Coalescent (PSMC) analysis uses information from a single genome to derive fluctuations in effective population size change over the last ~5 million years. Here we apply PSMC analysis to two European nightjar (*Caprimulgus europaeus*) genomes, sampled in Northwest and Southern Europe, with the aim of revealing the demographic history of nightjar in Europe. We successfully reconstructed effective population size over the last 5 million years for two contemporary nightjar populations. Our analysis shows that nightjar are responsive to global climate change, with effective population size broadly increasing under stable warm periods and decreasing during cooler spans and prolonged glacial periods. PSMC analysis on the pseudo-diploid combination of the two genomes revealed fluctuations in gene flow between the populations over time, with gene flow ceasing by the last-glacial maximum. This pattern of differentiation is in line with the species utilising different refugia during glacial maxima. We suggest that nightjar in Europe may show latitudinal (East-West) genetic structuring as a result of reduced gene flow between different glacial refugia. Finally, our results suggest that migratory behaviour in nightjar likely evolved prior to the last-glacial maximum, with long-distance migration seemingly persisting throughout the Pleistocene. However, further genetic structure analysis of nightjar from known breeding sites across the species' contemporary range is needed to fully understand the extent and origins of range-wide differentiation in the species.

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during glacial maxima. We suggest that nightjar in Europe may show latitudinal (East-West) genetic structuring as a result of reduced gene flow between different glacial refugia. Finally, our results suggest that migratory behaviour in nightjar likely evolved prior to the last-glacial maximum, with long-distance migration seemingly persisting throughout the Pleistocene. However, further genetic structure analysis of nightjar from known breeding sites across the species' contemporary range is needed to fully understand the extent and origins of range-wide differentiation in the species.

Keywords; PSMC, conservation genomics, refugia, *Caprimulgus europaeus* , demography

Introduction

Technological and methodological advancements have led to genomics playing an increasingly important role in conservation biology (Allendorf, 2017). Genomes provide a repository from which information on historic changes in genetic diversity, effective population size (N_e), speciation, and population structuring can be inferred and used to track adaptations to environmental change (Mather et al., 2019; Patil and Vijay, 2021). Specifically, sequence data from a single aligned genome can be used to track historic demographic patterns exhibited by a species or population (Li and Durbin, 2011). Pairwise sequentially Markovian coalescent (PSMC) analysis is a powerful tool which infers ancestral changes in N_e from a single genome. The analysis applies hidden-Markov modelling to the coalescence framework, treating a genome as multiple historic genealogies partitioned by recombination events (see Li and Durbin, 2011; Mather et al., 2019 for detailed explanation of the method). PSMC analysis has been used to determine ancestral (up to ~ 5 Mya) population trends and track time scales of species and population divergences (e.g. *Ficedula* flycatchers; Nadachowska-Brzyska et al., 2016, *Catharus* thrushes; Termignoni-Garcia et al., 2022), as well as shedding light on periods of gene flow among otherwise genetically structured populations (Sato et al., 2020). PSMC analysis can be applied to pseudo-diploid genomes constructed from two individuals from different populations to investigate changes in gene flow and timing of divergence (Li and Durbin, 2011; Prado-Martinez et al., 2013; Sato et al., 2020). For example, PSMC applied to pseudo-diploid genomes from three Golden eagle (*Aquila chrysaetos*) subspecies revealed the timing of divergence and gene flow change among the subspecies over a time scale of ~11 million years (Sato et al., 2020). When combined with geological and paleoclimate data, PSMC analysis can reflect a species' past ability to adapt to environmental change, and how different populations, or species, have been affected by broad climate trends (Nadachowska-Brzyska et al., 2015; Mather et al., 2019). Understanding a species' response to past environmental change allows for predictions to be made regarding vulnerability to contemporary and future climate change and how this may vary interspecifically under different life histories (Kozma et al., 2018, 2016; Chattopadhyay et al., 2019), or between populations at different locations across a species range (Sato et al., 2020).

Over the past ~5 million years the global climate has fluctuated dramatically, oscillating between periods of extensive glaciation and interglacial warming. Long glacial and short interglacial periods during the mid-Pleistocene revolution (MPR; ~1 Mya - 450 Kya) resulted in cooler interglacial temperatures than those presently recorded (Pisias and Moore, 1981). However, throughout the Mid-Brunhes Event (MBE; ~450-110 Kya), interglaciations were characterised by warmer temperatures, with comparatively less severe glacial periods compared with the mid-Pleistocene (Candy et al., 2010; Barth et al., 2018). During the last glacial maxima (LGM; ~110 Kya) the Fennoscandian ice sheet covered much of Western and North Western Europe, restricting temperate zones to contemporary Southern Eurasia (Denton and Hughes, 1981). These significant shifts in global climate have been shown to correspond with fluctuations in historic population size in a number of species (Nadachowska-Brzyska et al., 2015; Kozma et al., 2016, 2018). Over periods of cooling, temperate Western-Palaearctic species will have likely been restricted to southern refugia in Europe (Iberia, Apennines, and Balkans; Hewitt, 1999; but see Thorup et al., 2021). Restriction to different glacial refugia and subsequent northward expansion during interglacial periods have been linked to contemporary population structure and subspecies divergence in multiple species, including birds and aerial insects (e.g. Schmitt, 2007; Hansson et al., 2008; Nadachowska-Brzyska et al., 2016; de Greef et al., 2022). Occupation of separate glacial refugia by different populations is thought to have driven spatial patterns of genetic differentiation in temperate species, with many Palaearctic birds exhibiting contemporary East - West patterns

in genetic structure and speciation (e.g. Hansson et al., 2008; Lombardo et al., 2022; Väli et al., 2022).

The European nightjar (*Caprimulgus europaeus*), henceforth nightjar, is a long-distance migratory bird with a temperate breeding distribution ranging from Northwest Europe through to East Asia (BirdLife International, 2022). Nightjar likely originated from the Afrotropics (Han et al., 2010), with the most closely related extant species being an Afrotropic resident (Rufous-cheeked nightjar *Caprimulgus rufigena*) (Han et al., 2010). Nightjar are composed of six subspecies (*C.e. europaeus*, *meridionalis*, *sarudnyi*, *unwini*, *plumipes*, *dementievi*) broadly following an East-West clinal distribution (Cleere, 1998; Cleere et al., 2021), although mtDNA analysis has found little association between genetic variation and current subspecies classifications (Schweizer et al., 2020). Based on mtDNA analysis, Larsen et al., (2007) proposed that nightjar migratory behaviour evolved at the end of the LGM with glacial expansion and cooling temperatures likely restricting the availability of temperate breeding habitat in Eurasia. However, it seems unlikely that nightjar migratory behaviour developed post-LGM; with evidence of a range-wide phylogenetic divergence in nightjar between Eastern and Western clades, likely originating in ~2.5 Mya, predating the LGM (Schweizer et al., 2020). Suitable breeding habitat would have been available across temperate Eurasia throughout warm interglacial periods, with breeding birds able to seek refuge in Mediterranean or North Africa during glacial periods (Ponti et al., 2020). If nightjars exhibited an Afro-European migration strategy pre-LGM, paleoclimatic-driven periods of dramatic N_e change should be evident from PSMC analysis, following population expansion and contraction from refugia during global warming and cooling, respectively.

Nightjar have been subject to population decline across the NW of their range (Conway et al., 2007; Langston et al., 2007). Although current population trends are not a cause for concern (IUCN: Least Concern; BirdLife International, 2022), nightjar migratory behaviour and habitat specialisation leave them sensitive to environmental change as seen in other taxa ((Case et al., 2015; Bairlein, 2016). Its ancestral demographic history may leave nightjar vulnerable to contemporary and future environmental change, if, for example, populations have been subject to bottlenecks resulting in genetic variation depletion (Bürger and Lynch, 1995; Frankham et al., 2010; Nadachowska-Brzyska et al., 2015; Hohenlohe et al., 2021). The reference genome for the European nightjar was sequenced and assembled in 2021 from a bird captured in Southern Europe during the spring migration period (Secomandi et al., 2021). Here we use this published genome alongside a novel Pacbio HIFI sequence, sequenced by us, sampled from a population from the extreme North-western range limit in the UK. We apply PSMC analysis to determine the ancestral demography of nightjar from two contemporary populations in Europe to estimate the historic N_e change over time from 10 Kya to 5 Mya. Specifically, we aimed to;

1. Investigate historic N_e trends of two contemporary nightjar populations relative to past climate fluctuations. We hypothesised that, in both populations, decreases in N_e will have followed periods of global cooling (i.e LGM (110–10 Kya)), as well as the rapid global climate oscillations of the MPR (~1.2 Mya - 450 Kya). We predict that N_e increased following periods of warming (i.e the warmer interglacial periods during the MBE (430–110 Kya));
2. Compare N_e trends from the two contemporary populations and a pseudo-diploid combined genome to examine divergence between the two populations, addressing the hypothesis that the two populations will have diverged during the LGM.
3. Examine temporal N_e patterns to investigate the evolution of migratory behaviour in nightjar. We hypothesise that nightjar will have exhibited fluctuating N_e and population divergence before the LGM, suggestive of long distance migratory behaviour arising prior to the LGM.

Methods

Sampling Genetic Material, Extraction & Sequencing

A female nightjar from a breeding population in the East of England (latitude: 53.531, longitude: -0.953) was used to extract DNA for sequencing (population henceforth referred to as NW Europe or NWE). The bird died on 7th August 2019, so was assumed to have been part of the breeding population and not moving through on migration, with spring and autumn migration for nightjar occurring between April - June and

late August and September respectively. High molecular weight DNA was extracted from a blood clot in the individual’s heart using a modified version of the phenol-chloroform protocol outlined by Sambrook et al., (1989). Full extraction protocol details can be found in the supporting information. The high molecular weight DNA was then sent to the Centre for Genomics Research facility at the University of Liverpool for PacBio HiFi sequencing library preparation. The reference genome (for assembly details see Secomandi et al., 2021) and 10x Genomics Illumina sequence reads were sequenced from a single female nightjar captured in South West Italy in spring 2021 (latitude: 40.794, longitude: 13.427) provided by Secomandi et al., 2021) (population henceforth referred to as South Europe or SE).

Genome Alignment

Minimap (minimap2 v. 2.18-r101; Li, 2018) and BWA mem (arXiv:1303.3997v1 [q-bio.GN]; Li, 2013) software were used to align the reads from the NWE (HIFI reads) and SE (10 x Illumina reads) nightjars to the reference genome, respectively. Unmapped reads were then filtered from both files leaving only mapped reads.

PSMC Analysis

To understand ancestral changes in N_e a Pairwise Sequential Markovian Coalescent (PSMC) method was applied to the mapped bam files from the HIFI and 10x Illumina reads, for which the average coverage was 30.5x and 88.1x respectively. First, consensus sequences were generated from the aligned indexed bam files from the HIFI and 10x reads using SAMtools mpileup command and vcutils.pl as per Li and Durbin, (2011) (<https://github.com/lh3/psmc>). Consensus files were generated for each chromosome independently before being combined. For the Hifi data, from the NWE genome, four chromosomes (chromosome numbers; 3, 5, 25, and 32) failed to produce consensus files and reduced representations for two of the four chromosomes (3 and 5) were used, with two chromosomes (25 and 32) excluded from the analysis. This resulted in a loss of only ~1% of genomic material for analysis. Sex chromosomes were also excluded from the analysis for both HIFI and 10x genomes. This resulted in 89.8% of the NWE genome and 90.8% of the SE genome being retained for downstream analysis. Consensus files were then filtered for read depth and quality. In order to reduce the effects of low coverage and collapsed regions, consensus files were filtered by excluding reads $\sim < 1/3$ and $> 2x$ mean depth. This resulted in removing reads $< 10x$ and $> 60x$ for the Hifi data and < 30 and $> 120x$ for the 10x reads, respectively. Finally, filtering for base quality scores of < 20 for the HIFI reads and 10x reads were applied.

The PSMC analysis was then run on the combined consensus .fastq files using the PSMC software package (Li and Durbin, 2011; <https://github.com/lh3/psmc>). PSMC parameters used by Nadachowska-Brzyska et al., (2015) for demographic analysis of 38 different bird species were chosen for our analysis, where “-N” (30) is the number of iterations, “-t” (5) is the maximum time to the most recent common ancestor, “-r” (5) is the initial mutation/ recombination rate ($r = \vartheta/\rho$) and “-p” (4+30*2+4+6+10) denotes the distribution of atomic time intervals. In order to determine variation in PSMC predictions, the data were bootstrapped 100 times.

PSMC analysis can be applied to pseudo-diploid genomes formed from the fusing of haploid genomes from two separate populations or species. When PSMC is applied, deviations in N_e trends of the pseudo-diploid genome from the two parent populations can denote reductions in gene flow and points of divergence between the two populations signified by the N_e of the pseudo-diploid genome tending towards infinity (reducing coalescence events leading to an apparent increase in N_e) (Li and Durbin, 2011; Prado-Martinez et al., 2013; Sato et al., 2020). To determine the timing of divergence between the two sampled populations a pseudo-diploid genome was created by first generating pseudo-haploid genomes through randomly sampling heterozygous alleles using Seqtk V1.3 ‘randbase’ (-r) (<https://github.com/lh3/seqtk>; accessed Oct 25th, 2022) from both consensus sequence files as generated above. Pseudo-haploid files were then merged using Seqtk ‘mergfa’ to produce a single pseudo-diploid genome consensus file. PSMC analysis was then applied to the pseudo-diploid genome as described above.

Finally, all PSMC results were plotted using gnuplot (<http://www.gnuplot.info/>) with the -R flag applied to

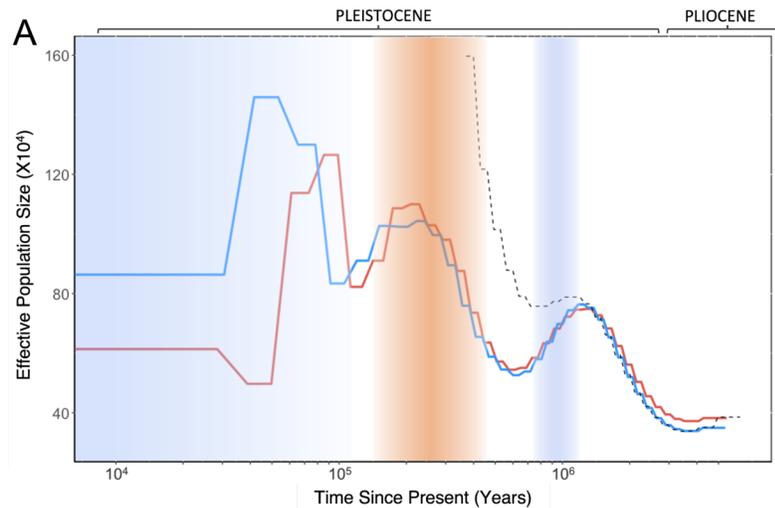
export .txt files. In order to plot the PSMC results, the data must be scaled to real-time by using mutation rate and generation time (Li and Durbin, 2011). A generation time of 2 (-g 2) was selected for nightjars following that used for Chuck-will's-widow (*Antrostomus carolinensis*) (Nadachowska-Brzyska et al., 2015), with birds able to breed in their second year (Cramp and Simmons, 1985). As no species-specific mutation rates were available for European nightjar, a mutation rate of $\mu = 4.6 \times 10^{-9}$ was used as per Sato et al., (2020). The mutation rate was initially estimated for collared flycatchers (*Ficedula albicollis*) (Smeds et al., 2016), but has since been successfully applied to other passerines (Ericson et al., 2022), raptors (Hanna et al., 2017; Sato et al., 2020) and waterfowl species (Ericson et al., 2017).

Results and Discussion

In this study we explored the demographic history of two modern nightjar populations (NW and S Europe). Using PSMC we found significant fluctuation in N_e in European nightjar over the last 5 million years, coinciding with major paleoclimatic events (Fig1a). The timing of initial divergence between the two nightjar populations was ~ 1.2 Mya (Fig1A), with final divergence found to coincide with the LGM (~ 110 Kya) (Fig1B).

Demographic History of European Nightjar

Our analysis suggests that nightjar have experienced significant fluctuations in N_e over the last ~ 5 million years. Two of the most significant N_e changes occurred during the Pleistocene, with both populations (NWE & SE) increasing throughout the early Pleistocene to a maximum N_e of $\sim 780,000$ individuals, before decreasing to $\sim 570,000$ individuals by 600 Kya during the MPR (~ 1 Mya - 450 Kya; Fig 1a). As hypothesised, the N_e of both populations then increased throughout the MBE to ~ 1 million individuals by ~ 240 Kya (Fig 1a). Both populations then decreased in N_e until ~ 100 Kya (Fig 1a). At the onset of the LGP, populations exhibited a peak in N_e , followed by a steep decline as the LGP progressed (Fig 1a). The N_e of the two populations then diverged in size (see below).



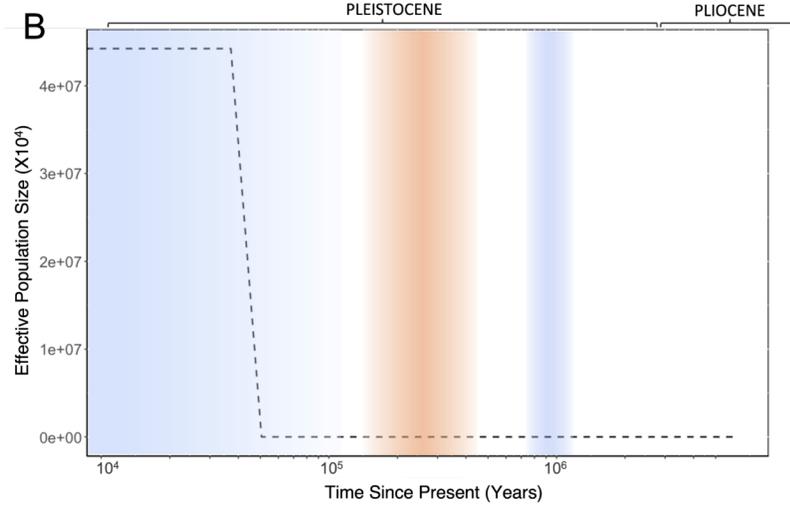


Fig 1 PSMC plots: A) NW Europe (Red line) and S Europe (Blue line) sampled European nightjar, as well as pseudo-diploid genome of NW/S Europe birds (dashed line), depicting demographic history (N_e change) over the last ~ 5 million years (bp), scaled with a mutation rate of 4.6×10^{-9} per site and generation time of 2 years. The x-axis depicts time (in years) on a log scale, with the y-axis showing effective population size. B) Estimated N_e for pseudo-diploid genome only (dashed line). Approximate timings of significant periods of global climate change are shown by shading along the x-axis. Light blue shading = last glacial period (LGP), orange shading = Mid-Brunhes event (MBE), and dark blue shading = Mid-Pleistocene Revolution (MPR).

Overall, historic nightjar N_e in Europe decreased and increased during periods of cooling and warming respectively (Fig 1a). Nightjar are insectivorous habitat specialists requiring clear fell, heathland, or woodland edge to breed (Cleere, 1998), feeding primarily on Lepidoptera (Mitchell et al., 2022). With reductions in temperature and glacial expansion, prey and habitat availability will have been constrained to more southerly latitudes (Schmitt, 2007), likely corresponding with a reduction in nightjar distribution and thus N_e . For example, the decrease in nightjar $N_e \sim 1.2$ Mya ~ 600 Kya (Fig1a) overlapped the MPR (~ 1 Mya - 450 Kya), which was characterised by shortened interglacial periods and cooler average temperatures which restricted the northward resurgence of temperate animal and plant communities (Pisias and Moore, 1981; Head and Gibbard, 2015). Conversely, warmer temperatures will have likely increased the availability of suitable habitat across northerly latitudes (Schmitt, 2007; Candy et al., 2010). Indeed the stable climate of the late Pliocene and early Pleistocene (Head and Gibbard, 2015), as well as the short glacial and warm interglacial periods of the MBE (Candy et al., 2010; Barth et al., 2018) associated with increases in nightjar N_e in our study (Fig 1a). Similarly, the dramatic N_e increase during the late Pleistocene prior to the LGP (Fig 1a) coincided with the Eemian warm phase (~ 127 Kya; Bergoing, 2017), which was characterised by the expansion and persistence of temperate plant communities into northerly latitudes (Van Andel and Tzedakis, 1996; Sanchez Goni et al., 1999; Lisiecki and Raymo, 2005). Following similar trends exhibited by other Afro-Palaearctic migrants (i.e. *Ficedula* flycatchers; Nadachowska-Brzyska et al., 2016), N_e of both nightjar populations greatly decreased as the LGP continued, likely restricting nightjar to Southern European refugia (Schmitt, 2007; Lombardo et al., 2022) or North Africa (Thorup et al., 2021). Bootstrapping indicates caution is required regarding exact timings of N_e fluctuations (Supporting Information). However, PSMC analysis in other Caprimulgids (i.e.: Chuck-will's-widow) and Afro-Palaearctic migrants (i.e.: Common cuckoo, *Cuculus canorus*) (Nadachowska-Brzyska et al., 2015), have shown similar fluctuating trends in N_e over the same timeframe, suggesting that the estimated timings of N_e change with paleoclimatic events in our study are reasonable.

Population Structure and Divergence in Nightjar

When applied to a pseudo-diploid genome derived from two different populations, PSMC analysis can be used to determine the timing of population divergence. This is signalled by the pseudo-diploid N_e diverging from the two parent populations and tending towards infinity (Prado-Martinez et al., 2013). This occurs because coalescence events between the two populations were severely reduced or ceased, leading to an increase in N_e as interpreted by the analysis. In our analysis, the pseudo-diploid N_e trend appeared to diverge from the NWE and SE populations ~ 1.2 Mya (Fig 1a). However, true divergence (the point at which N_e tends to infinity) does not occur until ~ 40 Kya (Fig 1b). Even taking into account the ~ 35 Ky error window suggested by the bootstrapping (Supporting Information), the main divergence event between the NWE and SE populations occurred within the LGP (Fig 1b).

The two modern populations used in this study are spatially distant and behaviorally distinct as they exhibit different migration strategies (Evens et al., 2017). Nightjars breeding in Western Europe typically migrate through Iberia during spring migration, with Eastern breeders migrating through Italy and SE Europe (Evens et al., 2017; Norevik et al., 2017). In other trans-Saharan migrants, such migratory behaviour is thought to be ancestral and ‘hard wired’ into populations, likely predating the Pleistocene (Thorup et al., 2021). Although the SE bird in our study was trapped during the spring migration period (Secomandi et al., 2021), it is probable that the individual’s breeding population was located within Central to Eastern Europe as suggested by recent tracking studies (e.g., Norevik et al., 2017).

As in other migratory Palearctic birds (e.g., Lesser whitethroat *Sylvia curruca* ; Olsson et al., 2013; Pied wagtail *Motacilla alba* ; Li et al., 2016), we suggest that nightjar may exhibit East-West genetic structuring, but investigation is required. Results from MtDNA analysis have suggested that nightjar can be divided into Eastern and Western lineages, with divergence being deeper (c. 2.9 Mya) (Schweizer et al., 2020) than that suggested by our PSMC analysis (initial divergence ~ 1.2 Mya, cessation of gene flow ~ 40 Kya \pm 35 Ky; Fig 1). However, the samples contributing to Schweizer et al.’s (2020) work spanned a much broader latitudinal range (encompassing W Europe to Asia) than those in our study and likely represented a deeper divergence. Much of the structure in contemporary Palearctic and Nearctic animal populations are fundamentally linked to past glacial and interglacial cycles, which have led to the contraction of temperate breeding populations into Southern refugia, and subsequent northward recolonisation during warmer interglacial periods (Hewitt, 2004; Nadachowska-Brzyska et al., 2016; Yao et al., 2022; de Greef et al., 2022). Previously panmictic populations may become isolated from one another in different refugia, during periods of glaciation, leading to genetic differentiation post-interglacial expansion (Hewitt et al., 2001).

Considering the timings of gene flow reduction and divergence between the two populations it is likely that the ancestral states of both populations utilised different refugia over historic glacial periods and most recently the LGM. Owing to the apparent divide in contemporary migration routes we suggest that the NWE population will have utilised the Iberia refugium and the SE sampled population the Italian refugia during glacial periods. Whilst most Western Palearctic avifauna (117 out of 131 studied by Parau and Wink, 2021) show admixture among populations, our study suggests that nightjar exhibit genetic structure within their Eurasian breeding range. With the sampling information available our results tentatively reflect East-West structuring in the European nightjar population, likely diverging during isolation in different glacial refugia during periods of glaciation. However, it should be noted that the SE individual may belong to the Southern European *C.e.meridionalis* subspecies and instead our results might reflect divergence between *C.e.meridionalis* and the nominate *C.e.europaeus* (NWE individual) subspecies. Nevertheless, validity of the *C.e.meridionalis* subspecies remains questionable, with as yet no genetic evidence to support the subspecies status (Del Hoyo et al., 2014; BirdLife International, 2022). Nightjars of the nominate race *C.e.europaeus* migrate through Italy and SE Europe (Evens et al., 2017; Norevik et al., 2017). Given the individual used in this study was sampled during spring migration, it is likely that the bird was migrating to breeding grounds at a higher latitude and belongs to *C.e.europaeus*. However, further work is required to resolve population genetic structure across the species range, and there is a wider need to resolve the molecular phylogeny of European nightjar and its subspecies.

Evolution of Migratory Behaviour in Nightjar

Following the timeline proposed by our study (Fig 1), it seems unlikely that nightjar migratory behaviour developed post-LGM as suggested by Larsen et al., (2007). The dramatically fluctuating N_e prior to the LGM throughout the Pleistocene may reflect periods of significant population expansion and contraction associated with climate driven changes in temperate breeding habitat availability (Ponti et al., 2020). If nightjar had exhibited a sedentary Afrotropic distribution prior to the LGM we might expect to see less severe fluctuation in N_e relative to global climate change (Kimmitt et al., 2023; but see Speckled mousebird; *Colinus striatus* in Nadachowska-Brzyska et al., 2015). Similarly, if migratory behaviour had not developed until ~20,000 ya, we would expect divergence between populations to occur exclusively post-LGM. However, our results highlight that whilst gene flow appeared to cease between the NWE and SE populations towards the LGM (Fig 1b), population divergence occurred as early as ~1.2 Mya (Fig 1a), with subsequent episodes of mixing ensued during periods of range expansion. Our results suggest that long-distance migratory behaviour in nightjar evolved prior to the LGM and was maintained throughout the Pleistocene, likely predating the initial divergence 1.2Mya recorded in our study (Fig 1a). This is corroborated by the deeper divergence (2.9Mya) between range-wide East-West lineages recorded by Schweizer et al., (2020), which may indicate a migratory divide. Our results contribute to the growing consensus that long distance migratory behaviour in contemporary Western-Palaearctic avifauna predates the LGM (i.e. Ponti et al., 2020; Ralston et al., 2021; Thorup et al., 2021; Kimmitt et al., 2023).

Limitations of PSMC analysis

Results of PSMC analysis are influenced by the scaling applied to plots, determined by mutation rate and generation time (Li and Durbin, 2011; Mather et al., 2019). However, the overall pattern of N_e change will remain the same independent of scaling parameters (Nadachowska-Brzyska et al., 2016). Data on both of these parameters are often limited for study species (e.g. see Sato et al., 2020; Chattopadhyay et al., 2019; Ericson et al., 2022), including nightjar. Thanks to the wealth of PSMC studies over a multitude of avian taxa (e.g., Nadachowska-Brzyska et al., 2015, 2016; Kozma et al., 2018; Sato et al., 2020; Bruniche-Olsen et al., 2021) parameters suited to a wide range of avifauna can be selected, such as those used in our study as per Sato et al., (2020). Therefore, whilst caution must be applied concerning the timings and magnitudes of N_e change, as highlighted by bootstrapping (see Supporting Information), we believe that PSMC analysis provides a valuable method to associate broad N_e trends concurrent climate cycles.

Conclusion

PSMC is a useful tool to characterise past demography and resolve timing of species and population differentiation, processes which underlie contemporary genetic and demographic patterns. Results from our PSMC analysis suggest that nightjar were highly susceptible to climatic variation, increasing in number during warm interglacials and long periods of relative climate stability. The historical context provided by our research suggests that the current climate best suits nightjar. Limitations on population size are likely primarily anthropogenic, with humans responsible for the mass deforestation and agriculturalisation of Europe from 8.2 Kya (Kaplan et al. 2009). Habitat loss, fragmentation, degradation and disturbance are reported as the primary drivers of contemporary population reduction in nightjar (Langston et al., 2007; Lowe et al., 2014; Ashpole et al., 2015). Although nightjar have been shown to persist through historic climate change, contemporary anthropogenic pressures may reduce the ability of the species to adapt to the current rapidly changing climate.

As in multiple other Palaearctic and Nearctic birds, our analysis suggests that restriction to different refugia during glacial cycles may have driven divergence within the European population of nightjar. Our analysis suggests a complete cessation of gene flow between the two populations by ~14 Kya during the LGM, although mixing under current interglacial conditions is likely. Genetic structure within the European population has significant conservation implications, potentially delimiting the current population into smaller conservation units. Our results also suggest that migratory behaviour in nightjar evolved prior to the LGM, persisting throughout the Pleistocene. However, further research is needed to understand the spatial context of this

apparent range-wide genetic structure, as well as to clarify timing of long-distance migration evolution, as well as current taxonomic assignments. We recommend a range-wide molecular analysis, including population genetics, of nightjar to better understand the extent and origins of divergence within the species. Such research would also aid ongoing taxonomic uncertainties surrounding subspeciation in nightjar (Schweizer et al., 2020). Finally, while caution is needed, here PSMC analysis has provided a useful insight into the demographic past of nightjar in Europe, which has highlighted the nightjar population genetics as a valuable future research direction.

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