



Ancestry and genetic differentiation of red foxes (*Vulpes vulpes*) on the Isle of Wight



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ABSTRACT

Foxes were presumed absent from the Isle of Wight, UK, until their introduction for sport hunting in the 19th century. We investigated the ancestry of red foxes on the Isle of Wight, UK, by generating a dataset of 53 concatenated D-loop and cytochrome-b mitochondrial DNA sequences which was then compared to a previously published database of European sequences. We also tested for genetic differentiation between the Isle of Wight and other populations throughout Europe. We found evidence that red foxes on the Isle of Wight likely originated from mainland Britain, but that the Isle of Wight population is genetically differentiated from adjacent populations in mainland central southern England. At >1 km across, The Solent serves as a natural barrier to gene flow between the mainland and the Isle of Wight.

INTRODUCTION

Although widespread across Great Britain, red foxes (*Vulpes vulpes*) are absent from many islands surrounding the mainland (Reynolds & Short 2003; Harris & Lloyd 1991). However, there are several islands where they have been introduced by humans, including the Isle of Wight. According to the hunting-with-hounds literature, foxes of unknown origin were introduced to the Isle of Wight during the mid-1800s for sport hunting (The Leverets 2012), where they are now common. Although there are no records of the transactions, foxes introduced to the Isle of Wight may have come from mainland Britain as well as imports from continental Europe (Carr 1976).

Regional fox populations typically exhibit low levels of genetic structure across mainland areas, with physical barriers such as rivers and small mountain ranges having relatively little or no isolating effects (Atterby et al., 2015; Galov et al., 2014), a testament to the species' ability to occupy a range of habitats and disperse over large

distances (Walton et al., 2018). However, there is a consistent effect of peninsularity and insularity in creating genetically differentiated fox populations (Amaike et al., 2018; Galov et al., 2014), indicating a limited ability for this species to traverse narrow land bridges and large bodies of water. Having been present on the island for nearly two centuries and separated from mainland Britain by 1.2 km of open water, the Isle of Wight provides an interesting case study for investigating the long-term effect of insularity in promoting genetically differentiated fox populations.

We investigated i) the geographic origins of foxes inhabiting the Isle of Wight, and ii) whether they are genetically differentiated from foxes at nearby locations on the mainland in central southern England. To achieve this, we used two fragments of the mitochondrial genome, the D-loop and cytochrome-b (Cytb) gene.

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METHODS

Ear tissue samples were collected opportunistically from 57 wild foxes culled from 2018-2021 on the Isle of Wight and across six locations in central southern England (Figure 1) by professional wildlife managers during their routine fox control activities to protect ground-nesting bird populations (i.e., not culled specifically for use in this study). Samples were stored in individually labelled plastic wallets in a -20°C laboratory freezer. Mitochondrial DNA (mtDNA) was extracted using the QIAGEN DNeasy® Tissue Kit. A 305 base pair (bp) fragment of the D-loop was amplified using primers and protocol from Aubry et al. (2009). A 348 bp fragment of the Cytb gene was amplified using the primers and protocol from Perrine et al. (2007). Samples were sequenced in both directions using Sanger sequencing technology. Reactions were outsourced to GENEWIZ®.

D-loop and Cytb sequences were concatenated to a final alignment of 653 bp. To provide phylogenetic context with foxes across Europe, these sequences were aligned to a previously published dataset of 286 sequences from 14 countries (Statham et al., 2014), using the 'MUSCLE' algorithm (Edgar 2004) in MEGA v11.0.10 (Tamura et al., 2021). Final alignment was inspected visually. To evaluate the adequacy of our sample size, a rarefaction curve based on 1000 permutations was fitted to the Isle of Wight allelic richness data using 'vegan' (Oksanen et al., 2022) in R (R Core Team 2022). Two and three-parameter asymptotic exponential models were fitted to the rarefaction curve, with the better-fitting model used to estimate the asymptotic number of haplotypes (Pacioni et al., 2015). A median-joining haplotype network was constructed using PopART (Leigh & Bryant 2015) and pairwise F_{ST} between populations represented by \geq five samples were calculated using Arlequin v3.5.2.2. (Excoffier & Lischer 2010).

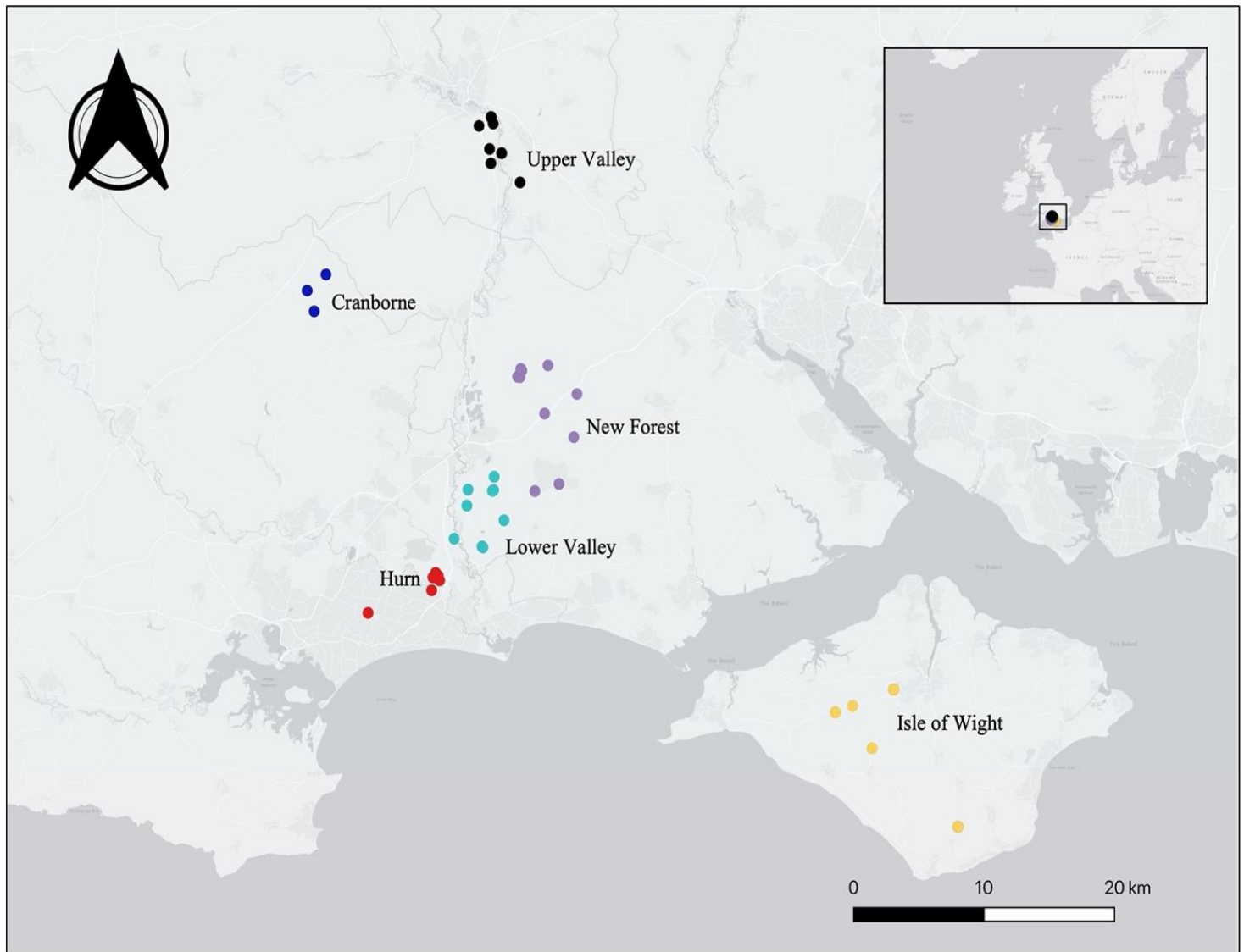


Figure 1: Map of central southern England with circles representing the locations of the 53 fox samples successfully sequenced

RESULTS

D-loop and Cytb sequences were successfully obtained from 53 out of 57 fox samples available in the study region. Four haplotypes were found on the Isle of Wight, of which two were private to the island considering the full European dataset, and both only a single mutational step away from their closest haplotypes (Figure 2). When fitted to the Isle of Wight rarefaction curve, the three-parameter model had a significantly better fit than the two-parameter model ($P < 0.0001$). The expected asymptotic number of haplotypes was 4.48 (95% CIs: 4.37 – 4.59), indicating that additional sampling would not reveal many additional haplotypes. There were two non-private haplotypes on the Isle of Wight, one of which was shared only with central England and the other with several locations in central southern England (Hurn, Cranborne, and the Lower Avon Valley) (Figure 2).

Pairwise F_{ST} values were relatively high (0.30-0.84; Mean: 0.59) between the Isle of Wight and all European populations (Figure 3). The pairwise F_{ST} values were significantly different between the Isle of Wight and adjacent populations in central southern England (mean pairwise $F_{ST} = 0.53$, SD = 0.16; Figure 3).

DISCUSSION

Our analysis of fox mtDNA sequences provides insight into the geographic origins of foxes on the Isle of Wight and the population genetic structure of the species in this region. Our results indicate that while the current population of foxes on the Isle of Wight were likely derived from mainland Britain, they are genetically differentiated from foxes in mainland central southern England.

There is no mention in the literature of foxes on the Isle of Wight until their introduction in the mid-19th century for sport hunting (The Leverets 2012). Inferring the geographic origins of these foxes is complicated given that foxes in Europe show a lack of phylogeographic structure based on mtDNA (Teacher et al., 2011), possibly due to relatively short periods of isolation during glacial maxima with gene flow occurring between regions during temporary warm phases (Kutschera et al., 2013). Nevertheless, our analysis indicates that the ancestry of the foxes sampled on the Isle of Wight can be pinpointed

to mainland Britain. The non-private haplotypes recorded on the Isle of Wight are only shared with central southern England and the Midlands.

It is surprising not to find more diverse haplotypes on the Isle of Wight, given the historically large numbers of foxes thought to have been imported from the continent (Carr 1976). It is possible that foxes from mainland Europe were introduced to the Isle of Wight but have gradually been supplanted by other lineages from mainland Britain due to the popularity of fox hunting on the island alongside other methods of population control. Additionally, despite indications that our sample size was adequate, samples were only collected from a relatively small part of the island; greater phylogenetic diversity might be uncovered through more widespread sampling.

Interestingly, the fox on the Isle of Wight shares a similar pattern to red squirrels (*Sciurus vulgaris*). Genetic analysis of red squirrels revealed a British origin for haplotypes sampled on the island despite the widespread presence of Scandinavian haplotypes on the mainland, indicating that in this case the existing population represents the remnants of the original British red squirrel population (Hardouin et al., 2019).

We found convincing evidence for genetic differentiation between the Isle of Wight and all mainland areas in central southern England. Elsewhere, genetic differentiation between mainland and insular fox populations, or those connected to larger areas via narrow land bridges, has been observed (Amaike et al., 2018; Langille et al., 2014). Foxes are capable swimmers - for example - a male fox was reported to have swum over 125 metres across a Danish fjord in flowing water (Olesen 2016). However, the minimum distance between the island and the mainland (1.2 km) in the strongly tidal waters of The Solent almost certainly represents a restrictive boundary to natural migration. Differences in haplotype frequencies between the Isle of Wight and mainland Britain are expected given that the island was likely founded by a relatively small number of individuals, with the barrier to gene flow preserving these genetic differences over time. In future work, a genome-wide approach will likely help to resolve the population genetic structure and biogeographic history of foxes in the region in more detail (McDevitt et al., 2022; Walton et al., 2021).

Table 1: Genetic variability of fox samples and pairwise F_{ST} values between sites in the present study.

Area	Location	No. samples	No. haplotypes	Haplotype diversity	Nucleotide diversity	Pairwise F_{ST}				
						Lower Avon Valley	Upper Avon Valley	New Forest	Hurn	Cranborne
		41								
Central southern England	Lower Avon Valley	10	4	0.80	0.010	-				
	Upper Avon Valley	9	3	0.67	0.009	0.25	-			
	New Forest	10	2	0.20	0.004	0.74	0.55*	-		
	Hurn	9	2	0.39	0.006	0.20	0.44*	0.60*	-	
	Cranborne	3	2	0.67	0.010	0.00	0.34*	0.03	0.18	-
Isle of Wight		12	4	0.65	0.004	0.49*	0.57*	0.74*	0.30*	0.53*

* $p < 0.05$

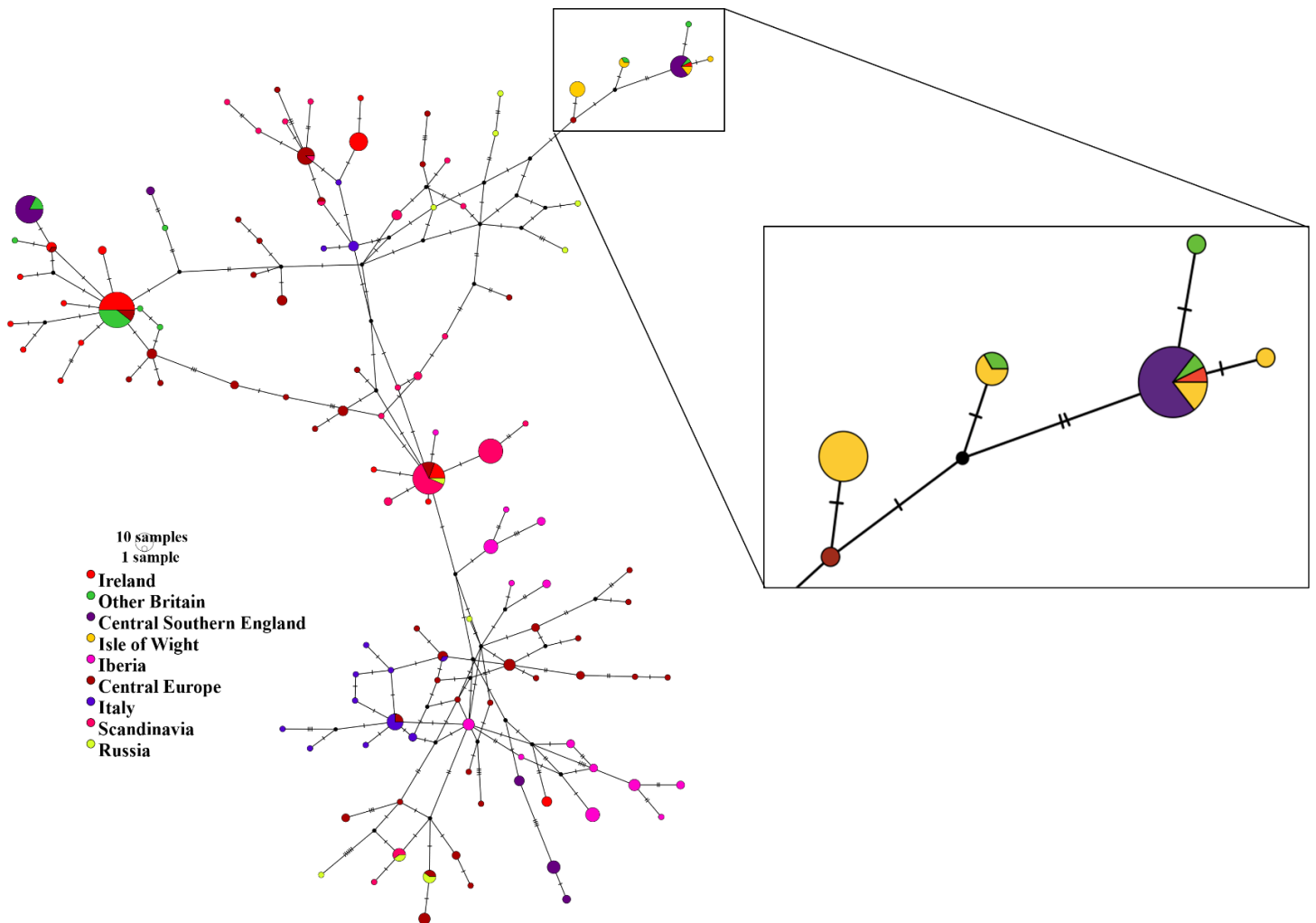


Figure 2: Concatenated D-loop and Cytb median-joining haplotype network for the entire dataset and an enlarged view of the branch with all the haplotypes sampled from the Isle of Wight. The size of the circle represents the frequency of the respective haplotype based on the 653 bp alignment, and the colours represent the populations of the individuals carrying a particular haplotype. 'Other Britain' refers to locations in Britain outside of the focal study region (Wiltshire, Hampshire, Dorset, and the Isle of Wight). Dashed lines represent number of base pair differences between haplotypes. Black circles represent internal nodes.

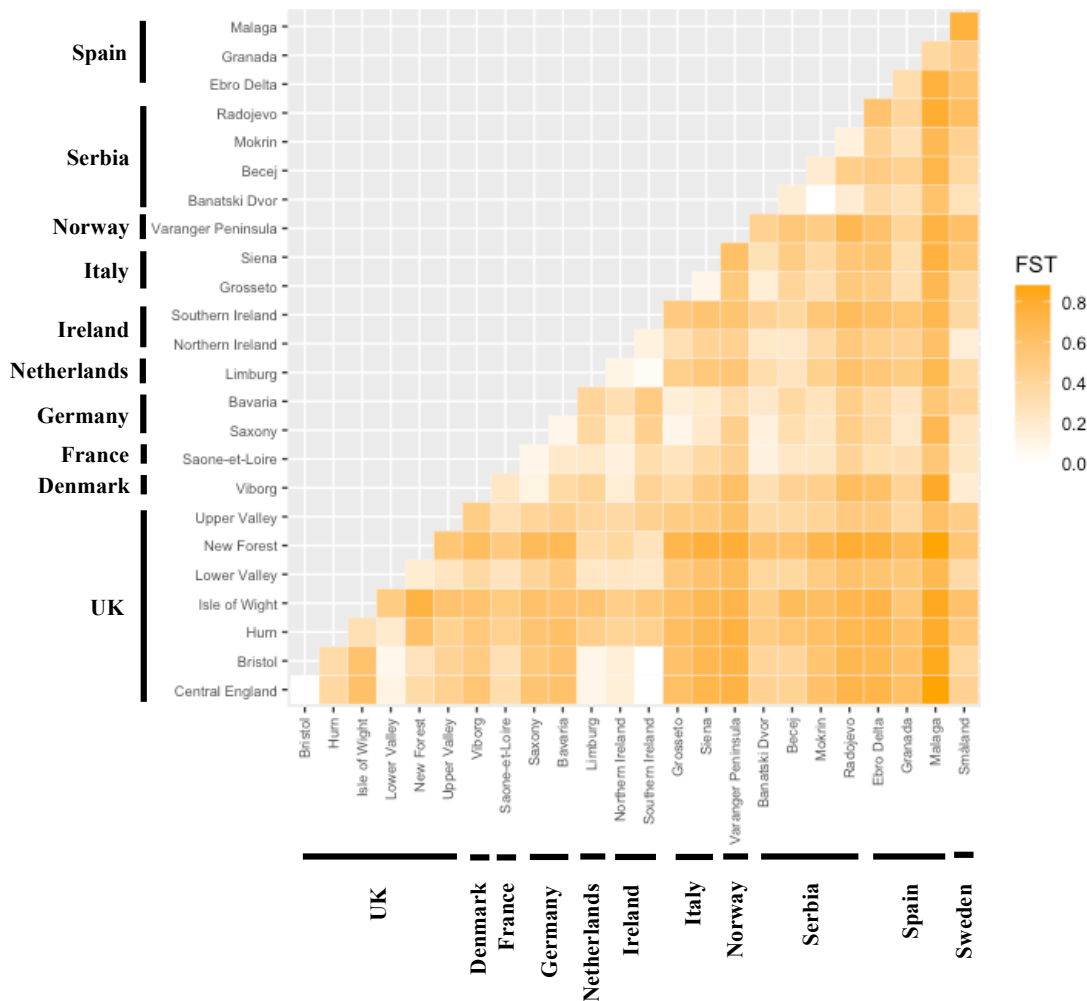


Figure 3: Pairwise F_{ST} heatmap for the populations across Europe with \geq five samples.

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