# Conservation genetics of regionally extinct peregrine falcons (Falco peregrinus) and unassisted recovery without genetic bottleneck in southern England

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**Original Research**

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**Title:**

**Conservation genetics of regionally extinct peregrine falcons (*Falco peregrinus*) and unassisted recovery without genetic bottleneck in southern England**

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**Abstract**

The peregrine falcon (*Falco peregrinus*) has been affected by persecution, pollution, trade, and habitat degradation, but it is considered a flagship conservation success story because of successful reintroductions and population recoveries across broad ranges. However, in the UK there were never formal reintroduction programmes for peregrine falcons, and it appears that UK populations – and specifically the Sussex peregrines of the English south coast – recently recovered from a population crash unassisted. To study this, we obtained samples from contemporary populations in southern England, Ireland, continental Europe, domestic-bred peregrine falcons, and from England pre-population crash. Using microsatellite and mtDNA control region data, the genetic diversity and structure, signatures of genetic bottlenecks, and potential origin of the Sussex peregrines was investigated. We found low levels of genetic diversity across all peregrine falcon groups, low but significant genetic differentiation, and a few private alleles, indicating some level of genetic structure among European peregrines. Although we could not pinpoint the origin of the Sussex peregrines, the data suggests that it is not likely to have originated from escaped domestic birds or from adjacent European populations. The results obtained here parallel other studies on peregrines elsewhere showing low genetic diversity but genetic structure. We conclude that not enough time elapsed for genetic erosion to occur due to the population bottleneck, and that at least for the Sussex peregrines there is no need for genetic conservation by wild-take and subsequent captive breeding programmes as long as current protection measures remain in place.

**Keywords** Conservation, genetic diversity, microsatellites, mtDNA control region, organochlorine pesticides, raptor

## Declarations

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**Authors' contributions:** All authors contributed to the study conception and design. Sample collection was done by MKN, JF and RV. Material preparation and analyses were done by AW, HAJ and RV. The first draft of the manuscript was written by AW and RV, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Introduction

The peregrine falcon (*Falco peregrinus*, Tunstall 1771) has a wide distribution and it can be found in all continents except Antarctica (Fig. 1) (White 1994, White et al. 2013a). Although it is considered as a Least Concern species by the International Union for the Conservation of Nature (IUCN), the peregrine falcon is a charismatic species, admired for its speed, agility, and hunting behaviour (Ratcliffe 1993), which has been severely affected by persecution, pollution, trade, and habitat degradation (BirdLife International 2019). The peregrine falcon is now considered a flagship conservation success story because of effective reintroductions, e.g. Canada, United States of America, Southern Scandinavia, Germany, and Poland, aiding its recovery from localised extinctions (Crick and Ratcliffe 1995; Ratcliffe 2003; Banks et al. 2010; Smith et al. 2015). However, although in the UK there were never formal reintroduction programmes for peregrine falcons, populations in southern England recovered from a population crash unassisted (Franklin and Everitt 2009). This brings up the question as to where do the re-established peregrines in southern England come from, and have they suffered the detrimental impacts associated with a genetic bottleneck?

During the middle of the 20$^{th}$ century, peregrine falcon populations in many regions of the northern hemisphere, including mainland UK, underwent dramatic declines (Ratcliffe 1993; Crick and Ratcliffe 1995; Horne and Fielding 2002). Prior to 1939, Sussex (Southern England) supported the densest breeding population in any part of the British Isles (Ratcliffe 1963; unpublished diaries John Walpole-Bond 1904-1954, see Franklin and Everitt 2009), with 12-14 breeding pairs in a 30 km stretch between Eastbourne and Brighton (Franklin and Everitt 2009). The wartime (1939-1945) persecution of peregrines, due to them being predators of homing pigeons (war or carrier pigeons) carrying military messages, saw many coastal UK peregrine populations decline (Ratcliffe 1963). The small number of fatalities in Sussex had no persisting effect on the local population, and the number of breeding pairs recorded immediately after the war between 1945 and 1951 were comparable with the records for the years leading up to 1940. After World War II, adult peregrine falcons were persecuted by grouse-moor gamekeepers and pigeon fanciers (Ratcliffe 1993; Humphreys et al. 2007).

Although organochlorine pesticides like dichloro-diphenyl-trichloroethane (DDT) and dichloro-diphenyl-dichloroethylene (DDE) may have contributed to peregrine falcon declines due to eggshell thinning and a reduction in breeding success, the population decline became obvious after the introduction of the more toxic cyclodienes dieldrin and aldrin causing large-scale mortality of seed-eating birds and their predators, including peregrine falcons (Ratcliffe 1984; Newton 1988; Wilson et al. 2018). The well-documented peregrine falcon population crash during the pesticide era showed a reduction of the total UK breeding population by 50% within a decade (Ratcliffe 2003). By 1958, for example, there were no occupied territories and no young produced in Sussex, and peregrine falcons were absent from the region as a breeding species for over three decades (Franklin and Everitt 2009). The decline and subsequent crash of this southern England population of peregrines paralleled changes in the rest of the country (Ratcliffe 1984, 2003; Franklin and Everitt 2009). Only the peregrine populations of the Scottish Highlands, outer UK islands (Ratcliffe 1963), and Northern Ireland remained relatively unaffected (Wells and Ruddock 2009).

Since the ban on organochlorine pesticides in the UK in the 1980s and 1990s (Meijer et al. 2001), UK surveys have recorded a steady increase of breeding populations (Ratcliffe 1984; Crick and Ratcliffe 1995; Horne and Fielding 2002; Wilson et al. 2018), making the recovery of peregrine falcons a flagship conservation success story (Crick and Ratcliffe 1995). In 2014, the breeding population estimates were 1769 pairs (Wilson et al. 2018), and by 2018, peregrine populations in England had increased by 250% of its former pre-war size (Wilson et al. 2018). National survey reports since 1972 document the progressive post-crash expansion of the peregrine populations notably in the north and west of the British Isles (Ratcliffe 1972, 1984; Crick and Ratcliffe 1995; Horne and Fielding 2002). However, the re-colonisation of Sussex by peregrine falcons lagged behind the rest of mainland UK, and it was not until 1990 when a single pair successfully bred (Franklin and Everitt 2009). The Sussex peregrine population had substantially increased from 8-12 breeding pairs recorded between 1904 and 1954 to more than 40 by 2016 (Franklin and Nicholls 2018). After an absence of more than 30 years, the remarkable and unaided recovery of the Sussex peregrine led conservation

managers to question where the founders originated from, and if the current thriving population shares its ancestry with surviving native peregrines or with peregrines from further afield.

Such a newly formed population of peregrine falcons deriving from a few migrant founders could be experiencing detrimental impacts associated with inbreeding, genetic drift, and low genetic variation (Maruyama and Fuerst 1984, 1985; Cornuet and Luikart 1996; Jacobsen et al. 2008). This could result in inbreeding depression (i.e. reduced biological fitness because of inbreeding) and deteriorated adaptive potential (Frankham 2015), including reduced egg clutch size and juvenile survival. As peregrine falcons have suffered unprecedented population bottlenecks and the occurrence of regional extirpations (Cade et al. 1988; Ratcliffe 1993, 2003; Humphreys et al. 2007), it would be expected for the re-established Sussex peregrine population to be genetically impoverished, with limited potential for long-term survival. Conversely, levels of genetic diversity of a newly established population could be increased through mechanisms including hybridisation, mutation, and gene flow (Amos and Harwood 1998).

As the origins of the current Sussex peregrine population is unknown, it is therefore reasonable to hypothesise that the founder population could have been the result of immigration from populations in relatively unaffected areas in mainland UK, from the island of Ireland, or from continental Europe (Nicholls et al. 2017). There are, however, other plausible explanations to the rapid recovery of the Sussex peregrines. Fleming et al. (2011) argued that escapes of large numbers of domestic birds into the wild derived originally from captive-bred peregrines of several subspecies, and that even interspecific falcon hybrids could have augmented the recovering UK population. Extrapolation of Fleming et al. (2011) data and information from a voluntary lost-found register for falconry birds (Barbara Royle pers. comm., The Independent Bird Register) indicate that on average around 30 peregrines and 30 peregrine hybrids (i.e. hybrids of peregrines with other *Falco* species) per year are reported as lost and unrecovered by mainland UK falconers since records began in 1980. This is likely an underestimate as there are no longer legal requirements to report escapes or recoveries of falcons as there was previously. Furthermore, Fleming et al. (2011) discussed that

peregrines and peregrine hybrids have been inadvertently released in numbers which, based on planned re-introduction programmes, are known to be sufficient for establishing wild populations (Saar 1988; Holdroyd and Banash 1990; Tordoff and Redig 2001; Jacobsen et al. 2008). Moreover, a gyrfalcon/saker falcon x peregrine (of domestic origin) produced viable hybrid offspring with a wild peregrine within the Sussex population (Everitt and Franklin 2009). This key observation demonstrated that domestic hybrids between peregrine and other falcon species can interbreed and produce offspring with wild peregrines, and that domestic hybrids lost into the wild could potentially have an impact on the genetic diversity of the post-pesticide wild populations of peregrine falcons.

In this study, we used a combination of microsatellite and mitochondrial (mtDNA) markers to examine the levels of genetic diversity and structure for the population of peregrine falcons in the English south coast before their local extinction between 1955 and 1958 (pre-pesticide extinction), and since their repopulation of the area (post-pesticide recovery), to explain the origins of the recently recovered Sussex peregrines. For this, we analysed genetic data of: 1) a historical sample of peregrine falcons from museum specimens collected from the English south coast before the population crash of the mid 1950's; 2) UK domestic-bred peregrines freely available for sale to falconers and therefore potentially vulnerable to escapes into the wild population; 3) a German group which had an assisted recovery from local extinction by the release of captive-bred birds (Saar 1988; Wink 2019); 4) wild peregrines from the Republic of Ireland which had also experienced a population depletion in the 1960s and subsequent recovery (Ratcliffe 2003), but which has had no releases of captive-bred birds; 5) a small sample of Mediterranean peregrines (*F. p. brookei*) which are a near geographical neighbour, and thought to have contributed in some part to the recovery of the German peregrine population (Wink 2019).

Our aims were: 1) to assess the levels of genetic diversity in the re-established Sussex peregrine population compared with a historical population of peregrine falcons before the population decline; 2) to test for signatures of genetic bottlenecks in the contemporary population; 3) to estimate the genetic structure among British, Irish, continental European, and

domestic-bred peregrine falcons, as well as between contemporary and historical groups; 4) to evaluate the potential phylogeographic origin of the Sussex peregrines from wild peregrines or from augmentation by domestic peregrines of mixed origin.

## Methods

### Sample collection and groupings

A total of 164 feather or toepad samples were obtained from various regions (Online Resource 1, Table S1). Contemporary feather samples of peregrine falcons (*F. p. peregrinus)* from the south of England were collected from wild birds by the Sussex Peregrine Study over the period of 2000 to 2015 from known peregrine territories and monitored nesting sites within the County of Sussex, and from London, Hampshire, Kent, and Surrey. Feather samples were obtained from Ireland (wild *F. p. peregrinus* or first generation bred from wild birds obtained in the Republic of Ireland) where there was a population decline of peregrines in the 1960's followed by a rapid, probably unassisted, re-establishment (Ratcliffe 1984; Norriss 1995; Madden et al. 2009) with a census of 425 pairs in the year 2017 (David Norriss pers. comm.). Feather samples were obtained from North-Rhine Westphalia in Germany (wild free-living peregrine falcons). In Germany, peregrines were extirpated during the 1950's and 60's with only around 40 pairs remaining in the south of the country (Saar 1988). There was an assisted reintroduction in southern Germany of just over 1000 captive bred birds comprising *F. p. peregrinus*, but also some *F. p. brookei* (Saar 1988; Wink 2019). Feathers samples were taken from randomly selected domestic birds from different breeding lines from various breeders (considered as 'peregrines' but bred from a mix of subspecies of *F. peregrinus*, including *F. p. pelegrionides* and *F. p. babylonicus*, of largely unknown provenance). The founder breeding population for domestic peregrines was around 200-250 breeding birds, and of these approximately 2/3 were native UK *F. p. peregrinus* and 1/3 imported birds of other subspecies (Online Resource 1). Other feather samples were obtained from Mediterranean peregrines (*F. p. brookei*) from Spain, Gibraltar, and Italy. The relative proximity of Mediterranean peregrine populations to southern England peregrine populations make them a potential source of

immigrants into the depleted post-pesticide UK population. Toepad samples of peregrine falcons from British museums were obtained from several counties in the south of England and the Channel Islands (Table S1). For this study, museum samples dated pre-1955 represented the pre-pesticide population crash, while toepad and feather samples with a collection date post-1955 represented the post-pesticide recovery. Samples were stored at 4 °C or at room temperature until analysis.

The sample was then divided into geographical and temporal groups for further analysis with microsatellite and mtDNA data (sample sizes per group are given in Table 1 and Table 2), namely: 'UK-post' (UK post-1955 ban on organochlorine pesticides, including museum samples dated post-1955, feathers from wild free living animals, and wild-injured *F. p. peregrinus* in captivity), 'UK-pre' (UK pre-1955 ban on organochlorine pesticides, including museum samples pre-1955 of *F. p. peregrinus*), 'Domestic' (all post-1955 feathers from birds bred in captivity in the UK, of possibly mixed subspecies of peregrines, as explained above), 'German' (all post-1955 feathers from wild free-living birds), 'Irish' (all post-1955 feathers from wild, wild-injured *F. p. peregrinus* in captivity and F1 from wild-take), and '*F. p. brookei*' (including feathers from wild-injured animals held in captivity for rehabilitation and captive bred). To explore the genetic polymorphism at the UK and at the species levels, the groups 'UK-peregrines' (including all pre- and post-1955 samples) and 'All-peregrines' (all *F. peregrinus* and subspecies from all sources) were also created.

**DNA extraction**

DNA from contemporary feathers was extracted using the GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific) or the Isolate II Genomic DNA Kit (Meridian Bioscience), and suspended in 50 µL elution buffer. DNA from historical museum toepad samples was extracted using the Isolate II Genomic DNA Kit (Meridian Bioscience), but suspending tissues in 400 µL lysis buffer with 25 µL proteinase k and incubated overnight at 55 °C. DNA was then washed through a spin column and eluted into 50 µL elution buffer. DNA extractions from historical museum toepad samples were carried out in a separate laboratory

dedicated to ancient DNA work, and in a UV-irradiated fume hood to ensure no contamination. Negative controls were included to ensure no contamination.

**Microsatellite genotyping**

We used a suite of ten polymorphic microsatellite markers developed for peregrine falcons (NVHfp5, NVHfp13, NVHfp31, NVHfp46-1, NVHfp54, NVHfp79-4, NVHfp82-2, NVHfp86-2, NVHfp89, NVHfp92-1; Nesje et al. 2000). The PCR procedure and amplification volumes followed Raisin et al. (2009) for all samples (feather and toepad), set at a total volume of 2 µL. Each PCR contained 2 µL of DNA that was air-dried, 1 µL of primer mix (with a fluorescently labelled forward primer) at 0.2 µM and 1 µL QIAGEN Multiplex PCR Master Mix (QIAGEN). PCR cycling conditions were 95 °C for 10 min followed by 35 cycles of 95 °C for 30 s, 55 °C for 90 s and 72 °C for 90 s, with a final incubation at 72 °C for 10 min. PCR products were separated using an Applied Biosystems 3730 DNA analyser (Applied Biosystems) using ROX 500 as a size standard. Alleles were scored on GeneMapper version 3.7 (Applied Biosystems).

Approximately 25% of contemporary samples were amplified twice at all 10 loci to determine genotyping error (Hoffman and Amos 2005; Pompanon et al. 2005), and 37.5% of museum samples were genotyped twice to confirm accurate scoring of alleles, and to allow identification of potential errors due to allelic dropout of larger alleles. No scoring differences between the two PCRs were observed. Owing to degradation and yield associated with historical DNA (Wandeler 2007), two microsatellite markers, NVHfp5 and NHVfp92-1, failed to amplify across all museum samples. Genotype error (mismatch alleles) was found for microsatellite marker NHVfp82-2 across all DNA samples and was subsequently removed from analysis. The resulting dataset comprised seven microsatellite markers and 149 individuals (available on Dryad https://doi.org/10.5061/dryad.mgqnk98wj).

**Microsatellite analysis**

Any potential genotyping errors due to microsatellite stuttering, allelic dropout and null alleles in the total sample were examined using Micro-Checker version 2.2.3 (Van Oosterhout et al. 2004). Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between loci were calculated using Genepop version 4.6 (Raymond and Rousset 1995; Rousset 2008).

To assess the levels of genetic diversity of peregrine falcons, the allelic richness (i.e. number of alleles, $N_a$), effective number of alleles ($N_e$), observed heterozygosity ($H_O$), expected heterozygosity ($H_e$; Nei's gene diversity), inbreeding coefficient ($F_{IS}$), and private alleles ($N_p$) per group were estimated in GenAlEx version 6.5 (Peakall and Smouse 2012). A Multivariate Analysis of Variance (MANOVA) on the per-locus genetic diversity values ($N_a$, $N_e$, $H_O$, and $H_e$) was done in SPSS version 24 (SPSS Statistics) to determine whether there were temporal differences between UK-post and UK-pre groups. The effective number of breeders ($N_{eb}$), or inbreeding effective number, and the jackknife confidence intervals were estimated for each group using the co-ancestry method of Nomura (2008), as implemented in NeEstimator version 2.01 (Do et al. 2014). The presence of genetic bottlenecks was tested by estimating heterozygosity excess based on the two-phase model (TPM; 70% proportion of stepwise mutation model and variance = 30) as implemented in Bottleneck version 1.2 (Piry et al. 1999). TPM is considered appropriate for detecting significant heterozygosity excess while using microsatellite markers (Di Rienzo et al. 1994; Cornuet and Luikart 1996; Piry et al. 1999). In populations undergoing a bottleneck, the allelic richness is reduced faster than heterozygosity (Nei's gene diversity) thus becoming larger than the expected heterozygosity at mutation-drift equilibrium (Cornuet and Luikart 1996), and the difference was tested with a Wilcoxon's test assuming that all loci fit the TPM under mutation-drift equilibrium.

Nei's unbiased pairwise genetic distances (Nei 1978) among peregrine groups were estimated using GenAlEx, which for neutral markers under an infinite-allele-model it is predicted to increase linearly with time. The extent of population subdivision was assessed by calculating Wright's pairwise differentiation values among populations ($F_{ST}$), and by a hierarchical Analysis of Molecular Variance (AMOVA) at three hierarchical levels (among

groups, among individuals within groups, and within individuals) using GenAlEx. Significance levels were obtained using 1000 permutations.

Genetic distances among individuals were estimated (Smouse and Peakall 1999), and the distance matrix was converted to a covariance matrix to perform a Principal Coordinate Analysis (PCoA) in GenAlEx. The detection of first-generation migrants was done with GeneClass2 (Piry et al. 2004) selecting likelihood ratio $L_{home}/L_{max}$, the Bayesian method by Rannala and Mountain (1997), 1000 Markov Chain Monte Carlo simulations (Paetkau et al. 2004), and a probability threshold alpha = 0.05. The assignment of individuals to groups (using the leave-one-out procedure) based on their relative likelihood scores and a quality index (computed as the mean value of the scores of each individual in the population it belongs to) were also estimated with GeneClass2. A Bayesian approach in Structure version 2.3.4 (Pritchard et al. 2000) was used to detect the most likely number of genetic clusters in the peregrine falcon sample. This was done with 10 replications, number of clusters K = 1–10 with 100,000 burn-in, 1,000,000 MCMC iterations after burn-in, and selecting the admixture model (using sampling locations as prior information) with correlated allele frequencies (Falush et al. 2003). The most likely number of K clusters was examined in StructureSelector (Li and Liu 2018) using log likelihood scores [mean LnP(K)] and $\Delta K$ values (Evanno et al. 2005). As these methods often underestimate clusters due to uneven sample sizes (Janes et al. 2017), we obtained estimates of K based on Puechmaille's method by subsampling the independent clusters previously identified, a technique which accounts for uneven sample size across groups (Puechmaille 2016). Likelihood scores and clusters were obtained using the CLUMPAK (Kopelman et al. 2015) function in StructureSelector, and individual probability plots were generated using Structure Plot (Ramasamy et al. 2014).

**Mitochondrial DNA sequencing**

PCR was performed to amplify approximately 650 bp of the domain I of the mtDNA control region of contemporary peregrine falcon feathers using primers L15206 and H15856 (Talbot et al. 2011). PCR amplifications were carried out in 50 µL final volume, consisting of 5 µL 10X

Maxima Hot Start Taq buffer, 5 µL dNTP Mix (2 mM each), 1 µM 10µM forward and reverse primers, 1.2 µL 25 mM $MgCl_2$, 0.3 µL 5U/µL Maxima Hot Start Taq DNA Polymerase (ThermoFisher Scientific), 1 µL DNA sample and 27.5 µL molecular grade water. PCR conditions were 95° C for 5 min followed by 35 cycles of 95° C for 60 s, 47° C for 60 s and 72° C for 90 s, and a final elongation step at 72° C for 10 min. PCR products were visualised under UV light after electrophoresis in 1% Agarose gels containing SYBR Safe DNA gel stain (Invitrogen). Successful amplifications were purified using the GeneJET PCR Purification Kit (ThermoFisher Scientific) and sent for Sanger sequencing to DBS Genomics (Durham University).

PCR amplification for museum toepad samples was carried out in a separate irradiation UV isolation hood dedicated to ancient DNA work to prevent contamination. Due to the fragmented nature of museum DNA, internal primers were specifically designed to amplify short overlapping fragments (175-198 bp) of the mtDNA control region of peregrine falcons (Table S2). PCR amplifications were carried out in 10 µL final volume, consisting of 5 µL MyTaq HS DNA Polymerase (Meridian Bioscience), 0.2 µL of each forward and reverse primer, 1 µL DNA and 3.6 µL $dH_2O$. Negative controls were included in each PCR replacing DNA with ultrapure $ddH_2O$. PCR cycling parameters were an initial hot start of 95 °C for 1 min, followed by 35 cycles of 95 °C for 15 s, 52 °C for 15 s and 72 °C for 10 s, followed by a final incubation period of 72 °C for 10 min. PCR products and negative controls were examined by agarose gel electrophoresis to ascertain amplification and to confirm no traces of contamination. PCR products were purified and sequenced using a 3730xl Analyser (Macrogen).

From the feather and toepad samples, 102 DNA sequences were newly obtained here, from which 100 were *F. peregrinus* and two were *F. rusticolus*, and all new sequences were deposited in GenBank (accession numbers: MT247108-MT247209). In addition, 83 DNA sequences of peregrine falcons (including sequences identified to the species level as *F. peregrinus*, and to the subspecies level *F. p. peregrinus* or other subspecies of peregrines) were retrieved from GenBank (accession numbers: AF090338, DQ165502-DQ165549,

JN400833-JN400846, JQ282801, JX029991, JX878245-JX878269, and NC000878), plus eight gyrfalcons *F. rusticolus* (accession numbers: EF517337-EF517344) used as an outgroup.

**Mitochondrial DNA analysis**

All control region sequences were aligned using ClustalW multiple alignment in BioEdit version 7.2.6 (Hall 1999). Considering alignment gaps, 628 bp were analysed in a total of 193 DNA sequences. DNA sequences were imported into DnaSP version 5.10.1 (Librado and Rozas 2009) and were separated into the main groups indicated above (i.e. UK-post, UK-pre, German, Domestic, Irish and *F. p. brookei*), and into 'GenBank-peregrines' (identified from online source as the subspecies *F. p. peregrinus*), and 'GenBank-others' (identified from online source as the species *F. peregrinus* and/or several other subspecies of peregrines but not *F. p. peregrinus* or *F. p. brookei*), and '*F. rusticolus*' (i.e. gyrfalcons, the outgroup to peregrine falcons; identified from online source, and feathers from captive bred animals).

DNA polymorphism indices were obtained in DnaSP to allow comparisons among the groups and published data, including total number of segregating sites (S), number of polymorphic sites (Pol), number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity ($\pi$), and average number of nucleotide differences (k). To explore the historical demography of the falcons, pairwise sequence polymorphisms were estimated to generate mismatch distributions, and the population size changes per group were evaluated using Ramos-Onsins and Rozas' $R_2$ (Ramos-Onsins and Rozas 2002) and Fu's Fs (Fu 1997) values calculated with DnaSP. Significance was tested with the coalescent process for a neutral infinite-sites model and assuming a large constant population size (1000 replications).

Pairwise genetic differentiation ($F_{ST}$) values were obtained using DnaSP to analyse the genetic structure of the groups. An AMOVA (among and within groups) was performed using Arlequin version 3.5 (Excoffier and Lischer 2010), with significance levels obtained using 1000 permutations. The phylogenetic relationship among the samples was assessed with a phylogenetic network using all the haplotypes, including those from museum samples,

generated with the software Network version 5.0.0.3 (Fluxus Technology). The Median Joining algorithm with the Greedy FHP criterion was selected.

## Results

### Microsatellite data analysis

There was no evidence of large allele dropout. Only one locus showed potential scoring errors due to stuttering but revising the genotype data showed no allele scoring errors. Four out of seven loci could have null alleles, but this was probably due to homozygosity excess for most allele size classes. Overall, estimated frequencies of null alleles for the full dataset ranged between 0.010%–0.15%, which was considered acceptable due to the origin of the feather and tissue samples. There were no deviations from HWE in the groups due to heterozygote excess or in a global test across all loci across all groups; however, deviations from HWE due to heterozygote deficiency were detected in four groups (UK-post, Domestic, German, and Irish, $P < 0.05$). There was no evidence of LD for each locus pair across all groups.

Overall, genetic diversity values were lower in the UK-post and UK-pre groups compared with other peregrine falcon groups, and the UK-post group had the lowest heterozygosity value and number of effective alleles ($N_e$) among all groups (Table 1). Comparing the two temporal groups, UK-post had higher mean number of alleles ($N_a$) and heterozygosity ($H_e$) than UK-pre, but lower observed heterozygosity ($H_O$) and number of private alleles ($N_p$), the Domestic group had the highest $N_p$ followed by the Irish group, and the *F. p. brookei* group had the highest level of heterozygosity followed by the Domestic and Irish groups (Table 1; Online Resource 1, Fig. S1). However, the differences in genetic diversity values were not statistically significant (MANOVA: Wilk's $\lambda$ $F = 0.933$, $P = 0.487$; Box's test of equality of covariances: $F = 1.834$, $P = 0.052$; Levene's tests of equality of variances: $P > 0.05$ for all variables). The Domestic group had the highest value of inbreeding ($F_{IS}$), while the *F. p. brookei* and UK-pre groups had the lowest levels of inbreeding (Table 1). The UK-post and German groups had the lowest effective number of breeders ($N_{eb}$), while the Domestic group had the highest $N_{eb}$ (Table 1). There was no evidence of genetic bottleneck

425 in peregrine groups under the TPM with all P-values > 0.05, except for *F. p. brookei* (P =
426 0.008). In all cases caution should be taken because of low sample size and few loci used.
427 There were high pairwise genetic distances between the UK-post and UK-pre groups
428 with any of the other groups, while the lowest genetic distance among all pairwise comparisons
429 was between the German and *F. p. brookei* groups, and between the UK-post and UK-pre
430 groups (Table S3). All pairwise comparisons (except between the German and *F. p. brookei*
431 groups) showed significant and moderate levels of genetic differentiation ($F_{ST}$) (Table S3); the
432 highest $F_{ST}$ values were observed in pairwise comparisons between the UK-post and UK-pre
433 groups with any other groups (Table S3). Based on the AMOVA, 13% of the total variation
434 was found among groups, while 30 and 57% was found among and within individuals,
435 respectively, and significant global F-statistics ($F_{ST}$ = 0.130, $F_{IS}$ = 0.348 and $F_{IT}$ = 0.433, all P
436 < 0.05).
437 A total of 37.9% accounted for the first three dimensions of the PCoA (PCo1 = 19.28%,
438 PCo2 = 10.02% and PCo3 = 8.6%). The analysis indicated two main clusters weakly
439 separated, one mainly including the UK-post and UK-pre individuals (except for individual UK-
440 post PF5) and two *F. p. brookei* individuals (BR5 and BR6), and another one mainly including
441 all other samples (Fig. 2). Eight individuals were detected as first-generation migrants with
442 likelihood probabilities below 0.05, including four from UK-post assigned to UK-pre, one from
443 UK-post (UK-post PF5) assigned to the German group, one from the Domestic group and one
444 from *F. p. brookei* group assigned to the Irish group, and one from *F. p. brookei* assigned to
445 the German group. Based on the genotypes, 71.14% of the individuals (106 out of 149
446 individuals) were correctly assigned to the group of origin; the German group had the highest
447 percentage of correct assignment of individuals (88.9%), followed by UK-post (76.9%), Irish
448 (72.4%), *F. p. brookei* (66.7%), Domestic (65.5%) and UK-pre (57.1%). Structure analysis
449 using sub-sampling with Puechmaille's method resulted in the most likely number of clusters
450 at K = 4 (Fig. 3). Assuming K = 4, the UK-pre and UK-post individuals assigned predominantly
451 to cluster 1, whilst Domestic individuals assigned to cluster 2, German and *F. p. brookei*
452 individuals predominantly assigned to cluster 3, and Irish individuals assigned to cluster 4 (Fig.

3). However, the log likelihood simulations indicated K = 2 clusters under Evanno's method, while mean LnP(K) indicated K = 3 clusters. Assuming K = 2 and K = 3, the UK-pre and UK-post peregrines also clustered together, demonstrating the genotypic similarities of the two temporal groups (Fig. 3). Likelihood results for all K values from the different Structure methods are shown in Online Resource 1 (Fig. S2). With Structure, one UK peregrine (UK-post PF5) appeared with a partial genotypic assignment to the German group; however, when K = 4, the highest estimated membership coefficient (q-value) for UK-post PF5 individual was for the cluster containing all other UK-post peregrines.

**Mitochondrial DNA data analysis**

A 628 bp fragment of the control region was obtained for all feather samples from contemporary birds. However, probably because of DNA degradation and amplification failure of 5' and/or 3' regions, it was not possible to obtain the full mtDNA control region fragment from some museum (toepad) samples (pre- and post-1955). This restricted the amount of nucleotide sites available for pairwise sequence comparisons when using museum samples in UK-pre and UK-post groups.

A total of 183 mtDNA sequences of peregrine falcons were obtained for analysis from all sources (including GenBank, museum and feather samples), but only 10 haplotypes were found (Table 2). Haplotype diversity varied considerably among peregrine groups, with the lowest values found in the UK-pre and UK-post groups, and the highest values found in the Irish group, followed by the Domestic, GenBank-peregrines, and German groups (Table 2). Nucleotide polymorphism was also generally low across all peregrine groups, with the lowest values observed in German and UK-post groups (Table 2).

No signatures of a recent population expansion were detected in any peregrine falcon group with $R_2$ or Fs (Table 2); however, *F. rusticolus* showed significant departures from neutrality using $R_2$. Based on Fs values, the groups GenBank-others, UK-peregrines, All-peregrines, and *F. rusticolus* showed significant departures from neutrality. Overall, there were very few pairwise differences among mtDNA sequences of peregrine falcons, and all

mismatch distributions were right skewed (Online Resource 1, Fig. S3); the UK-pre and UK-post groups showed high frequencies of 0 pairwise differences, and other peregrine falcon groups showed high frequencies of only 1-2 pairwise differences.

Pairwise genetic differentiation (Table S4) among all peregrine falcon groups (i.e. excluding *F. rusticolus*) showed a low average $F_{ST}$ = 0.064 (or 6.4% differentiation in the mtDNA control region), with values ranging from 0 to 20.4%, indicating low genetic differentiation. There was no differentiation (0%) between UK-post vs. Domestic, Irish vs. GenBank-others, and German vs. *F. p. brookei* groups, very low differentiation between UK-post vs. Irish groups (0.8%), and low differentiation between UK-post vs. UK-pre groups (5%). Higher differentiation values were obtained in pairwise comparisons with GenBank, GenBank-others, *F. p. brookei*, and *F. rusticolus* groups, as expected from their taxonomic diversity or evolutionary divergence. Based on the AMOVA, 43.86% and 56.14% of the total variation was found among and within groups, respectively, and there was a significant global $F_{ST}$ = 0.439 ($P < 0.05$).

The phylogenetic haplotype network (Fig. 4) reflected the genetic diversity and differentiation results shown above. Including museum samples increased the number of sites with gaps/missing data which cannot be utilised in phylogenetic haplotype network reconstructions; therefore, the network analysis was based on 238 nucleotide sites. There were 13 haplotypes in the total sample, all peregrine falcons belonged to 10 haplotypes, while all *F. rusticolus* individuals (the outgroup) belonged to three other haplotypes. Most peregrine falcon individuals (150) belonged to haplotype 1, which had a central position relative to all other peregrine haplotypes, and it was formed by a mix of individuals from various peregrine groups, including 10 UK-pre, 17 UK-post, 21 German, 20 Irish, 10 Domestic, 45 GenBank-peregrines, 22 GenBank-others, and five *F. p. brookei*. Other peregrine individuals (15) belonged to haplotype 2, which included samples from all peregrine falcon groups except UK-pre, German, and *F. p. brookei*. Overall, the group GenBank-others showed the highest number of haplotypes, including three haplotypes not found elsewhere, reflecting world-wide origin of the GenBank data set. The German population was the least diverse with only one

haplotype, while the Irish and Domestic populations only had two haplotypes. UK-post and UK-pre had four haplotypes both; notably, UK-pre was represented in four haplotypes, two shared with UK-post and two unique haplotypes (haplotypes 5 and 6), each separated from the central haplotype 1 by two mutational steps; UK-post had only one unique haplotype (haplotype 7) not shared with any other group.

## Discussion

Using microsatellite and mtDNA markers, we examined the levels of genetic diversity and structure of peregrine falcons before and after the documented post-pesticide extinction in the English south coast. We also compared the genetic affinities of UK peregrines with other European populations, with domestic-bred birds and with published falcon mtDNA data, and explored the phylogeographic origins of the recently recovered Sussex peregrines. Our results support the hypothesis of an unassisted recovery of a peregrine falcon population in Southern England, with no detected signature of a genetic bottleneck, and little change in the level of genetic diversity through time. This adds to the growing literature showing unassisted recoveries of peregrines in several countries (Sielicki and Mizera 2009) and, to our knowledge, this is the first study to explore this in the UK using molecular tools, with implications toward their conservation and protection.

### Spatial and temporal genetic diversity and structure

Successful breeding, good dispersal capabilities, and inter-population connectivity are fundamental for preventing inbreeding depression and subsequent loss of genetic diversity (Frankham 2015; Ponnikas et al. 2017). As evidenced by our population genetics and phylogeographic study, results broadly indicated that there was no overall temporal loss of genetic diversity or a genetic signature of a population bottleneck in peregrines from southern England, in spite of the relatively recent and documented population bottleneck. UK-pre and UK-post groups were more similar genetically with each other than with any other peregrine group, possibly reflecting that contemporary UK peregrines are native and not a recent arrival

537 from elsewhere. Although the differences were not statistically significant, nuclear genetic
538 diversity indices showed lower values of genetic diversity in the post-pesticide sample (UK-
539 post) than in the pre-pesticide sample (UK-pre). There was, however, one less private allele
540 in UK-post than in UK-pre group. Furthermore, the Domestic group had more private alleles,
541 likely reflecting its mixed origin from various subspecies of *F. peregrinus*. The higher
542 heterozygosity and number of alleles in the Irish group warrants further attention considering
543 that the peregrines in the Republic of Ireland underwent a population decline in the 1960s and
544 subsequent recovery (Ratcliffe 2003), and that peregrines in Northern Ireland were not
545 severely affected by pesticides (Wells and Ruddock 2009).

546 For the main peregrine falcon groups, no genetic signatures of population bottlenecks
547 were detected under the TPM. The TPM is considered more appropriate than other models
548 for detecting significant heterozygosity excess while using microsatellite markers (Di Rienzo
549 et al. 1994; Cornuet and Luikart 1996; Piry et al. 1999). The statistical power to detect a
550 bottleneck increases with number of loci and sample size, but at least 20 diploid individuals
551 are needed to achieve reasonably high power (Cornuet and Luikart 1996). Here we only had
552 a limited number of microsatellite loci, but our main populations of interest (UK-post, German,
553 Domestic, and Irish groups) had adequate sample sizes.

554 Based on mtDNA, UK-post showed lower number of polymorphic sites and nucleotide
555 diversity values than UK-pre, and UK-pre had two haplotypes not found elsewhere in our
556 samples, which could represent a significant loss of haplotype diversity in general terms. The
557 mismatch distribution also showed few pairwise differences within the groups, and there was
558 no evidence of sudden population expansion. There was one main haplotype with sequences
559 from all peregrine groups, including GenBank samples with peregrine sequences from other
560 subspecies from around the world. The overall low genetic diversity and differentiation in
561 mtDNA control region reflected what was previously described in the mitochondrial
562 cytochrome b gene in peregrine falcons (Wink 2019), which can be explained by a fast
563 expansion of a genetically deprived population of falcons in the late Pleistocene and early
564 Holocene (Nittinger et al. 2005; White et al. 2013b).

565 It was expected, based on population genetics theory, that the re-established Sussex
566 peregrine population would be genetically impoverished, in comparison with a historical
567 sample, due to being founded by a small number of individuals surviving the pesticide crash.
568 It was therefore somewhat surprising that the level of nuclear genetic diversity retained by the
569 newly established population was comparable to ancestral levels in the UK. This was also
570 remarkable considering that no specific population augmentation efforts (i.e. no formal
571 reintroductions) have been directed to peregrines in the UK, a conservation strategy that other
572 countries have employed (Brown et al. 2007; Jacobsen et al. 2008; Johnson et al. 2010).
573 The results found here are consistent with other spatial and temporal studies on
574 falcons using microsatellites and/or mtDNA control region (Table 3). Using a panel of 11
575 polymorphic microsatellite loci, Brown et al. (2007) showed similar levels of heterozygosity
576 (but slightly higher allelic richness) in historical and contemporary Canadian peregrine falcon
577 (*F. p. tundrius*) populations, no signatures of genetic bottlenecks, and weak but significant
578 population genetic structure; however, effective number of breeders in the Canadian falcon
579 populations were much higher than those reported here. Based on mtDNA, Brown et al. (2007)
580 found low nucleotide and haplotype diversities in historical and contemporary populations,
581 much lower than in the UK-post and UK-pre populations reported here. Overall, Brown et al.
582 (2007) concluded that contemporary and historical samples had similar genetic diversity
583 levels, and that the organochlorine-induced bottleneck did not likely have an impact on the
584 evolutionary potential of peregrines in Canada.
585 While studying captive and wild populations of peregrines in Scandinavia and potential
586 gene introgression from the captive breeding stock during a reintroduction programme using
587 11 microsatellite loci, Jacobsen et al. (2008) showed similar heterozygosity values among
588 contemporary, captive, and historical samples, which were comparable with the
589 heterozygosity values reported here; however, they found higher allelic richness than in the
590 wild samples of peregrines studied here. Jacobsen et al. (2008) therefore concluded that the
591 levels of genetic diversity in the contemporary wild population of peregrines in Scandinavia
592 remained higher than expected from the population bottlenecks, and that this could be due to

593 population admixture, considerable gene introgression, the release of a large number of young
594 from the captive breeding population, and increased survivorship of chicks and adults in the
595 wild, which could have altered the genetic composition of the contemporary wild population.
596 Ponnikas et al. (2017), using 10 microsatellite loci, also found similar heterozygosity
597 values and no genetic bottlenecks, but they found higher allelic richness in peregrine falcons
598 from Finland than in the UK groups reported here, and no genetic structure. They concluded
599 that the genetic diversity could have been maintained through high dispersal rates across
600 Finland without reintroductions. This prevented genetic structuring of populations and the
601 negative effects of the population decline due to the use of organochlorine pesticides, but
602 resulted in a low effective population size in need of genetic monitoring (Ponnikas et al. 2017).
603 Nesje et al. (2000), however, based on 12 microsatellite loci, found lower heterozygosity
604 values in Scottish, Scandinavian, Canadian, American, and Tasmanian populations of
605 peregrine falcons than those reported here, but the Scottish population had similar allelic
606 richness to the UK groups described in this study.
607 Island (*F. p. nesiotes*) and mainland Australian (*F. p. macropus*) peregrine populations
608 in the South Pacific, and mainland and island populations in the North Pacific (*F. peregrinus*)
609 described in Talbot et al. (2011) were also less genetically diverse than the peregrine groups
610 described here, but peregrine populations from Vanuatu (South Pacific) and Colville area of
611 northern Alaska showed higher number of private alleles than the European peregrines.
612 Similarly, Talbot et al. (2011) found much lower mtDNA haplotype and nucleotide diversities
613 in the South Pacific islands than in our study, but comparable with those from the North Pacific
614 islands. There was also significant genetic differentiation with both molecular markers among
615 their sampled populations in the South Pacific and elsewhere, suggesting isolation and long-
616 term residency due to strong philopatry by peregrines, which reduces the capacity for gene
617 flow and a limited ability for long-distance dispersal (Talbot et al. 2017).
618 There are several possible intrinsic and extrinsic factors, such as dispersal (natal and
619 breeding), life history, behaviour, habitat, and connectivity (Kozakiewicz et al. 2017),
620 influencing the genetic composition of the newly re-established Sussex population. It is

possible that not enough time elapsed for genetic erosion to occur or to be observed after the population bottleneck (Hailer et al. 2006; Johnson et al. 2010; Ponnikas et al. 2017). Studies have shown that longevity is instrumental to reducing genetic drift, working as an intrinsic buffer against loss of genetic diversity (Hailer et al. 2006; Ponnikas et al. 2017). Longevity is characteristic of many raptor species, as seen in White-tailed eagles (Hailer et al. 2006) and Golden eagles (Sonsthagen et al. 2012), often extending over twenty to thirty years. Comparably, peregrines in the wild have also been known to reach up to sixteen years of age (Tordoff and Redig 1997; London Peregrine Partnership), with a generation time of 6.8 years (BirdLife International 2019).

**The origin of the Sussex peregrine population**

Our phylogenetic analyses based on mtDNA was unable to detect the ancestral origins of the re-colonised population of peregrines; however, microsatellite data detected signatures of genetic structure demonstrating that the contemporary wild peregrine population in Sussex is genetically similar, but not identical, to the pre-pesticide UK population, and that it is genetically different from other European populations and from the domestic stock.

Despite UK peregrines (*F. p. peregrinus*) possessing no migratory behavioural traits, the peregrine falcon is a species of innate dispersal and colonising capability (Smith et al. 2015; McGrady et al. 2017; Ponnikas et al. 2017). Individuals dispersing from other refugial areas within the UK, such as Cornwall, Cumbria, Wales, and Scotland (Crick and Ratcliffe 1995; Horne and Fielding 2002; Franklin and Everitt 2009), or dispersing from continental Europe (i.e. France, the Scandinavian peninsula, or from further afield), could have contributed to the Sussex peregrine population. However, we lack the necessary samples to test this hypothesis. Only one UK individual (UK-post PF5) showed partial membership with the German group, but when K = 4, the highest estimated membership coefficient (q-value) of UK-post PF5 individual clustered it with UK-post peregrines. If Sussex peregrines have a German origin, we would have found several UK peregrines clustering with German peregrines or showing mixed German ancestry. Also, two mtDNA haplotypes were observed

649 in the UK-post group that were not present in any other group. Two *F. p. brookei* individuals
650 grouped closer to UK samples in the PCoA (a genetic distance-based method), but genetic
651 assignment and membership based on Structure analysis (a probabilistic approach based on
652 genotype data and allele frequencies) put them in the same cluster as all German individuals,
653 likely reflecting that Mediterranean peregrines were used in the captive breeding programme
654 in southern Germany (Saar 1988; Wink 2019).

655 Mature female peregrines may have contributed to the new population as some have
656 been observed to change territories each year, rather than returning year on year to the same
657 breeding territories (Mearns and Newton 1984). It is possible that non-breeders (floaters),
658 dispersing but sexually inactive birds, coming from regions not severely affected by
659 organochlorine pesticides had an effect on the structure, dynamics, persistence, and recovery
660 of UK peregrine falcon populations, including the Sussex peregrines, as has been
661 hypothesised for other avian populations (Penteriani et al. 2011). Further sampling of wild
662 peregrines from continental Europe, the island of Ireland, and the British Isles is needed,
663 especially from possible refuge areas in the Scottish Highlands, outer UK islands (Ratcliffe
664 1963), and Northern Ireland (Wells and Ruddock 2009), as well as a more comprehensive
665 sampling of domestic peregrines and wild subspecies of *F. peregrinus*.

666 There was no genetic evidence of informal reintroductions via falconry escapes of
667 domestic birds into the UK population. Based on microsatellite data, we reason that the
668 domestic falcons have not contributed to the genetic diversity of UK peregrines or to the re-
669 establishment of the Sussex peregrine population. There was significant genetic differentiation
670 between the Domestic group vs. the historical and the contemporary wild UK peregrines, the
671 Bayesian clustering method showed that the Domestic individuals were genetically different
672 from the UK and other European peregrine individuals, and the genetic assignment test did
673 not cluster UK-post peregrines and Domestic ones together.

674 The analysis of mtDNA data was less conclusive than with microsatellites due to the
675 restricted number of nucleotides and low haplotypic diversity, as shown in other studies on
676 peregrine falcons (e.g. Brown et al. 2007; Talbot et al. 2011; White et al. 2013b). The only two

haplotypes found in the Domestic group were also found in all other peregrine groups, and the UK-post group had two haplotypes not found in the Domestic group. If mtDNA from the domestic falcons (from other species of falcons or subspecies of *F. peregrinus*) had introgressed the Sussex peregrines, we would have expected to find more haplotypes in UK-post, or at least more shared haplotypes between Domestic and UK-post peregrines.

Other studies using microsatellite markers have suggested introgression of alleles due to re-introductions of individuals from distant geographic origins (Tordoff and Redig 2001; Brown et al. 2007), but in our study there was a clear genetic distinction of domestic, Mediterranean falcons (*F. p. brookei*), and gyrfalcons (*F. rusticolus*) from the wild peregrine falcons. MtDNA might not be the best marker to explore introgression due to being maternally inherited and having low genetic variation in falcons in general (e.g. Brown et al. 2007; Talbot et al. 2011; White et al. 2013b). However, there were shared haplotypes between Domestic and UK-post groups, but wild peregrines showed unique haplotypes demonstrating that some peregrines cannot be of domestic origin.

Conservation genetics has traditionally depended on the use of microsatellite markers as a molecular marker of choice (Ouborg et al. 2010). A few microsatellites, however, cannot provide genome-wide variation estimations, and as near-neutral markers microsatellites cannot be used to determine the effects of selection (Ouborg et al. 2010). Similarly, mtDNA has been the marker of choice in phylogeography for decades, but there are concerns due to the limitations of this marker (Zink and Barrowclough 2008). With the advent of high-throughput sequencing, thousands of genomic markers, e.g. Single Nucleotide Polymorphisms (SNPs) obtained from Genotyping-by-Sequencing methods like Restriction Site Associated DNA sequencing (RAD-seq), can be used for conservation genomics and phylogeography (Narum et al. 2013). RAD-seq SNPs can produce more precise and accurate estimations of genome-wide diversity and differentiation than with a few microsatellite loci or few mtDNA markers, and can be used to generate robust phylogenies. Although applying high-throughput sequencing techniques on historical samples with degraded DNA is

challenging, such work would further the understanding of genomic diversity and structure, introgression, and evolutionary history of peregrine falcons.

**Implications for the protection of peregrines in the UK**

Currently, the global populations of peregrines have made a successful recovery and the population trends appears to be stable (BirdLife International 2019). In the UK the peregrine falcon has subsequently been moved from amber to green on the Birds of Conservation Concern (Eaton et al. 2015). In Sussex, the newly established peregrine falcons are currently occupying all known historical nesting territories within the county, and other inland non-traditional sites, indicating retention of good-quality habitat, with no significant barriers to dispersal.

We recognise that there are implications for the protection of UK peregrines if it is accepted that there has not been a genetic bottleneck effect, and that contemporary and pre-pesticide populations contain similar levels of genetic diversity, as described here. Reestablishment of the contemporary population has been rapid, with conservation of the integrity of its genetic variability (in comparison with an historical sample), and without reintroductions from captive breeding and release schemes such as has occurred in North America, Scandinavia and Germany. We suggest that for Sussex peregrines there is no need for genetic conservation by wild-take and subsequent captive breeding programmes, and that the various legal conventions for peregrines already in place (e.g. UK Wildlife and Countryside Act 1981) and protection of breeding sites should be enough to prevent a new population decline in this recently recovered avian population. Although it appears that population growth rate of long-lived species (like the peregrine falcon) is more sensitive to changes in survival than to reproductive success (Smith et al. 2015; McGrady et al. 2017), any harvest for falconry of different age classes, if allowed by law, should be scientifically informed to assert minimal impacts to wild populations. Wild populations of peregrine falcons in the UK should be adequately monitored to determine any potential impact in population demography and survival (Millsap and Allen 2006).

732 Furthermore, it appears that the escape of domestic birds (presumably of mixed
733 origins) into the wild has not influenced the genetic diversity of UK peregrine falcons. Domestic
734 peregrines have been found to be genetically different from wild peregrines (at least as
735 supported by the Bayesian clustering analysis), therefore not supporting the hypothesis that
736 domestic birds could have contributed to the reestablishment of the Sussex peregrine
737 population. Nonetheless, continuous escapes of domestic birds into the wild could eventually
738 lead to the introduction of alleles and mtDNA haplotypes not typically belonging to *F. p.*
739 *peregrinus*. Careful monitoring and care while carrying out falconry should be advocated.
740 Although the estimated effective population sizes of peregrines studied here were
741 found to be below the critical threshold of 50 individuals to avoid short-term inbreeding
742 depression (Brook et al. 2002), at least in Sussex there is a healthy breeding population that
743 could safeguard its long-term survival. It is recommended that field monitoring is carried out
744 on a yearly basis to assess the Sussex peregrine population, and that genetic monitoring
745 should be performed at 8 to 10-year intervals, in line with generation lifespans of this species
746 in the wild, to ensure that the evolutionary potential is not being compromised by inbreeding
747 depression and genetic drift through intrinsic and anthropogenic factors.
748
749 **References**
750 Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations.
751 Phil Trans R Soc Lond B 353:177–186. https://doi.org/10.1098/rstb.1998.0200
752 Banks AN, Crick HQP, Coombes R, et al (2010) The breeding status of Peregrine Falcons
753 *Falco peregrinus* in the UK and Isle of Man in 2002. Bird Study 57:421–436.
754 https://doi.org/10.1080/00063657.2010.511148
755 BirdLife International (2019) *Falco peregrinus* (amended version of 2016 assessment). The
756 IUCN Red List of Threatened Species 2019: e.T45354964A155500538
757 https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T45354964A155500538.en.
758 Downloaded on 29 October 2020

759 Brook BW, Tonkyn DW, O'Grady JJ, Frankham R (2002) Contribution of inbreeding to
760 extinction risk in threatened species. Conserv Ecol 6:16. https://doi.org/10.5751/es-
761 00387-060116
762 Brown JW, Van Coeverden De Groot PJ, Birt TP, et al (2007) Appraisal of the consequences
763 of the DDT-induced bottleneck on the level and geographic distribution of neutral
764 genetic variation in Canadian peregrine falcons, *Falco peregrinus*. Mol Ecol 16:327–
765 343. https://doi.org/10.1111/j.1365-294X.2007.03151.x
766 Cade TJ, Enderson JH, Thelander CG, White CM (eds) (1988) Peregrine falcon populations:
767 their management and recovery. Peregrine Fund, Idaho
768 Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent
769 population bottlenecks from allele frequency data. Genetics 144:2001–2014
770 Crick HQP, Ratcliffe DA (1995) The Peregrine *Falco peregrinus* breeding population of the
771 United Kingdom in 1991. Bird Study 42:1–19.
772 https://doi.org/10.1080/00063659509477143
773 Di Rienzo A, Peterson AC, Garza JC et al (1994) Mutational processes of simple-sequence
774 repeat loci in human populations. Proc Natl Acad Sci USA 91:3166–3170
775 https://doi.org/10.1073/pnas.91.8.3166
776 Do C, Waples RS, Peel D, et al (2014) NeEstimator v2: Re-implementation of software for the
777 estimation of contemporary effective population size (Ne) from genetic data. Mol Ecol
778 Resour 14:209–214. https://doi.org/10.1111/1755-0998.12157
779 Eaton M, Aebischer N, Brown A, et al (2015) Birds of Conservation Concern 4: The population
780 status of birds in the UK, Channel Islands and Isle of Man. British Birds 108:708-746
781 Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using
782 the software STRUCTURE: A simulation study. Mol Ecol 14:2611–2620.
783 https://doi.org/10.1111/j.1365-294X.2005.02553.x
784 Everitt PJ, Franklin J (2009) First UK record of a wild free-living Peregrine Falcon female
785 breeding and producing young with a hybrid male falcon of domestic origin. In: Sielicki
786 J, Mizera T (ed) Peregrine Falcon populations – status and perspectives in the 21st

787 century, European Peregrine Falcon Working Group, Society for the Protection of Wild
788 Animals "Falcon". Turul/Poznań University of Life Sciences Press, Warsaw-Poznań,
789 pp 585–592
790 Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: A new series of programs to perform
791 population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567.
792 https://doi.org/10.1111/j.1755-0998.2010.02847.x
793 Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus
794 genotype data: Linked loci and correlated allele frequencies. Genetics 164:1567–1587
795 Fleming LV, Douse AF, Williams NP (2011) Captive breeding of peregrine and other falcons
796 in Great Britain and implications for conservation of wild populations. Endanger
797 Species Res 14:243–257. https://doi.org/10.3354/esr00352
798 Frankham R (2015) Genetic rescue of small inbred populations: meta-analysis reveals large
799 and consistent benefits of gene flow. Mol Ecol 24:2610–2618.
800 https://doi.org/10.1111/mec.13139
801 Franklin J, Everitt PJ (2009) Population trends of peregrine falcons on the Sussex coast of the
802 United Kingdom, 1904-2006. In: Sielicki J, Mizera T (ed) Peregrine Falcon populations
803 – status and perspectives in the 21st century, European Peregrine Falcon Working
804 Group, Society for the Protection of Wild Animals "Falcon". Turul/Poznań University of
805 Life Sciences Press, Warsaw-Poznań, pp 87–98
806 Franklin J, Nicholls MK (2018) Notes on the present-day population of Sussex Peregrines.
807 The Oologist 2:122–127
808 Fu YX (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking
809 and background selection. Genetics 147:915–925
810 Hailer F, Helander B, Folkestad AO, et al (2006) Bottlenecked but long-lived: High genetic
811 diversity retained in white-tailed eagles upon recovery from population decline. Biol
812 Lett 2:316–319. https://doi.org/10.1098/rsbl.2006.0453
813 Hall TA (1999) BIOEDIT: a user-friendly biological sequence alignment editor and analysis
814 program for Windows 95/98/ NT. Nucleic Acids Symp Ser 41:95–98

Hoffman JI, Amos W (2005) Microsatellite genotyping errors: Detection approaches, common sources and consequences for paternal exclusion. Mol Ecol 14:599–612. https://doi.org/10.1111/j.1365-294X.2004.02419.x

Holroyd GL, Banasch U (1990) The reintroduction of the peregrine falcon *Falco peregrinus* anatum into southern Canada. Can Field Nat 104:203–208

Horne G, Fielding AH (2002) Recovery of the Peregrine Falcon *Falco peregrinus* in Cumbria, UK, 1966–99. Bird Study 49:229–236. https://doi.org/10.1080/00063650209461270

Humphreys L, Wernham C, Crick HQ (2007) Raptor species conservation frameworks: The peregrine conservation framework project progress report-Phase I. British Trust for Ornithology, Scotland

Jacobsen F, Nesje M, Bachmann L, Lifjeld JT (2008) Significant genetic admixture after reintroduction of peregrine falcon (*Falco peregrinus*) in Southern Scandinavia. Conserv Genet 9:581–591. https://doi.org/10.1007/s10592-007-9373-4

Janes JK, Miller JM, Dupuis JR, et al (2017) The K = 2 conundrum. Mol Ecol 26:3594–3602. https://doi.org/10.1111/mec.14187

Johnson JA, Talbot SL, Sage GK, et al (2010) The use of genetics for the management of a recovering population: Temporal assessment of migratory peregrine falcons in North America. PLoS One 5:e14042. https://doi.org/10.1371/journal.pone.0014042

Kopelman NM, Mayzel J, Jakobsson M, et al (2015) CLUMPAK: A program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15:1179–1191. https://doi.org/10.1111/1755-0998.12387

Kozakiewicz CP, Carver S, Austin JJ, et al (2017) Intrinsic factors drive spatial genetic variation in a highly vagile species, the wedge-tailed eagle *Aquila audax*, in Tasmania. J Avian Biol 48:1025–1034. https://doi.org/10.1111/jav.01326

Li YL, Liu JX (2018) STRUCTURESELECTOR: A web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176–177. https://doi.org/10.1111/1755-0998.12719

842 Librado P, Rozas J (2009) DnaSP v5: A software for comprehensive analysis of DNA
843 polymorphism data. Bioinformatics 25:1451–1452.
844 https://doi.org/10.1093/bioinformatics/btp187
845 Madden B, Hunt J, Norriss D (2009) The 2002 survey of the peregrine *Falco peregrinus*
846 breeding population in the Republic of Ireland. Irish Birds 8:543–548
847 Maruyama T, Fuerst PA (1984) Population bottlenecks and nonequilibrium models in
848 population genetics. I. Allele numbers when populations evolve from zero variability.
849 Genetics 108:745–763
850 Maruyama T, Fuerst PA (1985) Population bottlenecks and nonequilibrium models in
851 population genetics. II. Number of alleles in a small population that was formed by a
852 recent bottleneck. Genetics 111:675–689
853 McGrady MJ, Hines JE, Rollie CJ, et al (2017) Territory occupancy and breeding success of
854 Peregrine Falcons *Falco peregrinus* at various stages of population recovery. Ibis
855 159:285–296. https://doi.org/10.1111/ibi.12443
856 Mearns R, Newton I (1984) Turnover and dispersal in a Peregrine *Falco peregrinus*
857 population. Ibis 126:347–355 https://doi.org/10.1111/j.1474-919X.1984.tb00255.x
858 Meijer SN, Halsall CJ, Harner T, et al (2001) Organochlorine pesticide residues in archived
859 UK soil. Environ Sci Technol 35:1989–1995. https://doi.org/10.1021/es0000955
860 Millsap BA, Allen GT (2006) Effects of falconry harvest on wild raptor populations in the United
861 States: theoretical considerations and management recommendations. Wildl Soc Bull
862 34:1392–1400. https://doi.org/10.2193/0091-7648(2006)34[1392:eofhow]2.0.co;2
863 Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA. (2013) Genotyping-by-
864 sequencing in ecological and conservation genomics. Mol Ecol 22:2841–2847.
865 doi:10.1111/mec.12350
866 Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number
867 of individuals. Genetics 89:583–590

868 Newton I (1988) Changes in the status of the peregrine falcon in Europe: an overview. In:
869 Cade TJ, Enderson JH, Thelander CG, White CM (eds) Peregrine populations, their
870 management and recovery. The Peregrine Fund, Boise, pp 227–234
871 Nesje M, Røed KH, Bell DA, et al (2000) Microsatellite analysis of population structure and
872 genetic variability in peregrine falcons (*Falco peregrinus*). Anim Conserv 3:267–275.
873 https://doi.org/10.1017/S1367943000000998
874 Nicholls MK, Franklin J, Vega R, Jackson H (2017) Return of the Sussex peregrine: where
875 have they come from? In: Proceedings of the International Conference on the
876 Stewardship of Biodiversity and Sustainable Use. Irish Hawking Club and International
877 Association of Falconry and Conservation of Birds of Prey, Moyvalley
878 Nittinger F, Haring E, Pinsker W, et al (2005) Out of Africa? Phylogenetic relationships
879 between *Falco biarmicus* and the other hierofalcons (Aves: Falconidae). J Zool Syst
880 Evol Res 43:321–331. https://doi.org/10.1111/j.1439-0469.2005.00326.x
881 Nomura T (2008) Estimation of effective number of breeders from molecular coancestry of
882 single cohort sample. Evol Appl 1:462–474. https://doi.org/10.1111/j.1752-
883 4571.2008.00015.x
884 Norriss DW (1995) The 1991 survey and weather impacts on the peregrine *Falco peregrinus*
885 breeding population in the Republic of Ireland. Bird Study 42:20–30.
886 https://doi.org/10.1080/00063659509477144
887 Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct,
888 real-time estimation of migration rate: A simulation-based exploration of accuracy and
889 power. Mol Ecol 13:55–65. https://doi.org/10.1046/j.1365-294X.2004.02008.x
890 Peakall R, Smouse PE (2012) GenALEx 6.5: Genetic analysis in Excel. Population genetic
891 software for teaching and research-an update. Bioinformatics 28:2537–2539.
892 https://doi.org/10.1093/bioinformatics/bts460
893 Penteriani V, Ferrer M, Delgado MM (2011) Floater strategies and dynamics in birds, and their
894 importance in conservation biology: Towards an understanding of nonbreeders in

avian populations. Anim Conserv 14:233–-241. https://doi.org/10.1111/j.1469-1795.2010.00433.x

Piry S, Alapetite A, Cornuet JM, et al (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. J Hered 95:536–539. https://doi.org/10.1093/jhered/esh074

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503. https://doi.org/10.1093/jhered/90.4.502

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: Causes, consequences and solutions. Nat Rev Genet 6:847–859. https://doi.org/10.1038/nrg1707

Ponnikas S, Ollila T, Kvist L (2017) Turnover and post-bottleneck genetic structure in a recovering population of Peregrine Falcons *Falco peregrinus*. Ibis 159:311–323. https://doi.org/10.1111/ibi.12460

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959. https://doi.org/10.1111/j.1471-8286.2007.01758.x

Puechmaille SJ (2016) The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627. https://doi.org/10.1111/1755-0998.12512

Raisin C, Dawson DA, Greenwood AG, Jones CG, Groombridge JJ (2009) Characterisation of Mauritius Parakeet (*Psittacula eques*) microsatellite loci and their cross-utility in other parrots (Psittacidae, Aves). Mol Ecol Resour 9:1231–1235. https://doi.org/10.1111/j.1755-0998.2009.02621.x

Ramasamy RK, Ramasamy S, Bindroo BB, Naik VG (2014) STRUCTURE PLOT: A program for drawing elegant STRUCTURE bar plots in user friendly interface. SpringerPlus 3:431. https://doi.org/10.1186/2193-1801-3-431

Ramos-Onsins SE, Rozas J (2002) Statistical properties of new neutrality tests against population growth. Mol Biol Evol 19:2092–2100. https://doi.org/10.1093/oxfordjournals.molbev.a004034

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. Proc Natl Acad Sci USA 94:9197–9201. https://doi.org/10.1073/pnas.94.17.9197

Ratcliffe DA (1963) The status of the Peregrine in Great Britain. Bird Study. https://doi.org/10.1080/00063656309476042

Ratcliffe DA (1972) The Peregrine population of Great Britain in 1971. Bird Study 19:117–156. https://doi.org/10.1080/00063657209476336

Ratcliffe DA (1984) The peregrine breeding population of the United Kingdom in 1981. Bird Study 31:1–18. https://doi.org/10.1080/00063658409476809

Ratcliffe DA (1993) The peregrine falcon, 2nd edn. T & AD Poyser, London

Ratcliffe DA (2003) The peregrine saga. In: Thompson DBA, Redpath S, Fielding AH, Marquiss M, Galbraith CA (eds) Birds of prey in a changing environment. The Stationary Office, Edinburgh, pp 91–98.

Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283. https://doi.org/10.2307/2410454

Rousset F (2008) GENEPOP'007: A complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Saar C (1988) Reintroduction of the peregrine falcon in Germany. In: Cade TJ, Enderson JH, Thelander CG, White CM (eds) Peregrine falcon populations: their management and recovery. Peregrine Fund, Idaho, pp 629–636

Sielicki J, Mizera T (2009) Peregrine Falcon populations – status and perspectives in the 21st century, European Peregrine Falcon Working Group, Society for the Protection of Wild Animals "Falcon". Turul/Poznań University of Life Sciences Press, Warsaw-Poznań

948 Smith GD, Murillo-García OE, Hostetler JA, et al (2015) Demography of population recovery:
949 survival and fidelity of peregrine falcons at various stages of population recovery.
950 Oecologia 178:391–401. https://doi.org/10.1007/s00442-014-3168-3
951 Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and
952 multilocus genetic structure. Heredity 82:561–573.
953 https://doi.org/10.1038/sj.hdy.6885180
954 Sonsthagen SA, Coonan TJ, Latta BC, et al (2012) Genetic diversity of a newly established
955 population of golden eagles on the Channel Islands, California. Biol Conserv 146:116–
956 122. https://doi.org/10.1016/j.biocon.2011.11.031
957 Talbot SL, Palmer AG, Sage GK, et al (2011) Lack of genetic polymorphism among peregrine
958 falcons *Falco peregrinus* of Fiji. J Avian Biol 42:415–428.
959 https://doi.org/10.1111/j.1600-048X.2011.05280.x
960 Talbot SL, Sage GK, Sonsthagen SA, et al (2017) Intraspecific evolutionary relationships
961 among peregrine falcons in western North American high latitudes. PLoS One 12:
962 e0188185. https://doi.org/10.1371/journal.pone.0188185
963 Tordoff HB, Redig PT (1997) Midwest Peregrine Falcon demography, 1982-1995. J Raptor
964 Res 31:339–346
965 Tordoff HB, Redig PT (2001) Role of genetic background in the success of reintroduced
966 peregrine falcons. Conserv Biol 15:528–532. https://doi.org/10.1046/j.1523-
967 1739.2001.015002528.x
968 Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER:
969 Software for identifying and correcting genotyping errors in microsatellite data. Mol
970 Ecol Notes 4:535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x
971 Wandeler P, Hoeck PEA, Keller LF (2007) Back to the future: museum specimens in
972 population genetics. Trends Ecol Evol 22:634–642.
973 Wells JH, Ruddock M (2009) Population dynamics of the peregrine falcon in Northern Ireland.
974 In: Sielicki J, Mizera T (ed) Peregrine Falcon populations – status and perspectives in
975 the 21st century, European Peregrine Falcon Working Group, Society for the

976 Protection of Wild Animals "Falcon". Turul/Poznań University of Life Sciences Press,
977 Warsaw-Poznań, pp 281–294
978 White CM (1994) Family Falconidae. In: del Hoyo J, Elliot A, Sargatal J (eds) Handbook of
979 birds of the world, Volume 2: New World Vultures to Guinea fowl. Lynx Edicions,
980 Barcelona, pp 216–275
981 White CM, Cade TJ, Enderson JH (2013a) Peregrine falcons of the world. Lynx Edicions,
982 Barcelona
983 White CM, Sonsthagen SA, Sage GK, et al (2013b) Genetic relationships among some
984 subspecies of the Peregrine Falcon (*Falco peregrinus* L.), inferred from mitochondrial
985 DNA control-region sequences. The Auk 130:78–87.
986 https://doi.org/10.1525/auk.2012.11173
987 Wilson MW, Balmer DE, Jones K, et al (2018) The breeding population of Peregrine Falcon
988 *Falco peregrinus* in the United Kingdom, Isle of Man and Channel Islands in 2014. Bird
989 Study 65:1–19. https://doi.org/10.1080/00063657.2017.1421610
990 Wink M (2019) Phylogeny of Falconidae and phylogeography of Peregrine Falcons. Ornis
991 Hungarica 26:27–37. https://doi.org/10.1515/orhu-2018-0013
992 Zink RM, Barrowclough GF (2008) Mitochondrial DNA under siege in avian phylogeography.
993 Mol Ecol 17:2107–2121. https://doi.org/10.1111/j.1365-294X.2008.03737.x
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**FIGURE CAPTIONS**

**Fig. 1** Geographic distribution of peregrine falcons (*Falco peregrinus*) and subspecies (adapted from White et al. 2013a). Ranges show only general areas where peregrines have been observed, but there are often areas of overlap among subspecies, and uncertainty in their relationships and distributions. The question mark (?) indicates a disputed area for *F. p. peregrinus* and *F. p. japonicus*

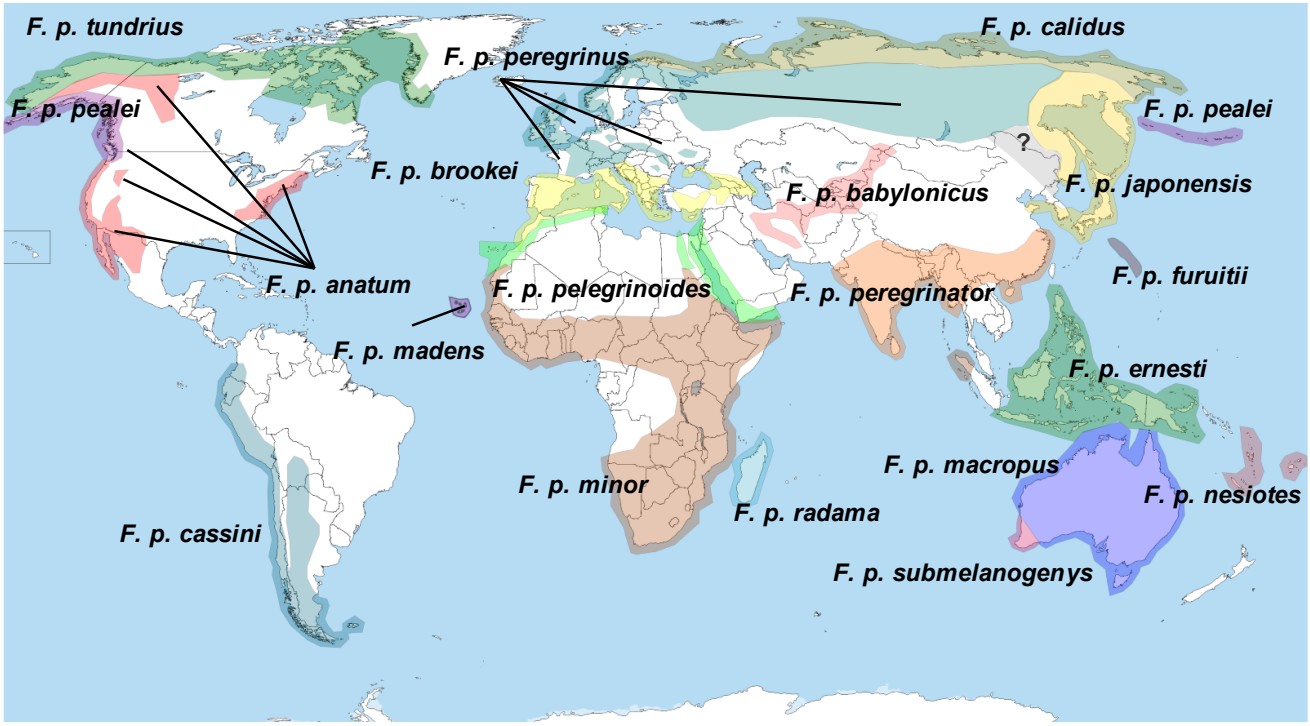
**Fig. 2** Principal coordinates analysis (PCoA) based on microsatellite genotypes of peregrine falcons (*Falco peregrinus*). Convex hulls illustrate the outline of the individuals from each group

**Fig. 3** Individual probability plots showing the most likely number of genetic clusters of peregrine falcons (*Falco peregrinus*) for K number of clusters (K = 4, K = 3, and K = 2) based on a Bayesian analysis of genetic structure of microsatellite data. The appropriate K clusters were selected by Puechmaille's, mean LnP(K), and Evanno's ΔK methods, respectively

**Fig. 4** Phylogenetic haplotype network showing the evolutionary relationships among peregrine falcon (*Falco peregrinus*) mtDNA control region haplotypes arranged into populations

## FIGURES

**Fig. 1**



1034 **Fig. 2**

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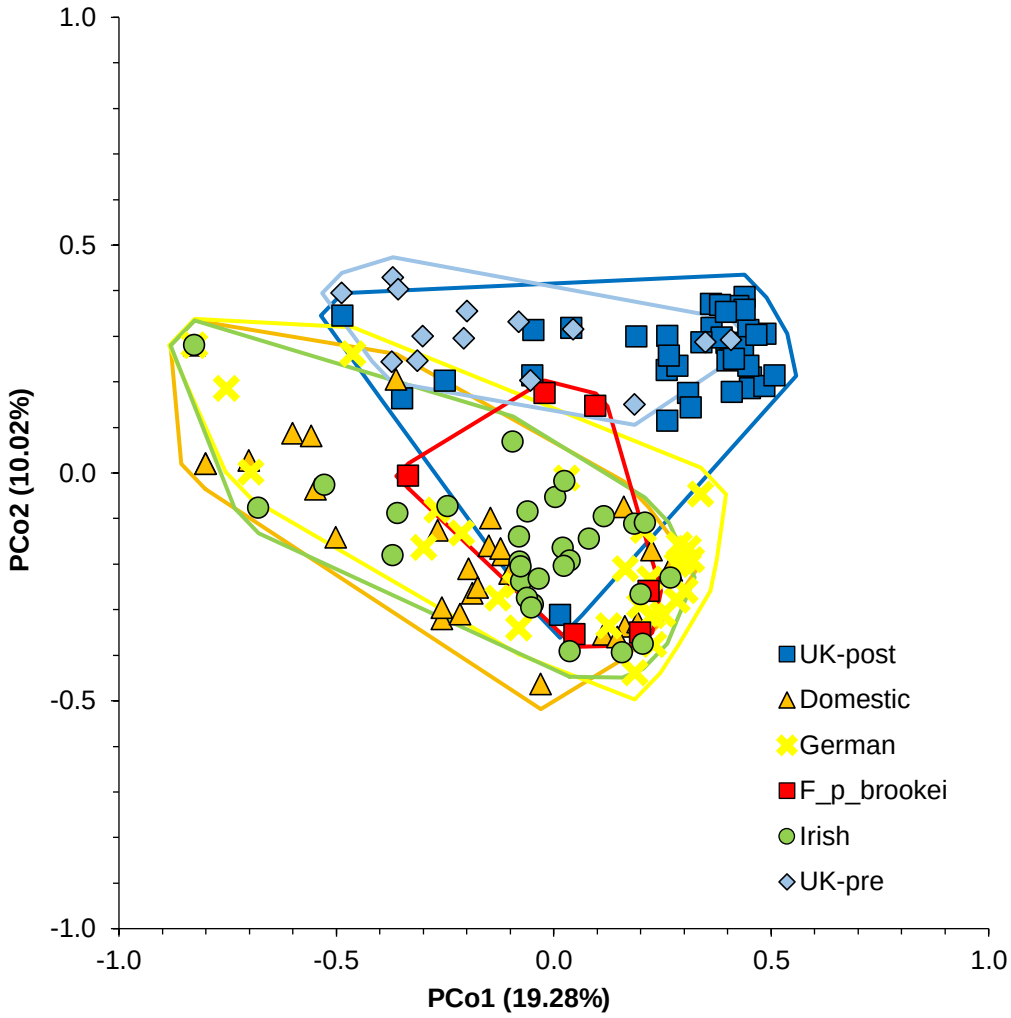


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**Fig. 3**

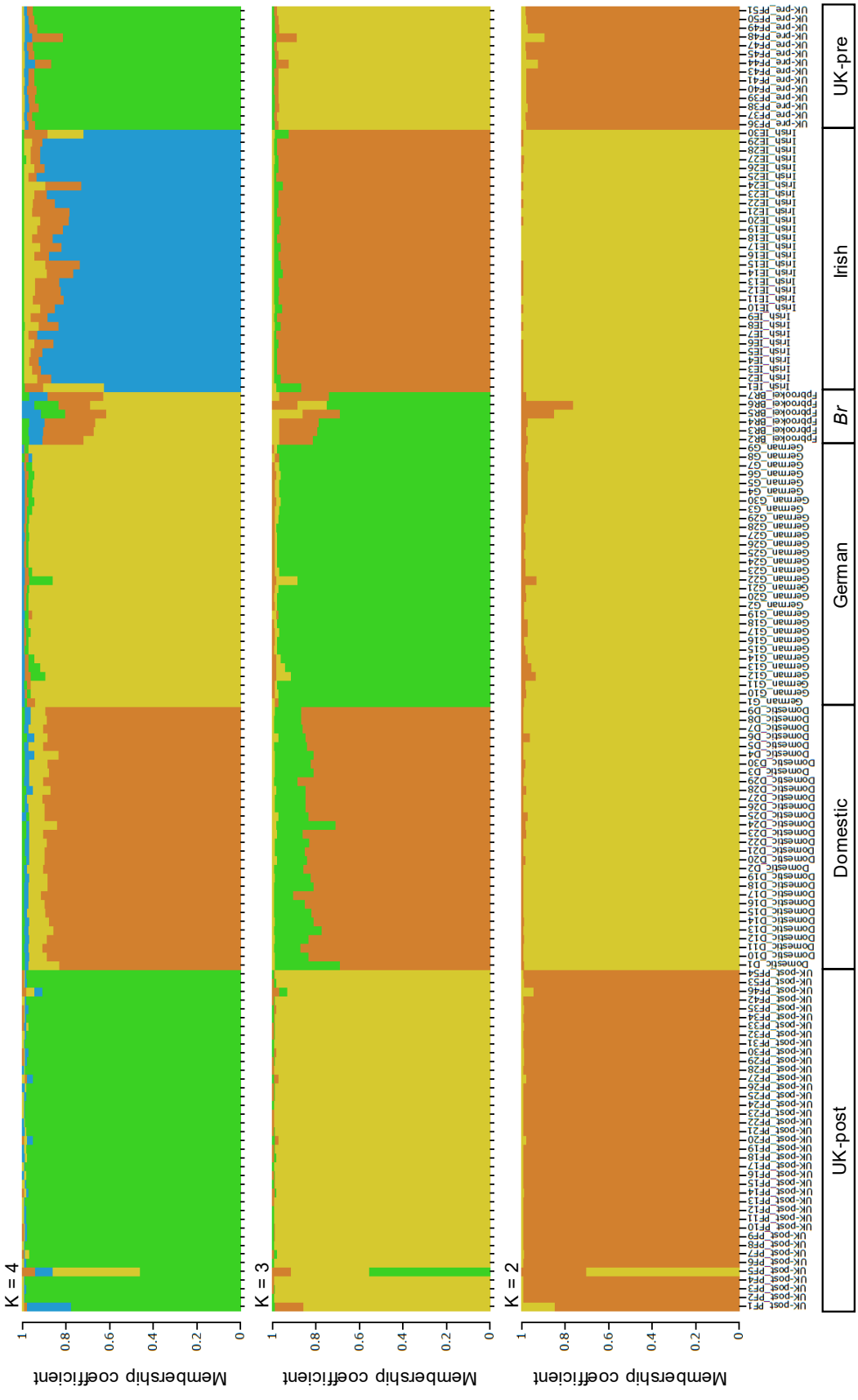
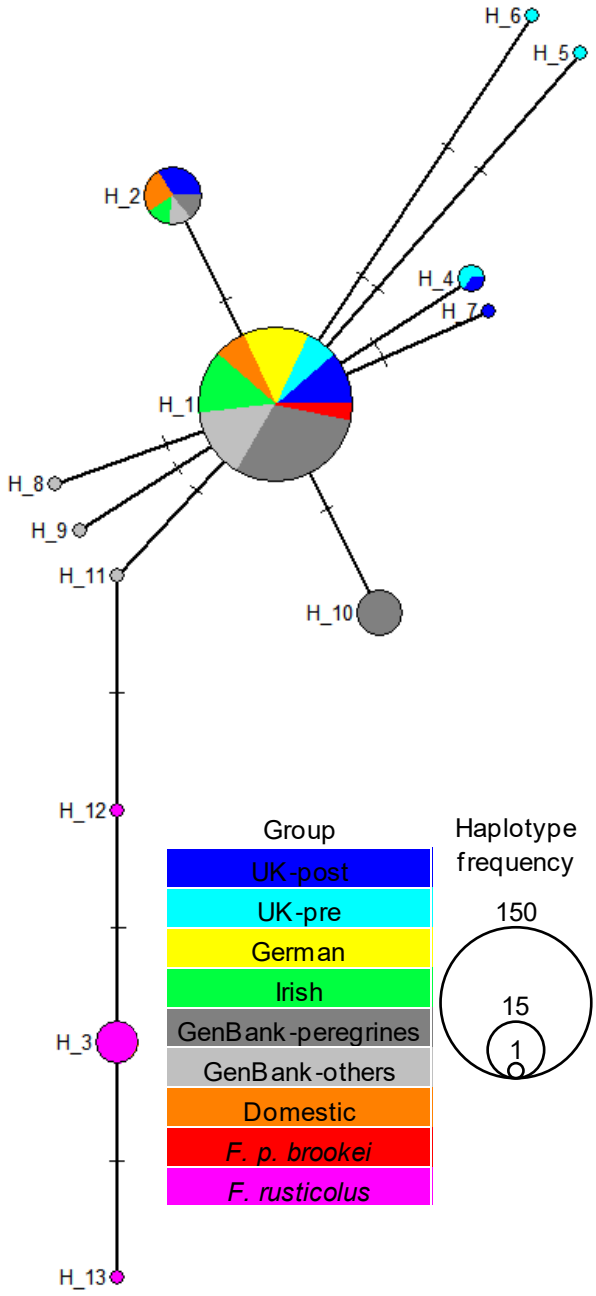

K = 4
K = 3
K = 2
Membership coefficient
1
0.8
0.6
0.4
0.2
0
UK-post
Domestic
German
Br
Irish
UK-pre

**Fig. 4**


H_6
H_5
H_2
H_4
H_7
H_1
H_8
H_9
H_11
H_10
H_12
H_3
H_13
Group
UK-post
UK-pre
German
Irish
GenBank-peregrines
GenBank-others
Domestic
F. p. brookei
F. rusticolus
Haplotype frequency
150
15
1

## Tables

**Table 1** Genetic diversity values in peregrine falcon (*Falco peregrinus*) populations based on microsatellite data

| Population | N | $N_{miss}$ | $N_a$ | $N_e$ | $H_o$ | $H_e$ | $F_{IS}$ | $N_P$ | $N_{eb}$ (CI) | $N_{assign}$ | % Assign |
|---|---|---|---|---|---|---|---|---|---|---|---|
| UK-post | 39 | 35.143 | 4.143 | 2.294 | 0.557 | 0.548 | -0.005 | 2 | 4.5 (1.4-9.6) | 30 | 76.923 |
| UK-pre | 14 | 8.571 | 3.286 | 2.297 | 0.587 | 0.545 | -0.097 | 3 | Infinite | 8 | 57.143 |
| German | 30 | 22.143 | 4.429 | 2.649 | 0.581 | 0.590 | 0.008 | 1 | 6.5 (2.9-11.6) | 24 | 88.889 |
| Irish | 30 | 23.143 | 6.000 | 3.481 | 0.634 | 0.669 | 0.029 | 3 | 19.3 (0.5-71.3) | 21 | 72.414 |
| Domestic | 30 | 19.857 | 7.000 | 4.122 | 0.624 | 0.706 | 0.114 | 10 | 81.7 (0.1-410) | 19 | 65.517 |
| *F. p. brookei* | 6 | 4.714 | 3.714 | 2.885 | 0.771 | 0.646 | -0.194 | 0 | 30.9 (0-155.4) | 4 | 66.667 |
| All UK-peregrines | 53 | 43.714 | 4.714 | 2.302 | 0.558 | 0.558 | 0.003 | 6 | 22.3 (0-111.7) | 52 | 98.113 |
| All-peregrines | 149 | 113.571 | 9.429 | 3.818 | 0.599 | 0.725 | 0.170 | - | 10.3 (5-17.6) | - | - |

N = sample size, $N_{miss}$ = sample size considering missing data across loci, $N_a$ = number of different alleles, $N_e$ = number of effective alleles, $H_o$ = observed heterozygosity, $H_e$ = expected heterozygosity, $F_{IS}$ = fixation index, $N_P$ = number of private alleles, $N_{eb}$ = Effective number of breeders (CI = jackknife confidence interval), $N_{assign}$ = Number of individuals used in assignment test, % Assign = percentage of individuals correctly assigned to the population of origin

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**Table 2** DNA polymorphism in peregrine falcon (*Falco peregrinus*) populations based on mtDNA control region data

| Population | N | S | Pol | H | Hd | π | k | $R_2$ | Fs | τ |
|---|---|---|---|---|---|---|---|---|---|---|
| UK-post | 24 | 363 | 6 | 5 | 0.529 | 0.002 | 0.837 | 0.101 | -1.349 | 0.356 |
| UK-pre | 14 | 295 | 9 | 5 | 0.505 | 0.005 | 1.407 | 0.132 | -0.786 | 0.153 |
| German | 21 | 627 | 2 | 4 | 0.710 | 0.001 | 0.924 | 0.231 | -0.140 | 0.924 |
| Irish | 22 | 627 | 5 | 8 | 0.892 | 0.003 | 1.792 | 0.179 | -2.349 | 1.792 |
| Domestic | 14 | 627 | 4 | 5 | 0.824 | 0.002 | 1.209 | 0.148 | -1.159 | 1.209 |
| GenBank-peregrines | 55 | 376 | 6 | 7 | 0.756 | 0.003 | 1.182 | 0.098 | -1.289 | 1.182 |
| GenBank-others | 28 | 556 | 26 | 19 | 0.971 | 0.006 | 3.198 | 0.087 | **-13.598** | 0.828 |
| *F. p. brookei* | 5 | 627 | 2 | 3 | 0.800 | 0.002 | 1.000 | 0.250 | -0.475 | 1.000 |
| UK-peregrines | 38 | 290 | 11 | 8 | 0.486 | 0.003 | 0.908 | 0.070 | **-3.908** | 0.095 |
| All-peregrines | 183 | 238 | 11 | 10 | 0.320 | 0.002 | 0.365 | 0.026 | **-9.361** | 0.365 |
| *F. rusticolus* | 10 | 627 | 8 | 7 | 0.911 | 0.003 | 1.867 | **0.116** | **-3.502** | 1.867 |

N = number of sequences used, S = total number of sites (excluding sites with gaps/missing data, or excluding them in pairwise comparisons), Pol = polymorphic sites, H = number of haplotypes, Hd = haplotype diversity, π = nucleotide diversity, k = average number of nucleotide differences, Fs = Fu (1997), $R_2$ = Ramos-Onsins and Rozas (2002), τ = 2ut. Values in bold ($P < 0.05$), simulations based on the coalescent process for neutral infinite-sites model assuming a large constant population size

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**Table 3** Genetic diversity values of falcons (including *Falco peregrinus* and other species) from various regions obtained from selected studies using microsatellite and mtDNA molecular markers

| | | Microsatellites | | | | | MtDNA control region | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Author | Species or subspecies, and location | N | $N_a$ | $H_o$ | $H_e$ | $N_p$ | N | H | Hd | π |
| Nesje et al. 2000 | *F. p. peregrinus* Scandinavian peninsula | 91 | 4.20 | 0.46 | 0.49 | - | - | - | - | - |
| | *F. p. peregrinus* Scotland | 5 | 2.60 | 0.46 | 0.45 | - | - | - | - | - |
| | *F. p. anatum* USA | 10 | 4.10 | 0.41 | 0.43 | - | - | - | - | - |
| | *F. p. tundrius* Canada | 15 | 3.30 | 0.42 | 0.46 | - | - | - | - | - |
| | *F. p. pealei* Canada | 6 | 2.90 | 0.48 | 0.43 | - | - | - | - | - |
| | *F. p. macropus* Tasmania | 4 | 1.40 | 0.15 | 0.17 | - | - | - | - | - |
| Brown et al. 2007 | *F. p. anatum*/*tundrius*/*pealei*/hybrids Historical (Canada) | 95 | 5.45 | 0.50 | | 5 | 82 | 3 | 0.38 | 0.001 |
| | *F. p. anatum*/tundrius/pealei Contemporary (Canada) | 184 | 5.82 | 0.54 | | 9 | 184 | 5 | 0.41 | 0.001 |
| Johnson et al. 2007 | *F. rusticolus* Alaska, Greenland, Canada and Iceland | 178 | 3.20 | 0.42 | 0.45 | - | 132 | 8 | 0.57 | 0.001 |
| | *F. cherrug* UAE | 20 | 5.40 | 0.47 | 0.57 | - | 20 | 11 | 0.87 | 0.005 |
| Jacobsen et al. 2008 | *F. peregrinus* Contemporary (southern Scandinavia and northern Fennoscandia) | 44 | 4.64 | 0.46 | 0.50 | 10 | - | - | - | - |
| | *F. peregrinus* Historical (Norway and Denmark, 1877-1951) | 38 | 5.27 | 0.53 | 0.56 | 10 | - | - | - | - |
| | *F. peregrinus* Captive (southern Scandinavia and northern Fennoscandia) | 20 | 4.82 | 0.51 | 0.54 | 1 | - | - | - | - |
| Johnson et al. 2011 | *F. p. pealei* Western Canada | 33 | 4.10 | 0.49 | 0.50 | - | - | - | - | - |
| | *F. p. tundrius* Alaska, Canada and Greenland | 153 | 4.90 | 0.47 | 0.53 | - | - | - | - | - |
| | *F. p. anatum* Alaska, Canada and contiguous USA | 163 | 4.78 | 0.53 | 0.53 | - | - | - | - | - |
| | *F. p. cassini* South America | 25 | 2.50 | 0.37 | 0.39 | - | - | - | - | - |
| | *F. p. macropus* Australia | 15 | 2.50 | 0.29 | 0.28 | - | - | - | - | - |
| Talbot et al. 2011 | *F. p. nesiotes* South Pacific | 22 | 2.15 | 0.15 | 0.21 | 7 | 17 | 2 | 0 | 0 |
| | *F. p. macropus* Australia | 18 | 2.8 | 0.31 | 0.36 | 4 | 13 | 10 | 0.95 | 0.007 |
| | *F. p. pealei* Alaska | 52 | 3.93 | 0.45 | 0.53 | 10 | 35 | 10 | 0.53 | 0.001 |
| Ponnikas et al. 2017 | *F. p. peregrinus* Finland | 145 | 5.60 | 0.55 | 0.58 | - | - | - | - | - |

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