1 Genomic consequences of colonisation, migration and 2 genetic drift in barn owl insular populations of the eastern 3 Mediterranean

4 **Short title:** Population genomics of insularity in barn owls

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30 Abstract

31 The study of insular populations was key in the development of evolutionary theory. The successful
 32 colonisation of an island depends on the geographic context, and specific characteristics of the
 33 organism and the island, but also on stochastic processes. As a result, apparently identical islands
 34 may harbour populations with contrasting histories. Here, we use whole genome sequences of 65
 35 barn owls to investigate the patterns of inbreeding and genetic diversity of insular populations in the
 36 eastern Mediterranean Sea. We focus on Crete and Cyprus, islands with similar size, climate and
 37 distance to mainland, that provide natural replicates for a comparative analysis of the impacts of
 38 microevolutionary processes on isolated populations. We show that barn owl populations from each
 39 island have a separate origin, Crete being genetically more similar to other Greek islands and
 40 mainland Greece, and Cyprus more similar to the Levant. Further, our data show that their
 41 respective demographic histories following colonisation were also distinct. On the one hand, Crete
 42 harbours a small population and maintains very low levels of gene flow with neighbouring
 43 populations. This has resulted in low genetic diversity, strong genetic drift, increased relatedness in
 44 the population and remote inbreeding. Cyprus, on the other hand, appears to maintain enough gene
 45 flow with the mainland to avoid such an outcome. Our work provides a comparative population
 46 genomic analysis of the effects of neutral processes on a classical island-mainland model system. It
 47 provides empirical evidence for the role of stochastic processes in determining the fate of diverging
 48 isolated populations.

49

50 Keywords

51 Demographic inference; Inbreeding; Population genomics; *Tyto alba*; Whole genome sequencing

52

53 Introduction

54 Given their discrete borders, geographical isolation and abundance, islands are ideal systems to
 55 study patterns of genetic diversity in natural populations (Losos & Ricklefs, 2009). Due to the
 56 combination of biotic, abiotic, and stochastic forces, no two insular populations share the same
 57 demographic history (MacArthur & Wilson, 1967). Their fate is shaped by the timing of colonisation,
 58 fluctuations in population size and connectivity to neighbouring populations. These are directly
 59 impacted by the characteristics of the island, like carrying capacity and distance to the mainland, as
 60 well as the circumstances of colonisation such as bottlenecks and founder effects. The combined
 61 actions of reduced gene flow, *in situ* genetic drift, selection and potentially mutation influence the
 62 degree to which insular populations diverge (Barton, 1996; Grant, 1998; Mayr, 1954). Small
 63 populations are particularly sensitive to the effect of genetic drift, accelerating divergence from the
 64 surrounding populations. While high levels of gene flow can counter this effect, the lack of it can
 65 facilitate local adaptation by maintaining locally advantageous alleles (Tigano & Friesen, 2016) but
 66 can also lead to inbreeding with detrimental consequences (Frankham, 1998).

67 In small isolated populations, without other sources for genetic diversity besides mutation and
 68 recombination, the relatedness among insular individuals increases over time under the effect of
 69 drift. As a result, levels of remote inbreeding may rise even with the avoidance of mating between
 70 close relatives. Although this is a common occurrence in island populations, mating between related
 71 individuals can lead to inbreeding depression (Keller & Waller, 2002) and, in extreme circumstances,
 72 local extinction (Frankham, 1997, 1998). As such, the study of the genetic makeup of insular
 73 populations can provide key information from a conservation perspective. Despite being widely used
 74 to estimate inbreeding and infer demographic histories, traditional genetic markers lack resolution
 75 to reconstruct particularly convoluted systems such as, for example, multiple islands or among
 76 modestly differentiated populations. Technological advances now provide more affordable high-
 77 representation genomic data such as the sequencing of whole genomes. Combined with increasingly

sophisticated methods, it allows for more accurate inferences, even for non-model species (Ellegren, 2014).

The eastern Mediterranean offers an excellent setting to study insular demographic history. A biodiversity hotspot (Médail & Quézel, 1999), the area is riddled with islands, the largest of which are Crete (CT) and Cyprus (CY). While fluctuating sea levels intermittently connected smaller islands to the mainland in the Quaternary, CT and CY have been isolated since the end of the Messinian salinity crisis (approx. 5 Mya.; Bache et al., 2012). They share many common features such as distance to mainland (95 and 75 km, respectively), surface area (8500 and 9200 km²) and a Mediterranean-subtropical climate with mild winters and warm summers. Their strategic position makes them pivotal stop-overs in the seasonal migration of many bird species, and movements of bird populations are widely studied (e.g. Emin et al., 2018; Panter et al., 2020). However, thus far they have been the subject of only few genetic studies, most on each island individually rather than comparatively, and typically focusing on human commensal small mammal species (Bonhomme et al., 2011; Cucchi, Vigne, Auffray, Croft, & Peltenburg, 2002; Dubey et al., 2007).

The Afro-European barn owl (*Tyto alba*) is a non-migratory bird of prey present across the African and European continents, as well as most of the surrounding islands and archipelagos (Uva, Päckert, Cibois, Fumagalli, & Roulin, 2018). In spite of being quite widespread and maintaining high gene flow overland (Antoniazza, Burri, Fumagalli, Goudet, & Roulin, 2010; Machado, Clément, Uva, Goudet, & Roulin, 2018), populations separated by water barriers appear to accumulate differentiation more quickly, with numerous insular subspecies (Burri et al., 2016; Machado et al., 2021; Uva et al., 2018). In the eastern Mediterranean, the continental European barn owl lineage meets the eastern subspecies *T. a. erlangeri* (W. L. Sclater, 1921) from the Levant (Burri et al., 2016; Cumer et al., 2021). Although Crete and Cyprus populations supposedly belong to *T. a. erlangeri* (Clements et al., 2019), the low resolution genetic data previously available was insufficient to clarify the history of each island and how they relate to the mainland. Barn owls from Crete appeared to be quite distinct

from all surrounding mainland, including the Levant (Burri et al., 2016), and the demographic history of the Cyprus owl population has never been studied.

Here, we investigate the genetic structure and past demographic history of insular and mainland barn owl populations in the eastern Mediterranean. We focus in particular on Crete and Cyprus, the two largest islands in the region, that have very similar intrinsic characteristics and are thought to harbour barn owls from the eastern subspecies found in the Levant (*T. a. erlangeri*). As such, the populations should have originated, independently or not, from the Levant. However, being closer to other Greek islands and the Greek mainland, Crete could have actually been colonised from there, which would be incompatible with it belonging to the same subspecies as Cyprus. Taking advantage of the whole genome sequences of 65 individuals and the recent publication of a high-quality reference genome (Machado et al., 2021), we address this by modelling the colonisation of both islands from the mainland. Lastly, we compare how their different demographic histories impacted their current genetic diversity and inbreeding levels.

Materials and Methods

Sampling, Sequencing and Genotyping

A total of 67 barn owl individuals from seven populations were used in this study (Table 1; Supporting Table 1): 10 in Italy (IT), 5 in islands of the Ionian Sea (IO), 10 in Greece (GR), 11 in islands of the Aegean Sea (AE), 11 in Crete (CT), 10 in Cyprus (CY) and 10 in Israel (IS). Of these, 47 were sequenced in Cumer et al. (2021; GenBank BioProject PRJNA727977; Sup. Table 1). One additional individual of the Eastern barn-owl species (*T. javanica* from Singapore; Uva et al., 2018) was used as an outgroup for specific analyses. The outgroup was sequenced in Machado et al., (2021; GenBank BioProject PRJNA700797). The remaining 20 samples followed the same protocol described in (Cumer et al., 2021; Machado et al., 2021). In brief, we extracted genomic DNA using the DNeasy

127 Blood & Tissue kit (Qiagen, Hilden, Germany) and prepared individually tagged 100bp “TruSeq DNA
128 PCR-free” libraries (Illumina) following the manufacturer’s instructions. Then, whole-genome
129 resequencing was performed on multiplexed libraries with Illumina HiSeq 2500 high-throughput
130 paired-end sequencing technologies at the Lausanne Genomic Technologies Facility (GTF, University
131 of Lausanne, Switzerland) with an expected sequence coverage of at least 15X.

132 The bioinformatics pipeline used to obtain analysis-ready SNPs from the raw sequenced of the 65
133 individuals plus the outgroup was the same as in (Machado et al., 2021) adapted from the Genome
134 Analysis Toolkit (GATK) Best Practices (Van der Auwera et al. 2013) to a non-model organism
135 following the developers’ recommendations. Briefly, we trimmed the reads to 70bp length with
136 Trimomatic v.0.36 (Bolger et al. 2014) and aligned them with BWA-MEM v.0.7.15 (Li and Durbin
137 2009) to the barn owl reference genome (GenBank accession JAEUGV000000000; Machado et al.,
138 2021). Then, we performed base quality score recalibration (BQSR) following the iterative approach
139 recommended for non-model species that lack a set of “true variants” in GATK v.4.1.3 using high-
140 confidence calls obtained from two independent callers: GATK’s HaplotypeCaller and GenotypeGVCF
141 v.4.1.3 and ANGSD v.0.921 (Korneliussen et al. 2014). Following BQSR, we called variants with
142 GATK’s HaplotypeCaller and GenotypeGVCFs v.4.1.3 from the recalibrated bam files.

143 For variant filtering we followed GATK hard filtering suggestions for non-model organisms, with
144 values adapted to our dataset and expected coverage using GATK v4.1.3.0 and VCFtools v0.1.15
145 (Danecek et al., 2011). A detailed documentation of the filters applied can be found in Sup. Table 2.
146 We also removed scaffolds that belong to the Z chromosome due to it being hemizygous in females
147 (Sup. Table 1). In preliminary analyses we corrected the origin of a sample, an injured owl found at
148 sea and reported to a port in mainland Greece but that was genetically of Cretan origin and
149 considered as such hereafter. We also removed one Italian (IT10) and one Israeli (IS10) individuals as
150 relatedness analyses revealed they were each part of a sibling pair. The final dataset contained
151 5’493’583 biallelic SNPs with a mean coverage of 16.4X (4.38 SD) across 65 individuals (Sup. Table 1).

152

153 **Mitochondrial DNA**

154 *Sequencing and assembly of mitochondrial genome*

155 We produced a complete mitochondrial reference genome for the barn owl, from the same
 156 individual used for the reference nuclear genome recently published (Machado et al., 2021). The
 157 mitochondrial genome was thus produced from the high molecular weight (HMW) DNA extraction
 158 described in detail in (Machado et al., 2021). Briefly, HMW DNA was extracted from a fresh blood
 159 sample using the agarose plug method as described in (M. Zhang et al., 2012). Then, 15-20 kb DNA
 160 fragments were obtained with Megaruptor (Diagenode, Denville, NJ, USA) and checked on a
 161 Fragment Analyzer (Advanced Analytical Technologies, Ames, IA, USA). 5 µg of the sheared DNA was
 162 used to prepare a SMRTbell library with the PacBio SMRTbell Express Template Prep Kit 2.0 (Pacific
 163 Biosciences, Menlo Park, CA, USA) according to the manufacturer's recommendations. The resulting
 164 library was size-selected on a BluePippin system (Sage Science, Inc. Beverly, MA, USA) for molecules
 165 larger than 13 kb. It was then sequenced on 1 SMRT cell 8M with v2.0/v2.0 chemistry on a PacBio
 166 Sequel II instrument (Pacific Biosciences, Menlo Park, CA, USA) at 30 hours movie length to produce
 167 HIFI reads.

168 After sequencing, we searched the circular consensus sequences (ccs) HIFI reads for sequences
 169 matching the 18128 bp mitochondrial genome of the previous assembly (NCBI Reference Sequence:
 170 NW_022670451.1; Ducrest et al., 2020) using minimap2 (Li, 2018) with the option -x asm5. We
 171 obtained twelve reads, which were reverse complemented as needed in order to be in the same
 172 orientation as our seed mitochondrial genome. No read was long enough to obtain a closed circular
 173 mitochondrial genome. Thus, we selected a ccs read of particularly high quality as an anchor and
 174 used two other overlapping reads to complete the circular sequence. From these three high quality
 175 reads, we manually assembled a full-length mitochondrial genome of 22461 bp. Mitochondrial ccs

are provided in supplementary material and the reference sequence has been deposited at GenBank under the accession MZ318036.

We annotated the mitochondrial genome using MitoAnnotator v3.52 (Iwasaki et al., 2013) and removed the hyper-variable D-loop for the subsequent analyses, yielding a 15'571bp sequence.

Mitochondrial population structure and genetic diversity

To obtain the mitochondrial sequences of each individual, we mapped their trimmed whole-genome resequencing reads onto the newly assembled barn owl mitochondrial genome using the BWA-MEM v.0.7.15 algorithm (Li & Durbin, 2009). We then called variants using the bcftools v1.8 (Danecek et al., 2011) mpileup (with mapping quality > 60, depth < 5000) and call (consensus calling, -c) for haploid data (ploidy=1). We then created a consensus fasta sequence with bcftools consensus, applying variants called above on the reference genome. We aligned individual fasta sequences using ClustalOmega v1.2.4 (Sievers et al., 2011) and manually checked the alignment for errors in MEGA X v10.1.7 (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). We generated a mitochondrial haplotype network using the R package pegas v0.14 (Paradis, 2010) and grouped similar haplotypes into haplogroups (Sup. Fig. 1). Finally, we quantified population diversity (nucleotide diversity, π) and divergence (Φ_{ST}) with Arlequin v3.5.2.2 (Excoffier & Lischer, 2010).

Population structure, diversity and inbreeding

To elucidate population structure in our dataset, we performed a principal component analysis (PCA) using the R-package SNPRelate v3.11 (Zheng et al., 2012) and inferred individual admixture proportions with the software sNMF v1.2 (Frichot, Mathieu, Trouillon, Bouchard, & François, 2014). sNMF was run for values of K ranging from 2 to 9, with 10 replicates for each K. Runs were checked visually for convergence within each K. For both analyses, we used a dataset of 603'496 biallelic

200 SNPs obtained by pruning our SNP dataset for linkage disequilibrium (LD) using PLINK v1.9 (--indep-
 201 pairwise 50 10 0.1; (Chang et al., 2015) as recommended by the authors. To investigate whether an
 202 island population was the product of admixture between two sampled populations, we used the f_3
 203 statistic (Patterson et al., 2012) and TreeMix (Pickrell & Pritchard, 2012) both calculated with the
 204 TreeMix v1.13 software. TreeMix was run in 20 replicates, using a bootstrap per 500 SNP interval,
 205 with 0 to 3 migration events, using the same LD-pruned dataset as above, to which any sites with
 206 missing data were removed yielding a total of 598'599 SNPs.

207 We used SNPRelate to calculate an allele sharing matrix between individuals (β ; (Weir & Goudet,
 208 2017) individual inbreeding coefficients relative to the total and then averaged per population (F_{IT}).
 209 We used the R package hierfstat v.0.5-9 (Goudet, 2005) to estimate population pairwise and
 210 population-specific F_{ST} as in (Weir & Goudet, 2017). Confidence intervals were obtained by
 211 bootstrapping 100 times 100 blocks of contiguous SNPs. We also used hierfstat to quantify individual
 212 inbreeding coefficients relative to their population of origin and then averaged per population (F_{IS}).
 213 For population genetic diversity, we calculated the observed individual observed heterozygosity and
 214 estimated the number of private alleles (i.e. alleles present in only one population) using custom
 215 made R scripts. To account for sample size differences in the estimation of private alleles, we
 216 subsampled 5 individuals (without replacement) from each population 100 times and calculated the
 217 mean number of private alleles in a population. When calculating the lineage-specific private alleles
 218 for $K=5$ from sNMF, we merged the populations of Greece, Ionian and Aegean islands and followed
 219 the same approach, this time sampling 9 individuals instead of 5 (corresponding to the new lowest
 220 sample size).

221 The Estimated Effective Migration Surface (EEMS) v.0.9 software (Petkova, Novembre, & Stephens,
 222 2016) was used to visualize relative gene flow over the sampled region. First, we used the tool
 223 bed2diff to compute the matrix of genetic dissimilarities for the LD-pruned dataset mentioned above
 224 and utilized the Google Maps API v.3 tool (<http://www.birdtheme.org/useful/v3tool.html>) to draw a

polygon outlining the study area. Then, EEMS was run with 700 demes in 3 independent chains of 2
 million MCMC iterations with a 1 million iterations burn-in. We tested convergence of the results
 through a plot of observed-fitted values and the trace plot of the MCMC chain as suggested by the
 authors and plotted the results using the accompanying R package (rEEMSPLOTS v.0.0.1).

 We inferred runs of homozygosity (ROH) in the dataset by using the plink command --homozyg with
 default parameters (minimum 1 Mb length and 50 SNP). Only autosomal scaffolds of length more
 than 1 Mb were considered in ROH inference (47/70 scaffolds) covering 92% of the total assembly
 length. Given that bird chromosomes are typically shorter than those of humans (G. Zhang et al.,
 2014), for whom such methods were developed, we also called ROH with a minimum of 100Kb
 length. As the qualitative results were unchanged (data not shown), we kept the standard 1Mb
 threshold in a conservative approach to identify only identity by descent (IBD) segments and to
 facilitate potential comparisons with other studies. To estimate the index F_{ROH} we divided the sum of
 lengths of ROH in an individual with the length of the scaffolds (McQuillan et al., 2008) used after
 subtracting the number of 'N's (gaps) in the assembly. To visualize the distribution of ROH lengths
 per population, we divided ROH into five length classes: i) from 1Mb to under 2Mb, ii) from 2Mb to
 under 4Mb, iii) from 4Mb to under 6Mb, iv) from 6Mb to under 8Mb and finally, v) 8Mb or longer.
 We then calculated the number of base pairs falling within each ROH length class for every individual
 and averaged the values for each population.

 To compare the levels of inbreeding, we tested whether F_{IT} , F_{ROH} and β differ significantly between
 populations using a non-parametric Kruskal-Wallis rank sum test since the normality assumption did
 not hold. Further, we performed a pairwise Wilcoxon rank sum exact test with a Bonferroni
 correction for multiple testing to assess significance in the differences between pairs of populations.
 Given the small sample sizes (Table 1), we excluded obvious hybrid individuals (AE01, CT06) to avoid
 biasing the average of their respective populations.

250 **Demographic history**

251 *Demographic scenarios and parameters*

252 To infer the origin and connectivity of the major insular barn owl populations (CT and CY), we used
 253 the software fastsimcoal2 (Excoffier, Dupanloup, Huerta-Sánchez, Sousa, & Foll, 2013). It uses
 254 coalescence simulations to estimate the composite likelihood of simulated demographic models
 255 under the observed site frequency spectrum (SFS). To model both island systems together, we would
 256 need to simulate the coalescence of the European and Levant lineages (sNMF K=2, Sup. Fig. 2) for
 257 which we have no time calibrating event and could be hundreds of thousands of generations in the
 258 past. Such inference would likely be unreliable as well as extremely consuming computationally.
 259 Thus, we inferred the demographic history of each island system separately, including their closest
 260 populations. For each island system, ‘Crete’ and ‘Cyprus’, we tested three demographic scenarios
 261 (Figure 2b).

262 To infer the history of ‘Crete’, we did not include IS in the simulated scenarios as population
 263 structure analyses show that CT’s origin is not in the Levant, but rather from the European lineage
 264 (Figure 1). As such, we only considered the populations of AE and GR. The first two scenarios assume
 265 that both the Aegean islands and the island of Crete were colonized independently from the Greek
 266 mainland population. In the first one, the colonization of Crete takes place after the colonization of
 267 the Aegean islands, while in the second scenario Crete is colonized first. The third demographic
 268 scenario assumes the islands are colonized in a stepping-stone fashion, with owls from mainland
 269 Greece reaching the Aegean islands first and from there colonizing Crete (Figure 2b). Due to the low
 270 sea levels at Last Glacial Maximum (LGM), the Aegean islands were part of a larger emerged land
 271 mass that allowed nearly continuous overland connectivity to the mainland (Simaiakis et al., 2017).
 272 As such, for every demographic scenario in ‘Crete’ we assumed that the colonization of the Aegean
 273 islands from Greece occurred at the LGM (rounded to 18’000 years BP, 6’000 generations with a 3-

year generation time). While the exact date is an approximation, allowing for migration between all populations after they split should reduce potential biases.

For ‘Cyprus’, in addition to IS as a representative of the Levant origin, a ghost population was incorporated in an attempt to represent the unsampled Turkish coast north of Cyprus, where the distance from the island to the mainland is the shortest. Including this ghost population in the model served two purposes. First, to account for unsampled sources of migrants into CY. Second, to avoid inflating artificially the effective population size of the CY population to justify the non-negligible admixture signal from AE (Figure 1a) that the simulator might interpret as *in situ* mutations. In the first two scenarios, both the Ghost and Cyprus populations originate from Israel, with the difference being the order in which they are colonized (same topology as Figure 2b). For the third scenario, owls from Israel would give origin to the Ghost population first and from there reach Cyprus.

Data preparation

Population sizes were reduced to the number of the smallest population in each model, resulting in 10 individuals per population for ‘Crete’ and 9 for ‘Cyprus’ (Sup. Table 1). To calculate the observed SFS for both systems, we filtered the data to a homogenous set of neutral markers. Specifically, we only kept sites with no missing data and with a depth of coverage less than 2/3 standard deviation from the mean. We also excluded CpG mutations (Pouyet, Aeschbacher, Thiéry, & Excoffier, 2018) and SNPs in genic regions. We inferred the ancestral state of the SNPs using the barn owl from Singapore, an outgroup to all our populations (Uva et al., 2018). Where the outgroup was homozygous for an allele, we marked that allele as the ancestral under rules of parsimony, while any other sites were removed. Population pairwise SFS were produced from the filtered datasets, giving 479’244 and 477’987 SNPs for ‘Crete’ and ‘Cyprus’, respectively.

298 *Demographic inference with fastsimcoal2*

299 For each system and each scenario, we specified a range of parameters from which the software
300 drew an initial number as input in the optimization cycle (Sup. Table 4, 6). We modelled population
301 splits with an instantaneous bottleneck in which the founding population size is a fraction of the
302 present size.

303 For each scenario and each island, we performed 100 software runs. For each run we set the
304 number of coalescent simulations to 500'000 and estimated the parameters through 50 expectation-
305 maximization (EM) cycles. As we do not currently have a good estimation of the barn owl mutation
306 rate, the end of the glaciation (rounded to 6000 generations ago) was fixed and all other parameters
307 were scaled relative to it using the -O option (based solely on polymorphic sites).

308 The best-fitting scenario was determined using Akaike's information criterion (AIC; Akaike 1974). For
309 the best scenario of each system, we performed non-parametric bootstrapping to estimate the 95%
310 confidence intervals of the inferred parameters. Specifically, we divided the SNP dataset in 100
311 blocks with an equal number of SNPs, from which we created 100 bootstrapped-SFS and performed
312 50 independent runs of the software for each, with 250'000 simulations. Due to computational
313 constraints we reduced the number of EM cycles to 10, an approach used previously and
314 characterized as conservative (Malaspinas et al., 2016). The highest likelihood run for each
315 bootstrapped replicate was used to calculate the 95% CI of the inferred parameters.

316

317 **Ancient population size inference**

318 For inference of past effective population sizes, we used the Pairwise Sequential Markovian
319 Coalescent (PSMC; Li and Durbin 2011). Specifically, we intended to estimate sizes in the distant past
320 as this method is inaccurate for recent events. We ran the software on every individual of every
321 population and calculated the median size for a population for each time interval. PSMC was

executed with the same parameters as in (Nadachowska-Brzyska, Li, Smeds, Zhang, & Ellegren, 2015) (-N30 -t5 -r5 -p 4+30*2+4+6+10). For plotting we used a mutation rate of 8.28×10^{-9} mutations per site per generation as estimated for avian species by (Smeds, Qvarnström, & Ellegren, 2016) and a generation time of 3.6 years (Altwegg, Roulin, Kestenholz, & Jenni, 2006).

Results

Population structure and divergence in the eastern Mediterranean

Mitochondrial DNA exhibited an overall Φ_{ST} of 0.13 (AMOVA) across all sampled individuals and a range of nucleotide diversity (0.0013 – 0.0023; Table 1). The mitochondrial DNA analyses failed to show consistent population structure in the dataset. The first two haplogroups constructed from the haplotype network (Sup. Fig. 1) were present in all populations, while haplogroup 3 which was missing from Israel despite being predominant in nearby Cyprus, and haplogroup 4 which was found only on the mainland populations (Figure 1a). Cretan owls had the lowest haplogroup diversity with mostly haplogroup 3 present and the lowest nucleotide diversity (0.0013).

Principal component analysis based on whole nuclear genome SNP separated the populations approximately from West to East along the first axis with individuals for each population clustering together (Figure 1b), similar to K=2 in sNMF (Sup. Fig. 2). The second axis separated the two islands (CT & CY) from the rest of the populations, with admixed individuals dispersing between sources of admixture. Admixture analyses with sNMF were consistent between runs up to K=5 (Sup. Fig. 2, Figure 1a). For K=3, Crete separates from the European lineage and for K=4 CY separates from the Levant lineage (Sup. Fig. 2). For K=5 (Figure 1a), Italy, Crete, Cyprus, and Israel formed separate clusters while owls from the Ionian islands, mainland Greece and the Aegean were grouped into a single population. Owls from the Aegean islands showed the highest proportion of admixture (mean=0.2, SD=0.1) with components from Crete, Cyprus, and Israel in addition to their majority

Greek component (Figure 1a). Some individuals from Crete and Cyprus appeared admixed between their respective island's and the Greek component (blue in Figure 1a).

Tests for population admixture with f_3 yielded a single slightly but significantly negative value ($f_3 = -0.00065$, $SE = 6e-05$, $Z = -10.375$), which showed the Greek population to be the product of admixture between the Aegean and the Ionian populations. None of the insular populations appeared to be the product of admixture between any population sampled in this study. The topology created by TreeMix was rooted at IS, with CY splitting first. CT displayed the longest branch of genetic drift and split before AE and the rest of the European populations (Figure 2a). The first migration event was from AE to GR (Sup. Fig. 3), and it was the only one consistent across runs.

Pairwise nuclear F_{ST} values ranged from 0.014 to 0.088 (Figure 1c), with the highest found between Cyprus and Ionian (0.088), followed by between Crete and Israel (0.087). Crete exhibited overall the highest pairwise values with any population (all above 0.056). Matching population divergence, the quantitative depiction of gene flow through EEMS identified a strong barrier to migration around the island of Crete and regions of reduced migration around the southern Ionian islands and the island of Cyprus (Sup. Fig 5).

Genetic diversity and inbreeding

Genetic diversity based on nuclear SNP was generally highest in Israel and lowest in Crete, with Cyprus bearing comparable levels to any mainland population (Table 1). This was consistent for nuclear heterozygosity, population specific F_{ST} and gene diversity as well number of polymorphic sites in mtDNA. Private alleles were lowest among the closely related populations of Greece, the Ionian and Aegean Islands with Israel boasting the highest number. When considering GR, IO and AE as a genetic cluster (Figure 1a), Crete actually had the lowest number of private alleles (Table 1).

F_{IS} , the average inbreeding coefficients of individuals relative to their population, was slightly negative in all populations (Table 1), as expected with random mating in a species with separate sexes (Balloux, 2004). A Cretan individual had a local inbreeding coefficient below -0.1, likely due to it being a F1 hybrid between CT and AE (see individual bars in Figure 1a and PC2 in Figure 1b) and two samples in the Aegean islands had a local inbreeding coefficient larger than 0.1 (Table 1; Figure 3b). F_{IT} values, the average inbreeding of individuals relative to the total set, but averaged per population, were highest on the island of Crete followed by the Aegean and Cyprus. Israel had significantly lower F_{IT} than all other populations, whereas Crete's was higher than all but the Aegean (Figure 3a; Table 1; $\chi^2 = 36.043$, $p < 0.001$). Thus, Cyprus had higher F_{IT} than Israel, smaller than Crete and similar to every other population. Individual relatedness (β) was highest between two Ionian individuals found to be half-sibs (Sup. Fig. 4). Otherwise, individuals from Crete were more related to each other than any other pair of individuals in the dataset (Table 1; $\chi^2 = 195.77$, $p < 0.001$). In its turn, individuals from Cyprus were only more related to each other on average than the populations of Israel and the Aegean.

Mean population F_{ROH} (i.e. proportion of the genome in runs of homozygosity) were also highest in Crete, followed by the Ionian, Aegean and Cyprus (Table 1). Individuals from Crete showed the highest proportion of ROH of all sizes (Figure 3c; Sup. Fig. 6), while individuals from Israel had the lowest proportion in all categories. Individuals from Cyprus and the Aegean were also enriched in ROH segments compared to their mainland origin in most length classes, but much less so than Crete (Figure 3c). Indeed, while F_{ROH} was significantly higher in Crete than in Greece, it was not the case between the Aegean and Greece nor between Cyprus and Israel ($\chi^2 = 11.862$, $p < 0.001$).

Demographic history

We simulated three different demographic scenarios for each island system, two where the island was colonized from the mainland either before or after the other population in the model (AE for

“Crete” and Ghost for “Cyprus”) and one where the populations are colonized in a stepping-stone manner (Figure 2b). The best demographic scenario inferred with fastsimcoal2 for the island of Crete was the stepping-stone model (Figure 2c; Sup. Table 3). Here, the Cretan population originates very recently from the Aegean islands 321 generations BP (68-1400 95% CI), itself colonized from mainland Greece at the fixed time of 6000 generations BP (Sup. Table 5). Estimated migration rates were higher towards the island from both GR and AE (6.7 and 3.7, respectively) and lower in the other direction (0.7 and 1.7). Inferred effective population sizes were highest for the Greek (1465 haploids; 509-7880 95% CI) mainland and lowest for Crete (373 haploids; 107-944 95% CI). Past instantaneous bottlenecks at colonisation were pronounced both for the Aegean and Cretan populations (48 [13-2922 95% CI] and 74 [6-243 95% CI] haploids, respectively).

For the island of Cyprus, the best-fitting scenario consisted of colonization from Israel after the colonization of the ‘Ghost’ population coinciding with a hypothesized mainland population residing on the southern coast of Turkey (similar topology as Figure 2b; Sup. Table 3). Colonization time for CY was much more recent than the last glaciation (986 generations, less than 3’000 years BP; Sup. Table 7). However, the Ghost population was estimated to have an unrealistic large effective population size (65’310 diploids), and CY an extremely small one (61). The migration rates inferred indicate a complete replacement of CY each generation by the Ghost, suggesting this model is far from being an accurate representation of reality. As such we interpret its results with caution.

PSMC identified a pronounced bottleneck for all populations (around 20’000 years BP) but failed to show a clean split for the two Islands, particularly Crete, and any mainland population (Sup. Fig. 7).

Discussion

Although insular populations have greatly contributed to the development of evolutionary theory (Grant, 1998; MacArthur & Wilson, 1967; Warren et al., 2015), the study potential of many of these

remains untapped. The colonisation and settlement of an island by a given organism depend not only on the geographic context and specific island characteristics but also on stochastic events. As such, seemingly identical islands may yield populations with contrasting fates. Here, we investigate the demographic history and current patterns of inbreeding and genetic diversity of insular barn owls in the eastern Mediterranean Sea. In particular, we are interested in owls from Crete and Cyprus which, alongside the Levant region, were thought to form a subspecies *Tyto alba erlangeri*. These two similar islands in terms of size, climate and distance to mainland provide natural replicates for a comparative analysis of the colonisation and ensuing demographic processes. Using whole genome sequences, we show how each island and archipelago have unique histories and exhibit different degrees of isolation and the effect this has on the genomes of individuals. Specifically, Crete and Cyprus were colonized from distinct mainland locations, each from a different ancestral lineage, inconsistent with them belonging to the same subspecies. The population in Crete originated from the European lineage, more precisely from the Aegean islands, while the population in Cyprus came from the Levant in the east. Additionally, Crete underwent stronger genetic drift and inbreeding than Cyprus, resulting in a smaller and less diverse population.

Insular populations in the eastern Mediterranean

In the broader context of the Western Palearctic, our study targets two islands in the region where the European and eastern lineages of barn owls meet (Cumer et al., 2021). This is clearly shown in the genomic PCA, where the mainland populations of Italy and Greece in southern Europe were opposed to that of Israel in the Levant, with insular populations placed along this west-to-east genetic gradient roughly according to their geographic position (Figure 1b, Sup Fig K=2). The main islands of Crete and Cyprus are the most genetically distinct populations (Figure 1a,c), consistent with previous results for Crete (Burri et al., 2016). Conversely, the Greek archipelagos – Ionian and Aegean – were genetically very similar to the Greek mainland population (Figure 1a,c) suggesting

they remain highly connected genetically. Such patterns of genetic differentiation reflect the geographical isolation of CT and CY, in contrast to the Aegean and Ionian archipelagos that are closer to the mainland through a network of adjacent islands and islets.

Overall, our results confirm that water bodies are strong barriers to barn owl movement (Cumer et al., 2021; Machado et al., 2021). For example, distant populations in the mainland, such as GR and IT, were much more similar to each other than any insular population, regardless of how distant each of them are (Figure 1). Nonetheless, all insular populations showed small signals of admixture with their neighbouring populations (Figure 1a). This likely reflects the intricate geographic setting, as well as the overall low differentiation within this species (overall F_{ST} in our dataset 0.03, and 0.047 in the whole Western Palearctic (Cumer et al., 2021), that mtDNA data lacked the resolution to detect (Figure 1a, Table 1, Sup. Fig. 1; Burri et al., 2016). Insular populations had generally lower levels of population private diversity, while displaying similar levels of heterozygosity (Table 1), and higher within-population relatedness compared to the mainland (Sup. Fig. 4), reflecting their isolation. However, despite all populations appearing to mate randomly within localities (F_{IS} slightly negative as is expected from a dioecious species (Balloux, 2004); Figure 3b, Table 1), the inbreeding levels of insular barn owls relative to the whole set of populations were quite large (F_{IT} and F_{ROH} ; Figure 3a,b, Table 1).

Crete and Cyprus

Despite the inherent physical similarities between the islands of Crete and Cyprus, their barn owl populations differ in many aspects. These natural replicates of island-mainland comparisons, with similar climatic conditions, supposedly harbour the subspecies *T. a. erlangeri* from the Levant (W. L. Sclater, 1921). However, while Cyprus' genetically closest mainland population is indeed Israel, Crete is actually most similar genetically to Greece (Figure 1b). This demonstrates that Crete is not home to barn owls of the eastern subspecies, but rather from the European mainland lineage (*T. a. alba*).

Not only they have separate geographic origins, but we also show that their *in situ* demographic histories are quite distinct.

Since its colonisation and founding bottleneck, Crete maintained a low population size with little gene flow with neighbouring populations (Figure 2c; Sup. Fig. 5), generating background relatedness among individuals (Sup. Fig. 4). The low gene flow it maintains with the surrounding populations (Sup. Fig. 5) may be due to the very strong winds that surround the island (Zecchetto & De Biasio, 2007) acting as a barrier by hindering flight. Thus, despite random mating within the island (low F_{IS}), remote inbreeding increased (high F_{IT} and F_{ROH}) due to high relatedness (high β), making CT the most inbred population by far in our dataset, as well as the least diverse (Table 1; Figure 3a,b). Accordingly, it carried the highest proportion of ROH compared to any other populations (Table 1). Notably, CT was enriched in ROH of all sizes (Figure 3c), suggesting a small effective size over a long time period until today (Ceballos, Joshi, Clark, Ramsay, & Wilson, 2018). This strong isolation coupled with small population size resulted in a very distinct genetic composition through the effect of genetic drift (Figure 2a) as well as high individual relatedness and inbreeding.

In contrast, Cyprus appears to have maintained enough gene flow with the mainland preventing it from accumulating remote inbreeding, while allowing for differentiation. Winds in this region are weaker than around Crete (Zecchetto & De Biasio, 2007), potentially facilitating the contact between Cyprus and Israel in the Levant, the most diverse population in our study. This could explain the surprisingly similar patterns of genetic diversity in CY to that of mainland populations (Table 1), which suggest a higher effective population size in spite of the inference from fastsimcoal2 (Sup. Table 7). Furthermore, CY had considerably less runs of homozygosity (ROH) than CT, carrying only a slight enrichment in short length classes, similar to the Aegean and Ionian islands (Figure 3c). Given the high inter-individual variability in relatedness and inbreeding coefficients (Table 1; Fig. 3a; Sup. Fig. 4), it appears that the gene flow with Israel and/or an unknown, unsampled population prevents the rise of population-wide inbreeding as observed in Crete. Interestingly, the most common

mitochondrial haplogroup in CY was found in European populations but absent in IS (haplogroup 3, Figure 1a). Although it could simply have been unsampled in the Levant, it may also be evidence of some gene flow between the European and eastern lineages as seen in the two admixed individuals of CY (Figure 1a; see also paragraph after next)

Overall, the different levels of connectivity (i.e. levels of gene flow) of each island appear to be the main driver of their diverging histories. However, insular specificities may also contribute to this effect. The carrying capacity of CT and CY for barn owls could be different due to cryptic differences in nesting or roosting site availability, for example, in spite of their similar surface area. In addition, the mountainous landscape in CT could restrict dispersal movements as well as reduce the suitable surface for breeding. Finally, intrinsic characteristics of the colonisation of both islands may also have contributed to their diverging histories.

On the one hand, CY was colonised directly from the highly diverse and large mainland population of IS (Sup. Table 7). As such, both the settlers of the island and subsequent immigrants were likely unrelated and diverse, preventing the insular population from increasing steeply in relatedness. Our simulations suggest that colonisation occurred about 3000 years BP (1900 – 10000 years BP). However, this result should be interpreted cautiously as the modelling for this island system yielded unreasonable population size estimates (Sup. Table 7) likely due to our use of a ghost population to represent mainland Turkey. This is suspected to be a contact zone between the European and eastern barn owl lineages with sporadic gene flow (Cumer et al., 2021). Our observations support this hypothesis as islands on both sides of Turkey, namely CY and AE, carried some small genetic components from the other (Figure 1a). In this context, our modelled ghost population would likely be admixed or even outbred which would explain its exaggerated population size. Sampling in Turkey will be key to clarify this hypothesis and fully describe the dynamics between barn owl populations in the eastern Mediterranean.

On the other hand, demographic simulations showed that CT was colonised from the AE archipelago rather than directly from mainland Greece (Figure 2c; Sup. Table 5). This was supported by the second axis in the PCA which placed individuals in a gradient from GR to AE and then CT (Figure 1b). Remarkably, one AE owl from the south-eastern island of Rhodes had approximately 50% Cretan origin hinting at how the patchwork of islands and islets in the region could have been used as stepping stones during colonisation. Thus, CT was colonised from what is already a less diverse insular population, which in turn came from the GR mainland, itself less diverse than Israel (Table 1). This cumulative loss of diversity through recurrent bottlenecks and possible expansion could contribute to the quick increase in relatedness in the island given its small population size, despite its recent colonisation. Indeed, CT was inferred to have been colonised by barn owls around 1000 years BP (204 – 4200 years BP; Figure 2c; Sup. Table 5). Accordingly, PSMC failed to uncover any signal of older divergence (Sup. Figure 7). Nonetheless, considering the geological age of the island (5 million years BP) and that agricultural practices have been established there for millennia (Greig & Warren, 1974), this estimation appears extraordinarily recent. Absent any other source of evidence, one can only speculate as to why this population is so recent. It is possible that a massive migration led to population replacement at a time when sea levels were lower and the surrounding islands closer, masking any trace of an earlier settlement. Alternatively, earlier settlers could have been extinct due, for example, to a natural disaster such as the catastrophic Minoan volcanic eruption (3'500 years BP) (Pareschi, Favalli, & Boschi, 2006).

Conclusion

Our work provides a comparative study on two natural replicates of island colonisation by the barn owl, a bird that despite being found in many islands avoids flying over open bodies of water. The use of whole genome sequences allowed us to demonstrate that Crete and Cyprus owls come from different genetic backgrounds, as each island originates from a distinct continental genetic lineage

(Figure 1). Further, their histories diverge resulting in noticeably different populations. Cyprus was colonised directly from the most diverse mainland population, accumulated differentiation but also remained sufficiently connected with it to maintain high levels of genetic diversity and prevent inbreeding (Table 1, Figure 3). Crete was reached by island hopping in the Aegean from a less diverse mainland population. The small size and isolation of this island population facilitated the impact of genetic drift which, along with inbreeding, led to it diverging considerably from its founders despite the recent colonisation (Table 1, Figure 2). Although further analyses would be necessary to study the functional consequences of inbreeding in Crete, this study shines a light on a real-life illustration of stochasticity in the classical island-mainland model systems.

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Author contributions

APM, AT, AR, JG designed this study; APM produced whole-genome resequencing libraries; AT, APM conducted the analyses with input from TC, EL; PB; VB, MC, NK, PL, FM provided samples; ALD, MD produced the mitochondrial reference genome and NG assembled it; APM, AT led the writing of the manuscript with input from all authors.

Data availability

The raw Illumina reads for the whole-genome sequences produced here have been deposited at GenBank BioProject PRJNA727915. In addition, we used data from BioProjects PRJNA727977 and PRJNA700797.

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574 **References**

- 575 Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic*
576 *Control*, 19(6), 716–723. doi: 10.1109/TAC.1974.1100705
- 577 Altwegg, R., Roulin, A., Kestenholz, M., & Jenni, L. (2006). Demographic effects of extreme winter
578 weather in the barn owl. *Oecologia*, 149(1), 44–51. doi: 10.1007/s00442-006-0430-3
- 579 Antoniazza, S., Burri, R., Fumagalli, L., Goudet, J., & Roulin, A. (2010). Local adaptation maintains
580 clinal variation in melanin-based coloration of European barn owls (*Tyto alba*). *Evolution*, 64(7),
581 1944–1954. Retrieved from <http://www.jstor.org/stable/40793087>
- 582 Bache, F., Popescu, S. M., Rabineau, M., Gorini, C., Suc, J. P., Clauzon, G., ... Çakir, Z. (2012). A two-
583 step process for the reflooding of the Mediterranean after the Messinian Salinity Crisis. *Basin*
584 *Research*, 24(2), 125–153. doi: 10.1111/j.1365-2117.2011.00521.x
- 585 Balloux, F. (2004). Heterozygote excess in small populations and the heterozygote-excess effective
586 population size. *Evolution*, 58(9), 1891–1900. doi: 10.1111/j.0014-3820.2004.tb00477.x
- 587 Barton, N. H. (1996). Natural selection and random genetic drift as causes of evolution on islands.
588 *Philosophical Transactions of the Royal Society B: Biological Sciences*, 351, 785–795. doi:
589 10.1098/rstb.1996.0073
- 590 Bonhomme, F., Orth, A., Cucchi, T., Rajabi-Maham, H., Catalan, J., Boursot, P., ... Britton-Davidian, J.
591 (2011). Genetic differentiation of the house mouse around the Mediterranean basin:
592 Matrilineal footprints of early and late colonization. *Proceedings of the Royal Society B:*
593 *Biological Sciences*, 278(1708), 1034–1043. doi: 10.1098/rspb.2010.1228
- 594 Burri, R., Antoniazza, S., Gaigher, A., Ducrest, A.-L., Simon, C., Fumagalli, L., ... Roulin, A. (2016). The
595 genetic basis of color-related local adaptation in a ring-like colonization around the

596 Mediterranean. *Evolution*, 70(1), 140–153. doi: 10.1111/evo.12824

597 Ceballos, F. C., Joshi, P. K., Clark, D. W., Ramsay, M., & Wilson, J. F. (2018). Runs of homozygosity:
598 Windows into population history and trait architecture. *Nature Reviews Genetics*, Vol. 19, pp.
599 220–234. doi: 10.1038/nrg.2017.109

600 Chang, C. C., Chow, C. C., Tellier, L. C., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-
601 generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, 4, 7. doi:
602 10.1186/s13742-015-0047-8

603 Clements, J. F., Schulenberg, T. S., Iliff, M. J., Billerman, S. M., Fredericks, T. A., Sullivan, B. L., &
604 Wood, C. L. (2019). *The eBird/Clements Checklist of Birds of the World: v2019*. Retrieved from
605 <https://www.birds.cornell.edu/clementschecklist/download/>

606 Cucchi, T., Vigne, J. D., Auffray, J. C., Croft, P., & Peltenburg, E. (2002). Introduction involontaire de la
607 souris domestique (*Mus musculus domesticus*) à Chypre dès le Néolithique précéramique
608 ancien (fin IXe et VIIIe millénaires av. J.-C.). *Comptes Rendus - Palevol*, 1(4), 235–241. doi:
609 10.1016/S1631-0683(02)00033-7

610 Cumer, T., Machado, A. P., Dumont, G., Bontzorlos, V. A., Ceccherelli, R., Charter, M., ... Goudet, J.
611 (2021). Landscape and climatic variations of the Quaternary shaped multiple secondary
612 contacts among barn owls (*Tyto alba*) of the Western Palearctic. *BioRxiv*. doi:
613 10.1101/2021.06.09.447652

614 Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., ... Durbin, R. (2011). The
615 variant call format and VCFtools. *Bioinformatics*, 27(15), 2156–2158. doi:
616 10.1093/bioinformatics/btr330

617 Dubey, S., Cosson, J. F., Magnanou, E., Vohralík, V., Benda, P., Frynta, D., ... Vogel, P. (2007).
618 Mediterranean populations of the lesser white-toothed shrew (*Crocidura suaveolens* group):
619 An unexpected puzzle of Pleistocene survivors and prehistoric introductions. *Molecular*

620 *Ecology*, 16(16), 3438–3452. doi: 10.1111/j.1365-294X.2007.03396.x

621 Ducrest, A.-L., Neuenschwander, S., Schmid-Siegert, E., Pagni, M., Train, C., Dylus, D., ... Goudet, J.

622 (2020). New genome assembly of the barn owl (*Tyto alba alba*). *Ecology and Evolution*, 10(5),

623 2284–2298. doi: 10.1002/ece3.5991

624 Ellegren, H. (2014). Genome sequencing and population genomics in non-model organisms. *Trends in*

625 *Ecology and Evolution*, Vol. 29, pp. 51–63. doi: 10.1016/j.tree.2013.09.008

626 Emin, D., Toxopeus, A. G., Groen, I. T. A., Kontogeorgos, I., Georgopoulou, E., & Xirouchakis, S.

627 (2018). Home range and habitat selection of long-eared owls (*Asio otus*) in Mediterranean

628 agricultural landscapes (Crete, Greece). *Avian Biology Research*, 11(3), 204–218. doi:

629 10.3184/175815618X15263010605630

630 Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V. C., & Foll, M. (2013). Robust Demographic

631 Inference from Genomic and SNP Data. *PLoS Genetics*, 9(10), 1003905. doi:

632 10.1371/journal.pgen.1003905

633 Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform

634 population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10(3),

635 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

636 Frankham, R. (1997). Do island populations have less genetic variation than mainland populations?

637 *Heredity*, 78(3), 311–327. doi: 10.1038/hdy.1997.46

638 Frankham, R. (1998). Inbreeding and extinction: Island populations. *Conservation Biology*, 12(3),

639 665–675. doi: 10.1111/j.1523-1739.1998.96456.x

640 Frichot, E., Mathieu, F., Trouillon, T., Bouchard, G., & François, O. (2014). Fast and efficient

641 estimation of individual ancestry coefficients. *Genetics*, 196(4), 973–983. doi:

642 10.1534/genetics.113.160572

643 Goudet, J. (2005). HIERFSTAT, a package for R to compute and test hierarchical F -statistics.
644 *Molecular Ecology Notes*, 5, 184–186. doi: 10.1111/j.1471-8278

645 Grant, P. R. (1998). *Evolution on Islands*. Oxford, UK: Oxford University Press.

646 Greig, J. R. A., & Warren, P. M. (1974). Early Bronze Age Agriculture in Western Crete. *Antiquity*,
647 48(190), 130–132. Retrieved from [https://search.proquest.com/docview/1293747375?pq-](https://search.proquest.com/docview/1293747375?pq-origsite=gscholar&fromopenview=true&imgSeq=2)
648 [origsite=gscholar&fromopenview=true&imgSeq=2](https://search.proquest.com/docview/1293747375?pq-origsite=gscholar&fromopenview=true&imgSeq=2)

649 Iwasaki, W., Fukunaga, T., Isagozawa, R., Yamada, K., Maeda, Y., Satoh, T. P., ... Nishida, M. (2013).
650 Mitofish and mitoannotator: A mitochondrial genome database of fish with an accurate and
651 automatic annotation pipeline. *Molecular Biology and Evolution*, 30(11), 2531–2540. doi:
652 10.1093/molbev/mst141

653 Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology and*
654 *Evolution*, 17(5), 230–241. doi: 10.1016/S0169-5347(02)02489-8

655 Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary
656 genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–
657 1549. doi: 10.1093/molbev/msy096

658 Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*, 34(18), 3094–
659 3100. doi: 10.1093/bioinformatics/bty191

660 Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform.
661 *Bioinformatics*, 25(14), 1754–1760. doi: 10.1093/bioinformatics/btp324

662 Li, H., & Durbin, R. (2011). Inference of human population history from individual whole-genome
663 sequences. *Nature*, 475(7357), 493–496. doi: 10.1038/nature10231

664 Losos, J. B., & Ricklefs, R. E. (2009). Adaptation and diversification on islands. *Nature*, 457(7231),
665 830–836. doi: 10.1038/nature07893

- 666 MacArthur, R. H., & Wilson, E. O. (1967). *The theory of island biogeography*. Princeton University
667 Press.
- 668 Machado, A. P., Clément, L., Uva, V., Goudet, J., & Roulin, A. (2018). The Rocky Mountains as a
669 dispersal barrier between barn owl (*Tyto alba*) populations in North America. *Journal of*
670 *Biogeography*, (45), 1288–1300. doi: 10.1111/jbi.13219
- 671 Machado, A. P., Cumer, T., Iseli, C., Beaudouin, E., Dupasquier, M., Guex, N., ... Goudet, J. (2021).
672 Unexpected post-glacial colonisation route explains the white colour of barn owls (*Tyto alba*)
673 from the British Isles. *BioRxiv*. doi: 10.1101/2021.04.23.441058
- 674 Malaspinas, A.-S., Westaway, M. C., Muller, C., Sousa, V. C., Lao, O., Alves, I., ... Willerslev, E. (2016).
675 A genomic history of Aboriginal Australia. *Nature*, 538(7624), 207–214. doi:
676 10.1038/nature18299
- 677 Mayr, E. (1954). Change of genetic environment and evolution. In J. Huxley, A. C. Hardy, & E. B. Ford
678 (Eds.), *Evolution as a Process* (pp. 157–180). London, UK: Allen & Unwin. Retrieved from
679 [http://www.sevin.ru/fundecology/speciation/mayr\(20\).pdf](http://www.sevin.ru/fundecology/speciation/mayr(20).pdf)
- 680 McQuillan, R., Leutenegger, A. L., Abdel-Rahman, R., Franklin, C. S., Pericic, M., Barac-Lauc, L., ...
681 Wilson, J. F. (2008). Runs of Homozygosity in European Populations. *American Journal of*
682 *Human Genetics*, 83(3), 359–372. doi: 10.1016/j.ajhg.2008.08.007
- 683 Médail, F., & Quézel, P. (1999). Biodiversity Hotspots in the Mediterranean Basin: Setting Global
684 Conservation Priorities. *Conservation Biology*, 13(6), 1510–1513. doi: 10.1046/j.1523-
685 1739.1999.98467.x
- 686 Nadachowska-Brzyska, K., Li, C., Smeds, L., Zhang, G., & Ellegren, H. (2015). Temporal dynamics of
687 avian populations during pleistocene revealed by whole-genome sequences. *Current Biology*,
688 25(10), 1375–1380. doi: 10.1016/j.cub.2015.03.047
- 689 Panter, C. T., Xirouchakis, S., Danko, Matušík, H., Podzemný, P., Ovčiariková, S., & Literák, I. (2020).

690 Kites (*Milvus* spp.) wintering on Crete. *European Zoological Journal*, 87(1), 591–596. doi:
691 10.1080/24750263.2020.1821801

692 Paradis, E. (2010). Pegas: An R package for population genetics with an integrated-modular
693 approach. *Bioinformatics*, 26(3), 419–420. doi: 10.1093/bioinformatics/btp696

694 Pareschi, M. T., Favalli, M., & Boschi, E. (2006). Impact of the Minoan tsunami of Santorini: Simulated
695 scenarios in the eastern Mediterranean. *Geophysical Research Letters*, 33(18), n/a–n/a. doi:
696 10.1029/2006GL027205

697 Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., ... Reich, D. (2012). Ancient
698 admixture in human history. *Genetics*, 192(3), 1065–1093. doi: 10.1534/genetics.112.145037

699 Petkova, D., Novembre, J., & Stephens, M. (2016). Visualizing spatial population structure with
700 estimated effective migration surfaces. *Nature Genetics*, 48(1), 94–100. doi: 10.1038/ng.3464

701 Pickrell, J., & Pritchard, J. (2012). Inference of population splits and mixtures from genome-wide
702 allele frequency data. *Nature Precedings*. doi: 10.1038/npre.2012.6956.1

703 Pouyet, F., Aeschbacher, S., Thiéry, A., & Excoffier, L. (2018). Background selection and biased gene
704 conversion affect more than 95% of the human genome and bias demographic inferences.
705 *ELife*, 7. doi: 10.7554/eLife.36317

706 Sievers, F., Wilm, A., Dineen, D., Gibson, T. J., Karplus, K., Li, W., ... Higgins, D. G. (2011). Fast, scalable
707 generation of high-quality protein multiple sequence alignments using Clustal Omega.
708 *Molecular Systems Biology*, 7(1), 539. doi: 10.1038/msb.2011.75

709 Simaiakis, S. M., Rijdsdijk, K. F., Koene, E. F. M., Norder, S. J., Van Boxel, J. H., Stocchi, P., ... Tjørve, E.
710 (2017). Geographic changes in the Aegean Sea since the Last Glacial Maximum: Postulating
711 biogeographic effects of sea-level rise on islands. *Palaeogeography, Palaeoclimatology,*
712 *Palaeoecology*, 471, 108–119. doi: 10.1016/j.palaeo.2017.02.002

713 Smeds, L., Qvarnström, A., & Ellegren, H. (2016). Direct estimate of the rate of germline mutation in
714 a bird. *Genome Research*, 26(9), 1211–1218. doi: 10.1101/gr.204669.116

715 Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation with gene flow. *Molecular Ecology*,
716 25(10), 2144–2164. doi: 10.1111/mec.13606

717 Uva, V., Päckert, M., Cibois, A., Fumagalli, L., & Roulin, A. (2018). Comprehensive molecular
718 phylogeny of barn owls and relatives (Family: Tytonidae), and their six major Pleistocene
719 radiations. *Molecular Phylogenetics and Evolution*, 125, 127–137. doi:
720 10.1016/j.ympev.2018.03.013

721 Warren, B. H., Simberloff, D., Ricklefs, R. E., Aguilée, R., Condamine, F. L., Gravel, D., ... Thébaud, C.
722 (2015, February). Islands as model systems in ecology and evolution: Prospects fifty years after
723 MacArthur-Wilson (F. Courchamp, Ed.). *Ecology Letters*, Vol. 18, pp. 200–217. doi:
724 10.1111/ele.12398

725 Weir, B. S., & Goudet, J. (2017). A unified characterization of population structure and relatedness.
726 *Genetics*, 206(4), 2085–2103. doi: 10.1534/genetics.116.198424

727 Zecchetto, S., & De Biasio, F. (2007). Sea surface winds over the Mediterranean basin from satellite
728 data (2000-04): Meso- and local-scale features on annual and seasonal time scales. *Journal of*
729 *Applied Meteorology and Climatology*, 46(6), 814–827. doi: 10.1175/JAM2498.1

730 Zhang, G., Li, C., Li, Q., Li, B., Larkin, D. M., Lee, C., ... Froman, D. P. (2014). Comparative genomics
731 reveals insights into avian genome evolution and adaptation. *Science*, 346(6215), 1311–1320.
732 doi: 10.1126/science.1251385

733 Zhang, M., Zhang, Y., Scheuring, C. F., Wu, C.-C., Dong, J. J., & Zhang, H.-B. (2012). Preparation of
734 megabase-sized DNA from a variety of organisms using the nuclei method for advanced
735 genomics research. *Nature Protocols*, 7(3), 467–478. doi: 10.1038/nprot.2011.455

736 Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance

737 computing toolset for relatedness and principal component analysis of SNP data.

738 *Bioinformatics*, 28(24), 3326–3328. doi: 10.1093/bioinformatics/bts606

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TABLES & FIGURES

Table 1 – Population genetic diversity, inbreeding and divergence estimates for barn owls of the eastern Mediterranean. The standard deviations of the values are provided between brackets for each parameter except for population specific F_{ST} where values are the standard error of the mean.

Pop	Abbr.	N	# PA	# PA lin.	# mtDNA	π mtDNA	H _O	Pop F_{ST}	F_{IS}	F_{IT}	F_{ROH}	β
Italy	IT	9	118'152 (202)	188'285	68 (23)	0.0021 (0.0011)	0.164 (0.002)	0.058 (0.005)	-0.024 (0.012)	0.014 (0.009)	0.028 (0.01)	0.112 (0.007)
Ionian Islands	IO	5	46'340		87	0.0021 (0.0013)	0.16 (0.004)	0.091 (0.004)	-0.039 (0.027)	0.019 (0.02)	0.067 (0.02)	0.143 (0.041)
Greece	GR	10	73'108 (220)	239'089 (294)	86 (19)	0.0021 (0.0012)	0.165 (0.005)	0.047 (0.003)	-0.02 (0.029)	0 (0.022)	0.038 (0.02)	0.101 (0.008)
Aegean Islands	AE	11	79'357 (198)		81 (18)	0.0023 (0.0012)	0.164 (0.01)	0.038 (0.002)	0 (0.059)	0.013 (0.049)	0.043 (0.03)	0.092 (0.014)
Crete	CT	11	82'202 (177)	124'440 (129)	51 (24)	0.0013 (0.0007)	0.153 (0.006)	0.115 (0.005)	-0.018 (0.037)	0.05 (0.034)	0.086 (0.04)	0.165 (0.024)
Cyprus	CY	10	121'550 (196)	177'675 (113)	72 (22)	0.002 (0.001)	0.165 (0.008)	0.061 (0.005)	-0.032 (0.05)	0.029 (0.039)	0.04 (0.03)	0.114 (0.024)
Israel	IS	9	271'400 (235)	413'375	43 (20)	0.0013 (0.0007)	0.175 (0.003)	0.007 (0.003)	-0.035 (0.016)	0.027 (0.008)	0.019 (0.01)	0.063 (0.012)

N: number of individuals in the population; #PA: private alleles per population, bootstrapped to the smallest N of 5 individuals; #PA lin.: private alleles per lineage of K=5 identified with sNMF, bootstrapped to the smallest N of 9 individuals; #mtDNA: mitochondrial polymorphic sites per population, bootstrapped to the smallest N of 5 individuals; π mtDNA: mitochondrial nucleotide diversity; H_O: observed heterozygosity; F_{ST} : population specific F_{ST} as in (Weir and Goudet 2017) bootstrapped over 100 blocks of contiguous SNP; F_{IS} : population level inbreeding coefficient; F_{IT} : mean individual inbreeding coefficient relative to the meta-population; F_{ROH} : mean inbreeding coefficient estimated from ROH; β : mean pairwise relatedness within population.

Figure 1 – Population structure of barn owls in the Eastern Mediterranean. **(a)** Nuclear and mitochondrial population structure. Horizontal bars indicate individual admixture proportions for $K=5$ as determined by sNMF. Black dots on map indicate the approximate centroid of each population; coloured pie charts represent the mean admixture proportions per population; pie charts in shades of beige represent mitochondrial haplogroup proportions per population. **(b)** PCA based on the pruned nuclear SNP set. Values in parenthesis indicate the percentage of variance explained by each axis. **(c)** Pairwise F_{ST} between sampled barn owl populations. Heat map illustrates the given values according to the legend.

Figure 2 – Demographic history of barn owl insular populations in the Eastern Mediterranean. **(a)** Treemix analysis with zero migration events. Population abbreviations follow Figure 1. **(b)** Hypothesized demographic topologies for the colonisation of Crete. The same topologies were tested for Cyprus, with IS instead of GR, "Ghost" instead of AE and CY instead of CT. **(c)** Best supported demographic model for the colonisation of Crete as determined by fastsimcoal2. Time is indicated in generations, confidence intervals at 95% are given between brackets. Population sizes (diploid) are shown at the bottom of each population bar; arrows indicate forward-in-time number of migrants per generation.

Figure 3 – Comparison of inbreeding in insular barn owls to their closest mainland counterparts in the Eastern Mediterranean. **(a)** F_{IT} measure of inbreeding calculated from individual allele matching proportions relative to the average in the dataset (dashed line is $F_{IT}=0$). **(b)** F_{IS} measure of inbreeding calculated from individual allele matching proportions relative to the average in the subpopulation (dashed line is $F_{IS}=0$). **(c)** Per population average length of ROH segments (in Mb) in each ROH length class.