Evolutionary genetics of two sister taxa, Rüppell's fox (*Vulpes rueppellii*) and red fox (*Vulpes vulpes*)

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Summary

Interspecific hybridization can lead to introgression, but its genomic impact depends on the interplay of selection, drift and gene flow. The arid-adapted Rüppell's fox (*Vulpes rueppellii*) is thought to be the sister species of the red fox (*Vulpes vulpes*), albeit presumably nested within its mtDNA diversity, rendering it paraphyletic. This non-monophyly could indicate recent divergence of *V. rueppellii*, questioning its classification as a distinct species.

In this thesis, I generated and analysed high-resolution mitochondrial and genome-wide ddRAD-seq and whole genome resequencing data from both species, with focus on sympatric areas (North Africa and the Near East). I identified five mitochondrial clades, confirming with high support the paraphyly of *V. vulpes*: all *V. rueppellii* individuals clustered in 'Palearctic' clade, intermingled but not shared with *V. vulpes*. Furthermore, I reported for first time two mtDNA subclades of *V. rueppellii*. In contrast, species trees of autosomal loci showed the two species as overall strongly differentiated sister lineages. Whole genome data showed an ancient signal of gene flow from *V. rueppellii* into *V. vulpes*, while ddRAD-seq data from a larger sample size of individuals revealed recent signals in the opposite direction (a putative F1 hybrid found in Egypt), along with gene flow among *V. vulpes* populations. Genetic diversity appeared higher within *V. vulpes* populations than in those of *V. rueppellii.* Demographic analyses showed independent trajectories and fluctuations of effective population size in the two species, especially since the mid-Pleistocene aridity phase of the Sahara, previously suggested as the divergence time of the two species.

My findings highlight novel aspects about the biogeography and habitat flexibility of *V. vulpes*. Furthermore, the obtained findings suggest an early divergence and extended time for adaptation in *V. rueppellii*, followed by introgression – supporting its classification as a distinct species.

Chapter 1: General Introduction

1.1 Speciation

Species delimitation and understanding the process of speciation are key components of evolutionary biology (Seifert 2009; Jowers et al. 2014; Moutinho et al. 2020). Historically, speciation has been described against the backdrop of specific geographical contexts, classifying speciation into three main modes: allopatric (presence of an extrinsic barrier during divergence), parapatric (partial extrinsic barrier), and sympatric (no extrinsic barrier) (Butlin et al. 2008). Allopatric speciation was long considered the most common mode of speciation (Coyne and Orr 2004), but more recently, sympatric speciation has received more attention (Bolnick and Fitzpatrick 2007). From a theoretical perspective, allopatric and sympatric speciation are the ends of a continuum of initial different levels of gene flow among diverging populations (Gavrilets 2004). When considering the entire duration of the speciation process until complete reproductive isolation has been attained, consistent maintenance of the extreme conditions of allopatry or sympatry has been argued to be unlikely (Butlin et al. 2008). Indeed, clear evidence of pure cases of allopatric or sympatric speciation has been found in at best few natural systems (Coyne and Orr 2004; Bolnick and Fitzpatrick 2007). In recent years, research has focussed more on the processes governing reproductive isolation and ecological differentiation (Templeton 1981), and the genetic basis of barriers to gene flow (Butlin et al. 2008). From a diagnostic (species delimitation) perspective, therefore, it is not surprising to find 34 definitions of species concepts (Zachos 2018), although these are still debated as to their applicability and theoretical suitability.

Over recent decades, much work has focused on understanding the evolution of reproductive isolation between populations, and how this is impacted by the geographic mode of speciation (i.e., allopatric, parapatric, or sympatric) (Kondrashov and Kondrashov 1999; Gavrilets 2000). Other work has investigated the evolution of reproductive isolation when populations occur in different environments (e.g., ecological speciation; see Schluter, 2009) versus similar environments (e.g., nonecological speciation; Nosil & Flaxman, 2011). Natural selection can favour reproductive isolation and consequently lead to speciation, by limiting the chances of mixing between reproductively isolated gene pools (Rundle and Nosil 2005; Schluter 2009; Butlin et al. 2014). However, gene flow can erode the divergence between the populations, given the absence of barriers to dispersal (Felsenstein 1981; Smadja and Butlin

2011; Ravinet et al. 2017). Secondary genetic contact and introgression among primarily isolated taxa can render species delimitation difficult, leading to underestimation of species boundaries. Hence, during speciation, the spatial context and the extent of gene flow among demes are vital factors determining the degree of their reproductive isolation (Butlin et al. 2008; Kulmuni et al. 2020; Hernández-Hernández et al. 2021). Therefore, the biogeographical and demographic history of populations have a strong influence on their local adaptation and speciation. Periods of main ecosystem fluctuations can be key extrinsic drivers of such phylogeographic structuring (Chan et al., 2019; Pauls et al., 2013; Smadja & Butlin, 2011).

1.2 Biogeography of the Sahara

The Sahara is one area of biogeographical interest, due to its habitat diversity, landscape heterogeneity, and its complex paleoclimatic and geological history (Brito et al., 2014; Carranza et al., 2008; Douady et al., 2003; Gonçalves et al., 2012). Furthermore, North Africa is a biodiversity hotspot, and the Mediterranean region is one of the 36 biodiversity hotspots (see Myers et al. 2000). Phylogeographic studies have found evidence of diversification of Saharan species, e.g., induced by climate shifts during the Pliocene-Pleistocene interval (~ 5 million years ago; Mya) and the successive range shifts of the Sahara Desert (Carranza et al., 2008; Douady et al., 2003; Gonçalves et al., 2012; J. V. Leite et al., 2015; Sarabia et al., 2021; Velo-Antón et al., 2018). Desert-adapted species expanded their range during dry periods, and experienced reductions in distribution and population size during humid periods (Tamar et al. 2018; Moutinho et al. 2020). On the other hand, mesic and thus more water-dependent species show the opposite pattern, expanding during humid periods and contracting during the dry ones (Bertola et al., 2016; Cosson et al., 2005; Dinis et al., 2019; Husemann et al., 2014; Iyengar et al., 2007; Leite et al., 2015; Lerp et al., 2011). These climate fluctuations resulted in new selective pressures and/or geographic isolation among populations, paving the way for genetic diversification, adaptation, and eventually speciation (Brito et al., 2014; Lisón et al., 2019; Velo-Antón et al., 2018) .

1.3 Molecular markers and approaches to study populations history

Technical advances and development of novel types of molecular markers have led to the flourishing of population genetic analyses over the past three decades (Wan et al. 2004).

1.3.1 Traditional markers

1.3.1.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been used extensively in molecular phylogenetics, and population genetics to understand the evolutionary relationships among individuals, populations and species (Murtskhvaladze et al. 2020). The popularity of mtDNA-based approaches results from many reasons: high copy number per cell, availability of universal primer sequences, its haploid nature and lack of genetic recombination, an accelerated mutation rate compared to nuclear DNA loci, short coalescence time (due to low effective population size compared to nuclear DNA), and its maternal inheritance (Hutchison et al. 1974; Brown et al. 1979; Boore 1999; Gissi et al. 2008; Meiklejohn et al. 2014; Mazzatenta et al. 2021).

One common application of mtDNA sequencing is DNA barcoding, which in animals typically relies on mtDNA genes such as Cytochrome c oxidase I subunit 1 (COI) or 16S ribosomal RNA (rRNA) (Hebert et al. 2003). Extracting mtDNA from museum collections is often feasible, owing to the high copy number of the organelle per cell. Museum specimen DNA barcoding is very important in taxonomy, as it directly links type material to its genetic identifier/barcode (Timmermans et al. 2016). Although some concerns about issues with contamination associated with conventional PCR-based methods have been raised, some of these limits can be overcome by new high-throughput sequencing (HTS) and assembly of full mitogenome with improved bioinformatics (Desalle et al. 2017). Full, or near-complete mitogenome assembly has become increasingly feasible in recent years, as large numbers of off-target mitogenomic reads are often generated by sequence capture or whole-genome sequencing efforts (Meiklejohn et al. 2014) without additional consumables costs or laboratory effort.

With recent advances in HTS approaches, reliance on mtDNA as a tool has decreased. For instance, a survey of phylogeographic literature by Garrick et al., (2015) showed that the use

of nuclear Single Nucleotide Polymorphism (SNP) markers has increased significantly. However, that survey also showed that mtDNA has remained an essential marker in phylogeographic studies, alongside with and as a comparison with nuclear markers.

1.3.1.2 Nuclear genes

Genic regions of the nuclear genome consist of exons (coding sequences) and introns (non-protein-coding). Exons typically present low levels of intraspecific variation and are therefore, rarely used for population genetic studies (Brito and Edwards 2009; Igea et al. 2010). In contrast, introns have long been used in multilocus phylogeographic analyses (Palumbi and Baker 1994; Friesen et al. 1997; Bensch et al. 2006) owing to their greater genetic variability and ease of PCR amplification with primers binding to the flanking exons (Igea et al. 2010). However, also some introns show a high degree of conservation, e.g., due to involvement in certain cellular/biochemical functions (Rodova et al. 2003; Gazave et al. 2007). Sequencing of relatively few intron loci has successfully resolved phylogenetic relationships at higher taxonomic levels (e.g., at subfamily, family, or sub-order level), for instance, in mammals: the family Ursidae (Pagès et al. 2008), superfamily Muroidea (Steppan et al. 2004), the infra-class metatheria (Meredith et al. 2008) and family Phocidae (Slade et al. 1994); birds: order Charadriiformes (Paton and Baker 2006) and infraclass Palaeognathae (Haddrath and Baker 2012); reptiles: superfamily Colubroidea (Lawson et al. 2005); amphibia: order Anura (Hoegg et al. 2004) and insects: superorder Holometabola (Wiegmann et al. 2009). However, inference of phylogeny/evolutionary signals among closely related species requires analysis of a larger number of loci to increase the resolution, as the number of informative sites in nuclear genes is typically small (Brito & Edwards, 2009). Moreover, this approach when based on PCR and Sanger sequencing is time consuming and costly, and tends to scale up less efficiently compared with HTS techniques (see below). Therefore, Sanger sequencing of nuclear loci has largely been replaced by SNPs obtained from HTS (Brito & Edwards, 2009).

1.3.1.3 Microsatellites

Microsatellites (or simple sequence repeats, SSRs) have been one of the workhorses of phylogeographic (Hodel et al. 2017) and population genetic studies (Zachos et al. 2006; Hajji et al. 2007; Zachos et al. 2008; Zachos et al. 2009; Shakarashvili et al. 2020) . They comprise short, tandemly repeated DNA motifs (typically one to six nucleotides) found in high frequency

throughout eukaryotic genomes (Li et al. 2002; Zane et al. 2002; Selkoe and Toonen 2006). Their high mutation rate (between 10^{-3} and 10^{-4} per locus per generation, Li et al., 2002) and thus polymorphism, render them a powerful tool for distinguishing between closely related conspecific individuals and taxa (Guichoux et al. 2011; Kalia et al. 2011; Hodel et al. 2016). However, there are some drawbacks to using microsatellites. Most importantly, their unusually high mutation rates do not reflect those across non-repetitive areas of the genome, and homoplasy and saturation can impact signals from long divergence times, making microsatellites challenging/unsuitable to use for evolutionary comparisons between distant species (Hodel et al. 2016; Hodel et al. 2017). Also, the large number of alleles per locus associated with microsatellites can inflate F-statistic estimates relative to biallelic markers, such as SNPs (Whitlock 2011). Additionally, genotyping errors can bias downstream analyses (Taberlet and Waits 1999; Hoffman and Amos 2005). Finally, only a limited number of loci (usually <25) is applied in a typical microsatellite-based study (Hodel et al. 2017), yielding only sparse coverage of the whole genome. Therefore, microsatellite markers are increasingly being replaced by HTS-based approaches such as reduced representation sequencing (RRS) for phylogeographic and population genetic inferences (Hodel et al., 2017; Seeb et al., 2011; Sunde et al., 2020).

1.3.2 High-throughput sequencing (HTS) techniques

Advances in high-throughput sequencing techniques have opened the door for evaluation of thousands to millions of genetic markers across genomes and populations (Timm et al. 2018). These approaches include RRS and whole genome sequencing.

1.3.2.1 Reduced-representation sequencing (RRS)

Reduced-representation sequencing (RRS) approaches are a family of methods which attempt to subsample the genome in a reproducible way, to obtain an ideally unbiased view of genomic variability. There are usually used in combination with HTS approaches, generating large amounts of sequence data. One commonly used RRS approach is restriction-site associated DNA sequencing (RAD-seq), a cost-effective method to obtain tens of thousands of genome-scale SNPs across fractions of the genome from non-model organisms (Davey et al. 2011; Lemmon and Lemmon 2013; Wright et al. 2019). The main idea of this approach is to cut the DNA using restriction enzyme(s) and then to sequence a specific size-selected

subset of the resulting fragments (Timm et al. 2018). Several versions of RAD-seq have been developed: single-digest RAD-seq (sdRAD-seq) uses one cutting restriction enzyme plus a sonication step to generate short fragments for sequencing (Miller et al. 2007; Baird et al. 2008); double-digest RAD-seq (ddRAD-seq) uses two restriction enzymes, omitting the sonication step (Peterson et al. 2012); but a diversity of others exist (see e.g., review by Andrews et al., 2016). Multiple studies have demonstrated the utility of RAD-seq for phylogenetic reconstruction and fine-scale population substructure (Jones et al. 2013; Keller et al. 2013; Ogden et al. 2013; Roda et al. 2013; Henning et al. 2014; Sutherland et al. 2016), identifying genomic regions involved in hybridization (Hohenlohe et al. 2013), speciation (Jones et al. 2013), and divergent adaptation (Keller et al. 2013).

Among RAD-seq methods, ddRAD-seq uses a combination of two restriction enzymes and library size selection to reproducibly recover fragments randomly from across the genome. Thus, it provides more uniformity and replicability across samples in the selection of fragments for sequencing than the other RAD-seq methods for generating reduced representation libraries (Andrews et al. 2016; Lavretsky et al. 2019). Furthermore, because ddRAD-seq hence generates libraries containing a greater portion of homologous fragments within and among individuals, it tends to produce higher sequencing depths at each locus, which is useful for accurate variant calling (i.e., rather than scoring sequencing errors or false homozygotes; Peterson et al., 2012; Valencia et al., 2018). ddRAD-seq has been used to identify hybrid individuals, founder events, population structure and genomic regions under divergent selection in birds (Lavretsky et al. 2015; Peters et al. 2016; Lavretsky et al. 2019).

Despite these advantages, also ddRAD-seq approaches have certain limitations: for instance, bias may be introduced at several stages in a RAD-seq protocol: (1) polymorphisms in restriction sites can affect the presence/absence of polymorphisms that are difficult to detect without very deep sequencing (Arnold et al. 2013; Andrews et al. 2016); (2) low sequencing depth and sequencing errors can lead to incorrect variant calling (Andrews et al. 2016); (3) preferential PCR amplification of some loci inevitably reduces coverage of other loci (Arnold et al., 2013), and (4) misassembly of paralogous reads can lead to inference of false heterozygote positions (Xu et al. 2014). The arguably biggest issue with RRS methods is that they only provide data from a small subset of the entire genome, hindering inferences where

increased resolution in terms of numbers of loci is required, or where the whole genomic needs to be characterised (see next section).

1.3.2.2 Whole genome resequencing

Whole genome sequencing can be categorized into de-novo whole-genome sequencing (WGS), which includes de-novo assembly of the data, and whole genome resequencing (WGR). WGS is the sequencing and assembly of a genome sequence for the first time, while WGR requires a reference genome for read mapping and variant calling (Fuentes-Pardo and Ruzzante 2017). WGR has been used to obtain millions of SNPs across the genome in several species, and has allowed researchers to address a variety of questions in molecular ecology and evolutionary genetics (Foote et al., 2016; Hohenlohe et al., 2010; Lamichhaney et al., 2017). Below is a summary of the contribution of WGR to some areas in molecular ecology and evolutionary genetics.

(A) Genome scans for selection/adaptive introgression:

Detecting population-specific signatures of natural selection require genome-wide sequencing or high-density SNP genotyping to provide sufficient statistical power to detect signatures of selection against a background of neutral variation (Nielsen 2005). High-density SNP genotyping or sequencing allow assessment of effects of neutral process (e.g., genetic drift and gene flow) across the genome, against which the detection of a different evolutionary forces, broadly including variations of selection or neutral genetic drift, becomes feasible, by parameter estimation in sliding windows across the genome. Many studies have highlighted the power of WGR in detecting candidate genes across the genome. In the Russian farm-fox experiment, Kukekova et al. (2018) found some outliers with high F_{st} (the fixation index F_{st} is defined as the proportion of the total genetic variance contained in a subpopulation – the S subscript, relative to the total genetic variance – the T subscript), containing candidate genes related to the tame and aggressive behaviour. Barbato et al. (2017) reported adaptive introgression from mouflon to domestic sheep of alleles related to immunity mechanisms. In hot-desert fat-tail sheep breeds, many candidate genes enriched

for fat metabolism, responses to heat and UV radiation, kidney function and DNA repair have been identified (Kim et al. 2016; Mwacharo et al. 2017).

(B) Population structure and admixture

Many studies have demonstrated the usefulness of WGR in the estimating of population structure and admixture. For instance, whole genome SNP data were used to access the population structures of Korean native pigs, wild boar and three European origin breeds, showing clear population clusters with respect to each breed (Choi et al. 2015). Parejo et al. (2016) found genetic differentiation between subspecies of bees that coincided with geography, and admixed individuals in protected areas. In birds, differentiation of four species of the Western Palearctic black-and-white flycatchers of the genus *Ficedula* has been estimated at 1-2 Mya using WGR (Nadachowska-Brzyska et al. 2016). Also, it has been found that few thousand SNPs provide a better resolution of genetic diversity and genetic differentiation among populations of the plant *Arabidopsis helleri* than 19 microsatellites (Fischer et al. 2017). Velasco et al. (2016) identified ~7-fold higher genetic diversity in peach (*Prunus persica*) than almond (*P. dulcis*) in a study of the effect of mating system and the domestication on their genetic diversity. Also, WGR has been used in detecting hybridization in many taxa. For example, vonHoldt et al., (2016) reported that the two endemic North American wolves, the red wolf (*Canis rufus*) and the eastern wolf (*C. lycaon*) represent hybrids of coyote (*C. latrans*) and grey wolf (*C. lupus*). Wall et al. (2016) identified multiple hybridization events between yellow baboons (*Papio cynocephalus*) and Anubis baboons (*P. anubis*) in the Amboseli ecosystem of Kenya with no indication of fitness reduction in hybrids.

(C) Phylogenomics and taxonomical species resolution

The aim of phylogenomics (i.e., the study of evolutionary relationships among taxa based on genomics) is to reconstruct the evolutionary relationship among focal taxa (Chan & Ragan, 2013; Delsuc et al., 2005). This can be achieved by WGR data which represent a more comprehensive record of the evolutionary history of the taxa than approaches which sample only a sparse subset of the genome (Fuentes-Pardo and Ruzzante 2017). For example, phylogeny of 48 modern bird species was reconstructed and obtained a highly resolved tree with a discrimination of closely related species (Zhang et al., 2014). Straub et al. (2011)

characterized the phylogenetic markers for the common milkweed (*Asclepias syriaca*), including the complete chloroplast genome, a partial mitochondrial genome sequence, and some single copy ortholog genes.

(D) Demographic history and historical effective population size

The study of demographic history of species helps identify historical events that have affected the genetic variability and structuring of present-day populations. WGR has been used to study the change of the effective population size of several species (Fuentes-Pardo and Ruzzante 2017). For example, Sarabia et al., (2021) studied the demographic history of African golden wolves (*Canis lupaster*) and detected a correlation between divergence times and the fluctuation of climate changes during the Pleistocene. Zhou et al. (2014) reconstructed species-specific demographic histories for snub-nosed monkey (*Rhinopithecus roxellana*) and other three closely related species. Also, analysis of a dataset of 34 panda genomes provided genetic evidence of multiple demographic events such as population expansion, bottlenecks and divergence (Zhao et al. 2013). Foote et al. (2016, 2019) found out that the pattern of differentiation between contemporary allopatric and sympatric ecotypes of the killer whale (*Orcinus orca*) most likely reflects their ecological divergence, but also genetic drift resulting from bottlenecks during past founder events.

1.4 Mito-nuclear discordance

Mito-nuclear discordance is defined as "Significant difference in the patterns of differentiation between mtDNA and nuDNA (nuclear DNA) markers , where either mtDNA is more structured than the nuDNA, or vice versa" (Toews and Brelsford 2012). Because lack of recombination, mtDNA is inherited as one single unit and therefore considered a single genetic locus. As such, it does not (necessarily) mirror genetic signals from the rest of the genome (Bidon et al., 2014; Hailer et al., 2012; Zhang & Hewitt, 2003), being subject to the issues inherent to individual gene tree of any locus (reviewed in Rubinoff & Holland, 2005).

Several reasons have been suggested to explain Mito-nuclear discordances, but many of them are speculative and difficult to prove (Toews and Brelsford 2012; Bonnet et al. 2017). (1) Sex-biased gene flow and/or introgression: As a maternally inherited marker, sex-biased dispersal

can create different spatial patterns for sex-linked loci (Walton et al. 2021), such as mtDNA, X and Y chromosomes in mammals. For example, emergence of female kin-structured populations due to lower female than male dispersal is expected to lead to lower effective population size (N_e) and higher levels of genetic drift in mtDNA than nuDNA (Bernardo et al. 2019). (2) Selection on mtDNA may result in a discordance signal with nuDNA (Bonnet et al. 2017). Such conflicting signals between mtDNA and nuDNA can, if the signal is strong, result in diagnosis as Mito-nuclear discordance. (3) Incomplete lineage sorting (ILS) and introgression (Toews and Brelsford 2012; Mutanen et al. 2016). Among all the previous, ILS may result in Mito-nuclear discordance, when not enough time has elapsed for differentiation of the lineages to occur. However, (4) Introgression may yield the same pattern, making it difficult to distinguish the two signals from each other (Funk and Omland 2003; Buckley et al. 2006; Peters et al. 2007; Wang et al. 2014). Introgression events become progressively more difficult to detect with increased time since hybridization, since geographical signals of introgression (e.g., shared haplotypes in areas of sympatry) are eroded by recombination, mutation and range changes (Funk and Omland 2003; Ivanov et al. 2018). Introgression and incomplete lineage sorting (ILS) have been proposed as the main factors leading to Mito-nuclear discordance (Scornavacca and Galtier 2017; Tamashiro et al. 2019). Additional factors behind Mito-nuclear discordance that have been proposed include (5) presence of pseudogenes in nuclear DNA (NUMTs) (Leite 2012; Song et al. 2014), and (6) unresolved phylogenetic polytomies which may falsely be taken as evidence of discordance among loci (Caraballo et al. 2012).

1.5 Natural history of *V. vulpes* and *V. rueppellii*

The red fox *Vulpes vulpes* and Rüppell's fox *V. rueppellii* are sister species occurring in sympatry in the Middle east and North Africa (Geffen et al., 1992; Leite et al., 2015; Lindblad-Toh et al., 2005). *V. vulpes* has the widest natural distribution of any terrestrial carnivore and possibly any terrestrial wild mammal in the world (Wozencraft 2005; Macdonald and Reynolds 2008). Its range spans much of Europe, Asia and North America, and extends into North Africa, where it occupies mesic habitats along the Nile River, Mediterranean region and desert oases. There is also an introduced population in Australia (Macdonald and Reynolds 2008) (Fig. 1.1).

Lariviere and Pasitschniak-Arts (1996) recognized 44 subspecies of *V. vulpes* based on the morphological data, although many are doubtful (Sillero-Zubiri et al., 2004). In 2010, another distinct subspecies, which inhabits the grasslands of California, USA in the Sacramento Valley was identified based on a combination of mtDNA and morphological data (Sacks et al., 2010). The species occupies a wide variety of ecosystems, including grasslands, forests, deserts and agricultural and human-dominated environments (Lariviere and Pasitschniak-Arts 1996). The outstanding adaptability of this opportunistic omnivores is clearly manifested in its capability to feed on a wide variety of food items including small mammals, birds, fishes, invertebrates and fruits, which allows it to survive in a broad diversity of environments (Basuony et al., 2005; Flower, 1932; Macdonald, 1979; Osborn & Helmy, 1980; Sillero-Zubiri et al., 2004).

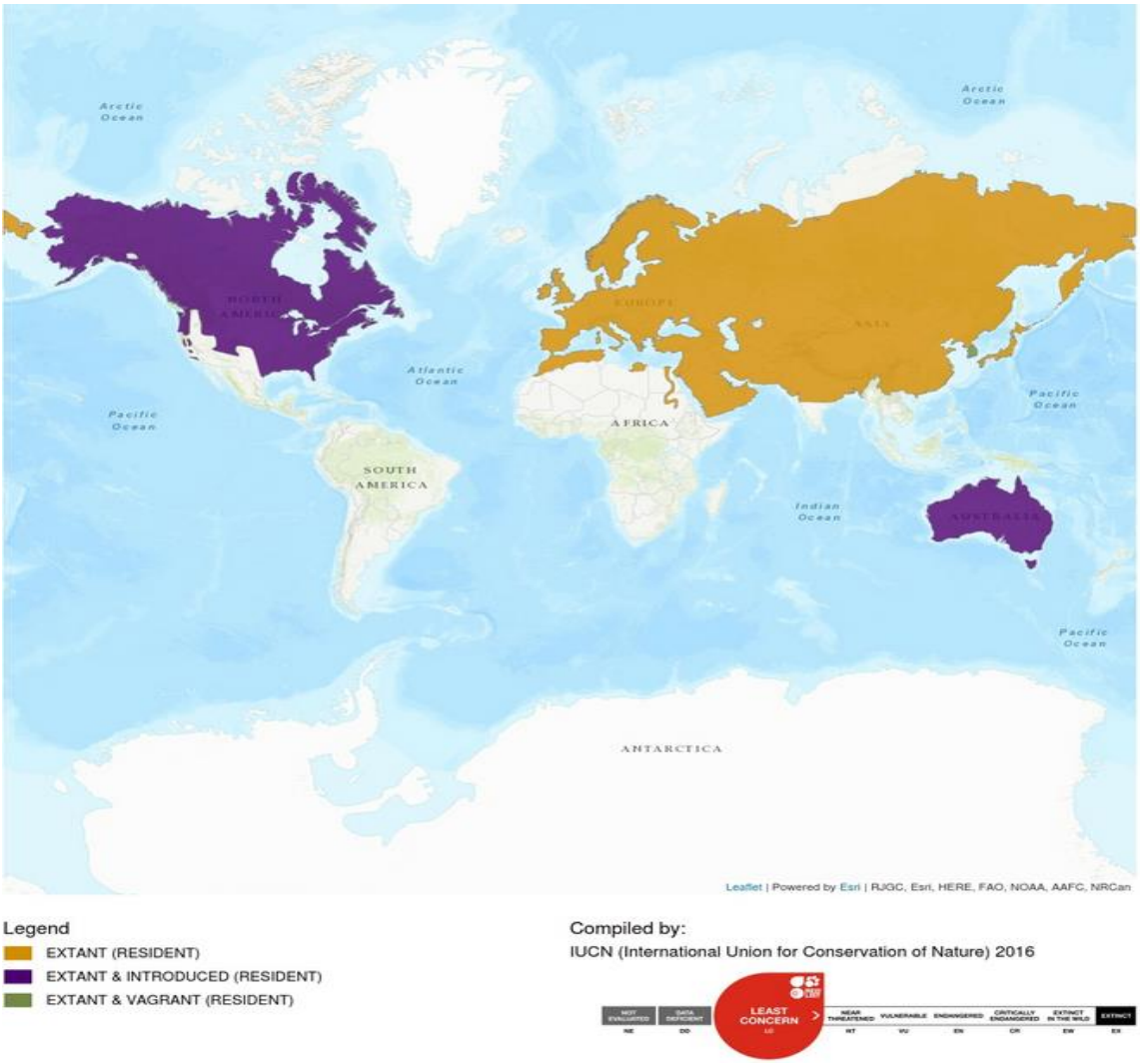

Figure 1.1: Global distribution of *V. vulpes*. (Modified from: IUCN, 2016)

On the other hand, *V. rueppellii* is an inhabitant of the desert zone of North Africa and Asia. Its geographical range comprises deserts and semi-deserts of North Africa from Mauritania to Somalia, and of Asia from the Arabian Peninsula to Iran and Pakistan (Fig. 1.2), with up to six described subspecies (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004; Mallon et al. 2015). The habitats of *V. rueppellii* are characterized by a low vegetation cover and few grass species, and receive only little rainfall (Mallon et al. 2015). These habitats include sand dunes, sand sheets and gravel plains (Murdoch et al. 2007), stony habitats (Lenain 2000) and coastal areas (Mallon et al. 2015). Considered an opportunistic species (Olfermann 1996; Lenain 2000) , *V. rueppellii* feeds on rodents, lizards, birds, snakes, wild fruits as well as a wide range of invertebrates (Kingdon, 2015; Kowalski, 1988; Lindsay & Macdonald, 1986; Osborn & Helmy, 1980; Valverde, 1957).

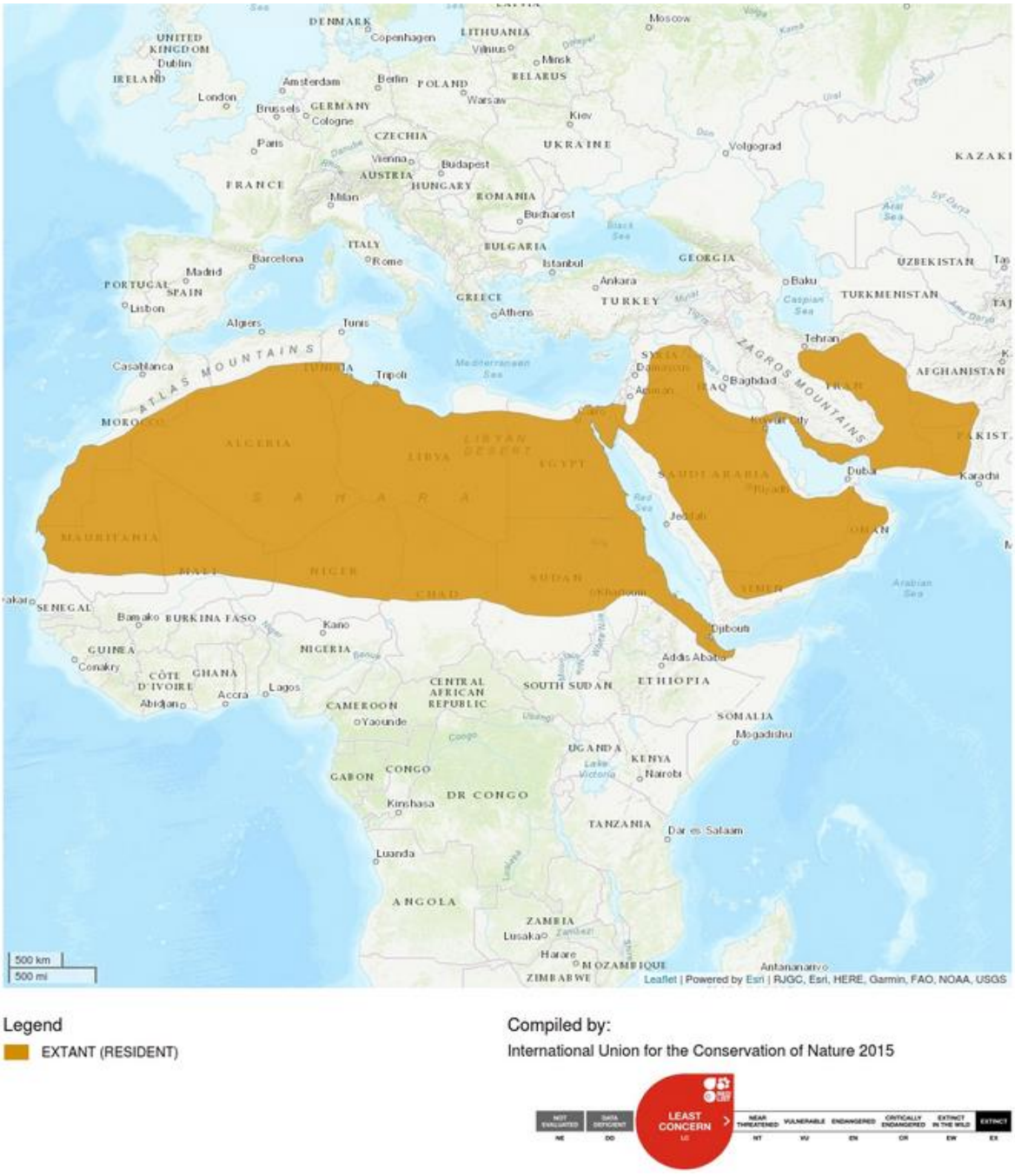

Figure 1.2: Global distribution of *V. rueppellii*. (Modified from: IUCN, 2015.)

The two species are morphologically different. An analysis of external measurements including head and body length, tail length, ear length, shoulder height and weight of *V. rueppellii* from Arabia (Lenain 2000) and Egypt (Osborn and Helmy 1980), and *V. vulpes* from across its distribution except North Africa (UK, Hattingh, 1956; Australia, McIntosh, 1963; Canada, Voigt, 1987; Japan, Zhan et al., 1991 and several studies from Cavallini, 1995) showed

a considerable difference between the two species (Sillero-Zubiri et al. 2004). *V. rueppellii* is smaller, and has shorter hind legs, a shorter tail, longer ears, and a smaller and more delicate skull than *V. vulpes* (Lariviere and Seddon 2001). The ability of *V. rueppellii* to survive in hyper-arid environments where the water is extremely rare, is believed to be facilitated by various mechanisms. These include morphological adaptations (e.g., large ears, coat colour, hair on feet), and behavioural (e.g., nocturnal activity), which assist in thermoregulation (Williams et al. 2002; Sillero-Zubiri et al. 2004), besides acquiring most of its moisture requirements by feeding on plant materials (Rosevear 1974; Lenain 2000). Although a competition for food resources has been reported between the two fox species (Cuzin 2003; Sillero-Zubiri et al. 2004), *V. rueppellii* tends to occupy areas which are more arid and marginal for *V. vulpes* (Wacher & Attum, 2005).

1.6 Previous genetic studies of *V. vulpes* and *V. rueppellii*

As a widespread and common species, *V. vulpes* has received much more attention and been the focus of many more phylogenetic and population genetic studies than *V. rueppellii.* During the past four decades, *V. vulpes* has been studied extensively in various regions across its range, while *V. rueppellii* has been much less studied. There are comprehensive studies dealing with the phylogeographic structure and pattern of genetic diversity in *V. vulpes* populations, using allozymes (Frati et al. 1998; Simonsen et al. 2003), random amplified polymorphic DNA (RAPD) markers (Gachot-Neveu et al., 2009; Stepniak al., 2002), mitochondrial DNA (Aubry et al., 2009; Belda & Larriba, 2017; Edwards et al., 2012; Fernandes et al., 2008; Frati et al., 1998; Galov et al., 2014; Goldsmith et al., 2016; Ibiş et al., 2014; Inoue et al., 2007; Karssene et al., 2019; Kirschning et al., 2007; Kutschera et al., 2013; Leite et al., 2015; Norén et al., 2017; Perrine et al., 2007; Sacks et al., 2010; Statham et al., 2011, 2012, 2014; Teacher et al., 2011; Telcİoğlu et al., 2019; Valière et al., 2003; Volkmann et al., 2015; Wallén et al., 2018; Yannic et al., 2017; Yu et al., 2012), microsatellite data (Lade et al. 1996; Wandeler et al. 2003; Kukekova et al. 2004; Wandeler and Funk 2006; Sacks et al. 2010; Oishi et al. 2011; Sacks et al. 2011; Mullins et al. 2014; Atterby et al. 2015), and single nucleotide polymorphisms (Sacks et al. 2011; Johnson et al. 2015; Sacks et al. 2018; McDevitt et al. 2021;

Walton et al. 2021). Most previous studies were conducted on *V. vulpes* in North America or Europe, and to a lesser extent in Asia (Inoue et al. 2007; Yu et al. 2012; Statham et al. 2014; Leite et al. 2015; Telcİoğlu et al. 2019). Meanwhile, North African populations of *V. vulpes* have received little attention, with only three studies: Statham et al. (2014) based on cytochrome b and D-loop; Leite et al., 2015 (cytochrome b, D-loop and microsatellites); Karssene et al., 2019 (cytochrome b and D-loop).

From this work, several mtDNA phylogroups of *V. vulpes* have been identified: a Nearctic clade (found only in North America; (Inoue et al. 2007; Aubry et al. 2009; Yu et al. 2012; Kutschera et al. 2013; Statham et al. 2014), Holarctic clade (distributed across Eurasia, North Africa and North America; Statham et al., 2014), African clade (restricted to North Africa; Statham et al., 2014; Leite et al., 2015), and the 'Palearctic basal haplotypes'- a group of haplotypes found in North Africa and Asia, but receiving insufficient statistical support to be robustly defined as a distinct clade (Statham et al. 2014). In fact, both African and Palearctic basal haplotypes received relatively low support (Bayesian posterior probability, BPP: 0.79 for African and <0.50 for the Palearctic basal haplotypes, Statham et al. (2014)), which could be related to the small sample size of haplotypes from these clades across previous studies. Importantly, Statham et al. (2014) emphasized that the North African range remains only relatively sparsely characterized to date. Among the three previous study, only Leite et al. (2015) looked at the phylogenetic relationships between the two species based on mtDNA (cytochrome b and D-loop) and microsatellites. Based on mtDNA, Leite et al. (2015) reported the clustering of *V. rueppellii* with African *V. vulpes*, leading to paraphyly of the latter. This finding casts doubt on the status of *V. rueppellii* as a distinct species.

1.7 Aims and structure of the thesis

The reconstruction of the evolutionary history of the two focal fox species requires a combination of data from different genetic markers, bearing in mind that different parts of the genome might reflect different evolutionary histories. In this thesis, I studied the evolutionary history of *V. vulpes* and *V. rueppellii* by gathering evidence from two main classes of markers, each with different inheritance modes: maternally inherited mtDNA, and

biparentally inherited (autosomal) single nucleotide polymorphisms (SNPs). The layout of the chapters is as follows:

Chapter 2: I sequenced longer fragments (than in most previous studies) of the mtDNA loci cytochrome b and D-loop from 116 samples, focusing the sampling on previously poorly sampled or unsampled geographic regions across North Africa and the Middle East. I combined the newly obtained data with formerly published sequences from GenBank.

Aims

(2.1) Synthesise the mtDNA phylogeny of the two species and see if analysis of longer sequence fragments will improve the support of the poorly supported clades from previous studies.

(2.2) Assess the validity of the previously reported paraphyly of *V. vulpes*.

Hypotheses

The paraphyly is correct and *V. rueppellii* is a subset of *V. vulpes* variation. Alternatively, the two species might be more clearly differentiated than previously thought, and the supposed paraphyly resulted from either lack of spatial sampling (e.g., undetected mtDNA lineages in *V. rueppellii*) and/or too low resolution due to sequencing of short fragments in previous studies.

Chapter 3: I sequenced, assembled and characterized the first near-complete mitogenome of *V. rueppellii*, using this sequence along with other fox mitogenomes from Genbank.

Aim: To study the phylogenetic relationship of *V. rueppellii* with *V. vulpes.*

Hypotheses

Increased phylogenetic resolution from whole mitogenome sequences could support or contradict the results from chapter 2.

Chapter 4: I used ddRAD-seq to generate thousands of genome-wide SNPs for ca. 100 individuals of both species, focussing mainly on populations from North Africa and the Middle East.

Aims

(4.1) Investigate the degree of genomic differentiation between the two species.

(4.2) Determine the levels of the genome-wide genetic variability within each species, and among presumably geographically isolated populations of each of the two foxes, using biparentally inherited markers.

Hypotheses

The two species could be reciprocally monophyletic in nuclear genomic species trees, genomically strongly differentiated, supporting that *V. rueppellii* is a distinct species. In this case, the reported mtDNA paraphyly could reflect introgressive gene flow after the original speciation.

Alternatively, similar as for mtDNA, nuclear genomic markers might confirm the phylogenetic placement of *V. rueppellii* within a broader genetic diversity of *V. vulpes.*

Genetic variability of *V. vulpes* could be higher than that of *V. rueppellii*, owing to the wide distribution range of the former and its high adaptability to different habitats.

Chapter 5: I used whole-genome resequencing, generating millions of SNPs from nine representative individuals (seven *V. vulpes* and two *V. rueppellii*, chosen to achieve a broad geographic representation of the main populations, and of mitochondrial clades from chapter 2). Also, I extracted whole mitogenome sequences from the whole genome sequencing data, comparing results from different bioinformatic approaches.

Aims

(5.1) Estimate the autosomal genomic differentiation of the two species at a high-resolution level that might clarify weaker/older signals that might have been missed or underestimated based on ddRAD-seq in chapter 4.

(5.2) Reconstruct changes in effective population size of both species during the climatic fluctuations of the Quaternary.

(5.3) Attempt to obtain (near-)complete mitogenome sequences for all major mtDNA clades identified in chapter 2.

(5.4) Compare the efficiency and accuracy of four de-novo and reference-based read mapping approaches for mitogenome recovery from whole-genome shotgun sequencing data.

(5.5) Use these mitogenome sequences to obtain a well-resolved mtDNA phylogeny of all major clades/lineages, allowing a re-assessment of *V. vulpes* paraphyly.

Hypotheses

The signal of the genomic differentiation between the two species from whole genome SNPs might likely reflect the signal from ddRAD-seq, assuming none of the two approaches would yield biased sets of SNPs. Improved resolution from whole-genome SNPs might improve the statistical power to detect old and/or weak signals of introgression among the two species.

The demographic history of the two species has been significantly affected by the climate oscillations during the Pleistocene, with the two species possibly showing different/independent evolutionary trajectories since their separation.

Whole mitogenome sequences would be predicted to significantly improve the statistical support for branches with low supported in analysis of shorter sequences. The obtained tree would, if based on full mitogenome sequences, offer maximum resolution for phylogenetic re-assessment of *V. vulpes* paraphyly.

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Chapter 2: Paraphyly of The Widespread Generalist Red Fox (*Vulpes vulpes*): Introgression Rather Than Recent Divergence of The Arid-Adapted Rüppell's Fox (*V. rueppellii*)?

2.1 Introduction

Except for unusual cases such as hybrid speciation (Lavrenchenko 2014; Lamichhaney et al. 2018; Masello et al. 2019), the evolution of distinct species is typically considered a process that, given enough time of reproductive isolation, will lead to reciprocally monophyletic lineages. During the Pleistocene, populations of many mammalian species were separated into distinct refugia and evolved pronounced phylogeographic structuring (Avise et al., 1998; Lister, 2004; Stewart, 2009; Morales-Barbero et al., 2017). This differentiation has in some cases warranted recognition either at the subspecies level, e.g., key deer *Odocoileus virginianus clavium* (Lister 1995) and marmots *Marmota sp.* (Polly, 2003), or at the species level, e.g., polar *Ursus maritimus* and brown *U. arctos* bears (Talbot and Shields 1996); *Lynx sp.* (Kurtén & Anderson, 1981; Johnson & O'Brien, 1997) and shrews *Sorex sp.* (Hoffmann 1981; Conroy and Cook 2000).

Coalescent theory predicts that the lineage sorting process – which depends on effective population size (N_e) (Nichols 2001) – is slow, implying that certain alleles in one species may appear more closely related to alleles from different species than to other conspecific alleles (Funk and Omland 2003; Hailer et al. 2013). This deviation from species-level monophyly can result in paraphyly. Paraphyletic patterns have been reported previously and related to (1) incomplete lineage sorting (ILS), e.g., in birds (Suh et al., 2015), European bison *Bison bonasus* (Wang et al. 2018), and salmonids (Campbell et al. 2020); or (2) introgression, e.g., chipmunks *Tamias ruficaudus* and *T. amoenus canicaudus* (Good et al. 2008), hares *Lepus granatensis* and *L. timidus* (Melo-Ferreira et al., 2005; Seixas et al., 2018), and possibly also polar and brown bears (Edwards et al., 2011; Hailer et al., 2012; Hassanin, 2015; Hailer & Welch, 2016).

One further prominent mammalian example of mitochondrial paraphyly comprises the red fox (*Vulpes vulpes*) and Rüppell's fox (*V. rueppellii*), which are considered sister taxa (Lindblad-Toh et al., 2005; Leite et al., 2015) and occur in sympatry in North Africa and the Middle East. *V. vulpes* has the widest natural distribution of any terrestrial carnivore (Wozencraft 2005; Macdonald and Reynolds 2008). The species occupies a wide variety of ecosystems, including forests, grasslands, deserts and agricultural and human-dominated environments (Lariviere and Pasitschniak-Arts 1996). Forty-five *V. vulpes* subspecies are currently recognized (Lariviere and Pasitschniak-Arts 1996; Sacks et al. 2010)). Previous work has resulted in the

identification of several main mtDNA phylogroups, which were classified as the Holarctic clade (distributed across Eurasia, North Africa and North America; Statham et al., 2014), Nearctic clade (found only in North America; (Inoue et al. 2007; Aubry et al. 2009; Yu et al. 2012a; Kutschera et al. 2013; Statham et al. 2014), African clade (restricted to North Africa; Statham et al., 2014; Leite et al., 2015), plus the 'Palearctic basal haplotypes', a group of haplotypes with hitherto insufficient statistical support to conclusively be defined as a distinct clade (Statham et al. 2014).

In contrast, the much less extensively studied *V. rueppellii* is a species of xeric conditions, occupying arid habitats from North Africa to Pakistan, with up to six described subspecies (Rosevear, 1974; Sillero-Zubiri et al., 2004). Analysis of mitochondrial (cytochrome b and D-loop) and 33 autosomal microsatellite markers in *V. rueppellii* from Northwest Africa and one sample from Northeast Africa (Egypt) did not reveal any clear genetic structuring (Leite et al. 2015), although this finding could have resulted from limited geographic coverage and small sample size (Leite et al. 2015). Based on mtDNA analysis, Leite et al. (2015) revealed paraphyly of *V. vulpes* and clustering of *V. rueppellii* within *V. vulpes*, with *V. rueppellii* being most closely related to two *V. vulpes* clades found in Morocco. The authors therefore proposed that *V. rueppellii* could represent an ecotype of *V. vulpes*, or that past introgression from *V. vulpes* into *V. rueppellii* could have occurred.

Although *V. vulpes* is a well-studied taxon in Eurasia and North America (e.g., Frati et al., 1998; Inoue et al., 2007; Perrine et al., 2007; Aubry et al., 2009; Teacher et al., 2011; Edwards et al., 2012; Yu et al., 2012a; Kutschera et al., 2013; Ibiş et al., 2014), the authors of the most comprehensive phylogeographic study of *V. vulpes* to date (Statham et al. 2014) emphasized that the North African range remains only relatively sparsely characterized to date. Indeed, several previous studies of *V. vulpes* phylogeography highlighted that sampling gaps in biogeographically important regions still remain (Frati et al. 1998; Inoue et al. 2007; Perrine et al. 2007; Aubry et al. 2009; Teacher et al. 2011; Edwards et al. 2012; Yu et al. 2012a; Kutschera et al. 2013). Hence, previous work in North Africa and the Middle East lacked a comprehensive representation of ecoregions that are occupied by the two species. Cryptic or shared lineages within either species might therefore have remained undetected in previous studies.

The reported paraphyly of *V. vulpes* and hence the absence of reciprocally monophyletic mtDNA of *V. rueppellii* could result from various mechanisms. These include (1) ILS, (2) introgressive hybridization, (3) insufficient spatial sampling and low sample size in key biogeographic areas, and (4) analysis of short mtDNA sequences. First, ILS can contribute to non-monophyly when within-species polymorphism persists longer than the time between two successive speciation events (Funk and Omland 2003; Lopes et al. 2021). Second, introgressive hybridization during a secondary contact of the two species, possibly during periods of fluctuating climate (Barton and Hewitt 1985; Melo-Ferreira et al. 2005; Rieseberg et al. 2007) might have contributed to that paraphyly. Indeed, prominent cases of mammalian hybridization occur in scenarios of secondary contact of previously allopatric species (Colella et al. 2018). Third, increased sampling can affect the inference of phylogenetic relationships (Nabhan and Sarkar 2012; Figueroa et al. 2016). Since *V. rueppellii* has so far only been sampled from Northwest Africa, a small part of its range (Fig. 2.1), mtDNA lineages distinct from those in *V. vulpes* might have remained undetected in previous work. Fourth, analysis of relatively short mtDNA sequences in previous work resulted in phylogenetic trees with partly low branch support, possibly masking true phylogenetic relationships between the two species. Analysis of longer sequences, e.g., of cytochrome b and D-loop, could hence increase resolution and help identify accurate phylogenetic and phylogeographic structuring (Keis et al. 2013).

Here, I present novel mtDNA data (cytochrome b and D-loop) for *V. vulpes* and *V. rueppellii* from North Africa and the Middle East. My goals were to: 1) investigate the phylogeographic relationship between disjunct populations of *V. vulpes* and *V. rueppellii* in North Africa and the Middle East within the context of previously published data; 2) assess the validity of the reported paraphyly of *V. vulpes* based on longer DNA sequence alignments and improved sampling in key biogeographic regions in the sympatric range of both species.

2.2 Materials and Methods

2.2.1 Sample collection

A total of 128 fox samples were newly obtained for this study (Fig. 2.1). My sampling included 88 samples from Egypt (65 *V. vulpes* and 23 *V. rueppellii*); seven from road-killed animals from Libya (five *V. vulpes* and two *V. rueppellii*); four road-killed *V. vulpes* from Algeria; 24 from road-killed animals from the Middle East (seven *V. vulpes* tissue samples, 11 *V. vulpes* hair samples and six *V. rueppellii* hair samples); and five road-killed *V. vulpes* obtained from the Vale of Glamorgan Council and Cardiff Council (Wales, UK) (supplementary file 2).

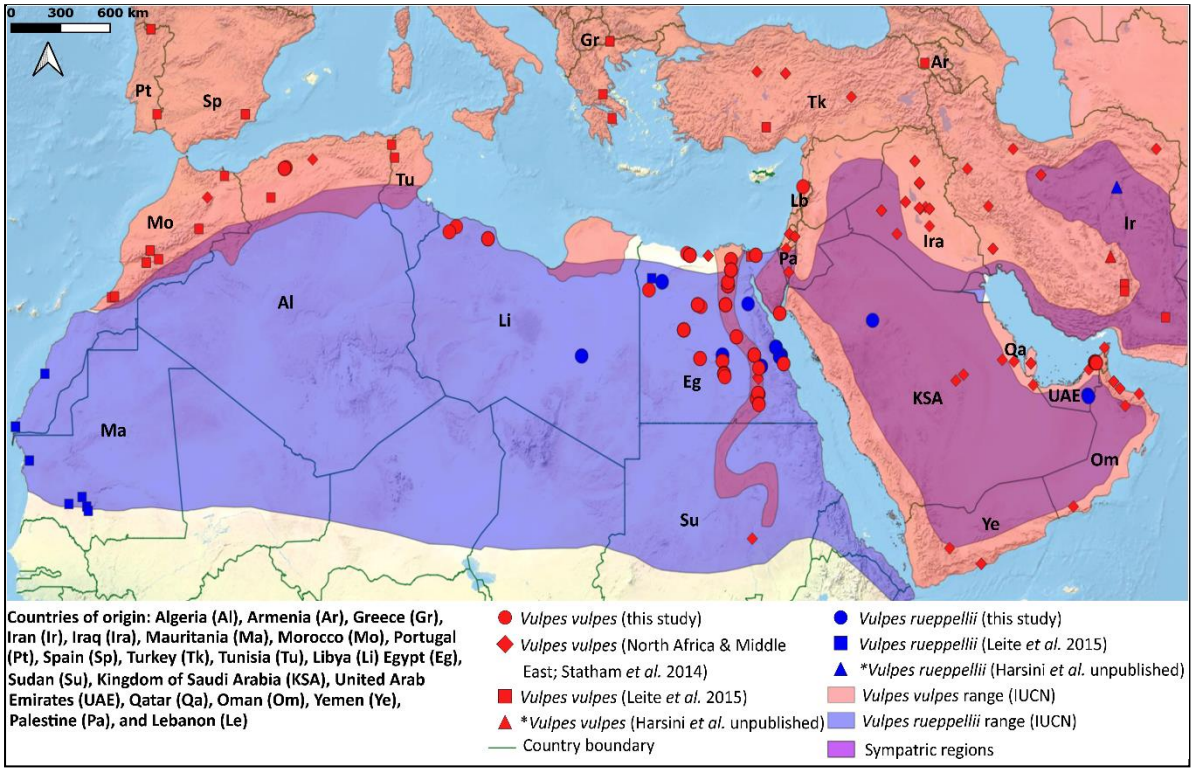


Figure 2.1: Sampling distribution of *V. vulpes* and *V. rueppellii* from North Africa, the Middle East and southern Europe. Additional samples from outside this region are not shown here, but were included in some analyses, e.g., the Bayesian tree. (*unpublished GenBank sequences, precise coordinates for these samples are unknown). Not all samples are discernible, due to spatial overlap of symbols (for details see supplementary file 2). Prepared using QGIS 3.8.3 (http://www.qgis.org).

2.2.2 Laboratory procedures

2.2.2.1 DNA extraction

Genomic DNA was extracted from tissue samples using a salting-out protocol modified from Rivero et al. (2006), which in turn was based on the Puregene^{TM} DNA extraction kit (Qiagen, Hilden, Germany). DNA extractions from hair samples were conducted using DNeasy Blood & Tissue Kits, following the manufacturer's recommendations, and quality was assessed by electrophoresis in 1% agarose gels.

2.2.2.2 Primer design

Among the previous studies of the two *Vulpes* species that included more than one gene or mtDNA fragments, most sequenced fragments spanned various and often non-overlapping regions of cytochrome b and the D-loop (Appendix 2.2). To include as many as possible of the previously published sequences for the geographical regions of interest, especially those of Statham et al. (2014) and Leite et al. (2015) for both cytochrome b and D-loop, I designed new primers for both loci using primer3 v4.1.0 (http://primer3.ut.ee/) (Table 2.1). For cytochrome b, three primer pairs were initially designed. All of them produced a strong band with PCR reaction, but only one pair (Vv.CY14144AF and Vv.CY15117AR) consistently produced clear and reliable Sanger sequences. For the D-loop, I designed a primer pair (Vv.CR2AF and Vv.CR2AR) which produced a strong band in PCRs and consistently high-quality Sanger sequences. For hair samples, the designed cytochrome b primers did not amplify, likely due to DNA degradation, so I used the primer pair L14724 and H15149 (Kocher et al., 1989; Irwin et al., 1991) that targets a 464 bp amplicon of cytochrome b. Locations of the sequenced fragments are shown in Appendix 2.2.

Table 2.1: Mitochondrial primers utilized in this study

Primer name	Primer length (bp)	Sequence (5'- 3')	Fragment length (bp) including primers	Locus	Reference
Vv.CR2AF	25	GCCAACCATTAGCATTATCGAAAAC	615	D-loop	This study
Vv.CR2AR	21	ACCAAATGCATGACACCACAG			
Vv.CY14144AF	26	GACATGAAAAATCATCGTTGTATTTC	974	cytochrome b	This study
Vv.CY15117AR	20	TTTGAGGTGTGTAGGTGRGG			
L14724	20	GATATGAAAAACCATCGTTG	464		Kocher *et al*. 1989; Irwin *et al*., 1991
H15149	20	CAGAATGATATTTGTCCTCA			

2.2.2.3 PCR Amplification and Sequencing

I amplified a 615 bp fragment from the 5' end of the mitochondrial D-loop (for both tissue and hair samples), and for cytochrome b, 974 or 464 bp fragments, respectively, for tissue and hair samples (Table 2.1). PCR amplification for tissue samples for both markers was performed in 15 µl reaction mixtures for each marker separately, containing: 1x GoTaq Flexi buffer (Promega, Madison, USA), 167 µM of each dNTP, 0.017 U GoTaq G2 polymerase (Promega), 2 mM $MgCl_2$, 200 µM of each primer for cytochrome b, 400 µM of each D-loop primer, and 1 µl DNA extract. PCR cycling conditions were 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, and 1.5 min at 72°C, followed by a 7 min step at 72°C. For hair samples, PCRs for both D-loop and cytochrome b were performed in 20 µl reaction mixtures containing 1x GoTaq Flexi buffer (Promega), 163 µM of each dNTP, 0.023 U GoTaq G2 polymerase, 4.0 mM $MgCl_2$, 300 µM of each primer, and 3 µl DNA extract. Cycling conditions were 3 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 50°C and 1.5 min at 72°C, followed by a final 10 min step at 72°C. The quality of PCR products was verified by electrophoresis in 2% agarose gels. Sanger sequencing of PCR products was performed by Eurofins Genomics (Wolverhampton, UK) on an ABI 3100 Genetic Analyzer.

2.2.3 Data analysis

Electropherograms were checked manually, and sequences were aligned using Geneious Prime 2020.1.1 (https://www.geneious.com). Ten individuals were sequenced in both directions to confirm any ambiguous polymorphism, especially in the D-loop. Previously published DNA sequences from *V. vulpes* and *V. rueppellii* were downloaded from GenBank, including 257 *V. vulpes* haplotypes from Statham et al. (2014), 9 haplotypes from 10 *V. rueppellii* individuals and 24 haplotypes from 31 *V. vulpes* individuals from Leite et al. (2015), 6 *V. rueppellii* (Accession numbers, cytochrome b: KU378368- KU378373, D-loop: KU378374-KU378379) and 90 *V. vulpes* (Accession numbers, cytochrome b: KU378491- KU378580, D-loop: KU378398- KU378486) haplotypes (Harsini *et al.*, *unpublished*), five complete mitogenomes (Accession numbers: KF387633 (Zhang et al. 2015), AM181037 (Arnason et al. 2006), GQ374180 (Zhong et al. 2010), KP342452 (Sun et al. 2016b), JN711443 (Yu et al. 2012b) and 25 *V. vulpes* haplotypes from Inoue et al. (2007) (supplementary file 2). I used *Vulpes lagopus* (Accession no. KP342451, (Sun et al. 2016a)) as an outgroup, which has been used previously by Kutschera et al., (2013) to study the phylogeography of *V. vulpes* in Europe. Geneious Prime was used to generate alignments using MUSCLE v3.8 (Edgar 2004), and to concatenate cytochrome b and D-loop sequences.

Bayesian phylogenetic analysis was conducted using BEAST V 2.6.0 (Bouckaert et al. 2019). I partitioned the data set into four regions: 1st, 2nd and 3rd codon positions of the cytochrome b gene, and the D-loop, and determined the most appropriate models of DNA substitution using the Akaike Information Criterion (AIC) in jModelTest 2.1.10 (Darriba et al. 2012). For the cytochrome b partitions of the data set, the GTR+G model was used, and GTR+I+G for the D-loop partition. In BEAST, I used the coalescence constant size model (Coalescence exponential model showed a qualitatively similar result) as a tree prior, with default values for other parameters. I conducted and combined five independent BEAST runs for 50 million generations each, sampling every 1000 generations, and subsequently combined these for further analyses. Trace plots were verified using TRACER v1.7 (Rambaut et al. 2018), confirming good mixing of chains. A burn-in of 10% was found to be suitable, and effective sample size (ESS) above 200 indicated convergence for all posterior parameter estimates. A maximum clade credibility tree with posterior probabilities for each node was obtained using

TREEANNOTATOR v2.6.0 (Bouckaert et al. 2019), and visualized using FIGTREE 1.4.4 (https://github.com/rambaut/figtree/releases).

I reconstructed statistical parsimony haplotype networks using the TCS algorithm (Clement et al., 2000) as implemented in PopArt v1.7 (https://popart.maths.otago.ac.nz/) , using a 95% minimum connection probability limit, and excluded gaps and missing data. Haplotype frequencies, haplotype and nucleotide diversity, Fu's F_S (Fu 1997), Tajima's D (Tajima 1989) and the average number of nucleotide substitutions per site between groups (D_{XY}) were calculated using DnaSP v6.12.03 (Rozas et al. 2017).

2.3 Results

Out of the 128 novel samples, 10 hair samples failed to amplify, and two (one tissue and one hair) were excluded due to signals of heteroplasmy and/or nuclear mitochondrial copies (see Appendix 2.1), leaving 116 newly obtained sequences (supplementary file 2). The ten individuals sequenced in forward and reverse directions did not reveal any discordant base calls. Most new sequences represented novel haplotypes, except three *V. vulpes* sequences from Egypt that were identical to the Egyptian haplotype from Leite et al. (2015). The concatenated sequences comprised 109 longer sequences (1400 bp: 864 bp cytochrome b + 536 bp D-loop), and seven shorter sequences from lower-quality samples (939 bp: 403 bp cytochrome b + 536 bp D-loop) (supplementary file 2). The alignment of the longer (1400 bp) sequences contained 129 segregating sites that formed 37 haplotypes (26 for *V. vulpes* and 11 for *V. rueppellii*). In addition, I encountered five haplotypes (two for *V. vulpes* and three for *V. rueppellii*) for the seven short sequences, across 39 polymorphic sites (supplementary file 2). Tajima's D deviated non-significantly from zero (p>0.5) for a total dataset of 148 individuals comprising 664 bp of concatenated sequences (cytochrome b: 360 bp; D-loop: 304 bp) and for each species separately, being -0.104 for 34 individuals of *V. rueppellii*, and 0.133 for 114 *V. vulpes* individuals, consistent with neutral evolution of the sequences (Table 2.2). Fu's F_S was non-significant for all investigated geographic groupings except for the Northwest African *V. rueppellii*, for which a significantly negative value was observed (Table 2.2).

2.3.1 Main phylogenetic clades of *V. vulpes* and *V. rueppellii*

A Bayesian phylogenetic tree of 459 mtDNA haplotype sequences grouped *V. rueppellii* inside the diversity of *V. vulpes* with high support (Bayesian Posterior Probability; BPP >0.99), showing paraphyly of *V. vulpes* (Fig. 2.2A). Figure 2.2C shows the distribution of *V. vulpes* and *V. rueppellii* clades in North Africa and Middle East and their sample frequencies. I obtained high support (BPP >0.99) for the 'Holarctic' and 'Nearctic' clades described by Statham et al. (2014), and also obtained such high support (BPP >0.99) for a clade containing newly obtained sequences along with previously published 'Palearctic basal haplotypes' from Statham et al. (2014). This clade, henceforth referred to as 'Palearctic clade', contains sequences from *V. vulpes* from North Africa and Asia, along with all sequences from *V. rueppellii* that have been generated to date – from across North Africa, Saudi Arabia, United Arab Emirates, and Iran. Further, I obtained high support (BPP >0.99) for two African clades (Africa 1 and Africa 2), which in turn clustered together with high support (BPP >0.99). These two African clades correspond to Maghreb 1 and Maghreb 2 described by Leite et al. (2015) for Northwest Africa. The support for the two African clades to cluster with the joint Holarctic/Nearctic clades was moderate (BPP: 0.82) and did not increase when we restricted the analysis to long sequences only, nor when cytochrome b and D-loop were analyzed separately (details not shown). Haplotype networks showed groupings consistent with these main clades, both for shorter (Fig. 2.2B) and longer (Appendix 2.4) alignment lengths.

All analyzed *V. rueppellii* sequences clustered into two main sub-clades within the Palearctic clade, each receiving high support (BPP >0.99). The average number of nucleotide substitutions per site between the two subclades was D_{XY} = 2.1%. Subclade 1 was restricted to North Africa, and subclade 2 was found in Iran, Arabia, and East of the Nile (Egypt) (Fig. 2.2A/B). The two subclades were sympatric only in one region, east of the Nile in Egypt.

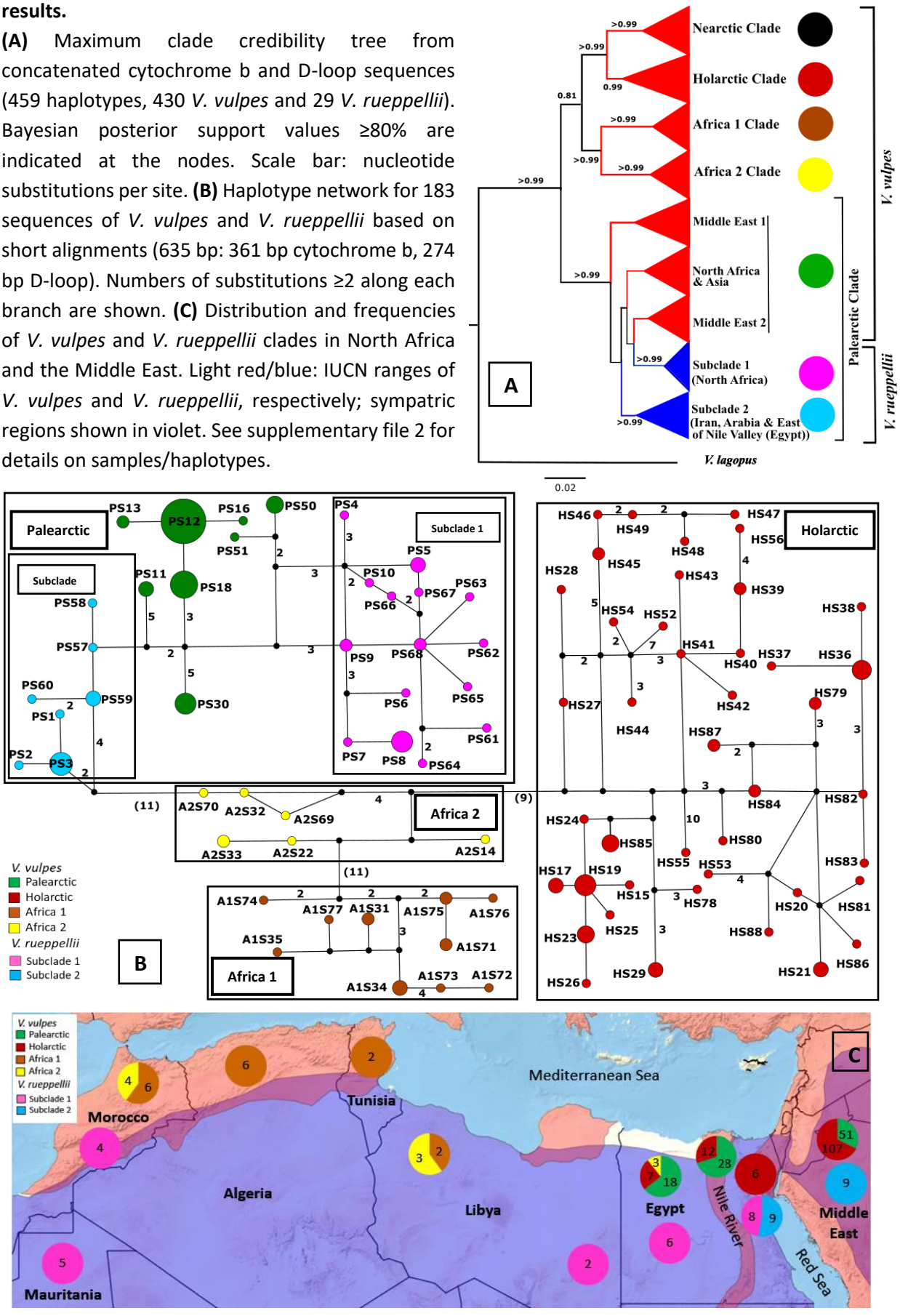
V. vulpes sequences were found within all major clades. The Palearctic clade is of particular interest, since it contains both *V. vulpes* and *V. rueppellii*, so it will be presented in greater detail. The Palearctic-clade *V. vulpes* comprised 8 haplotypes from North Africa, Middle East, East Asia (Japan) (Fig. 2.2B/C). Two haplotypes (PS12 and PS18) were widely distributed along

the Nile and western desert oases in Egypt (27 and 10 samples respectively), one (PS30) was found in 6 samples from United Arab Emirates, one (PS50) in 4 samples for from Japan, and 4 additional haplotypes were rare and geographically restricted (three in Egypt, one in Japan; see supplementary file 2). Appendix 2.3 shows the divergence between the main clades of short (Fig. 2.2B) and long (Appendix 2.4) sequences. The haplotype network for a subset of longer sequences (Appendix 2.4) showed the same overall topology, but with increased divergence between the main clades.

The Holarctic clade contained the greatest number of haplotypes and individuals, and was also the geographically most widely distributed, occurring in North Africa, Europe, Asia, and North America. Most newly obtained haplotypes within the Holarctic clade were from Europe, West Asia and Sinai Peninsula, along with a few from North Africa (supplementary file 2). The Nearctic clade only contained samples from North America, as found previously (Kutschera et al. 2013; Statham et al. 2014). The Africa 1 clade was restricted to central and Northwest Africa (Libya, Tunisia, Algeria, and Morocco). The Africa 2 clade was found in samples from the Mediterranean coastal desert in Egypt, Libya and the western Atlas, comprising two newly obtained Egyptian haplotypes, two Libyan haplotypes and two previously described haplotypes from Morocco (‘Maghreb 2’ subclade of Leite et al., 2015).

Figure 2.2: Phylogenetic and phylogeographic results.

(A) Maximum clade credibility tree from concatenated cytochrome b and D-loop sequences (459 haplotypes, 430 *V. vulpes* and 29 *V. rueppellii*). Bayesian posterior support values ≥80% are indicated at the nodes. Scale bar: nucleotide substitutions per site. **(B)** Haplotype network for 183 sequences of *V. vulpes* and *V. rueppellii* based on short alignments (635 bp: 361 bp cytochrome b, 274 bp D-loop). Numbers of substitutions ≥2 along each branch are shown. **(C)** Distribution and frequencies of *V. vulpes* and *V. rueppellii* clades in North Africa and the Middle East. Light red/blue: IUCN ranges of *V. vulpes* and *V. rueppellii*, respectively; sympatric regions shown in violet. See supplementary file 2 for details on samples/haplotypes.



2.3.2 Genetic diversity

To infer the genetic diversity within and among *V. vulpes* and *V. rueppellii* populations, I trimmed the data according to Leite et al. (2015), a dataset of particular interest since it includes *V. vulpes* and *V. rueppellii* from Africa, and *V. vulpes* from Europe and the Middle East. This combined data set contained 148 individuals (109 from this study, 39 from Leite et al., 2015), comprising 664 bp of concatenated sequences (cytochrome b: 360 bp; D-loop: 304 bp; table 2.2). The number of haplotypes for the 145 longer sequences (concatenated data of 1150 bp: 822 bp cytochrome b and 382 bp D-loop) was higher than for the trimmed data (summarised in Table 2.2), yielding 53 haplotypes among the 115 *V. vulpes* sequences, and 16 among the 30 *V. rueppellii* sequences.

Table 2.2: Diversity and neutrality indices of *V. rueppellii* and *V. vulpes* based on 664-bp concatenated sequence dataset (cytochrome b and D-loop, excluding sites with gaps). N number of sequences, *S* polymorphic sites, η number of mutations, *H* number of haplotypes, π nucleotide diversity, Hd haplotype diversity, with standard deviation for the latter two in brackets. Statistical significance: *P < 0.05. NW= North West, NE= North East, NC= North Central, Pt= Portugal, Sp= Spain, Gr= Greece, UK= United Kingdom, Ar= Armenia, Tk= Turkey, Ir= Iran, UAE= United Arab Emirates.

Species	Population	Subpopulation	N	*S*	η	*H*	π (SD)	Hd (SD)	Fu's Fs	Tajima's D
V. rueppellii	All		34	32	32	20	0.011 (0.00072)	0.938 (0.025)	-4.662	-0.104
	NW Africa (Morocco and Mauritania)		9	13	13	8	0.005 (0.00090)	0.972 (0.064)	-3.977*	
	NE Africa	All	25	26	26	12	0.012 (0.00062)	0.877 (0.041)	0.130	
		West of the Nile (Egypt, Libya)	8	10	10	5	0.005 (0.00093)	0.857 (0.108)	-0.005	
		East of the Nile (Egypt)	17	17	17	7	0.011 (0.00094)	0.779 (0.073)	2.659	
V. vulpes	All		114	82	85	42	0.025 (0.00081)	0.885 (0.027)	-2.640	0.133
	NW Africa (Algeria, Tunisia, Morocco)		15	34	34	11	0.015 (0.00276)	0.952 (0.040)	-0.946	
	NC Africa (Libya)		5	23	23	3	0.019 (0.00391)	0.800 (0.164)	4.390	
	NE Africa (Egypt)	All	66	46	46	14	0.018 (0.00163)	0.672 (0.063)	6.331	
		West of the Nile	26	42	42	6	0.020 (0.00294)	0.649 (0.094)	10.699	
		Nile Valley & Delta	34	28	28	7	0.015 (0.00273)	0.570 (0.094)	8.388	
		East of the Nile	6	13	13	4	0.009 (0.00275)	0.800 (0.172)	1.657	
	Europe Pt, Sp, Gr, UK		14	24	25	9	0.011 (0.00154)	0.923 (0.050)	-0.189	
	Near/ Middle East. Ar, TK, Ir, UAE		14	31	31	5	0.021 (0.00161)	0.758 (0.084)	7.695	

Consistent with the deeply divergent clades in *V. vulpes*, this species showed higher nucleotide diversity and numbers of variable sites than *V. rueppellii*, although the latter showed slightly higher haplotype diversity (Table 2.2). To avoid biases in the summary statistics of genetic diversity derived from differences in sample size between the two species, a bootstrap resampling approach was carried out. Each bootstrap replicate consisted of ten randomly chosen samples for which the haplotype diversity and nucleotide diversity was estimated; a total of 100 bootstrap replicates were carried out for each species separately and the distributions of each summary statistic was compared between species to assess if these overlapped. The haplotype diversity did not differ significantly between the two species, while nucleotide diversity was significantly higher in *V. vulpes* (Appendix 2.6). The high nucleotide diversity among *V. vulpes* populations along and west of the Nile coincides with clade admixture in these populations (west of the Nile: Africa 2, Holarctic and Palearctic clades; along the Nile: Holarctic and Palearctic clades). In contrast, *V. vulpes* populations from Northwest Africa, Europe and east of the Nile contained only one clade - the African clade for Northwest Africa, and Holarctic clade for both Europe and east of the Nile - yielding lower nucleotide variability estimates.

2.4 Discussion

I here provide a comprehensive phylogenetic and phylogeographic analysis of *V. vulpes* and *V. rueppellii,* allowing me to evaluate their matrilineal evolutionary history. This study incorporates newly obtained sequences from both species, along with previously published homologous mtDNA data from across their geographic ranges. Based on longer sequence alignments than most previous studies (Appendix 2.2), the obtained phylogeny demonstrates that the "Palearctic basal haplotypes" by Statham et al. (2014) form a distinct Palearctic clade that is shared between *V. vulpes* and *V. rueppellii*. Importantly, I showed that all analyzed *V. rueppellii*, sampled across North Africa and the Middle East, are nested within this Palearctic clade, rendering *V. vulpes* paraphyletic. These findings are consistent with previous work by (Leite et al. 2015), who found *V. rueppellii* to cluster with two African clades (Maghreb 1 and 2) of *V. vulpes*. My results link this paraphyly to Palearctic clade sharing with *V. vulpes* populations across North Africa and Asia.

2.4.1 Evolutionary history of *V. rueppellii* and paraphyly of *V. vulpes*

The results lead me to propose three evolutionary scenarios for the phylogenetic relationships of the two species (Fig. 2.3). Edwards et al. (2011) proposed similar scenarios to explain the paraphyly of brown bears.

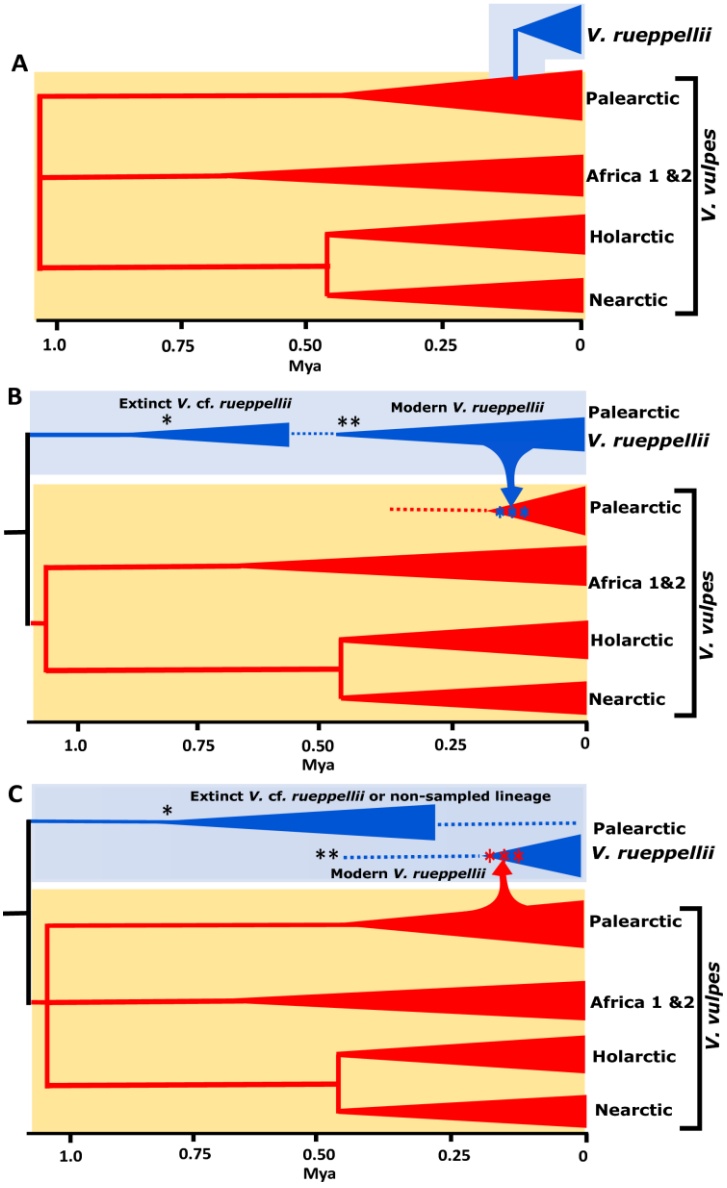


Figure 2.3: Three hypothetical scenarios for the evolution of *V. rueppellii* and current paraphyly of *V. vulpes*. **A**) 'Ecotype scenario': rapid evolution of *V. rueppellii* from Palearctic-clade *V. vulpes*; **B/C)** Old divergence and recent introgression of mtDNA between the two species. **B**) Introgression of *V. rueppellii* mitogenome into *V. vulpes,* (**C**) Introgression of *V. vulpes* mitogenome into *V. rueppellii*. Divergence times within *V. vulpes* are based on Statham et al. (2014). Interspecific divergence time in B/C is hypothesised based on the fossil record: * and ** are *V. cf. rueppellii* (0.8 Mya) and *V. rueppellii* (0.5 Mya) fossils, respectively, from Geraads (2011). Background colors indicate the *V. vulpes* (yellow) and *V. rueppellii* (light blue) gene pools, while red and blue foreground colors denote their mtDNA and black is the ancestor. *** refers to introgression of *V. rueppellii* into *V. vulpes* (blue in B) and vice versa (red in C).

Scenario 1: *'Ecotype scenario' – rapid evolution of V. rueppellii from Palearctic-clade V. vulpes (Fig. 2.3A)*

A parsimonious explanation for *V. vulpes* paraphyly and the low divergence of *V. rueppellii* from Palearctic clade *V. vulpes* sequences would be a recent and rapid evolution of *V. rueppellii*. This scenario could support the classification of *V. rueppellii* as a desert ecotype of *V. vulpes* (see Leite et al., 2015). The term ecotype is typically used to describe genetically distinct forms within a species that are highly adapted to a specific environment (Begon et al., 2005). Indeed, other species of canids have previously been suggested to contain distinct ecotypes, such as wolves (Carmichael et al., 2007; Leonard et al., 2007; Musiani et al., 2007; Muñoz-Fuentes et al., 2009; Hendricks et al., 2019) and arctic foxes (Dalén et al. 2005; Norén et al. 2011). However, I consider this scenario to be unlikely for *V. rueppellii*, for several reasons:

(a) The fossil record suggests that *V. rueppellii* as a species is much older than suggested by nesting of mtDNA within *V. vulpes* diversity. Geraads (2011) recorded two *V. rueppellii* fossils from Tighenif, Algeria (Northwest Africa): one of them dating to about 0.5 Mya and showing a similar morphotype as *V. rueppellii* today, and the other form from 0.8 Mya was interpreted as a fossil precursor species to *V. rueppellii*, suggesting an even earlier divergence from *V. vulpes.*

(b) The morphological and physiological differentiation between the two species is considerable, and well supported: *V. vulpes* is overall larger, with longer hind legs, longer tail, and proportionally shorter ears than the sympatric *V. rueppellii* (Lariviere and Seddon 2001). Ecologically, behaviorally and physiologically, *V. rueppellii* is adapted to xeric conditions (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004), while *V. vulpes* avoids such habitats, is distributed throughout the Holarctic and shows a wide plasticity in terms of habitat requirements (Sillero-Zubiri et al. 2004; Soulsbury et al. 2010). An analysis of external measurements (head and body length, tail length, ear length, shoulder height and weight) showed a large difference between the two species (Sillero-Zubiri et al. 2004). That dataset included *V. rueppellii* from Arabia (Lenain 2000) and Egypt (Osborn and Helmy 1980), and *V. vulpes* from across its distribution except North Africa (UK, Hattingh, 1956; Australia, McIntosh, 1963; Canada, Voigt, 1987; Japan, Zhan et al., 1991 and several studies from

Cavallini, 1995). These results appear comparable to those from other mammalian sister species pairs, which according to a meta-analysis by Avise *et al.* (1998) typically diverged more than one Mya. Hence, the significant physical differentiation between *V. rueppellii* and *V. vulpes* tentatively suggests a longer time since speciation than suggested by mtDNA.

(c) Nuclear microsatellite data show a relatively strong differentiation between *V. rueppellii* and *V. vulpes* (F_{ST} = 0.14; Leite et al., 2015) showing larger interspecific differences than mtDNA. Such mito-nuclear discordance has been found in other paraphyletic mammals and their sibling species, where paraphyly at mtDNA is accompanied by significant differentiation at nuclear loci (Good et al. 2008; Hailer et al. 2012). However, I caution that this pattern for microsatellites in *V. vulpes* and *V. rueppellii* could hypothetically result from strong/rapid genetic drift, rather than long evolutionary time. Under such a scenario one would predict decreased intrapopulation variability. However, when compared to their North African and Eurasian counterparts of *V. vulpes*, unbiased expected heterozygosity and allelic richness in *V. rueppellii* are ca. 105% and 102% for allelic richness and 90% and 87% of expected heterozygosity, respectively, (Leite et al. 2015). These findings do not reveal clear evidence of strong and recent genetic drift but are consistent with the long time frames indicated by the fossil record of *V. rueppellii* (Geraads 2011).

(d) For red foxes, Statham et al. (2014) estimated the time to most recent common ancestor (T_{MRCA}) of the Palearctic group at ca. 70–98 kya (kilo (thousand) years ago). Hence, *V. rueppellii* would have evolved from a lineage within the Palearctic *V. vulpes* clade, subsequently adapting rapidly to arid habitats. If the *V. rueppellii* lineage indeed were this young, the vast current geographic range (Fig. 2.1) would predict clear signals of demographic growth. However, my analyses only revealed signals of population growth for NW African *V. rueppellii* sequences, but not for any other regions studies (or all sequences combined) (Table 2.2).

Scenario 2: *incomplete lineage sorting (ILS) explains intermingled lineages*

The oldest fossil remains of *V. rueppellii* are from northwest Africa, dating back to ca. 0.8 Mya (Geraads 2011). The divergence between *V. vulpes* and *V. rueppellii* therefore likely occurred in or before the mid-Pleistocene. ILS can cause species-level non-monophyly if divergence between the species was too recent for ancestral polymorphisms to have sorted into

reciprocally monophyletic lineages (Funk and Omland 2003; McKay and Zink 2010). ILS has previously been suggested to cause non-monophyly in European bison (*Bison bonasus*) (Wang et al. 2018). Structuring within Eurasian and Nearctic *V. vulpes* populations has so far been interpreted as the result of biogeographic barriers, or isolation-by-distance (Kutschera et al. 2013; Statham et al. 2014). Therefore, if ILS explains lineage branching patterns between *V. vulpes* and *V. rueppellii*, then perhaps the intraspecific phylogeographic patterns of *V. vulpes* would need to be re-evaluated as well.

Lineage sorting for mtDNA requires on average 1 x N_{fe} generations (where N_{fe} is the effective female population size; Nichols, 2001). Indeed, in *V. vulpes*, this corresponds to only ca. 100–200 kya - based on an ancestral N_{fe} of 91,000 (Statham et al. 2014) and a generation time of 2 years (Statham et al. 2018). ILS therefore appears unlikely to impact red foxes mtDNA beyond few 100 kyr (thousand years), a time frame younger than the divergence time suggested by the fossil record (Geraads 2011).

Scenario 3: *Old divergence and recent introgression of mtDNA between the two species (Fig. 2.3B/C)*

There are numerous examples of introgressive hybridization in the genus *Canis,* e.g., between the Ethiopian wolf (*C. simensis*) and domestic dogs (*C. familiaris*) (Gottelli et al. 1994), and between red wolves (*C. rufus*) and coyotes (*C. latrans*; Adams et al., 2003; Hailer & Leonard, 2008). Even hybridization between taxa with differing chromosome numbers has been described for mammals (Horn et al. 2012; Giménez et al. 2016). Interspecific hybridization in *Vulpes* has been described for *V. vulpes* and the kit fox (*V. macrotis*) (Creel and Thornton 1974), and between *V. macrotis* and swift fox (*V. velox*) (Dragoo and Wayne 2003). The previous two cases suggest that hybridization between *V. vulpes* and *V. rueppellii* should not be excluded, despite the differences in their chromosome number: 2n = 34 plus 0–8 B chromosomes for the former (Graphodatsky et al., 2000) and 2n=40 for the latter (Ewer, 1973). Behaviourally, *V. vulpes* typically dominates other fox species, especially smaller species such as *V. lagopus* (Tannerfeldt et al., 2002), *V. corsac* and *V. macrotis* (Sillero-Zubiri et al. 2004). However, prezygotic interspecific barriers can break down under e.g., Allee effects (i.e., “individual fitness (or components thereof) could be positively related to population size or density (e.g., Allee 1931)”; Courchamp et al. 1999) acting at low population

densities or other population pressures (Adams et al. 2003; Hailer and Leonard 2008; Seehausen et al. 2008). Hybridization between *V. vulpes* and *V. rueppellii* therefore remains a reasonable scenario, although its occurrence has not been described to my knowledge.

If introgression indeed explains the Palearctic clade sharing between the two species, then I might expect to also see clade sharing for the other three clades occurring in sympatry (Holarctic, Africa 1 and Africa 2). Given the extended sample size across sympatric areas in North Africa and the Middle East included in this study, I consider the absence of clade sharing among those three clades to be robust. A more likely scenario therefore involves an ancient divergence between *V. vulpes* clades (including the Palearctic group that contain current *V. rueppellii*) at ca. 1.15 Mya (Statham et al. 2014), and a secondary contact leading to a gene flow at around 70-98 Kya. This introgression is consistent with the estimated time of the diversity of the Palearctic haplotypes (Statham et al. 2014). There are two possible directions of introgression, as follows:

3a: Introgression of V. rueppellii mtDNA into V. vulpes (Fig. 2.3B)

The Palearctic clade may originally have evolved in *V. rueppellii*, having diverged from other *V. vulpes* clades at ca. 1.15 (0.85-1.45) Mya (Statham et al. 2014). Broadly consistent with this timing, Geraads, (2011) recorded two *V. rueppellii* fossils from Tighenif, Algeria (see above), *V. cf. rueppellii* (0.8 Mya) and *V. rueppellii* (0.5 Mya). The latter is closer to the modern *V. rueppellii* than to any other species Geraads, (2011). Furthermore, Geraads, (2011) recorded *Vulpes hassani* (2.5 Mya) as a precursor of *V. rueppellii*, suggesting even earlier divergence of *V. rueppellii* from *V. vulpes*. *Vulpes rueppellii* may therefore have evolved from *V. cf. rueppellii* (Geraads 2011), and subsequently passed on its mitogenome to some *V. vulpes* populations currently found in parts of North Africa and Eurasia (the Palearctic clade). That may have been related to *V. vulpes* colonizing arid habitats and/or persisting in low densities, which can favor introgressive hybridization in canids (Hailer and Leonard 2008).

3b: Introgression of V. vulpes mitogenome into V. rueppellii (Fig. 2.3C)

If I instead assume that the Palearctic clade originally evolved in *V. vulpes*, clade sharing between the two species today could result from introgression of this clade from *V. vulpes* into *V. rueppellii.* The original *V. rueppellii* mtDNA would thus have been lost (mtDNA

replacement), as suggested for e.g., hare *Lepus* species (Melo-Ferreira et al. 2012) and *Ursus* bears (Hailer et al. 2012). Such replacement events can be due to a combination of strong genetic drift or potentially driven by selective advantage of introgressed lineages. Alternatively, the original *V. rueppellii* mtDNA lineage may persist, undetected despite our increased sampling.

Without additional evidence, we consider scenarios 3a and 3b to be of equal likelihood. Fossil, ancient DNA or modern genomic evidence from biparentally or male-inherited markers may shed further light on these scenarios.

2.4.2 Phylogeography of *V. rueppellii*

Only one previous study by Leite et al. (2015) has evaluated the phylogeography of *V. rueppellii*, finding no clear structuring at mitochondrial and nuclear markers (based on 10 samples mainly from Northwest Africa: 3 from Morocco, 6 from Mauritania and one from Egypt). My results extend these findings by revealing a second mtDNA clade within the species, and by showing population genetic structuring for these clades across the species' range (Fig. 2.2C). My findings demonstrate that the genetic structuring of *V. rueppellii* is shallower than that of the *V. vulpes*, with no deeply divergent lineages present.

My findings demonstrate, for the first time, the presence of two subclades within the species. These subclades show a predominantly western and eastern distribution, respectively. Populations of *V. rueppellii* are distributed through three main geographical regions, 1) North Africa (west of the Nile to the Atlantic Ocean), 2) An intermediate North Africa/ Middle East (east of the Nile) and 3) Middle East (from Sinai Peninsula through Arabia to Pakistan). Subclades 1 and 2 correspond to the geographical regions 1 & 3, respectively, while the east Nile populations in Egypt (geographical region 2) share mtDNA haplotypes with both subclade 1 and subclade 2 (Appendix 2.5).

This clear but relatively shallow genetic structuring between populations of *V. rueppellii* resembles that of the sand cat *Felis margarita*, which occupies nearly the same habitats and geographic range. Howard-McCombe et al. (2019) investigated the phylogeny of the four established populations (subspecies) of *F. margarita; F. m. margarita* (North Africa), *F. m.*

harrisoni (Arabia), *F. m. thinobia* (west/central Asia) and *F. m. scheffeli* (Pakistan), detecting a significant genetic differentiation between the African subspecies and the other three subspecies, and only low differentiation among the Asian subspecies.

The geological record suggests that arid habitats were widespread and largely contiguous across North Africa and extending into the Middle East at 1.2-0.8 Mya (deMenocal 2004). Leite et al. (2015) suggested that *V. rueppellii* might have evolved during the Pleistocene and colonized its existing range while the Sahara was connected to the Arabian and Syrian deserts. Subsequent climatic oscillations introduced more humid and mesic conditions, fragmenting these arid zones. At ca. 12 kya, the modern Nile River formed (Said 1981; Said 1993), its mesic habitats likely posing a barrier to gene flow for arid-adapted taxa such as *V. rueppellii*, splitting the populations to the west and east of the Nile. In contrast, these mesic habitats may have allowed more generalist species to colonize, perhaps explaining the arrival of Holarctic clade red foxes to North Africa. Similarly, climatic and sea level fluctuations would have created temporary barriers around the Gulf of Suez. Derricourt, (2005) suggested that during drier periods of the Pleistocene, the Gulf of Suez was reduced in area and the Sinai Peninsula was readily accessible from the Eastern Desert, merging these two regions into an arid mountainous zone. Until about 14–15 kya when sea levels rose above about -50 m.a.s.l., the Sinai Peninsula was therefore presumably connected to the Eastern Desert (Derricourt 2005; Bailey et al. 2007). This could explain the admixture of *V. rueppellii* subclade 1 and 2 haplotypes east of the Nile. The Eastern desert of Egypt and Sinai Peninsula may therefore represent a transitional region for *V. rueppellii*. Indeed, the Sinai Peninsula played an important role in the faunal exchange between Africa and Eurasia, linking these regions during periods of low sea level. Such conditions likely occurred frequently throughout the Pliocene and Pleistocene, facilitating multiple dispersion waves (Saleh et al. 2018). Existence of Pleistocene fossils of African mammalian fauna in the Levant dating back to 1.8-1.4 Mya (Tchernov 1992) suggests the activity of this Afro-Asian route during the Pleistocene.

2.5 Conclusion

This study solidifies our understanding of the phylogeography of both *V. rueppellii* and *V. vulpes*, documenting for the first time two subclades and phylogeographic structuring within *V. rueppellii*. While Holarctic, Nearctic, Palearctic and two African clades had previously been robustly defined for *V. vulpes*, I here obtained robust statistical support for the previously so-called 'Palearctic basal haplotypes' as a 'Palearctic clade'. I also report the first mtDNA data for *V. rueppellii* from Northeast Africa and the Middle East. My extended sampling across previously poorly sampled and unsampled regions reinforces that *V. rueppellii* is matrilineally rooted inside the diversity of the paraphyletic *V. vulpes.* This paraphyly may have resulted from introgressive hybridization rather than recent speciation of *V. rueppellii*, consistent with evidence from morphometrics and the fossil record. Although my study included *V. rueppellii* from different ecoregions across its range, additional sampling would be desirable, in particular from the Asian part of the range. The occurrence of the three *V. vulpes* clades (Holarctic, Palearctic and Africa 2) and both subclades of *V. rueppellii* in Northeast Africa, indicates that this region is a biogeographic diversity hotspot.

As a matrilineal marker that may not reveal genetic differentiation of the rest of the genome (Zhang and Hewitt 2003; Hailer et al. 2012; Bidon et al. 2014), mtDNA evidence should be revisited with information from independently inherited genetic markers (e.g., autosomal and Y-chromosome), to shed further light on the possible scenarios for the evolutionary history of the ecologically and morphometrically distinct *V. rueppellii* and *V. vulpes*.

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Chapter 3: First Complete Mitogenome of Rüppell's Fox (*Vulpes rueppellii*) Confirms Phylogenetic Placement Within the Palaearctic Clade of Its Sister Taxon, the Red Fox (*V. vulpes*)

3.1 Introduction

The Rüppell's fox (*Vulpes rueppellii*; Carnivora: Canidae; IUCN category of 'Least Concern'; Mallon et al., 2015) is widespread in desert regions across North Africa, the Arabian Peninsula and southwestern Asia, comprising up to six described subspecies (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004; Mallon et al. 2015). The typical habitat of *V. rueppellii* includes sand dunes, sand sheets, gravel plains (Murdoch et al. 2007), stony habitats with few grass species that receive little rainfall (Lenain 2000), and coastal areas with low vegetation cover (Mallon et al. 2015). The species' range partly overlaps with the red fox (*V. vulpes*), that is considered its sister taxon (Geffen et al. 1992; Lindblad-Toh et al. 2005; Leite et al. 2015). Although exploitative competition has been reported between the two species (Cuzin 2003; Sillero-Zubiri et al. 2004), *V. rueppellii* tends to utilise more arid habitats than *V. vulpes* (Wacher & Attum, 2005).

The mitochondrial genome has long been used as standard marker for inference of evolutionary and phylogeographic processes (DeSalle et al., 2017), but while this has been fully sequenced for *V. vulpes* (Arnason et al. 2006), the mitogenome of *V. rueppellii* is not available to date, and the mitochondrial phylogenetic relationships of *V. rueppellii* with other fox species, especially *V. vulpes*, remain poorly understood. Short fragments of various mtDNA loci have been used to investigate the evolutionary history of the two species (e.g., Leite et al., 2015; chapter 2), revealing putative clustering of *V. rueppellii* within the variation of *V. vulpes*, leading to paraphyly of the latter.

Due to the higher phylogenetic resolution provided by longer sequences (Keis et al. 2013; Anijalg et al. 2018), complete mitogenomes provide more robust and detailed insights in phylogenetic relationships on various taxonomic levels than short mtDNA (Finstermeier et al., 2013). Various bioinformatic approaches have been developed for retrieval of mitogenome sequences from whole-genome sequencing data, e.g., mapping of sequencing reads directly against a (typically closely related reference) genome (reviewed in Briscoe et al. 2016) , or approaches involving de-novo assembly of the reads, reducing or removing reliance on a reference genome, e.g., NOVOPlasty; **(**Dierckxsens et al. 2017) and MITObim (Hahn et al. 2013). These approaches now allow researchers to efficiently harvest mitogenomes from whole genome sequencing data.

The aim of this study was to obtain and characterize the first complete mitochondrial genome of *V. rueppellii* to 1) better understand its phylogenetic relationship with its sister species, *V. vulpes*, and 2) compare the performance of four different mitogenome assembly approaches (de novo, two different reference-based approaches, and baiting and iterative mapping) for obtaining mitogenome sequence data from Illumina whole-genome shotgun sequencing data.

3.2 Materials and Methods

3.2.1 Sampling and data generation

I extracted DNA from a male *V. rueppellii* tissue sample collected from Wadi om-Khiag, Eastern Desert, Egypt (25° 36’ 55.01”N 34° 23' 58.99"E), using a salting-out protocol modified from Rivero et al. (2006), which in turn was based on the Puregene™ DNA extraction kit (Qiagen, Hilden, Germany). I assessed the quality and concentration of the genomic DNA by electrophoresis in 1% agarose gels and a Qubit fluorometer v.3.0, respectively. DNA was subsequently sent to Neogen (Ayr, Scotland, UK) for library preparation and whole-genome sequencing. DNA was randomly sheared into short fragments, size selected to ca. 350 base pairs (bp), A-tailed, ligated with Illumina adapters (5'AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT-3' and 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGATGACTATCTCGTATGCCGTCTTCTGCTTG-3'), PCR amplified, and purified. After subsequent quantification and checks for fragment size distribution using Qubit, real-time PCR and a bioanalyzer, the library was sequenced on an Illumina Novaseq instrument using paired-end reads (2x 151 bp).

I used FASTQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to assess the quality of the reads, and TRIMMOMATIC v0.39 (Bolger et al. 2014) to remove adaptors and to trim low-quality reads (settings: minimum length 50 bp, sliding window 10:15).

3.2.2 Mitogenome assembly

I used four parallel approaches to obtain the *V. rueppellii* mitogenome. Approach 1: ***De novo* assembly, the program NOVOPlasty** (Dierckxsens et al. 2017) was used with the raw reads as an input and using default parameter settings, except setting insert size to 350 and K-mer to 33. As a seed to initiate the assembly, I used the Genbank-curated mitochondrial reference genome of the sister taxon, *V. vulpes* (GenBank accession: NC_008434), noting the completeness and the reliability of the PCR-based approach used to generate this sequence; Arnason et al. 2006). Approach 2: Using **baiting and iterative mapping approaches implemented in MIRA v4.0.2** (Chevreux et al. 1999) **and MITObim v1.9.1** (Hahn et al. 2013): based on default parameter settings, first, MIRA was used to build an initial reference by mapping the raw reads to the mitochondrial reference genome of *V. vulpes* (Arnason et al. 2006). Next, the MITObim.pl script was used to iteratively retrieve additional reads from the shotgun sequence data and to map them against the reference obtained from the previous iteration. This was repeated until gaps were closed, and a stationary number of reads was reached for the mitogenome. The approach only returns a single-padded consensus sequence, but any sequence fragments are connected by 'N' to indicate that the fragments are not connected by reads and therefore not contiguous in the MITObim assembly (Machado et al. 2016). Approaches 3 and 4: **Reference-based read mapping** was performed using two different parameter settings (see below) by aligning the trimmed data against the *V. vulpes* reference genome (assembly version: GCF_003160815.1_VulVul2.2; Kukekova et al., 2018) using BWA-MEM v0.7.17 (Li and Durbin 2009) in paired-end mode with default parameters. I then used SAMTOOLS v1.10 (Li et al. 2009) to obtain sorted bam files, followed by using GATK (https://gatk.broadinstitute.org/hc/en-us) to remove PCR duplicates using MARKDUPLICATESSPARK and to filter out bad read mates, reads with mapping quality zero and reads which mapped ambiguously (Nater et al. 2017). Then I used SAMTOOLS to extract the mitochondrial reads that mapped to the mtDNA scaffold (NC_008434.1, Arnason et al., 2006) of the reference genome. I ran HAPLOTYPECALLER in GATK to call variants using two different parameter settings, using as values for the flag *--sample-ploidy:* 1 for haploid (ploidy 1; approach 3), and 2 for diploid (ploidy 2, approach 4), each yielding a separate VCF file. Finally, FastaAlternateReferenceMaker from GATK was used to convert the two VCF files from approaches 3 and 4 to FASTA format.

Geneious Prime 2022.2.2 (http://www.geneious.com) was used to align and annotate the genes of all obtained mitogenome sequences to the mitogenome of *V. vulpes* (NC_008434.1, Arnason et al., 2006) and for trimming poorly-aligned and incompletely assembled tandem repeat region within the D-loop (see Results).

3.3.3 Phylogenetic analysis

To determine the phylogenetic relationship of *V. rueppellii* and *V. vulpes*, I downloaded representative haplotypes from Statham et al. (2014), Leite et al. (2015), and seven *V. vulpes* complete mitogenome sequences from GenBank, along with *V. lagopus* (KP342451.1, Sun et al. (2016) which was used as an outgroup. Details on included haplotypes/samples are provided in table 3.1. Then, MUSCLE v3.8 (Edgar 2004) as implemented in Geneious Prime 2022.2.2 was used for aligning the sequences and to generate FASTA file. I used W-IQ-TREE (Trifinopoulos et al. 2016) to construct a phylogenetic tree using a maximum likelihood approach based on the Hasegawa-Kishino-Yano (Hasegawa et al., 1985) model of sequence evolution, including an invariant sites parameter and a discrete Gamma model with 4 rate categories (HKY+F+I+G4), which had been determined as the optimal model using Modelfinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. The obtained tree was subjected to 1000 ultrafast bootstrap replications (Minh et al. 2013), and visualized using FIGTREE 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 3.1: Samples/haplotypes/sequences included in the phylogenetic analysis. Mitochondrial clades and subclades follow the terminology of Statham et al. (2014).

Sample ID/Haplotype/ Accession number	**Mitochondrial**		**Reference**
	Clade	**Subclade**	
V. rueppellii			
375 (MITObim)	Palearctic	n/d	This study
375 (NOVOPlasty)	Palearctic	n/d	This study
375 (Reference-based, ploidy 1)	Palearctic	n/d	This study
375 (Reference-based, ploidy 2)	Palearctic	n/d	This study
* V.ruRMo1 (KJ597994.1 and KJ597968.1)	Palearctic	n/d	Leite *et al*. 2015
V. vulpes			
* Oo24	Nearctic	I	Statham et al., 2014
* Fo12	Nearctic	II	Statham et al., 2014

* Ao63	Nearctic	III	Statham et al., 2014
* B2o106	Holarctic	I	Statham et al., 2014
* Uo211	Holarctic	II	Statham et al., 2014
* Go78	Holarctic	III	Statham et al., 2014
* Wo156	Holarctic	IV	Statham et al., 2014
* W4o175	Holarctic	V	Statham et al., 2014
* U35o98	Holarctic	VI	Statham et al., 2014
* U32o107	Holarctic	VII	Statham et al., 2014
* U12o115	Holarctic	VIII	Statham et al., 2014
* U8o118	Holarctic	IX	Statham et al., 2014
* Xo244	Africa	n/d	Statham et al., 2014
* X2o252	Africa	n/d	Statham et al., 2014
* X3o262	Africa	n/d	Statham et al., 2014
* V.vuMO4 (KJ598014.1, KJ597980.1)	Maghreb 1	n/d	Leite *et al*. 2015
* V.vuMO1 (KJ597977.1, KJ598009.1)	Maghreb 2	n/d	Leite *et al*. 2015
* Y2o197	Palearctic	I	Statham et al., 2014
* Yo202	Palearctic	II	Statham et al., 2014
* Yo155	Palearctic	III	Statham et al., 2014
* Y9o117	Palearctic	IV	Statham et al., 2014
KP342452.1	Nearctic	n/d	Sun et al., 2016
GQ374180.1	Holarctic	n/d	Zhong et al., 2010
KF387633.1	Holarctic	n/d	Zhang et al., 2015
JN711443.1	Holarctic	n/d	Yu et al., 2012
AM181037.1	Holarctic	n/d	Arnason et al., 2006
MN122913.1	Holarctic	n/d	DNAmark project, unpublished
KT448287.1	Holarctic	n/d	Koepfli et al., 2015

* Fragments of cytochrome b and D-loop (each <400 bp long), included in the present phylogenetic analysis to anchor analysed mitogenomes to existing *Vulpes* clade terminology. Genbank accession numbers for Statham et al. (2014) haplotypes are provided in their supplementary information. n/d: not determined.

3.3 Results and Discussion

I obtained a total of 216,237,628 read pairs for the sequenced *V. rueppellii* individual. The number of assembled mitogenome reads were 418,834 (average mitogenome coverage: 4,176) for NOVOPlasty, 361,475 (coverage: n.d) for MITObim, and 855,917 (coverage: 7,401)

for reference mapping (same for ploidy 1 and 2). The resulting total mitogenome length for *V. rueppellii* was 16,517 bp (NOVOPlasty), 20,6111 bp (MITObim) and 16,813 bp (reference mapping).

The sequences from the four approaches matched to the coding and non-coding regions of the V. vulpes mitochondrial reference genome (Arnason et al. 2006), yielding an overall identical organization, number and length of 13 protein-coding, two rRNA and 22 tRNA genes and D-loop. A 711 bp portion of the D-loop (positions 16,103 to 16,813 in the *V. vulpes* mtDNA reference genome; Arnason et al. 2006) contained a repetitive region known to comprise tandemly repeated variations of a ca. 12 bp repeat, and showed unreliable alignment characteristics (indels, uneven read coverage and apparent heterozygous sites in the raw read data, despite mtDNA being a haploid genome). I attributed this to the failure of short-read based sequencing methods to properly assemble the complete D-loop (see Formenti et al. 2021), especially around the tandem repeat region, based on the 151 bp read length used here. Following the trimming of this 711 bp region of the D-loop, we retained a 16,102 bp alignment for phylogenetic analysis.

Across this remaining alignment, the four mitogenome sequences obtained from different bioinformatic approaches yielded identical sequences. No previous studies have specifically compared the performance of the four assembly approaches used here to extract the whole mitogenome of *V. rueppellii*. One study by Machado et al. (2016) on frogs compared de novo and reference-based mapping using different software and pipelines than I used here and found the baiting and iterative mapping approach by MIRA/MITObim to be the best approach to extract the mitogenome, even from a low number of reads. However, in this study the outperformance of MIRA/MITObim over other approaches could be specific for frogs mitogenome (Machado et al. 2016). In Dierckxsens et al. (2017), NOVOPlasty outperformed MITObim slightly in terms of accuracy and memory usage, although its benefits may be especially prominent for AT-rich genomic regions. My analyses suggest that, at least when sufficient coverage is obtained and repeat-rich regions are excluded, the investigated approaches can yield identical results.

Maximum likelihood phylogenetic analysis yielded a tree (Fig. 3.1) in which *V. rueppelli* clustered inside the variation of *V. vulpes*, falling within the previously identified Palearctic

haplotypes/clade (Statham et al., 2014; chapter 2), rendering *V. vulpes* paraphyletic. This clustering is in accordance with previous work by Leite et al. (2015) who demonstrated clustering of *V. rueppellii* with *V. vulpes.* The support for most of the main clades in the tree was high, except for the Palearctic clade (bootstrap value, BV = 48). This clade received higher support (Bayesian posterior probability, BPP: p=0.99) in chapter 2, where I analysed shorter sequences, suggesting impact of small sample size in the present analysis.

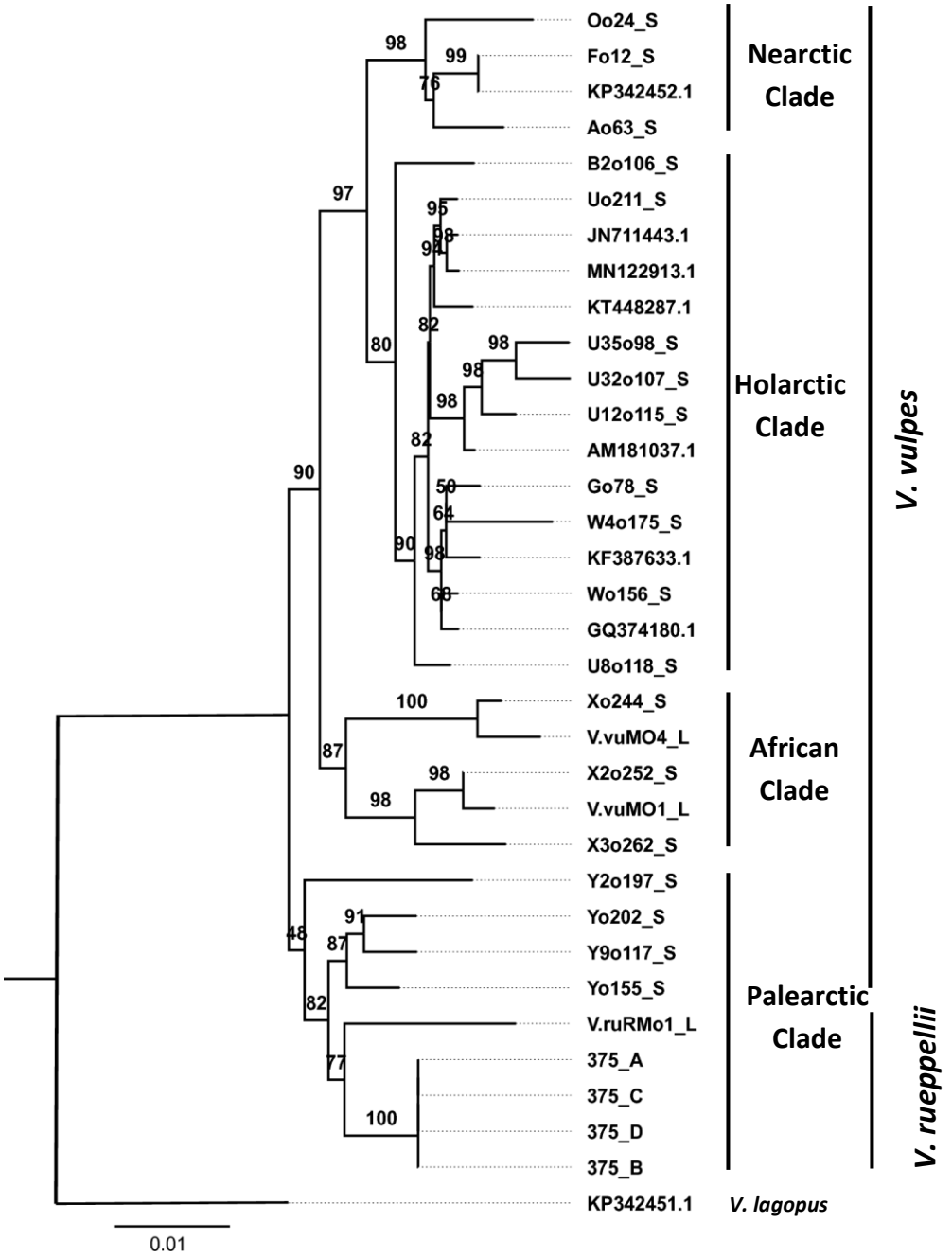

Figure 3.1: Maximum likelihood tree obtained from IQ-TREE based on an alignment of 16,147 bp with 1,000 bootstrap replicates and *V. lagopus* (KP342451.1) as an outgroup. Sample names are followed by **S** for sequences from Statham *et al.* (2014), and by **L** for those from Leite *et al*. (2015). The newly sequenced *V. rueppellii* (375) is followed by a letter for each assembly approach: A= MITObim, B= NOVOPlasty, C= reference-based-ploidy_1 and D= reference-based-ploidy_2. IDs that are not followed by a letter are Genbank accession numbers. Numbers on branches are bootstrap values; scale bar shows nucleotide substitutions per site. See table 3.1 for details on sample IDs.

3.4 Conclusion

I here report the first mitochondrial genome sequence of *V. rueppellii*, termed here as 'near-complete' due to incomplete characterisation of the tandem repeats in D-loop. This genome will be useful for future phylogenetic and other evolutionary studies of the little-studied *V. rueppellii* and its relatives. My results showed consistency of the de novo and reference-based approaches in extracting near-complete mitochondrial genomes, at least when excluding the tandem repeats. Assembling highly repetitive regions such as this will likely require read lengths which span across the entire repeated region, e.g., using Pacbio or Nanopore approaches, or long-range Sanger sequencing. Future sequencing of more individuals across the range of both species, combined with sequencing of long mitochondrial fragments will be required to improve the current low support of the Palearctic clade and to shed further light on the evolutionary history of *V. rueppellii* and *V. vulpes.*

3.5 References

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Chapter 4: Genomic Differentiation Between Red Fox (*Vulpes vulpes*) And Rüppell's Fox (*V. rueppellii*), Despite Signals of Past and Recent Introgression

4.1 Introduction

Closely related taxa frequently share genetic polymorphisms across their genomes, due to e.g., incomplete linage sorting (ILS) or post-speciation gene flow (Seehausen 2004; Rheindt and Edwards 2011; Mallet et al. 2016; Malinsky et al. 2018; Lavretsky et al. 2019). Such shared polymorphism complicates species delimitation and the reconstruction of species trees. Phylogenetic approaches have traditionally been applied to datasets involving only small numbers of loci or even only one marker, nevertheless aiming to obtain phylogenetic trees that should inform about past speciation events (Felsenstein 2004; Nater et al. 2015). Most phylogenetic methods perform best in cases of strictly bifurcating trees and pronounced reproductive isolation, but interpretations are less straightforward in cases such as gradual allele frequency changes among closely related species (Nater et al. 2015). Moreover, phylogenetic signal from a single gene/marker can cause bias interpretation, because single-locus genealogies often will not reflect the evolutionary history (i.e., species tree) of the populations or species (Edwards and Beerli 2000; Orozco-terWengel et al. 2011; Sequeira et al. 2011). Sequencing data from numerous, unlinked and hence statistically independent genomic regions can improve phylogenetic inference (Knowles and Maddison 2002; Knowles 2009; Carstens et al. 2013; Cozzolino et al. 2020). Such approaches are therefore promising for assessments of phylogenetic relationships of closely related species, potentially helping to overcome the known limitations arising from of analysis of single/few markers (Cozzolino et al., 2020; Edwards & Beerli, 2000; Edwards, 2009; Hipp et al., 2014).

For a long time, mitochondrial DNA (mtDNA) has been used as the marker of choice for examining the evolutionary history and relationships of the closely related species (Avise 2009; Sequeira et al. 2011). Unlike biparental nuclear DNA (nuDNA), mtDNA is an advantageous genetic marker because its maternally inherited, mutates fast, haploid and does not undergo genetic recombination (Hutchison et al. 1974; Brown et al. 1979; Mazzatenta et al. 2021). However, mtDNA is known to suffer from some drawbacks in terms of its suitability for inference of species/population trees: (a) in most animals, dispersal is often male-biased, with males dispersing further away and more frequently than females (Greenwood 1980; Bidon et al. 2014; Li and Kokko 2019; Walton et al. 2021). Such sex-biased dispersal can lead to emergence of kin-structured populations, creating different spatial

patterns between the two sexes (Walton et al. 2021). (b) As a consequence of the low dispersal and low effective population size, mtDNA is expected to experience a higher level of genetic drift than nuDNA (Bernardo et al., 2019). Therefore, mtDNA is more likely to introgress across species boundaries (Petit and Excoffier 2009; Melo-Ferreira et al. 2011). (c) Selection on the haploid and gene-dense mtDNA molecular can be strong and rapid, which favours emergence of discordance signals between nuDNA and mtDNA (Bonnet et al. 2017); (d) The mitogenome represents only a very limited fraction of the entire genomic information, e.g., in mammals typically ca. 15 – 20 kbp of mtDNA compared with ca. 1.6 – 6.3 Gbp nuclear genomes (Gissi et al. 2008; Kapusta et al. 2017). Therefore, evolutionary conclusions drawn from mtDNA will only represent a small fraction of the entire genome, and hence capture only a fraction of the diverse phylogenetic signals that describe the evolutionary history of an organism (Saccone et al. 1999).

The red fox (*Vulpes vulpes*) and Rüppell's (*Vulpes rueppellii*) occur in sympatry in North Africa and the Middle East, and are considered sister taxa (Geffen et al. 1992; Lindblad-Toh et al. 2005; Leite et al. 2015). The two species are morphologically, behaviourally, and physiological different (Lariviere and Seddon 2001). *Vulpes vulpes* has the widest natural distribution of any terrestrial carnivore (Wozencraft 2005; Macdonald and Reynolds 2008), with 45 described subspecies (Lariviere and Pasitschniak-Arts 1996; Sacks et al. 2010). the species occurs in Europe, Asia, North America and is in North Africa mainly found in relatively humid areas of desert oases and along the Nile River (Macdonald & Reynolds, 2008). Beyond this, the species occupies a wide variety of ecosystems, including grasslands, forests, deserts and agricultural and human-occupied environments (Lariviere and Pasitschniak-Arts 1996). In contrast, *V. rueppellii* is an arid adapted species distributed from across North Africa to Pakistan, with up to six described subspecies (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004; Mallon et al. 2015).

Based on an analysis of mitochondrial cytochrome *b* and D-loop markers, the two species did not appear well differentiated (Leite et al., 2015) (chapter 2)**.** Five mitochondrial clades (Holarctic, Nearctic, Palearctic, Africa 1 and Africa2) have been identified for *V. vulpes* (Statham et al. 2014; Leite et al. 2015) (chapter 2) with *V. rueppellii* splitting into two subclades and clustering within the Palearctic clade, leading to paraphyly of *V. vulpes* (chapter 2). In

chapter 2, I sequenced cytochrome b and D-loop markers for *V. rueppellii* from Arabia and Northeast Africa combining them with previously published sequences from Northwest Africa (Leite et al., 2015), and suggested that this non-monophyly could potentially arise from gene flow through a secondary contact. In contrast to these mtDNA findings, autosomal microsatellites support the differentiation between *V. vulpes* and *V. rueppellii* from North Africa (Leite et al., 2015), with a high genetic differentiation between European and North African *V. vulpes*, but gene flow signals among North African *V. rueppellii* populations. Support for nuclear genomic distinction of *V. rueppellii* has been found for slow-mutating nuclear SNPs (Sacks et al., 2018), although the are some limitations from these previous studies: (1) insufficient spatial sampling: Leite et al., (2015) sampled most of the studied foxes from Northwest Africa (only one sample from each species from Egypt) and Sacks et al., (2018) sampled only one *V. rueppellii* from Arabia. Increased sampling can improve the reliability of phylogenetic inference (Nabhan and Sarkar 2012; Figueroa et al. 2016); (2) the microsatellite data could be obscured by more recent population processes (Bohling et al. 2019): genomic differentiation might reflect recent population isolation/drift rather than long-term population isolation (McDevitt et al. 2021). Differing results from mtDNA and nuclear markers have been obtained in a range of other carnivore sister species, e.g., polar (*Ursus maritimus*) and brown (*Ursus arctos*) bears (Cronin and MacNeil 2012; Hailer et al. 2012; Miller et al. 2012; Liu et al. 2014), Iberian (*Lepus granatensis)* and Mountain (*L. timidus*) hares (Seixas et al. 2018).

Discordant phylogenetic signals between mtDNA and nuDNA are found in many taxa, including mammals (Toews and Brelsford 2012). Such cases of discordance between mtDNA and nuDNA genes can be attributed to incomplete lineage sorting (ILS) of ancestral polymorphism when within-species polymorphism lasts longer than the time between two successive speciation (Scornavacca and Galtier 2017), recent admixture, sex-biased gene flow or natural selection (Ballard & Whitlock, 2004; Funk & Omland, 2003; Hinojosa et al., 2019; Toews & Brelsford, 2012). In some mammals, interspecific hybridization is sex-biased, so signals from mtDNA may differ from those at biparentally inherited loci which is dispersed (more) through males (see Bidon et al. 2014 and references therein), e.g., brown and polar bears (Hailer et al. 2012), Iberian and Mountain hares (Seixas et al. 2018). Distinguishing between ILS and introgression using uniparentally inherited markers (e.g., mtDNA) is difficult,

because they leave similar genetic signatures (Buckley et al., 2006; Peters et al., 2007; Wang et al., 2014). Analysis of multiple independent markers/loci is needed to infer robust phylogenetic relationships (Cozzolino et al., 2020; Edwards & Bensch, 2009; Toews & Brelsford, 2012).

Although *V. vulpes* and *V. rueppellii* have been extensively studied using mtDNA (Frati et al., 1998; Inoue et al., 2007; Perrine et al., 2007; Aubry et al., 2009; Teacher et al., 2011; Edwards et al., 2012; Yu et al., 2012a; Kutschera et al., 2013; Ibiş et al., 2014; Statham et al. 2014 Leite et al. 2015; chapter 2), no previous studies have examined genome-wide biparentally autosomal loci across North Africa and the Middle East. In fact, the suggested signals of ILS and/or gene flow for mtDNA (Leite et al. 2015) and chapter 2) among *V. vulpes* and *V. rueppellii* have so far not been investigated for biparentally inherited DNA. Hence, using high-throughput sequencing technologies to produce genome-scale DNA polymorphism data would enable a comprehensive assessment of any mito-nuclear discordance and more broadly the evolutionary history of the two species.

High-throughput sequencing methods have become established, due to their economic and efficient ability for scanning thousands of representative loci across the entire genome (Funk et al., 2012; Lavretsky et al., 2019; Oyler-McCance et al., 2016; Rice et al., 2011). Such methods may provide sufficient coverage of the genome to detect genetic regions involved in phenotypic divergence and speciation (Seehausen 2004; Wu and Ting 2004; Wolf et al. 2010; Nosil and Schluter 2011; Rice et al. 2011; Abbott et al. 2013), in addition to providing sufficient power for multi-locus diagnosis of closely related species and populations (Ellegren 2008; Stapley et al. 2010; Toews et al. 2015) that might have not achieved with single/limited markers (Emerson et al. 2010; Jeffries et al. 2016; Puckett et al. 2016; Marková et al. 2020). Of the various reduced-representation sequencing techniques available to date, a well-established approach for non-model organisms is double digest restriction-site associated DNA sequencing (ddRAD-seq, Peterson et al. 2012), a technique modified from the original RAD-seq (Baird et al. 2008). ddRAD-seq involves the digestion of whole genomes using two restriction enzymes (Miller et al. 2007; Baird et al. 2008; Lavretsky et al. 2015) and subsequent shotgun sequencing, allowing identification of SNPs from sequenced genomes (Peterson et al. 2012). A key advantage of ddRAD-seq over the other RAD-seq approaches is that it generates

libraries containing a greater portion of homologous fragments, resulting in higher sequencing depths and less missing data at each locus, which is useful for accurate variant calling (i.e., rather than scoring sequencing errors or false homozygotes; Peterson et al., 2012; Valencia et al., 2018). I here used ddRAD-seq to obtain genome-wide, bi-paternally inherited SNPs, aiming to:

1) Investigate whether the previously reported nesting of *V. rueppellii* within the genetic variation of *V. vulpes* (i.e., mtDNA paraphyly of *V. vulpes*) is also discernible for nuclear genomic markers. Specifically, this involved testing of three hypothetical evolutionary scenarios outlined in chapter 2. a) *V. rueppellii* as an ecotype of *V. vulpes*: If mtDNA paraphyly is representative of genome-wide signals, we expect *V. rueppellii* to be clustered within the variation of *V. vulpes,* and *V. rueppellii* representing recently evolved form of *V. vulpes* that has adapted to arid habitats; b) Incomplete lineage sorting (ILS): recent divergence of *V. rueppellii*, implying that more evolutionary time might be needed for shared ancestral polymorphisms to be sorted into reciprocally monophyletic lineages, and c) Introgression: the two species might across most of their genomes be differentiated and *V. rueppellii* represent a distinct species, while the reported mitochondrial paraphyly could reflect gene flow postdating the original speciation and genomic differentiation, leading to secondary similarity for mtDNA.

2) Characterize the levels the genome-wide genetic variability among populations of both fox species, where many populations might be fragmented and hence at risk of genomic erosion (Díez-del-Molino et al. 2018).

4.2 Materials and Methods

4.2.1 Sample collection

A total of 100 fox samples were sequenced as part of this study (Fig. 4.1), including 70 tissue samples from Egypt (51 *V. vulpes* and 19 *V. rueppellii*) seven samples from road-killed animals from Libya (five *V. vulpes* and two *V. rueppellii*); four road-killed *V. vulpes* samples from Algeria; six *V. vulpes* samples from road-killed animals from UAE; eight *V. vulpes* from Portugal

and five road-killed *V. vulpes* obtained from the Vale of Glamorgan Council and Cardiff Council (Wales, UK).

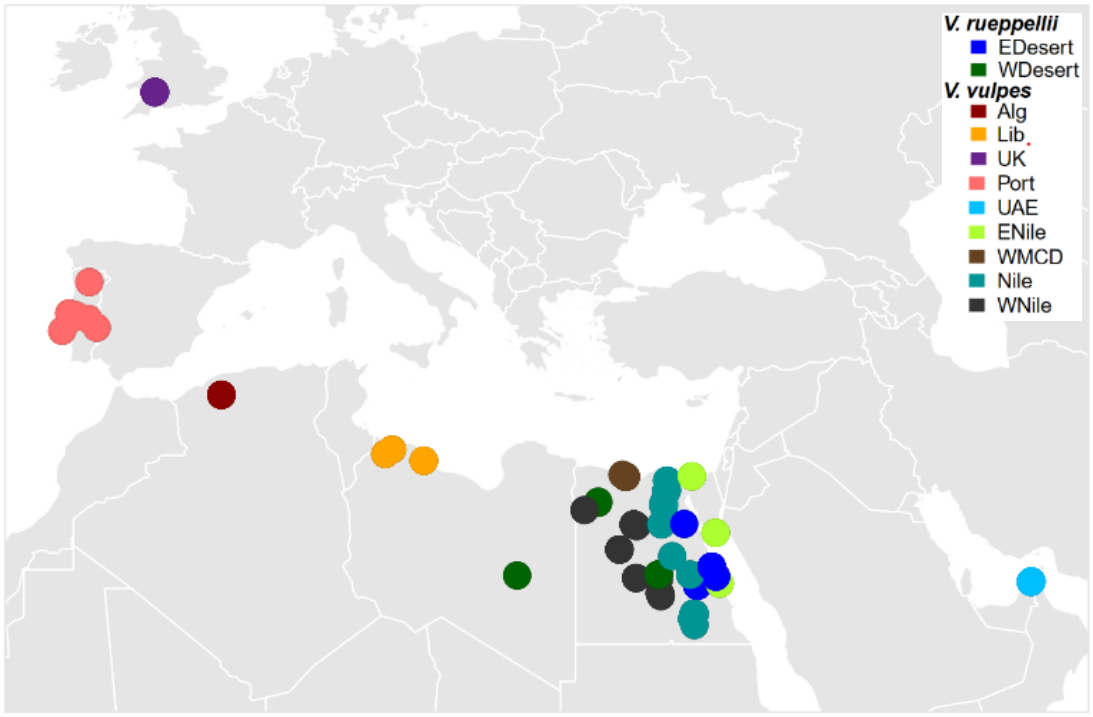


Figure 4.1: Distribution of in total of 100 *V. vulpes* and *V. rueppellii* samples analysed in this study. Dots correspond to approximate sample locations, coloured by geographic grouping as used throughout this chapter. Sample sizes are given in the main text; Table 4.2. For further details on samples, see Appendix 4.1.

4.2.2 Laboratory procedures

4.2.2.1 DNA extraction

Genomic DNA was extracted from tissue and samples using a salting-out protocol modified from (Rivero et al. 2006), which in turn was based on the Puregene™ DNA extraction kit (Qiagen, Hilden, Germany), with the addition of RNase A (Thermo Fisher Scientific) following the lysis step. DNA quality and quantity were assessed by electrophoresis in 1% agarose gels and a Qubit fluorometer v.3.0, respectively.

4.2.2.2 Library preparation

Approximately 200 ng of high molecular weight DNA for each sample was submitted to the Plateforme d'Analyse Génomique (Institut de Biologie Intégrative et des Systèmes IBIS, Université Laval, Québec, Canada) for library preparation. Two ddRAD-seq libraries, one for 96 individuals and one for the remaining eight (in total 100 individuals plus four technical replicates), were constructed: DNA was digested with NsiI (ATGCAT) and MspI (CCGG) restriction enzymes (New England Biolabs) – which were found to produce a large number of fragments compared to other restriction enzymes based on an *in silico* restriction analysis of the *V. vulpes* genome. Digestion was followed by library preparation following the protocol of Poland et al. (2012), adding adapters to both ends of each fragment, along with unique individual identifiers. Next, libraries were pooled, and fragment size was selected to ~ 375bp (expected DNA insert size: 200– 500 bp) using a Blue Pippin (Sage Science). The adaptor-ligated fragments were then PCR amplified in 25 µL volumes with 8µl H_2o, 10 µl of DNA fragment pool, 5µl of 5 × NEB Master Mix (New England Biolabs Inc.), and 2 µl of 10 pmol of each of the following Illumina primers: 5´-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT and 5´-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAACCGCTCTTCCGATCT.
Temperature cycling consisted of 98°C for 30 s followed by 18 cycles of 95°C for 30 s, 62°C for 20 s, and 68°C for 30 s, with a final extension step at 72°C for 5 min. The libraries (now containing ID tags and Illumina flow cell adapters) were purified using the QIAquick PCR Purification Kit (Qiagen). An aliquot was run on the BioAnalyser 2100 to verify fragment sizes. Library DNA was then quantified on a Nanodrop 2000 (Thermo Fisher Scientific) and subsequently sequenced on an Illumina NovaSeq S4 instrument (Génome Québec, Montréal, Québec, Canada).

4.2.3 Data analysis

4.2.3.1 Data processing and SNP calling

I used FASTQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to check the quality of reads. STACKS v2.54 (Rochette et al. 2019) was used for demultiplexing,

sorting, adaptor removal and for filtering out of low quality reads, using the *process_radtags* script. I mapped the remaining adaptor-free and high-quality reads against the chromosome-level genome assembly of dog (*Canis lupus familiaris*; assembly ROS_Cfam_1.0; Field et al., 2020) using BWA-MEM v0.7.17 (Li and Durbin 2009) with the default parameters. I ran *flagstst* in SAMTOOLS v1.10 (Li et al. 2009) to assess the proportion of single-end and not properly mapped reads. All samples showed paired-end reads mapping success ≥ 97 %. I then used SAMTOOLS to create sorted bam files from the previously obtained .sam files. The *Gstacks* program implemented in STACKS was used to build an initial data catalog, using all the reference mapped samples. Next, a SNP dataset was generated using the *populations* program in STACKS , using the following filters: (a) p=10 (loci genotyped in at least 10 out of the total 11 populations, to limit the amount of missing data), (b) -r=0.8 (loci found in 80% of samples, to limit missing data), (c) --min-maf 0.05 (only SNPs with minor allele frequency greater than or equal to 5% used, to ensure that rare SNPs, possibly resulting from errors in SNP calling, were excluded), and (d) --max-obs-het 0.5 (avoiding false positively called SNPs and sites resulting from paralogous loci; Rochette and Catchen 2017). Next, I identified and excluded loci located in stacks with more than three SNPs, to avoid genomic regions of low mapping/assembly or sequencing quality, by making a blacklist and running populations again with the above options (a-d) with the flag *--blacklist* to exclude those loci. Because of the expected effect of SNPs that are out of both Hardy Weinberg equilibrium (HWE) and linkage disequilibrium (LD) on genetic structuring and admixture analysis, steps were undertaken: SNPs that did not conform HWE were identified and excluded by running the *populations* program with the flag *–blacklist*, to exclude the corresponding loci using the same filtering criteria above in addition to the flag (e) --write-single-snp (only one SNP per locus, to avoid extreme linkage between SNPs), resulting in 39,035 SNPs and retaining all individuals (n=96). To assess the LD pattern, the correlation coefficient (r^2) between any two loci in each population was calculated using VCFTOOLS v0.1.16 (Danecek et al. 2011). Parameters were set as follows: --ld –window -bp 1000000, –geno -r^2 and --min-r^2 0.001. Then I used a custom R script to plot the LD decay curve. Next, PLINK v 1.07 (Purcell et al. 2007) was used to filter out linked SNPs based on the setting: --indep-pairwise 50 5 0.2, where 50, 5 and 0.2 are window size in kbp, step size and correlation coefficient (r^2), respectively. This filtering resulted in pruning and keeping of 24,420 and 14,615 SNPs, respectively. PLINK was then used

to convert PED files to *PLINK* format to be used in SambaR (de Jong et al. 2021), within R version 4.2.0 (R Core Team 2022), for downstream analysis. Finally, I applied flags indmiss= 0.25 (maximum allowed proportion of missing data points per sample) and snpmiss= 0.1 (maximum allowed proportion of missing data points per SNP) of the *filterdata* command inSambaR. Following this filtering, I retained 96 individuals with 12,601 SNPs (*combined dataset*). I ran another analysis for the *combined dataset* following the previous steps, but without filtering for HWE, resulting in 14,101 SNPs for final analysis.

The populations of *V. rueppellii* showed lower genetic structuring and heterozygosity compared to *V. vulpes* (see below). This could result from a bias due to using more *V. vulpes* than *V. rueppellii* samples, with possible effects on SNPs calling. Therefore, I split the samples by species, generating another two SNP datasets: *Vv77 dataset* (77 *V. vulpes* individuals), and *Vr19 dataset* (19 *V. rueppellii*) to look at signals within each species separately. Following the same filtering steps for the *combined dataset* (except p=8 for *Vv77 dataset* and p=1 for Vr19 dataset), 17,564 and 4,890 SNPs were obtained for the *Vv77* and *Vr19 datasets* respectively. All downstream analyses were done on the *combined dataset*, unless specifically mentioned otherwise.

4.2.3.2 Genetic structure

Genetic structuring analysis was performed in R, using wrapper functions of the R package SambaR. The data was imported into R and stored in a genlight object provided by the R package adegenet (Jombart 2008; Jombart and Ahmed 2011). Principal coordinate analyses (PCoA) were performed using the function 'pcoa' of the R package ape (Paradis and Schliep 2018), based on a matrix of Nei's genetic distances. Cluster analysis was done using ADMIXTURE v1.3.0 (Alexander et al. 2009) using map/ped files obtained from PLINK. Admixture was run with K (the number of clusters to be inferred) set to values from 1-11 (up to the maximum number of populations studied here), for five iterations each, enabling –cv flag to estimate cross-validation errors (set to five-fold). It has been reported that the most likely value of K is that with the lowest cross-validation error (Alexander and Lange 2011), although there is ongoing debate about the ability of this approach to detect the best value

of K (Lawson et al. 2018; Carlen and Munshi-South 2021). The outputs from ADMIXTURE were plotted using the *plotstructure* function in SambaR. Pairwise F_{ST} estimates of population differentiation were calculated according to (Weir and Cockerham 1984) using wrapper functions in SambaR in turn depending on the 'stamppFst' function of the R package StAMPP (Pembleton et al. 2013).

4.2.3.3 Genetic diversity

I used the *populations* script in STACKS (Rochette et al. 2019) to calculate genetic diversity statistics across all sites (variants and non-variants) of the entire dataset (*combined dataset;* 96 individuals), after excluding loci located in stacks with more than three SNPs and after filtering for HWE. Using the *--fstats* flag in *populations* script, I calculated indices of genetic diversity including expected heterozygosity (H_E), observed heterozygosity (H_O), nucleotide diversity (π). Additionally, the function "*kinship"* in SambaR wrapper was used for calculations of individual inbreeding coefficient based on the probability that the two alleles at any locus of a diploid individual are identical by descent (IBD), (Kardos et al., 2015).

4.2.3.4 Inference of population divergence and admixture

To jointly infer population splitting and gene flow events, I used TreeMix v1.13 (Pickrell and Pritchard 2012) including *Vulpes lagopus* and *V. zerda* as an outgroup. TreeMix uses genomic-scale allele frequency data, to infer the maximum likelihood tree including gene flow (migration) events. The nodes in the tree represent population splits, the horizontal branch lengths are proportional to the amount of genetic drift that has occurred, while the coloured arrows connect populations inferred to be admixed due to directional gene flow (Pickrell and Pritchard 2012; Demos et al. 2015). Whole genome data of two *V. lagopus* (ERR5417968 and ERR5417974; (Hasselgren et al. 2021) and two *V. zerda* (SRR14750349, SRR14750511; Phase One Resequencing for 10,000 Dog Genome Consortium) were downloaded from the Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) using the SRA Toolkit (https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software; SRA Development Team), and combining them with the *combined dataset* as follows: I downloaded *.bam* files from SRA

and then used *fastq-dump* implemented in SRA toolkit to generate forward and reverse FASTQ files. I ran FASTQC v0.11.9 to check the quality of reads and TRIMMOMATIC v0.39 (Bolger et al. 2014) to remove adaptor sequences and low-quality reads. The resulting reads were then mapped to the same dog reference genome mentioned above (ROS_Cfam_1.0), using BWA-MEM v0.7.17 with default parameter settings. PCR duplicates were removed using the MARKDUPLICATESSPARK program implemented in the GATK pipeline (https://gatk.broadinstitute.org/hc/en-us). After that, I used HAPLOTYPECALLER from GATK to call variants for each sample and performed joint genotyping across samples using GATK by running GENOMICSDBIMPORT program to combine the resulting GVCFs from HAPLOTYPECALLER into a single file and then GENOTYPEGVCFS to produce a multi-sample variant call-set. Then I used the SELECTVARIANTS program in GATK to extract SNPs only. BCFTOOLS (Li, 2011; Li et al., 2009) was used to extract SNPs from whole genome data, corresponding to the same chromosome positions variable in the ddRAD-seq data (*combined dataset*). Next, I applied the *–merge all* flag in BCFTOOLS to merge the datasets, producing a joint VCF file. I used PLINK to filter SNPs for linkage disequilibrium with the setting: --indep-pairwise 50 5 0.2 and to generate stratified allele frequencies for all populations. Following this filtering, I retained 14,485 SNPs for downstream analyses. Then I used the python script "plink2TreeMix.py" downloaded from https://bitbucket.org/nygcresearch/treemix/downloads to convert the allele frequencies output from PLINK into TreeMix format. After that, I ran TreeMix for twelve separate runs with the number of migration events (m) from 0 to 11 (number of the populations), assigning *V. zerda* as an outgroup (-root *V. zerda*). Then to identify the information contribution of each migration vector added to the tree (i.e., variance explained), I ran TreeMix with a global set of rearrangements (-global), and a randomly selected window size (-k) of between 100 and 1000 SNPs (50 SNP increments). The number of migration events (-m) varied between 1 (gene flow between two populations) and 11 (the total number of populations) and 10 replicates were performed for each value of "m". The value of "m" with the highest reproducibility and consistency, among the 11 tested, and which also had the highest composite log- likelihood value of 99.8% (recommended threshold for stopping the addition of migration edges, by Pickrell and Pritchard 2012), was chosen as the most optimal migration edge. Finally, I used custom R scripts in R-4.2.0 to plot the TreeMix maximum likelihood trees, and the R package

OptM (Fitak 2021) was used to plot the composite likelihood for each migration edges. To check further for admixture, I performed the three- and four-population (*f3* and *f4)* tests implemented in TreeMix. The *f3*-statistics (A, B, C) were to determine if 'A' was a mixture of populations 'B' and 'C'; a significantly negative value (cut-off Z scores < -3) of the *f3*-statistics would suggest population 'A' is admixed. In the four-population test, the expectation of *f*4 is zero under the null hypothesis whereas deviation from zero either positively or negatively indicate the presence of admixture. Given four taxa (*A*, *B)*, (*C, D)*, a significantly positive scores indicate gene flow between populations related to either 'A' and 'C' or 'B' and 'D', while a significantly negative scores suggest gene flow between populations related to either 'A' and 'D' or 'B' and 'C'. Since I was interested only in the gene flow between the two focal species, I excluded all the combinations that had both *V. lagopus* and *V. zerda*. Z-scores were reported for these tests (cut-off, Z> 3 for significantly positive scores and Z< -3 for significantly negative scores) with either *V. lagopus* or *V. zerda* as an outgroup or without both (only the populations of the two studied species). Standard errors of *f*3 and *f*4 statistics were computed using a block jack-knifing procedure with data split into blocks of 500 SNPs.

4.3 Results

A total of 1,306,414,734 paired-end reads were obtained from the sequencing provider. Technical replicates did not reveal any discordant signals when initially included in analyses detailed below (e.g., yielding near-identical results in PCoA and Admixture), so we excluded the four replicates, along with four additional samples that only had low average sequencing coverage (<10x), leaving in total 96 individuals of the two focal fox species for subsequent data analysis. For these 96 samples, following trimming and filtering, the per-sample coverage was on average 106.5x (SD=81.0; minimum coverage=10.9x, and maximum coverage=379.0x). For the single-species *Vv77* and *Vr19 datasets,* the per-sample mean coverage was 105.5x and 109.0x, respectively (stdev=79.2x and 87.0x; minimum coverage=10.9x and 12.2x; and maximum coverage=378.2x and 321.2x). Appendix 4.2 shows a summary statistic of the data used in this chapter.

Henceforth, the studied populations will be referred to by the following abbreviations: for *V. rueppellii*: Western Desert (WDesert), Eastern Desert (EDesert), for *V. vulpes*: Algeria (Alg), Libya (Lib), West of the Nile (WNile), Nile, East of the Nile (ENile), United Arabic Emirates (UAE), Portugal (Port), United Kingdom (UK), as shown in Figure 4.1.

4.3.1 Genetic structure

Principal coordinate analysis (PCoA) of the *combined dataset* (77 *V. vulpes* and 19 *V. rueppellii*) clearly separated *V. vulpes* from *V. rueppellii*, with PC1 separating the two species and explaining almost 90% of the variance (Fig. 4.2A). One *V. rueppellii* individual (ID:377), a female *V. rueppellii* from the Western desert of Egypt, was positioned in an intermediate position between the two species. Figure 4.2D shows the LD distribution decay curve of the two species. Given the large proportion of variance explained by just one coordinate, I next filtered the dataset for LD (cut-off r^2 at 0.2), which yielded a similar result (Fig. 4.2B) as before, but with PC1 now explaining only ca. 15% of the variance (A more restrictive r^2 =0.1 with 4,503 SNPs showed a clear separation along PC1 between the two species (Appendix 4.3)). The analysis of the *combined dataset* without filtering for HWE yielded a similar PCoA pattern as for Fig. 4.2B (see Appendix 4.4). The populations of *V. vulpes* clustered into four groups, placed along a gradient roughly corresponding to their geographic locations: Europe (UK and Port), Arabia (UAE), Northeast Africa (all studied Egyptian populations: Nile, WNile, ENile and WMCD) and Northwest Africa (Alg and Lib). The four individuals from Sinai (here included in the ENile population) were in an intermediate position between UAE and Northeast African populations, again corresponding to their geographic location. Next, to investigate fine-scale structuring within *V. rueppellii,* a PCoA for the *Vr19 dataset* only was conducted, showing clear genetic structuring among populations west (WDesert) and east (EDesert) of the Nile River, respectively, and with sub-structuring among the east of the Nile River populations (Fig. 4.2C). PCoA for *Vv77 dataset* (excluding *V. rueppellii*) showed the same patterns as for the *combined dataset* (Appendix 4.5).

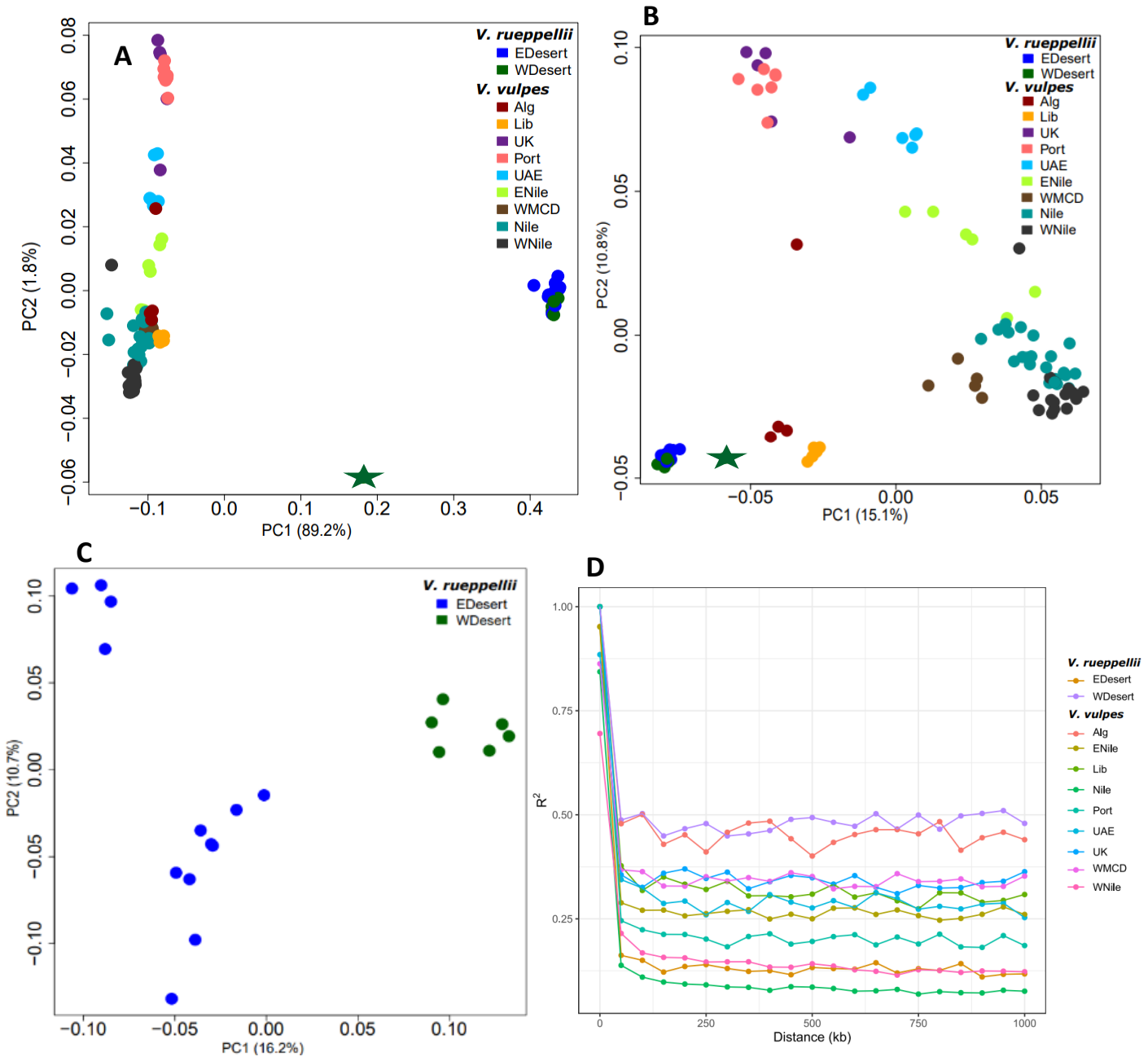


Figure 4.2: Principal Coordinate Analysis results. (A) *combined dataset*, based on 34,783 SNPs, not filtered for LD **(B) *combined dataset***, based on 12,601 SNPs, filtered for LD (r^2 cut-off: 0.2). The asterisk denotes a putatively admixed *V. rueppellii* individual (ID:377) from WNile (Egypt). **(C) *V. rueppellii* analyzed separately (*V.r19* dataset),** based on 4,890 SNPs, filtered for LD (r^2 cut-off: 0.2). **(D)** LD pattern in *V. vulpes* and *V. rueppellii*.

Admixture analysis showed a clear genomic differentiation between the two species (Fig. 4.3). At K = 2, *V. vulpes* and *V. rueppellii* were grouped into two largely separate groups. As in PCoA, *V. vulpes* showed a larger genetic structuring than *V. rueppellii*: with increasing K values, *V. vulpes* populations split off into geographically restricted subpopulations, while *V. rueppellii* remained clustering as a one group. An exception to this occurred at K = 7 & 8, where some admixture of the two populations east of the Nile River was detected, but the signal disappeared at K ≥ 9 (details not shown). The populations of *V. vulpes* at K = 4, the solution indicated as the best K value based on the cross-validation error (Fig. 4.3), were separated into three geographically defined subpopulations, i.e., Eurasia (UK, Portugal and UAE), Northeast Africa (Egypt) and Northwest Africa (Algeria and Libya). At K = 5, Eurasian populations of *V. vulpes* were split into Europe (UK and Portugal) and Arabia (UAE). There was a consistency of an admixed *V. rueppellii* individual from west of the Nile River, showing affinity to Algerian *V. vulpes* at different values of K (Fig. 4.3). Admixture analysis of *V. rueppellii* separately (*Vr19 dataset*) showed a signal of genetic structuring west (WDesert) and east (EDesert) of the Nile River, with sub-structuring among the east of the Nile River populations at K=3 (Appendix 4.6), consistent with the result from PCoA (Fig. 4.2C).

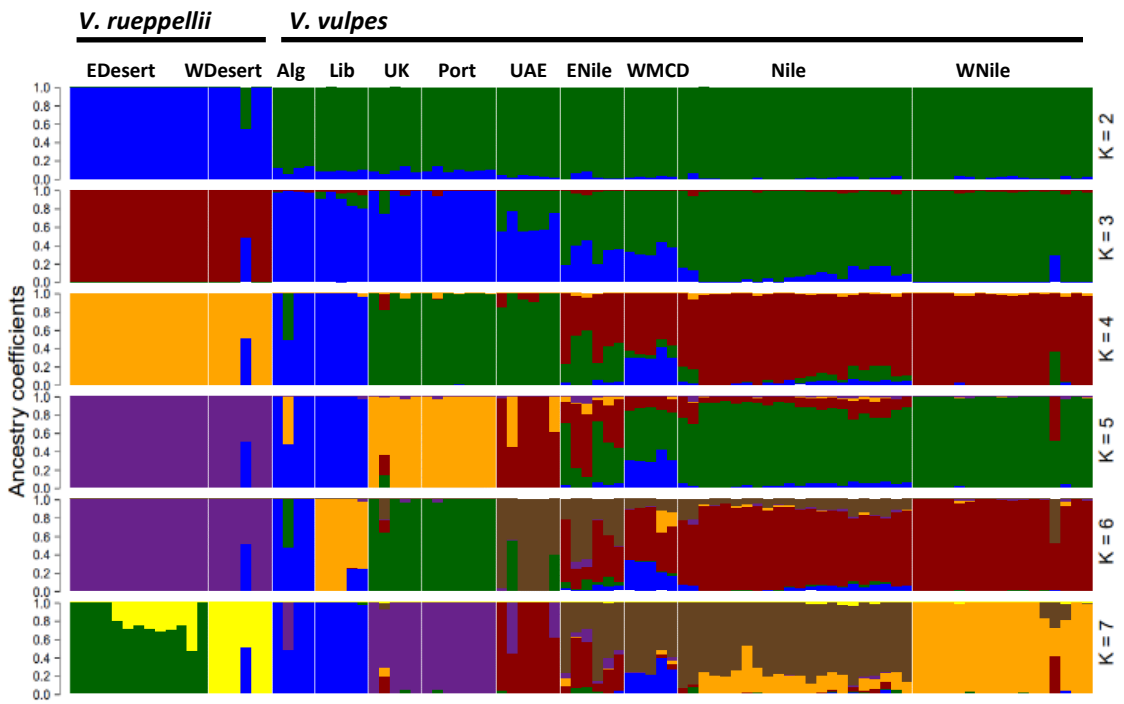
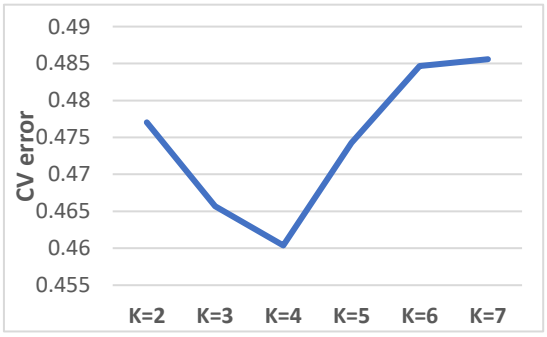


Figure 4.3: Admixture analysis of *combined data* of *V. vulpes* and *V. rueppellii* at K = 2-7, based on 12,601 SNPs. Corresponding cross-validation (CV) error values are shown bottom right.



Pairwise F_{ST} values between *V. vulpes and V. rueppellii* populations ranged from 0.206 to 0.550, indicating strong population genetic differentiation between the two species. The lowest and highest values among *V. vulpes* populations were 0.04 (between Nile and ENile) and 0.257 (Alg vs. UAE), respectively, while F_{ST} between the two *V. rueppellii* populations (EDesert and WDesert) was 0.062 (Table 4.1). The *p*-values for all the reported pairwise F_{ST} values were zero, indicating significant results.

Table 4.1: Pairwise F_{ST} values of the *combined dataset* of *V. vulpes* and *V. rueppellii* estimated based on Weir & Cockerham (1984). Bonferroni method has been used to correct for the *p*-values (significance threshold, *p*= 0.05).

	EDesert	WDesert	Alg	Lib	UK	Port	UAE	ENile	WMCD	Nile	WNile
EDesert	0.062	0.55	0.513	0.509	0.439	0.476	0.407	0.459	0.275	0.318	
WDesert		0.412	0.381	0.384	0.325	0.361	0.282	0.325	0.206	0.24	
Alg			0.137	0.254	0.213	0.257	0.183	0.174	0.152	0.182	
Lib				0.247	0.224	0.249	0.175	0.144	0.147	0.176	
UK					0.104	0.182	0.127	0.183	0.139	0.17	
Port						0.149	0.122	0.168	0.129	0.158	
UAE							0.093	0.169	0.116	0.147	
ENile								0.078	0.04	0.071	
WMCD									0.062	0.095	
Nile										0.038	
WNile											

4.3.2 Genetic diversity

Based on all the sites (variants and invariant sites), the estimated values of observed heterozygosity (H_o), expected heterozygosity (H_e), and nucleotide diversity (π) for *V. vulpes* populations were higher than those for *V. rueppellii* (Table 4.2). Based on segregating sites only (i.e., excluding invariant sites), the WDesert population of *V. rueppellii* had a non-significant ($p \geq 0.05$) positive inbreeding coefficient (0.3), while all remaining populations of both focal species had values close to zero, with a few outliers showing negative values (Alg, Nile, WNile, UK and UAE *V. vulpes* populations; Fig. 4.4). The admixed *V. rueppellii* individual from WDesert and one *V. vulpes* individual from Alg (which showed affinities to the Portuguese population at higher K values in the admixture analysis) showed high negative inbreeding coefficients (-1.80 and -1.30, respectively, Fig. 4.4).

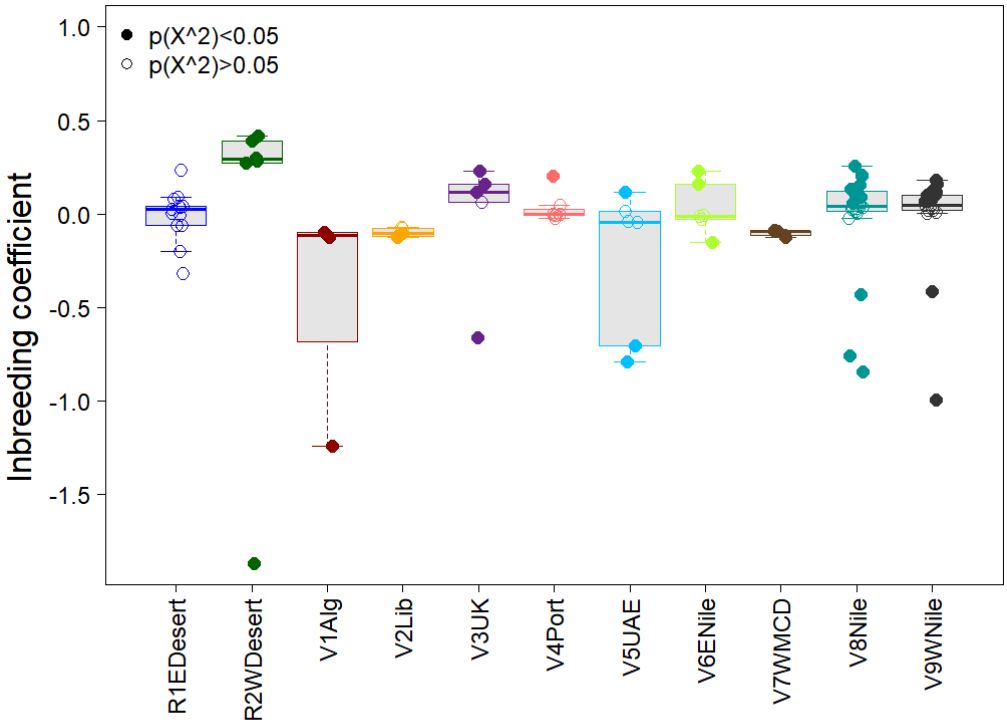


Figure 4.4: Individual inbreeding coefficients for *V. vulpes* and *V. rueppellii* individuals grouped by populations. Open circles are significantly different from zero ($p \leq 0.05$), while closed circles are non-significant ($p \geq 0.05$), based on Chi-square tests.

Table 4.2: Indices of genetic diversity of *V. vulpes* and *V. rueppellii* populations calculated for all sites (Variants and non-variants), after filtering for low-quality loci and HWE. n= number of individuals, H_O =observed heterozygosity and H_E =expected heterozygosity.

Population	Pop ID	*Species*	n	H_O	H_E	Pi (variance)
Western Desert*	WDesert	*V. rueppellii*	6*	0.00033	0.00035	0.00038 (0.00014)
Eastern Desert	EDesert	*V. rueppellii*	13	0.00027	0.00027	0.00028 (0.00012)
Nile	Nile	*V. vulpes*	22	0.00050	0.00050	0.00051 (0.00019)
West of the Nile	WNile	*V. vulpes*	17	0.00048	0.00047	0.00049 (0.00019)
Western Mediterranean Costal Desert	WMCD	*V. vulpes*	5	0.00046	0.00042	0.00047 (0.00020)
East of the Nile	ENile	*V. vulpes*	6	0.00042	0.00044	0.00048 (0.00019)
United Kingdom	UK	*V. vulpes*	5	0.00034	0.00033	0.00037 (0.00016)
United Arab Emirates	UAE	*V. vulpes*	6	0.00046	0.00038	0.00042 (0.00017)
Portugal	Port	*V. vulpes*	7	0.00035	0.00036	0.00039 (0.00016)
Algeria	Alg	*V. vulpes*	4	0.00039	0.00028	0.00032 (0.00014)
Libya	Lib	*V. vulpes*	5	0.00038	0.00035	0.00039 (0.00017)

*Indices of genetic diversity shown for this population (WDesert) are including the admixed *V. rueppellii* individual. When excluding this individual, all indices for this population were closer to values observed in the other *V. rueppellii* population (EDesert) (details not shown).

4.3.3 Population divergence and admixture

When using TreeMix without inference of any migration events, the obtained maximum likelihood tree showed *V. vulpes* and *V. rueppellii* as a reciprocally monophyletic, with *V. lagopus* and *V. zerda* clustered close to each other and outside the variation of the two focal species (Fig. 4.5A). Incrementally adding one to five migration events did not change this tree topology- only at m=3, ENile *V. vulpes* clustered with UAE instead of the consistent clustering with the Egyptian (Nile, WNile,WMCD) *V. vulpes* populations (Fig. 4.5 B-F). The first added migration edge suggested gene flow from UAE *V. vulpes* to the basal point of the Egyptian populations; ENile, WNile and Nile. Similarly, adding the second, third and fourth migration edges retrieved gene flow signals among *V. vulpes* populations; from UAE to east of Nile, from Libya to WMCD, and from Portugal to Algeria, respectively. The fifth migration edge showed the weakest signal of admixture, connecting Algerian *V. vulpes* to western desert *V. rueppellii*. Increasing the number of the migration edges to six led to a clustering of *V. rueppellii* within *V. vulpes*- a signal that was not observed at any other investigated values of 'm' (Fig. 4.5G). However, adding a 7th migration edge retrieved the previous tree topologies and gene flow signals (i.e., from m= 0-5), and added a gene flow signal from *V. lagopus* to WNile *V. vulpes* (Fig. 4.5H). This number of migration edges had the highest composite log-likelihood value, reaching 99.8% (recommended by Pickrell and Pritchard (2012) (Appendix 4.7), suggesting that no more edges should be added. Significantly negative *f3*-statistics (Z score < -3) showed *V. rueppellii* from west of the Nile (WDesert population) to contain admixture of *V. rueppellii* from east of the Nile (EDesert population) and any one of all remaining nine *V. vulpes* populations (Alg, Lib, WMCD, WNile, ENile, Nile, Uk, Port, UAE). The most extreme Z scores were -18, -10 and -7 for the admixture with Algerian (Alg), Libyan (Lib), Western Mediterranean costal desert (WMCD) *V. vulpes* populations, respectively (Appendix 4.8). These results are consistent with the presence of a putative recent hybrid observed in the PCoA (Fig. 4.2A, B) and admixture analysis (Fig. 4.3). Furthermore, *f4*-statistics suggested gene-flow signals between all the studied populations of the two focal species with either *V. lagopus* or *V. zerda* as an out group and when only in the absence of both (i.e., only the populations of the *V. vulpes* and *V. rueppellii*). *f4*-statistics showed a mix of significantly

positive (Z> 3) and negative (Z< -3) values when either *V. lagopus* or *V. zerda* assigned as an outgroup and when only the populations of the two species studied together (Appendix 4.9).

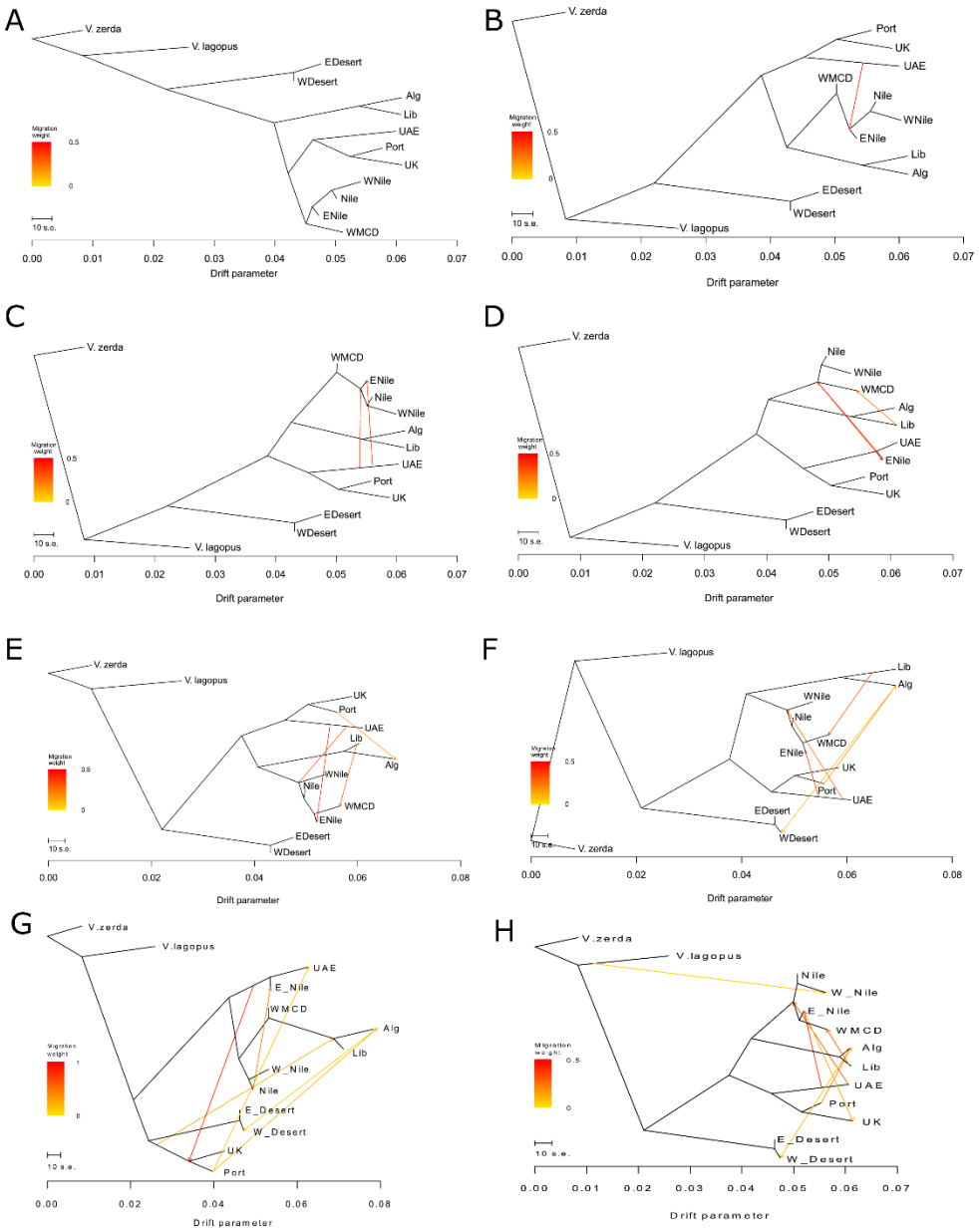


Figure 4.5: Maximum likelihood trees inferred by TreeMix, depicting the phylogenetic relationship of *V. vulpes* and *V. rueppellii* populations. Panels A-H show results for 0-7 inferred migration edges, respectively. The x-axis reflects the extent of genetic drift experienced by each branch in the graph. Colours of migration edge arrows: red indicates high migration weight, while yellow refers to low migration weight. *V. zerda* and *V. lagopus* were set as outgroups.

4.4 Discussion

Based on analysis of genome-wide SNPs, this study shows the two fox species as strongly genetically differentiated at nuclear loci, despite non-reciprocal monophyly for mtDNA. The rooted TreeMix phylogeny showed the two species as sister lineages, separated by a long divergence branch, consistent with ancient speciation rather than rapid, strong selection. The genomic SNPs also identified fine-scale population genetic structuring within each species, separating the populations according to their geographic locations. Notably, the analyses also showed some evidence for rare, likely recent hybridization. Altogether, these findings suggest that mtDNA paraphyly of *V. vulpes* (reported by Leite et al., 2015 and in chapter 2) could best be explained by ancient mitochondrial post-speciation introgression.

4.4.1 Mito-nuclear discordance and mtDNA paraphyly of *V. vulpes*

Mito-nuclear discordance is a widespread phenomenon in animals, including mammals (Toews and Brelsford 2012). Several reasons for the susceptibility of mtDNA to introgress across species borders have been suggested, e.g., sex-biased interspecific mating, neutral genetic drift in post-hybridization bottlenecks, or strong directional selection (reviewed by Toews & Brelsford, 2012). In the past 10 years, several studies have highlighted the adaptive retention of introgressed mtDNA, in species with deleterious mutations in their mtDNA (Llopart et al. 2014; Hulsey et al. 2016). Another neutral demographic mechanism that has received increasing attention recently in the introgression literature is range expansion of populations, e.g., during past climatic changes (polar bear, Cahill et al., 2013; Iberian hare, Marques et al., 2017), which can favour extreme and sometimes sex-specific bottlenecks which favour the emergence of phylogenetic discordance among loci. I next discuss three possible explanations (derived in chapter 2) for discrepancy between mtDNA and nuclear phylogenies reported for the two focal fox species:

Scenario 1: *V. rueppellii is an ecotype of V. vulpes*

The term ecotype refers to a genetically distinct population within a species that is adapted to a particular environment (Begon et al. 2005). Other species of canids contain ecotypes, e.g.,

wolves (Carmichael et al., 2007; Leonard et al., 2007; Musiani et al., 2007; Muñoz-Fuentes et al., 2009; Hendricks, Schweizer, & Wayne, 2019; Sarabia et al., 2021) and arctic foxes (Dalén et al. 2005; Norén et al. 2011). Possibly analogous to the focal taxa of this thesis, a generalist which shows adaptation to different habitats and occurs in many habitats across North America and Eurasia is the gray wolf *Canis lupus* (Hendricks et al. 2019), for which three distinct ecotypes have been described in North America (costal, forest and arctic wolves). In North Africa, a distinct ecotype of the African golden wolf (*Canis lupaster*) has been described (Sarabia et al., 2021).

If *V. rueppellii* is a subset of *V. vulpes* nuDNA variation, one could expect *V. rueppellii* and *V. vulpes* populations from North Africa and the Middle East to be less differentiated than *V. vulpes* populations compared between North African and Europe (here, UK and Portugal). Differentiation between *V. rueppellii* and all studied *V. vulpes* populations (F_{ST} range: 0.206-0.550) was higher than that between North African and Middle Eastern *V. vulpes* and European populations (F_{ST} range: 0.122- 0.254). It could be argued that *V. rueppellii* is a distinct form of *V. vulpes* that has experienced strong genetic drift, leading to larger inter-'specific' differentiation. However, the two populations of *V. rueppellii* do not show strongly reduced diversity compared with *V. vulpes* populations (Table 4.2), and the TreeMix results showed the two species as reciprocally monophyletic lineages with an ancient splitting event that occurred prior to diversification of *V. vulpes* populations across the study area (Fig. 4.5). Another piece of evidence against the ecotype scenario is the presence of considerable morphological (Lariviere and Seddon 2001; Sillero-Zubiri et al. 2004), ecological and behavioural (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004) differences between the two species. Based on the above reasoning and findings, the ecotype scenario is not a likely explanation for *V. vulpes* paraphyly.

***Scenario 2**: Incomplete lineage sorting (ILS)*

Species-level paraphyly can result from ILS if divergence occurred recently. In such cases, more time is required until ancestral polymorphisms will have sorted into reciprocally monophyletic lineages (Funk & Omland, 2003; McKay & Zink, 2010). ILS has been suggested as a cause of paraphyly across many taxa, e.g., European bison *Bison bonasus* (Wang et al., 2018), salmonids (Campbell et al. 2020) and birds (Suh et al. 2015). However, the obtained

TreeMix results in this chapter for *V. vulpes* and *V. rueppellii* based on genome-wide SNPs show the two species as reciprocally differentiated lineages, each at the end of a long drift branch, which points towards an old species divergence (Fig 4.5), although branch lengths may have been increased by drift within each lineage. Consistent with these TreeMix results, PCoA and admixture results (Figs. 4.2 & 4.3) showed a clear differentiation of the two species. Furthermore, the fossil record suggests an old divergence between the two species, where the oldest fossil remains of *V. rueppellii* have been recorded from Northwest Africa and dating back to ca. 0.8 Mya (Geraads 2011).

For mtDNA, ancestral polymorphisms should be lost earlier by means of within-lineage fixation of lineages, so the process of lineage sorting is predicted to be completed faster than for nuDNA (Funk & Omland, 2003). The reason for this is that mtDNA has an effective population size which is only ¼ compared with nuDNA (Hudson and Turelli 2003; Zink and Barrowclough 2008; Toews and Brelsford 2012), leading to more rapid drift and fixation for mtDNA. In *V. vulpes*, ILS might only extend across ca. 100–200 kya (discussed in chapter 2), based on a generation time of 1-2 years and an ancestral N_{fe} (the effective female population size) of 91,000 (Statham et al., 2018; Statham et al., 2014). ILS therefore appears unlikely to impact red fox mtDNA beyond a few 100 kya, a time frame younger than the divergence time suggested by the fossil record (Geraads 2011) . Hence, for these and for the same reasons mentioned above for the ecotype scenario, ILS appears to be an unlikely explanation for red fox paraphyly.

Scenario 3: *Old divergence of the two species, followed by secondary introgressive hybridization and paraphyly*

TreeMix analysis showed the two fox species as mutually monophyletic, with a weak signal of (likely recent) gene flow from *V. vulpes* into Western desert *V. rueppellii* (Fig. 4.5). Introgressive hybridization has been reported before in canids, e.g., between Ethiopian wolves (*C. simensis*) and domestic dogs (*C. familiaris*) (Gottelli et al. 1994), and between red wolves (*C. rufus*) and coyotes (*C. latrans*; Adams et al. 2003; Hailer and Leonard 2008). Interspecific hybridization in *Vulpes* has been described for *V. vulpes* and the kit fox (*Vulpes macrotis*) (Creel and Thornton 1974), and between *V. macrotis* and swift fox (*Vulpes velox*) (Dragoo and Wayne 2003). Analogous to these findings, admixture analysis (Fig. 4.3) found

one admixed *V. rueppellii* from the western desert (Egypt) at all values of K≥2. This individual showed ca. 50/50 admixture, which for K=6 best matched the *V. vulpes* gene pool from Algeria (Fig. 4.3). The same individual had an intermediate position between *V. vulpes* and *V. ruppellii* populations in PCoA (Fig. 4.2). In combination, these findings suggest that *V. rueppellii* and *V. vulpes* represent genomically strongly differentiated 'good' species, with signals of some interspecific gene flow.

Hence, mtDNA paraphyly of red foxes could likely be a consequence of introgressive hybridization. If true, this gene flow would likely have occurred in the distant past, since the two species do not share any mtDNA haplotypes (chapter 2), and since the shared Palearctic clade lineages are geographically widespread in both species – suggesting that whichever species was the recipient, post-introgression gene flow has had ample time to disperse the lineages across the range. The admixed *V. rueppellii* individual found in this present chapter in the Egyptian western desert likely represents recent admixture, and nuDNA introgression from *V. vulpes* into *V. rueppellii*. This thesis therefore presents tentative evidence of both past and present gene flow between the two species.

There are many reported mito-nuclear incongruence associated with low/negligible levels of nuclear introgression (Good et al. 2015), such as in elephants (Roca et al. 2005), hares (Melo-Ferreira et al. 2009) and chipmunks (Good et al. 2015). In contrast to nuDNA, mtDNA tend to introgress more readily (Doiron et al., 2002; Ferris et al., 1983; Powell, 1983; Roca et al., 2005; Shaw, 2002; Sota & Vogler, 2001), and most of reported cases of introgression in animals that involve the mtDNA (Toews and Brelsford 2012) involve high frequencies of introgressed lineages across extended geographic regions (Melo-Ferreira et al. 2005; Good et al. 2008; Sequeira et al. 2011), mirroring the case documented here for foxes. Many of the described cases of introgression have been linked climate fluctuations in the Pleistocene, which caused range shifts and population replacements, thus presumably a change in interspecific interactions which facilitated introgression (Marques et al. 2017).

It has been suggested that the mid-Pleistocene transition led to speciation events in North Africa (deMenocal, 2004), the time when *V. vulpes* first appears in the North African fossil record (Geraads 2011). Consequently, *V. vulpes* and *V. rueppellii* may have diverged in North Africa during the mid-Pleistocene 1.2-1.4 Ma (Leite et al., 2015), a time associated with

increasing aridity of the Sahara at 1.44 ± 0.2 Ma (Trauth et al. 2009). This time also coincides with the proposed speciation event of African golden wolves (Sarabia et al. 2021), the emergence of several clades of rodents (*Praomys rostratus*; Nicolas et al., 2008; *genus Acomys*; Nicolas et al., 2009; desert-adapted *Gerbillus tarabuli*, Ndiaye et al., 2012), and appearance of haplogroups of scimitar-horned oryx (Iyengar et al. 2007). The fossil record of *V. vulpes* is richer than that for *V. rueppellii,* and the former species has been found in several geographic regions. In North Africa, *V. vulpes* fossils have been recorded from the early mid-Pleistocene onwards (Geraads 2011). In Europe, the species has been recorded from the mid-Pleistocene at many sites. Those are, Lunel-Viel, France, (Bonifay 1971); Schöningen, Germany (van Kolfschoten 2003); Britain (Kurten, 1968) and from late Pleistocene from Belgium (Szuma and Germonpré 2019). Also, *V. vulpes* has been found in Choukoutien, China (Kurten, 1968). Conversely, *V. rueppellii* has been reported only from mid-Pleistocene onwards from North Africa (Geraads 2011) – suggesting that this might be the region where the two species initially diverged (Leite et al. 2015).

Giving the wide-range distribution of the two focal fox species, our sampling might not have captured some important lineages and possible signals of introgression; more individuals need to be sampled. One important factor to be considered here is the sample size of *V. rueppellii* from west of the Nile where we reported the admixed individual: sample size here was very small (n=6), which likely doesn't accurately portray the true extent of the nuDNA introgression signal. Therefore, sampling more *V. rueppellii* may be necessary to confirm the introgression, but the sample size was reasonably large from east of the Nile where we did not find any admixed individuals. In general, the average hybridization rate is relatively low in animals (ca. 10%; Mallet, 2005) and much lower in mammals (6% in European mammals, Grant & Grant, 1992; Mallet, 2005). Even if the frequency of the admixed individuals is rare in the studied fox population, even low introgression levels can have large effects in recipient gene pools, when coupled to strong drift or selection on introgressed alleles (Schwenk et al. 2008).

4.4.2 Phylogeographic structure and gene flow of *V. vulpes* and *V. rueppellii*

A generalist and a highly adaptable species such as *V. vulpes* is expected to have a larger population size, and hence to harbour more genomic variation than a species with a geographically restricted distribution and narrower ecological niche such as *V. rueppellii*, which is reflected in the obtained genomic diversity estimates.

Furthermore, the results showed a strong genetic structuring and clear biogeographic signal of *V. vulpes* populations. All *V. vulpes* populations studied here were splitting into four main geographical populations, Northwest Africa: Algeria and Libya; Northeast Africa: Egypt; Arabia: UAE and Europe (UK and Portugal) (Figs. 4.2& 4.3). I attributed this signal to a) High adaptability, habitat heterogeneity and large geographical area that is covered by the species b) Refugial effect: In the Sahara, a species of mesic and semi-arid habitats like *V. vulpes* would likely have persisted in isolated refugia (e.g., oases and humid areas e.g., along the Mediterranean) during Pleistocene and Holocene arid periods (Rato et al. 2007; Nicolas et al. 2009; Husemann et al. 2014; Dinis et al. 2019). The strong population genetic structuring among North African and Middle Eastern populations is in accordance with findings from a wide-range study of European *V. vulpes* that highlighted the role of refugial regions in terms of endemism (e.g., Iberia) and post-glacial expansion (e.g., Carpathian and Balkan region) (McDevitt et al. 2021). In contrast, *V. rueppellii* individuals clustered as one group (Fig. 4.2A&B) with an F_{ST} across the Nile of ca. 0.06 (a similar level of cross-Nile differentiation as between WNile and ENile *V. vulpes*), but when analysed separately (Vr19 *dataset*) they split into west and east of the Nile with a sign of sub-structuring even within the latter group (Fig. 4.2C). The desert adapted *V. rueppellii* would likely have expanded its range during drier climatic periods (Tamar et al. 2018; Moutinho et al. 2020), possibly enhancing genetic connectivity (Leite et al. 2015). This could explain the lack of genetic structuring among the populations of *V. rueppellii* west of the Nile (with both *combined* and *Vr19 datasets*), although they were sampled from three distant localities (see Appendix 4.1) – although the small sample size could also be a factor. On the other hand, the genetic structuring east of the Nile (*Vr19 dataset*) could be explained by the effect of the mountain chain of the Eastern desert acting as a barrier among populations in different wadis (shallow depressions in the Sahara). The effect of mountains as a physical barrier has been reported in numerous previous studies

(e.g., Atlas Mountains, Coelho et al., 2014; Central Mountain in Taiwan, Huang et al., 2004; Black Mountain, Australia, Schneider, Cunningham, & Moritz, 1998).

In addition to its relatively large genetic structuring and variation reported here, a high morphological variability has been documented among many populations of *V. vulpes* (Szuma, 2000, 2003, 2004, 2007, 2008 a,b), as for other relatively widely distributed *Vulpes* species (e.g., *V. lagopus*, Daitch & Guralnick, 2007; Szuma, 2008c; *V. corsac*, Gimranov, 2017). This variability likely results from the combination of several factors, e.g., habitat productivity, differential food availability, geographic factors, genetic diversity, population density and competition (Szuma, 2008b and references therein). These factors affect more strongly the morphology of sympatric species of *Vulpes* (e.g., *V. vulpes* - *V. rueppellii* or *V. vulpes* - *V. lagopus*), emerging the variability (Szuma 2008b, 2011) . Therefore, *V. rueppellii* is expected to be influenced negatively by its competition with *V. vulpes* in the sympatric zones, considering the large geographic range of *V. vulpes* and high adaptability to different habitat types.

TreeMix results showed evidence/signals of five gene flow events (Fig. 4.5). The high detected gene flow between *V. vulpes* populations of Arabia and east of the Nile could be explained by the absence of barriers and habitat homogeneity between Sinai and Arabia. Indeed, several previous studies have highlighted genetic affinity between the fauna of Sinai and Arabia, e.g., Agama, *Pseudotrapelus aqabensis* (Tamar et al. 2016), and Sinai and Levant *Gazella dorcas* (Lerp et al. 2011). Gene flow between Arabia and Northeast Africa populations would more recently have been interrupted by the construction of Suez Canal ca. 150 years ago, (Fletcher 1958), although gene flow between faunas of Africa and Asia across the Isthmus of Suez and northern Sinai after the postglacial uplift of that area was also suggested for lowland species such as *V. vulpes* (Saleh et al. 2018). The detected gene flow between *V. vulpes* from Northwest Africa (Algeria and Libya) and WMCD in Egypt likely reflects the absence of any clear barriers across Mediterranean habitats in North Africa.

This chapter also found evidence of gene flow between *V. vulpes* from Portugal and Algeria. Considering the old splitting of Gibraltar at ca. 5 Mya (Bianchi and Morri 2000; Patarnello et al. 2007; Lejeusne et al. 2010), a possible explanation for the obtained result is human translocation of foxes across the Mediterranean, consistent with previous evidence of human

mediated transport of species between North African taxa and Iberia, e.g., reptiles *Podarcis vaucheri* (Renoult et al. 2010) and *Chamaeleo chamaeleon* (Paulo et al., 2002), and mammals, Egyptian mongoose (*Herpestes ichneumon*) (Barros et al. 2021).

4.5 Conclusion

The analysis showed the two species as genetically distinct with a higher differentiation than that reported from microsatellite markers by (Leite et al. 2015), emphasizing the power of genome-wide SNP data to resolve complex phylogenetic relationships of closely related species. Consequently, the use of multiple independently inherited loci is preferrable for inference of species trees.

Levels of genetic structuring and variability were higher in *V. vulpes* than in *V. rueppellii*. These findings are consistent with the well-known adaptability of *V. vulpes*, allowing this generalist to cope with a wide range of environmental conditions and changes in food availability. In contrast, the desert specialist *V. rueppellii* is likely more vulnerable to habitat and environmental changes, with its higher dependence on limited resources promoting population fragmentation. The dominance of *V. vulpes* over the other fox species such as *V. lagopus* (Tannerfeldt et al. 2002), *V. corsac* and *V. macrotis* (Sillero-Zubiri et al. 2004) has been reported, so the species will likely also dominate over *V. rueppellii* when competing for food resources, which could lead to population declines in the latter, or possibly to increased introgression due to e.g., Allee effects (Courchamp et al. 1999; Hailer and Leonard 2008). The mito-nuclear discordance reported here suggests an early divergence and extended time for adaptation in *V. rueppellii*, likely followed by mtDNA introgression – supporting its classification as a distinct species. Ongoing hybridization between the two species might be very limited, but future studies that investigate larger numbers of samples especially from putative contact zones are needed to test this further.

4.6 References

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Chapter 5: Whole Genome Resequencing Reveals Genomic Differentiation, Ancient Introgression and Different Demographic Histories of the Red Fox (*Vulpes vulpes*) and Rüppell's Fox (*V. rueppellii*)

5.1 Introduction

The two canid sister taxa, the red fox *Vulpes vulpes* and Rüppell's fox *V. rueppellii* occur in sympatry in the Middle East and North Africa (Geffen et al. 1992; Lindblad-Toh et al. 2005; Leite et al. 2015). They occupy different ecological habitats, with *V. vulpes* having a wide distribution across Europe, Asia, North America; in North Africa typically found in mesic (e.g., Nile River) or semi-arid habitats (e.g., oases across the Sahara and along the Mediterranean) (Macdonald and Reynolds 2008), as well as human-inhabited areas (Lariviere and Pasitschniak-Arts 1996). On the other hand, *V. rueppellii* is an arid- adapted specialist with a distribution across desert habitats from the African Atlantic coast across the Arabian Peninsula to Pakistan (Rosevear 1974; Williams et al. 2002; Sillero-Zubiri et al. 2004; Mallon et al. 2015). It has been suggested that *V. rueppellii* is a sister species of *V. vulpes*, albeit presumably nested within its mtDNA diversity, rendering *V. vulpes* paraphyletic (Leite et al. 2015) and chapter 2. This non-monophyly could indicate recent divergence of *V. rueppellii*, casting doubt on its classification as a distinct species.

Analysis of genome-wide SNPs generated by double digest restriction-site associated DNA sequencing (ddRAD-seq) in chapter 4 revealed a pronounced genomic differentiation of *V. vulpes* and *V. rueppellii*. The analysed SNPs also showed a signal of recent admixture between the two species incl. a potential F1 hybrid, plus extensive gene flow and biogeographic structuring among *V. vulpes* populations. These findings were supported by three independent methods (e.g., principal coordinate analysis (PCoA), Admixture (Alexander et al. 2009) and TreeMix (Pickrell and Pritchard 2012).

Although the recognized advantages of ddRAD-seq and other reduced-representation sequencing techniques (RRS) as that they allow cost-efficient screening of loci across the genome, several limitations have been pointed out (Davey et al. 2013; Andrews et al. 2016; Lowry et al. 2017). The main concerns are related to both laboratory and bioinformatic procedures associated with the method (Puritz et al. 2014; Mastretta-Yanes et al. 2015; Shafer et al. 2017). RRS approaches typically evaluate a small fraction (ca. 1-5%) of the genome, leaving many loci uncharacterized (Ozsolak and Milos 2011; Warr et al. 2015). Therefore, RRS approaches are not effective at identifying fine-scale genomic regions which show an elevated divergence or signal of selection. With regard to ddRAD-seq, the distribution and frequency

of restriction sites in the genome can vary considerably, depending on the study species and the pair of enzymes being used (Herrera et al. 2015). To achieve an extensive and ideally unbiased representation of the total genome, choice of enzymes is therefore a critical decision in the ddRAD-seq procedure. For instance, this choice will strongly influence the size distribution of the digested fragments, their locations across the genome and their total number (Burns et al. 2017; Wang et al. 2017). Also, recovery of SNPs can be significantly affected by the quality of DNA, as degraded DNA will lead to a lower efficiency of restriction enzyme-based techniques, by inducing a loss of recovered fragments (Graham et al. 2015). Another critical laboratory issue during library preparation is the non-homogenous amplification of RAD fragments which can lead to considerable loss of alleles resulting from unbalanced fragment coverage (Andrews et al. 2014; Andrews and Luikart 2014; Puritz et al. 2014). The bioinformatic treatment of the resulting data is another important concern about RAD-based methods. For instance, the technique depends on the identification of homologous loci among individuals. These loci are typically identified using distance-based (Rochette et al. 2019) or global alignment (e.g., pyRAD; Eaton 2014) methods. Setting a stringent parameter can help avoiding the clustering of paralogs, but can also split highly divergent single-copy loci into different clusters (Eaton 2014; Rochette et al. 2019). Another common drawback of any genotyping technique is missing data among individuals (Arnold et al. 2013; Gautier et al. 2013; Malinsky et al. 2018). This can result from heterogeneity of laboratory methods (experimental lack of reproducibility), but is thought to result more frequently from polymorphism in restriction sites (Cumer et al. 2021). This polymorphism results in allelic drop-out for the individuals/alleles missing those restriction sites. Also, sequencing depth of coverage play an important role in reconstructing of loci. Setting a high threshold of the minimum number of reads will lead to increasing amounts of missing data, (insufficient coverage), while a low minimum depth setting will not dispose of rare sequences produced by e.g., PCR or sequencing errors (Paris et al. 2017). In summary, whole genome resequencing (WGR) approaches are preferable over RRS approaches in terms of obtaining signals from across the entire genome, and hence a more detailed picture of the genomic polymorphism and divergence landscape, even based on only low or moderately high coverage of limited numbers of individuals (Szarmach et al. 2021).

Based on cytochrome b and D-loop markers, five mtDNA clades (Nearctic, Holarctic, Palearctic, Africa1 and Africa 2) have been identified for *V. vulpes*, with *V. rueppellii* clustering within the variation *V. vulpes* in the Palearctic clade, leading to the paraphyly of the latter (chapter 2). In fact, some of these clades received low branch support in previous analyses, e.g., the joint clade including the two African clades (Africa 1 and 2) appeared to cluster with the Holarctic and Nearctic clades (chapter 2, Fig. 2.2A), but their precise phylogenetic relationships remained unresolved. Also, within the Palearctic clade, all *V. rueppellii* individuals formed two well-supported subclades (Bayesian Posterior Probability, BPP: 0.99), but support was insufficient to determine whether these two subclades grouped together, or whether either of them was more closely related to the Palearctic subclades of *V. vulpes* (chapter 2, Fig. 2.2A). In cases when short sequence cannot resolve ancient evolutionary relationships (Kocher et al., 1989), analysis of longer sequences might be helpful (Meiklejohn et al. 2014; Murtskhvaladze et al. 2020). In particular, whole mtDNA (mitogenome) sequences should provide a high resolution for inference of phylogenetic relationships at various taxonomic levels, compared with analyses of short mtDNA fragments (Finstermeier et al. 2013). For instance, phylogeographic processes and population structuring of brown bears (*Ursus arctos*) were revealed based on whole mitogenome sequences (Keis et al. 2013; Anijalg et al. 2018), finding signals that had not been detected previously using shorter sequences (Davison et al. 2011). Another example are gray wolves (*Canis lupus*), for which sequencing of whole mitogenomes reveal clear spatio-temporal population structuring that had not been found before from analysis of shorter mtDNA sequences (Koblmüller et al. 2016). Many studies have highlighted the importance of using whole mitogenomes in phylogenetic analysis as an alternative to individual loci/genes that should be used in caution. Mitogenomic analyses therefore show great promise in phylogeographic studies (Laurimäe et al. 2018).

Considering all the aforementioned drawbacks of both ddRAD-seq and short mtDNA sequences, taking advantage of decreasing costs of WGR presents a promising alternative to survey not just subsets of the genome, but the entire genome (but see Peona et al. (2018) for a discussion of remaining challenges in genome assembly and hence reference genome completeness). WGR is powerful approach to investigate in depth many questions in evolutionary biology and ecology that have not been fully resolved using traditional methods (e.g., mitochondrial DNA (mtDNA), Y-chromosome and lower numbers (e.g., thousands) of

autosomal SNPs (Fuentes-Pardo and Ruzzante 2017). WGR provides the highest marker density among the current genomic methods, generating millions of SNPs in typical mammalian genome, while simultaneously allowing to extract the whole mitogenome (Ellegren 2014). Another advantage of WGR, is that data even from a single individual can be used to infer the demographic history of the population/species. The most commonly used model to infer the past demography is the pairwise sequentially Markovian coalescent (PSMC), which uses the local density of heterozygosity in diploid genome for inferring changes in effective population size over time (Li and Durbin 2011). PSMC has been applied to many taxa, e.g., wolves (Sarabia et al. 2021), deer (Yi et al. 2020), pandas (Zhao et al. 2013), bears (Miller et al. 2012), flycatchers (Nadachowska-Brzyska et al. 2013; Nadachowska-Brzyska et al. 2016), grouse (Kozma et al. 2016) and falcons (Wang et al. 2013).

Except for whole genome sequencing work on *V. vulpes* from the silver fox domestication experiment (Kukekova et al., 2018), no whole genome data has been generated for either of the two species, leaving an important gap in our understanding of their evolutionary history. In this study, I sequenced the whole genomes of representative individuals for *V. rueppellii* and *V. vulpes*, aiming to represent the main mtDNA clades (chapter 2) and populations (identified from ddRAD-seq analysis; chapter 4). The research aims were as follow: (A) Generating a dataset of whole-genome SNPs to (1) estimate the intraspecific variability and interspecific divergence at a whole-genome level; (2) study the effects of the Quaternary climatic fluctuations on demographic history of the two species; and (3) look for signals of gene flow between the two species and within each species. (B) Extracting complete mtDNA genomes to (1) re-assess the reported paraphyly of *V. vulpes* based on analysis of longer sequences; and (2) improve the statistical support for poorly supported clades reported in chapter 2.

5.2 Materials and Methods

5.2.1 Sample collection

Nine foxes were sequenced as part of this study: seven from *V. vulpes* and two from *V. rueppellii* (Fig. 5.1). In addition, raw sequence reads of two *V. zerda* individuals (from Phase

One Resequencing for 10,000 Dog Genome Consortium), two *V. lagopus* (Hasselgren et al. 2021) and one *V. vulpes* from the Russian farm-fox experiment (Kukekova et al., 2018) were downloaded from the Sequence Read Archive (SRA). See table 5.1 for details on samples.

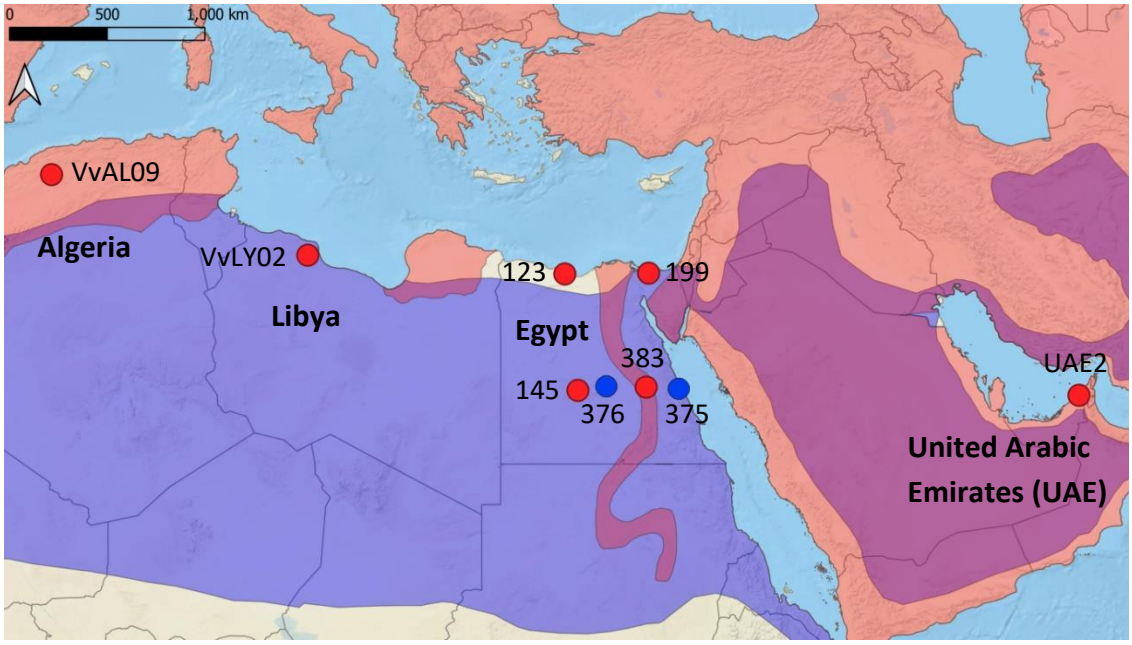


Figure 5.1: Samples collected and sequenced as part of this study. Red circles: *V. vulpes,* blue circles: *V. rueppellii*. Light red, light blue and violet backgrounds are IUCN range of *V. vulpes*, *V. rueppellii*, and sympatric regions, respectively.

Table 5.1: Details on samples used for whole genome resequencing. Unk denotes samples with unknown sex.

Sample ID	Sex	Species	Coordinates		Locality	Reference
			Y (latitude)	X (longitude)		
123	male	*V. vulpes*	30.96028	28.35278	El Daba Matrouh, Western Desert, Egypt	(1)
145	male	*V. vulpes*	25.54533	29.0472	Dakhla Oasis, Western Desert, Egypt	(1)
199	male	*V. vulpes*	30.9875	32.78889	Rabaa, North Sinai, Egypt	(1)
383	male	*V. vulpes*	25.72056	32.67333	Elkarnak El Kadem, Luxor, Nile Valley, Egypt	(1)
UAE2	male	*V. vulpes*	55.52444	25.33667	Sharjah, UAE	(1)

VvAL09	male	*V. vulpes*	35.33973	1.2405059	Tagdemt communale, Algeria	(1)
VvLY02	male	*V. vulpes*	31.82178	14.81388889	Misrata, Libya	(1)
375	male	*V. rueppellii*	25.61528	34.39972	Wadi om-Khiag, Eastern Desert,Egypt	(1)
376	male	*V. rueppellii*	25.72639	30.555	kharga Oasis, Western Desert, Egypt	(1)
SRR5328110	Unk	*V. vulpes*	NA	NA	Novobirzik, Siberia, Russia	(2)
ERR5417968	Unk	*V. lagopus*	NA	NA	Sweden	(3)
ERR5417974	Unk	*V. lagopus*	NA	NA	Sweden	(3)
SRR14750349	Unk	*V. zerda*	NA	NA	Unknown	(4)
SRR14750511	Unk	*V. zerda*	NA	NA	Unknown	(4)

References for the data: (1) this study, (2) Kukekova et al. (2018), (3) Hasselgren et al. (2021), (4) Phase One Resequencing for 10,000 Dog Genome Consortium.

5.2.2 Laboratory procedures

5.2.2.1 DNA extraction

Genomic DNA was extracted from tissue samples using salting-out protocol modified from (Rivero et al. 2006), which in turn was based on the Puregene™ DNA extraction kit (Qiagen, Hilden, Germany), with the addition of RNase A (Thermo Fisher Scientific) following the lysis step. DNA quality and quantity were assessed by electrophoresis in 1% agarose gels and Qubit fluorometer v.3.0, respectively.

5.2.2.2 Library preparation and sequencing

Approximately 600 - 1,400 ng of high molecular weight DNA of six *V. vulpes* samples (123, 199, 383, UAE2, VvAL09 and VvLY02) was sent to Novogene (Cambridge, UK) for whole genome shotgun sequencing. All samples underwent further qualitative and quantitative quality checks by Novogene, based on agarose gel electrophoresis and Qubit concentration assessment. Library construction was as follows: genomic DNA was randomly sheared into short fragments which were end repaired, A-tailed and ligated to Illumina adapters (5' Adapter: 5'AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCA-

TT-3' and 3' Adapter: 5'-GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGATGACTATCTCG-TATGCCGTCTTCTGCTTG-3'). Next, fragments with adaptors were PCR-amplified, size-selected to approximately 350 bp and purified. Qubit, real-time PCR as well as a bioanalyzer were used to quantify and to check library size distributions. The libraries were then pooled and sequenced on an Illumina NovaSeq instrument, using 150 bp paired-end (PE) reads. The remaining three samples (145, 375 and 376) were sequenced with Neogen (Ayr, Scotland, UK) following the above protocol, except the read lengths were 2x151 bp.

5.2.3 Data analysis

5.2.3.1 Nuclear genome

5.2.3.1.1 Data processing and SNP calling

FASTQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to evaluate the quality of the reads, and TRIMMOMATIC v0.39 (Bolger et al. 2014) to remove adaptors and to trim off low-quality bases (settings: minimum length 50 bp, sliding window 10:15). The trimmed reads were mapped using BWA-MEM v0.7.17 (Li and Durbin 2009) to the dog (*Canis lupus familiaris*) reference genome (assembly version: ROS_Cfam_1.0; Field et al., 2020) with default parameters. We used the highly contiguous, chromosome-level dog genome highly contiguous, chromosome-level assembly as a reference, instead of the much less contiguous *V. vulpes* assembly (Kukekova et al., 2018), because mapping of fox reads against an outgroup reference avoid reference bias signals (Günther and Nettelblad 2019) at the inference stage. Then, SAMTOOLS v1.10 (Li et al. 2009) was used to generate sorted bam files, followed by removal of PCR duplicates using MARKDUPLICATESSPARK from the GATK pipeline (https://gatk.broadinstitute.org/hc/en-us). Next, HAPLOTYPECALLER in GATK was used to call variants and to output a VCF (variant call format) file for each sample. GenomicsDBImport in GATK was used to merge VCF files from all the samples into one datastore. This datastore transposes sample-centric variant information across genomic loci to make data more accessible to bioinformatics tools and pipeline. Then GenotypeGVCFs in GATK was used to create a final VCF, allowing joint variant calling for all 14 samples. Then I used BCFTOOLS (Li et al. 2009; Li 2011) to (1) extract autosomal biallelic SNPs excluding any

variants on the Y chromosome, X chromosome, mtDNA and unplaced scaffolds, in addition to removing indels; and (2) subset the main VCF file to different datasets to be used in the corresponding analyses. One subset "*allallsamples14"* included all 14 sample (the two focal fox species, along with the two individuals each from the two outgroups, *V. zerda* and *V. lagopus*), and the "*samples10"* dataset included only the ten individuals from the two focal/ingroup species (details datasets/samples given in Appendix 5.1).

5.2.3.1.2 Filtering and handling VCFs

To determine appropriate filtering parameters, I used BCFTOOLS (Li et al. 2009; Li 2011) in a combination with the *vcfrandomsample* tool from the vcflib pipeline (Garrison et al. 2022) to randomly subsample the large VCF file of each dataset (allowing a faster quality check than if using 100% of the sites). Then I used VCFTOOLS v0.1.16 (Danecek et al. 2011) to run various summary statistics (e.g., genotype quality, minimum and maximum depth and missingness of variants) on the subsetted VCF files. After that I used a custom R script to plot these statistics in R (R Core Team 2022). Based on these investigations, the number for each filtering parameter were chosen: each dataset was filtered by running VCFTOOLS with the following flags: --max-missing (maximum missing variants at each site), --minQ (minimum quality score required for a site to pass the filtering threshold), --min-meanDP (minimum mean depth for a site), --max-meanDP (maximum mean depth for a site), --minDP (minimum depth allowed for a genotype - any individual failing this threshold was marked as having a missing genotype) and --maxDP (maximum depth allowed for a genotype - any individual failing this threshold was marked as having a missing genotype. I filtered dataset *samples10* with the following parameters: --max-missing = 0.95, --minQ= 50, --min-meanDP = 8 and --max-meanDP= 30. Dataset '*allsamples14*' was filtered with the following parameters: --max-missing = 0.90, --minQ= 50, --min-meanDP = 8 and --max-meanDP= 32. Because most of the analysis approaches below assume SNPs to be unlinked, when needed (see below), linked variants were identified and pruned from each dataset using PLINK v1.9 (Purcell et al. 2007) with the setting --indep-pairwise 50 10 0.3, where 50, 10 and 0.3 are window size in kbp, step size and correlation coefficient (r^2), respectively.

5.2.3.1.3 Genetic structuring

Signals of genetic structuring and admixture were investigated for the dataset "*sample10*" which included only *V. vulpes* and *V. rueppellii*. Both principal component analysis (PCA) and admixture analyses (Alexander et al. 2009) assume SNPs to be independent, therefore variants in linkage disequilibrium were filtered from the dataset using PLINK stated above. Following this filtering, 2,684,467 SNPs were kept for downstream analyses. For PCA, I enabled the flag *-pca* in PLINK to generate eigenvec and eginval files. These two files were plotted using custom R scripts. Admixture analysis was conducted using ADMIXTURE v1.3.0 (Alexander et al., 2009), based on the binary bed file generated by PLINK. I ran Admixture for values of K (the number of populations to be modelled) of 2- 7 for five iterations each, enabling the –cv flag to estimate cross-validation (CV) errors (based on 5-fold calculation). Although some authors have suggested that the best K value is the one with the lowest CV error (Alexander and Lange 2011), there is still ongoing dispute about this topic (Lawson et al. 2018; Carlen and Munshi-South 2021), so results from the full range of explored K values are reported. The outputs from ADMIXTURE, Q (the ancestry fractions), and P (the allele frequencies of the inferred ancestral populations) were plotted using custom R scripts.

5.2.3.1.4 Genetic diversity

I calculated nucleotide diversity for the *allallsamples14* dataset using VCFTOOLS, enabling the flag --*window-pi*, and the graph was plotted using a custom R script. Calculation was done on 200,000 kbp wide non-overlapping windows. Then I used a two-sample Kolmogorov-Smirnov test to test for differences in the level of the nucleotide diversity of *V. vulpes* and *V. rueppellii*.

5.2.3.1.5 Population admixture and divergence

I used TreeMix v1.13 (Pickrell and Pritchard 2012) to infer population relationships for the dataset *allsamples14*. TreeMix allows description of patterns of splitting and admixture in the history of populations based on genome-wide allele frequency data, by jointly inferring the maximum likelihood tree and addition of gene flow (termed 'migration events' in the software). For this analysis, I grouped the individuals by geographical region into six populations, following initial runs that indicated that TreeMix did not perform reliably when

using single individuals as groups/populations (details not shown). Individuals were grouped as follows: (1) *V. lagopus* (ERR5417968 and ERR5417974), (2) *V. zerda* (SRR14750349 and SRR14750511), (3) *V. rueppellii* (375 and 376), and (4) *V. vulpes*_North Africa (383, 145, 123, VvLY02 and VvAl09) and (5) *V. vulpes*_Asia (UAE and 199), and (6) the Russian individual (SRR5328110) grouped on its own. I used PLINK to filter SNPs in linkage disequilibrium with the setting: --indep-pairwise 50 10 0.3 and to generate stratified allele frequencies for all the populations. I kept 6,570,819 SNPs after previous filtering. Then I used a python script "plink2treemix.py" downloaded from https://bitbucket.org/nygcresearch/treemix/downloads to convert the allele frequencies output from PLINK into TreeMix format. After that, I ran TreeMix for seven separate runs with number of migration events (m) from 0 to 6 (number of the populations), assigning *V. zerda* as an outgroup (-root *V. zerda*). Then to identify the contribution of each migration vector to the variance explained to the tree, I ran TreeMix with a global set of rearrangements (-global), and a randomly selected window size (-k) of between 100 and 1000 SNPs (50 SNP increments). The number of migration events (-m) varied between one (migration between two populations) and six (migration between all the populations) and 10 replicates were performed for each value of m. The value of "m" with a composite log-likelihood value of 99.8% (recommended threshold for stopping the addition of migration edges, by Pickrell and Pritchard (2012), was chosen as the optimal number of migration edges. Finally, I used custom R script and the R package OptM (Fitak 2021) in R-4.2.0 to plot the TreeMix maximum likelihood trees and the composite likelihood for each migration edges, respectively. To test the TreeMix results, I ran *f3* and *f4* statistics (see chapter 4 for details).

5.2.3.1.6 Reconstructing the past population dynamics

I conducted the analysis of historical demography for the dataset "*samples10*" using pairwise sequentially Markovian coalescent (PSMC; Li & Durbin, 2011). The input VCF file underwent all filtering criteria mentioned above in the "Genetic structuring" section, except for Linkage disequilibrium, since PSMC is working on each sample separately and doesn't take population-level linkage into account, and since its inference is based on density of variable sites – which would be distorted if removing some variants based on population-level trends of linkage. Following this filtering, I kept 60,105,074 SNPs for the input VCF file for PSMC. I used

BCFTOOLS to convert VCF files into consensus FASTA files (input for PSMC). First, *fq2psmcfa* using a quality cut-off (-q=20) was used to split the sequences into 100 bp-long nonoverlapping bins, where each bin was marked as heterozygous ('1') if >10 bp were called and there was at least one heterozygous base, or homozygous ('0'), if >10 bp were called but no heterozygous position was present, or missing ('.'), if ≥ 90 bases were filtered or not called. After that, I carried out PSMC on the *psmcfa* sequences (output from the previous step), based on the following default options: -N25 -t15 -r5 -p '4+25*2+4+6', where -N = number of iterations, -t= maximum time ($T_{max)}$, -r= initial mutation/recombination ratio and -p= atomic time interval pattern. I applied 100 iterations of bootstrapping per genome to represent the variance of the results. To rescale the results of the demographic inference into years and numbers, it is critical to fix the mutation rate and generation time, neither of which is known precisely for *V. vulpes* and *V. rueppellii*. I used a mutation rate of 4.5×10^{-9} (Koch et al., 2019), which should apply reasonably well to foxes because the rate was estimated for wolves by identifying de-novo mutations from whole genome data in a pedigree of seven wolves. Furthermore, these de novo mutations were verified for the parents and offspring by Sanger sequencing. For the generation time setting, Aubry et al., (2009) estimated mtDNA divergence times in North American *V. vulpes* based on a generation time (average age of reproducing parents) of one year, but this was criticized by (Goddard et al. 2015) as the absolute minimum theoretically possible for foxes. This estimate is smaller than the average age (1.46 years) of *V. vulpes* from Egypt based on the dentine layers counting method (Younes & Basuony, 2015), and the most recent generation time estimate for foxes, which to my knowledge is two years, estimated for gray (*Urocyon cinereoargenteus*) and island (*U. littoralis*) foxes (Goddard et al. 2015; Sacks et al. 2022). Therefore, I used a generation time of two years to plot the PSMC results.

5.2.3.2 Mitogenome assembly

5.2.3.2.1 Data processing

Short read sequencing data from eleven samples was used to obtain mitogenome sequences, including the nine newly collected samples (seven *V. vulpes* and two *V. rueppellii*), plus two low coverage (ca. 2.5x) *V. vulpes* from the Russian farm-fox experiment (Kukekova et al. 2018),

which were downloaded from the Sequence Read Archive (SRA) (Table 5.2). Due to the difference in coverage between the two sets of samples, I opted for testing the four approaches to assemble the mitogenome to address any differences that may arise due to the sequencing coverage variation.

Table 5.2: Details on samples used for whole mitogenome assembly and phylogeny

Sample ID, SRA/ Genbank accession #	Mitochondrial Clade	Species	Locality	Reference for mitogenome sequence / raw data
375	Palearctic	*V. rueppellii*	Egypt	This study
376	Palearctic	*V. rueppellii*	Egypt	This study
123	Africa 2	*V. vulpes*	Egypt	This study
145	Palearctic	*V. vulpes*	Egypt	This study
199	Holarctic	*V. vulpes*	Egypt	This study
383	Holarctic	*V. vulpes*	Egypt	This study
UAE2	Palearctic	*V. vulpes*	UAE	This study
VvAL09	Africa 1	*V. vulpes*	Algeria	This study
VvLY02	Africa 2	*V. vulpes*	Libya	This study
SRR5280494	Nearctic	*V. vulpes*	Russia	SRA data from Kukekova et al., 2018
SRR5280501	Nearctic	*V. vulpes*	Russia	SRA data from Kukekova et al., 2018
KP342452.1	Nearctic	*V. vulpes*	China	Sun et al., 2016
GQ374180.1	Holarctic	*V. vulpes*	China	Zhong et al., 2010
KF387633.1	Holarctic	*V. vulpes*	China	Zhang et al., 2015
JN711443.1	Holarctic	*V. vulpes*	South Korea	Yu et al., 2012
AM181037.1	Holarctic	*V. vulpes*	Sweden	Arnason et al., 2006
MN122913.1	Holarctic	*V. vulpes*	Denmark	Margaryan *et al.* (unpublished)
KT448287.1	Holarctic	*V. vulpes*	Unknown	Koepfli et al., 2015

FASTQC v0.11.9 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for evaluating the quality of the reads. I used TRIMMOMATIC v0.39 (Bolger et al. 2014) to remove adaptors and to trim the low-quality reads (settings: minimum length 50 bp, sliding window 10:15). Following this, I used four different approaches to extract the whole mitogenome as below.

(1/2) **Reference-based read mapping** was performed using two different parameter settings (see below). The trimmed data was aligned with *V. vulpes* reference genome (assembly version: GCF_003160815.1_VulVul2.2; Kukekova et al., 2018) using BWA-MEM v0.7.17 (Li and Durbin 2009) in paired-end mode with default parameters. SAMTOOLS v1.10 (Li et al. 2009)

was used to obtain sorted bam files, followed by GATK (https://gatk.broadinstitute.org/hc/en-us) to remove PCR duplicates using MARKDUPLICATESSPARK and to filter out bad read mates, reads with mapping quality zero and reads which mapped ambiguously (Nater et al. 2017). Then I used SAMTOOLS to extract the mitochondrial reads that mapped to the mtDNA scaffold (NC_008434.1, Arnason et al., 2006) of the reference genome. HAPLOTYPECALLER in GATK was used to call variants using two different parameter settings, specifically using as values for the flag --sample-ploidy: **1** for haploid (ploidy 1), and **2** for diploid (ploidy 2), each yielding a VCF file. Finally, FastaAlternateReferenceMaker from GATK was used to convert the two VCF files into FASTA format.

(3) **Baiting and iterative mapping approaches implemented** in MIRA v4.0.2 (Chevreux et al. 1999) and MITObim v1.9.1 (Hahn et al. 2013): First, an initial reference was built using MIRA (part of the MITObim package), by mapping the raw reads to the mitochondrial reference genome of *V. vulpes* (GenBank accession: NC_008434.1, Arnason et al., 2006). Next, the MITObim.pl script was used to iteratively recapture additional hitherto unmapped reads to the reference obtained from the previous iteration. This procedure was repeated, closing remaining gaps until a stationary state was reached. This approach only returns a single-padded consensus sequence, and any sequences of fragments that are probably not contiguous will be connected by 'N' (Machado et al. 2016).

(4) **De novo assembly** with NOVOPlasty (Dierckxsens et al. 2017): I used the raw reads as an input with the default parameters except for insert size which set to 350 and K-mer to 33. Then, the mtDNA reference genome of *V. vulpes* (noting its completeness and the reliability of the PCR-based approach used to generate it; Arnason et al., 2006) was used as a seed to initiate the assembly.

Sequence alignment was performed with Geneious Prime 2022.2.2 (http://www.geneious.com). All gene annotations and boundaries of each discrete segment of mtDNA were located by sequence comparison with their counterparts in the published whole mitogenome of *V. vulpes* (NC_008434.1, Arnason et al., 2006). Trimming of poorly-aligning sections in and around the tandem repeat within the D-loop was also conducted in Geneious.

5.2.3.2.2 Phylogenetic analysis

To construct the phylogeny of the two species, I combined the newly obtained sequences with all available whole mitogenome sequences of *V. vulpes* and *V. rueppellii* from GenBank, using *V. lagopus* (KP342451.1,Sun et al., 2016) as an out group (Table 5.2). Then, MUSCLE v3.8 (Edgar 2004) as implemented in Geneious Prime 2022.2.2 was used to align the newly obtained sequences with those downloaded from GenBank and to produce a FASTA file. I used a maximum likelihood approach in W-IQ-TREE (Trifinopoulos et al. 2016) to reconstruct a phylogenetic tree. As substitution model, an invariant sites plus discrete Gamma model with 4 rate categories (TPM2u+F+I+G4) was used, which had been determined as the optimal model using the Modelfinder algorithm (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE. The trees were subjected to 1000 ultrafast bootstrap replications (Minh et al. 2013). The resulting maximum likelihood tree was visualized using FIGTREE 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

5.3 Results

5.3.1 Nuclear genome

5.3.1.1 Genetic structuring

The PCA revealed a clear separation of *V. vulpes* and *V. rueppellii*, along with stronger separation among the individuals of the former than the two individuals of the latter species (Fig. 5.2). There was a geographical gradient of sub-structuring within *V. vulpes* from Russia (ARR5328110), Arabia (UAE2, 199), Northeast Africa (383, 145, 123) to Northwest Africa (VvLY02, VvAlg09). This structuring was consistent with PCoA from the ddRAD-seq data (chapter 4, Fig. 4.2A&B).

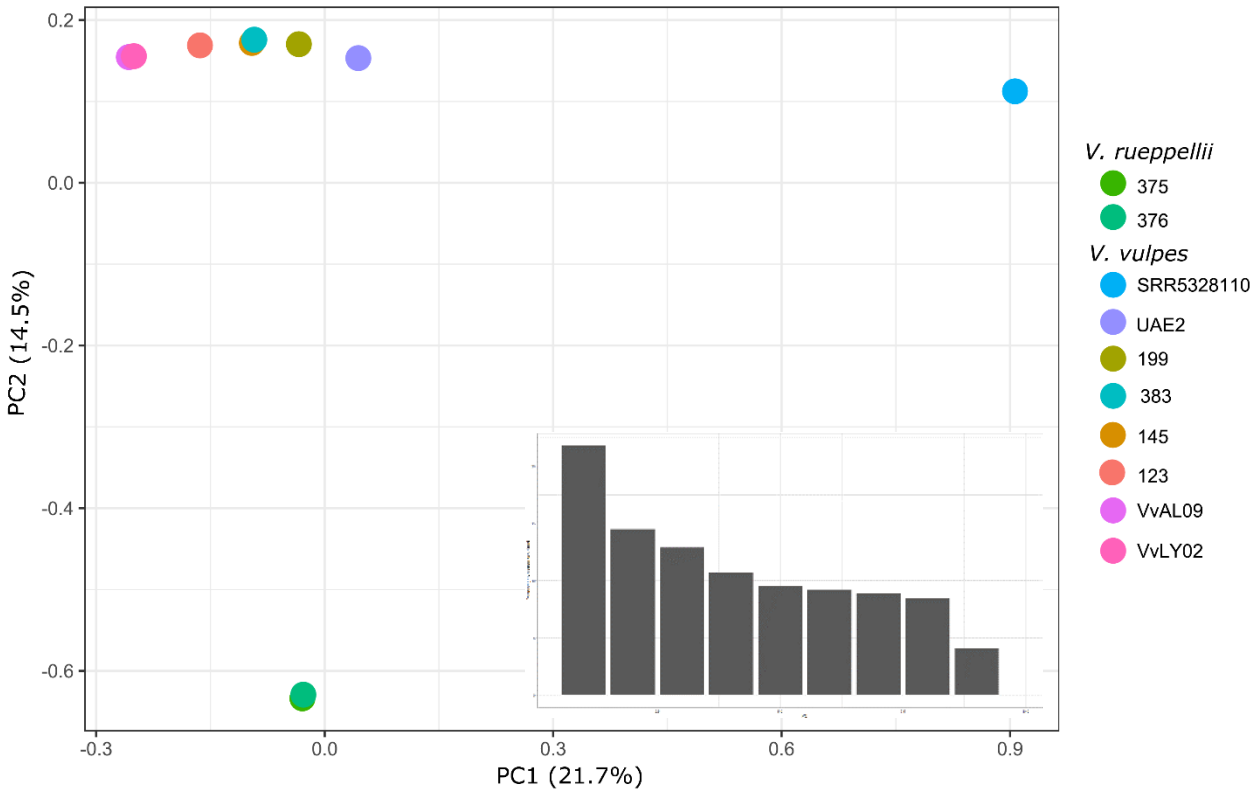
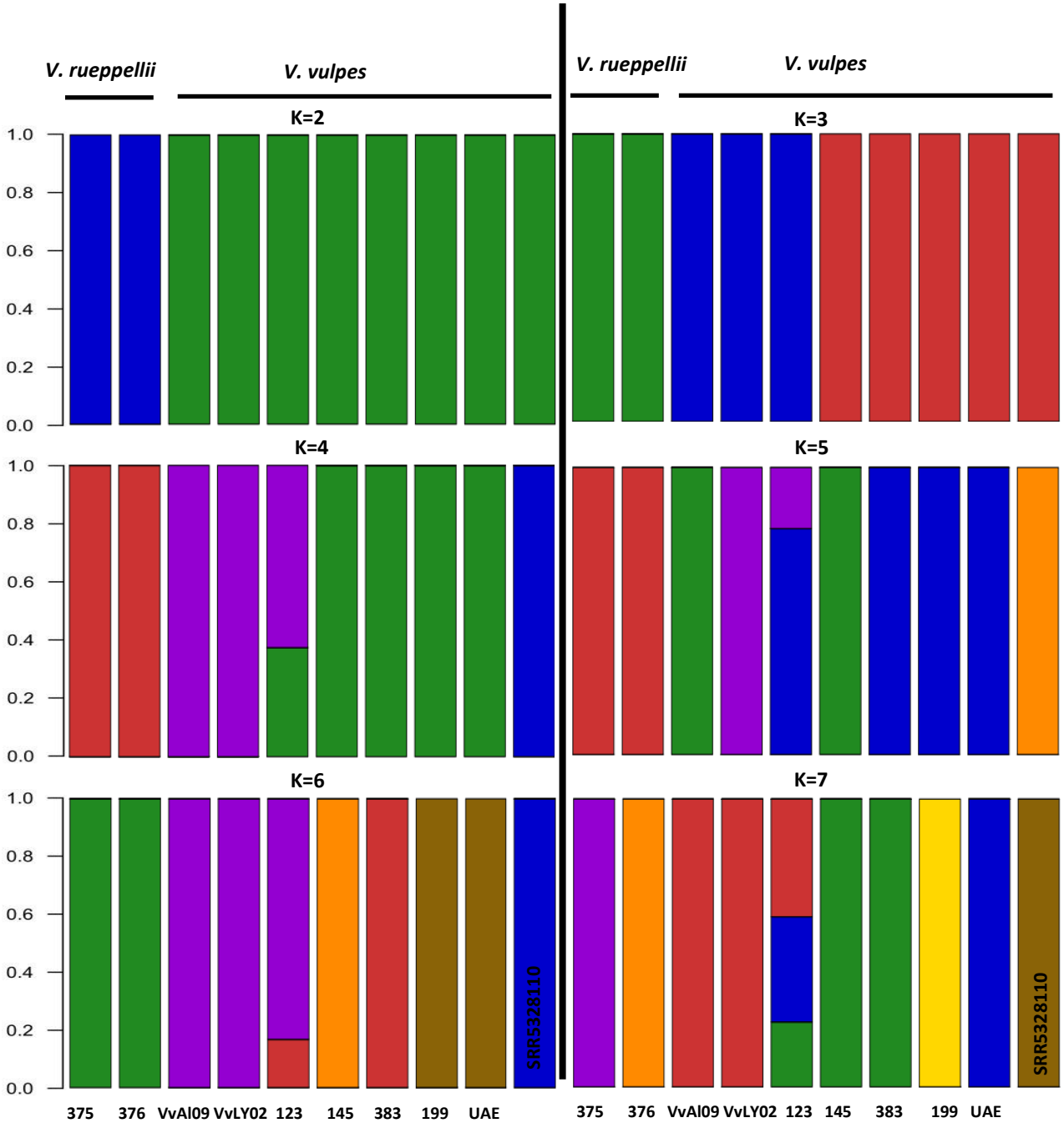


Figure 5.2: PCA results of *V. vulpes* and *V. rueppellii* based on 2,684,467 autosomal SNPs (dataset, "*samples10*"). The bar chart in the background shows the percentage of variance explained by nine PC axes.

Genetic clustering analysis in Admixture clearly separated *V. vulpes* and *V. rueppellii* into two genetic clusters at K=2 (Fig. 5.3). The two *V. rueppellii* individuals clustered together at all K values from 2-6. At K=3, *V. vulpes* was split into two groups, Group 1: Algeria, Libya and Western Mediterranean Costal Desert (WMCD) in Egypt and group 2: Russia, UAE, Sinai, Nile valley (Egypt) and Kharga oasis (west of the Nile, Egypt). At K=4, the Russian individual constituted a separate group, and the individual from WMCD was admixed, showing affinity to the Egyptian Nile valley, UAE individuals and Northwestern Africa individuals (Algeria and Libya). By increasing the K values, the inferred signals became inconsistent, consistent with the fluctuating values of the cross-validation error that showed a deviation from the normal pattern, as e.g., visible for the ddRAD-seq data (Chapter 4, Fig. 4.3). Admixture is a population-based analysis, thus the unreliable cross-validation error values (i.e., cv> 1) (Fig. 5.3) could be a result of the small sample size analysed here. However, to my knowledge, this has not been investigated deeply in the literatures and need more investigations.



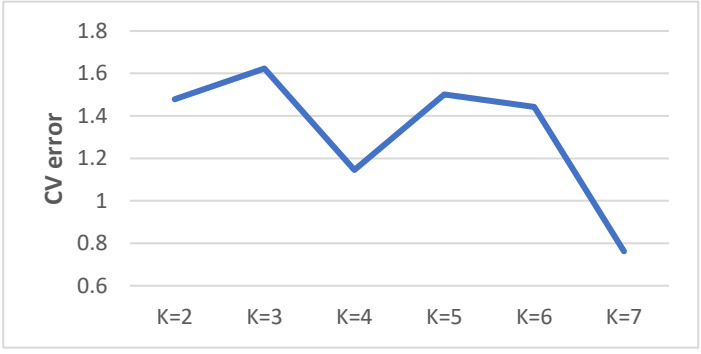


Figure 5.3: Admixture results showing inferred genetic structuring of *V. vulpes* and *V. rueppellii* based on 2,684,467 autosomal SNPs (dataset, *samples10*) at K=2-7, Y-axis is the ancestry coefficient. The bottom right panel shows the cross-validation (CV) error for each value of K.

5.3.1.2 Genetic diversity

When grouping the individuals of each species, the nucleotide diversity of *V. vulpes* (eight individuals) was relatively higher than *V. rueppellii* (two individuals) (Fig. 5.4A). The difference in nucleotide diversity between the two species was significant, based on a two-sample Kolmogorov-Smirnov test (D = 0.32302, p < 2.2e-16). However, when analysed each individual separately, the nucleotide diversity of the tow *V. rueppellii* appeared close to most of *V. vulpes* individuals. Figure 5.4B shows the nucleotide diversity for each individual of *V. vulpes* and *V. rueppellii* in addition to other fox species. North African *V. vulpes* (VvAl09, VvLY02, 123, 145, 383) had a higher nucleotide diversity than the Asian individuals (199, UAE, SRR5328110). One of the two *V. rueppellii* expressed a similar nucleotide diversity as the North African group of *V. vulpes*, while the other had a lower nucleotide diversity, slightly higher than the Asian *V. vulpes* individuals. Nucleotide diversity of *V. lagopus* was relatively similar to that of Asian V. *vulpes*. *Vulpes zerda* showed the lowest genetic diversity compared to the other three species (Fig. 5.4B). Importantly, however, the variance as estimated by data points for individual genomic windows (shown in gray in Fig. 5.4B) overlapped between most individuals of all four species, indicating low significance of the nucleotide diversity differences reported above.

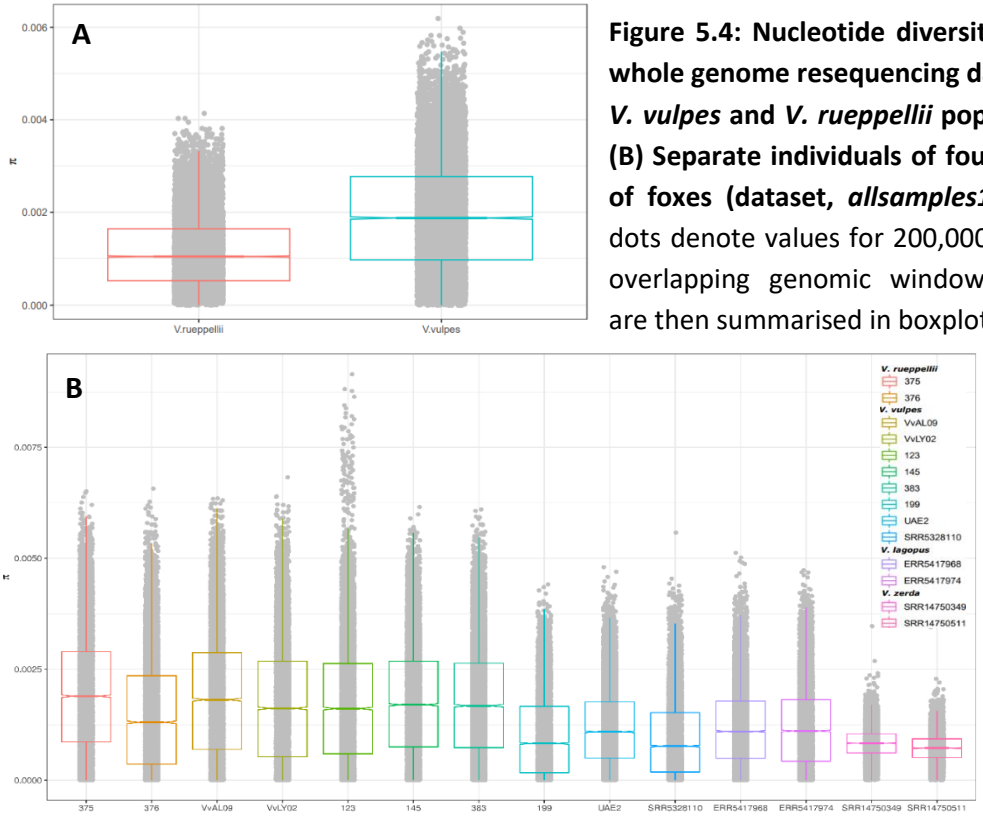


Figure 5.4: Nucleotide diversity (π) for whole genome resequencing data in (A) *V. vulpes* and *V. rueppellii* populations, (B) Separate individuals of four species of foxes (dataset, *allsamples14*). Grey dots denote values for 200,000 bp non-overlapping genomic windows, which are then summarised in boxplots.

5.3.1.3 Population admixture and divergence

The maximum likelihood tree from TreeMix indicated the splitting of *V. vulpes* and *V. rueppellii* into two mutually monophyletic groups, each separated from their common ancestor by a long branch, indicating a long-term divergence (Fig. 5.5A). By adding up to six migration events (m=1-6), the topology of the tree did not change (Fig. 5.4B-G). At m=1, there was a gene flow from *V. rueppellii* to north African *V. vulpes*. Gene flow from Russian *V. vulpes* to *V. lagopus* was found at m=2. Adding third and fourth migration edge suggested introgression from the ancestor of *V. vulpes* and *V. rueppellii* to the Russian *V. vulpes*, and from *V. lagopus* to *V. vulpes* from Asia, respectively. The fifth migration edge indicated introgression from *V. zerda* to north African *V. vulpes*, and a sixth edge suggested gene flow from the ancestor of *V. vulpes* from North Africa and Asia to the Russian *V. vulpes*. However, already with m=0 migration edges, the variance explained reached the 99.8% threshold suggested by Pickrell and Pritchard (2012), indicating that gene flow signals are unnecessary to better explain the tree (Appendix 5.2). All *f3*-statistics were positive and hence did not reveal any clear evidence of gene-flow among the analysed populations (Appendix 5.3). In contrast, *f4*-statistics showed a signal of gene flow between all the studied populations with either *V. lagopus* or *V. zerda* as an outgroup and in the absence of both. When using *V. lagopus* as an outgroup, *f4*-statistics for all *V. vulpes* populations (*V.vulpes*_NorthAfrica, *V.vulpes*_Asia and *V.vulpes*_Russia) and *V. rueppellii* showed extreme significant negative Z scores (Z<-3)- ranging from -7 to -168, while when assigning *V. zerda* as an outgroup, *f4*-statistics for all *V. vulpes* populations and *V. rueppellii* showed an extreme positive significant Z scores (Z> 3)- from 6 to 175. Without assigning either *V. lagopus* or *V. zerda* as an outgroup, the three *V. vulpes* populations and *V. rueppellii* also suggested putative instances of admixture (Appendix 5.4).

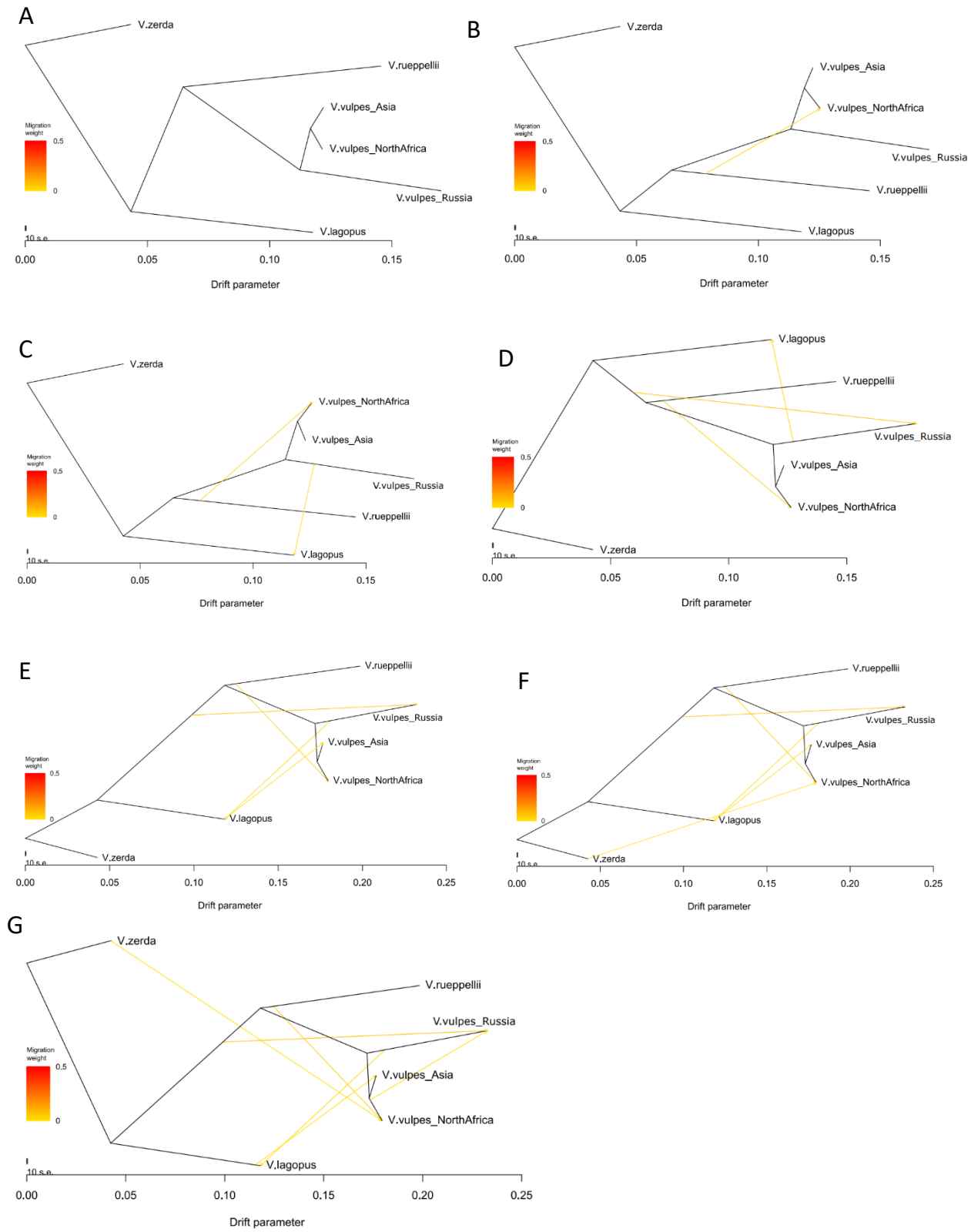


Figure 5.5: TreeMix results based on 6,570,819 autosomal SNPs (dataset, *allsamples14*), estimated using the pairwise correlation of allele frequencies between all groups. Shown trees illustrate from zero to six migration edges (panels A-G), with heat colours of arrows indicating signal intensity. *Vulpes zerda* was used as outgroup.

5.3.1.4 Demographic inference

The PSMC method allowed me to reconstruct changes in effective population size (N_e) over time (Figs. 5.6& 5.7). Four *V. vulpes* individuals from Egypt (199, 383, 145, 123) showed a decline in N_e from approximately 1.1 Mya until 700 to 800 kya, followed by a slight increase until around 200-250 kya. After that time, the four individuals showed different patterns of N_e: 123 and 145 declined in N_e until a sharp increase around 50 – 20 kya, followed by another decline. The population size of individual 383 declined gradually, stabilized at 30 – 10.5 kya and then increased slightly, reaching ~ 90,000, the largest inferred N_e among all analyzed individuals. Individual 199 decreased gradually until around 30 kya, and then declined to reach an N_e of ~ 19,000 – the lowest value among all ten individuals. The other three *V. vulpes* individuals (VvAL09, VvLY02 and UAE2) showed a slight decline in N_e between 1.1 Mya – ca. 750 kya, followed by slight increase until ca. 300 kya. In contrast, the two *V. rueppellii* individuals showed an opposite pattern of increasing population size from 1.1 Mya – ca. 750 kya, followed by a sharp decline in N_e until 40 kya. From that time until 10 kya, they showed a stable population size of ~ 35,000 individuals.

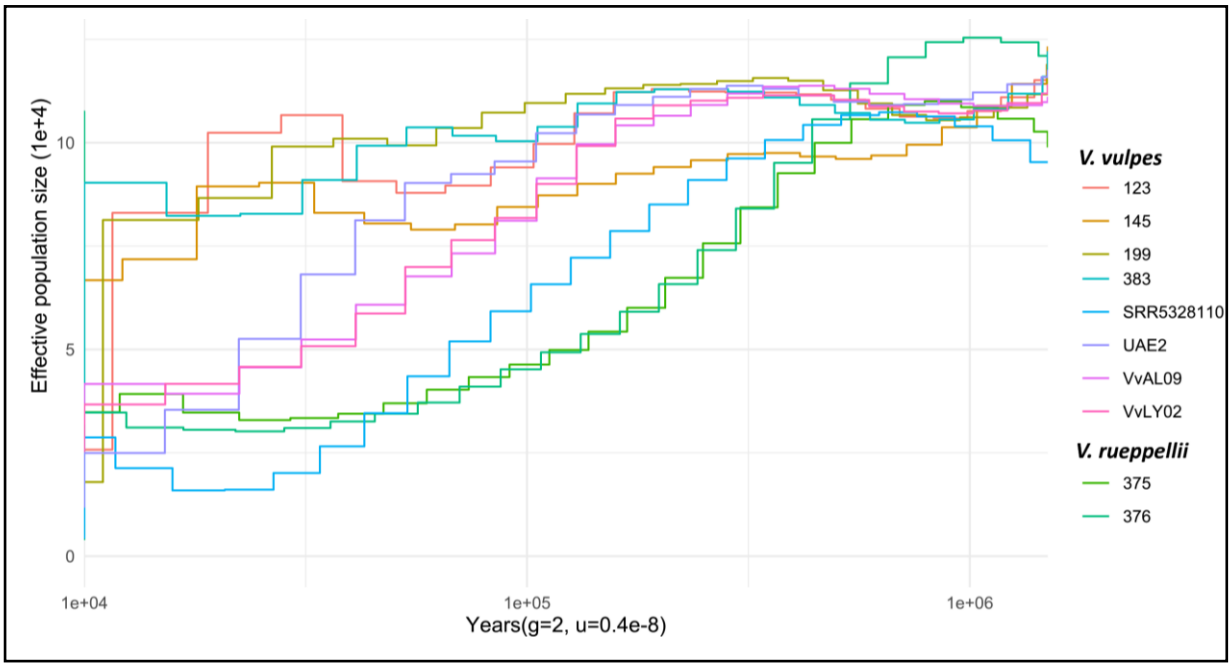


Figure 5.6: PSMC results for *V. vulpes* and *V. rueppellii*. For details on samples, see table 5.1.

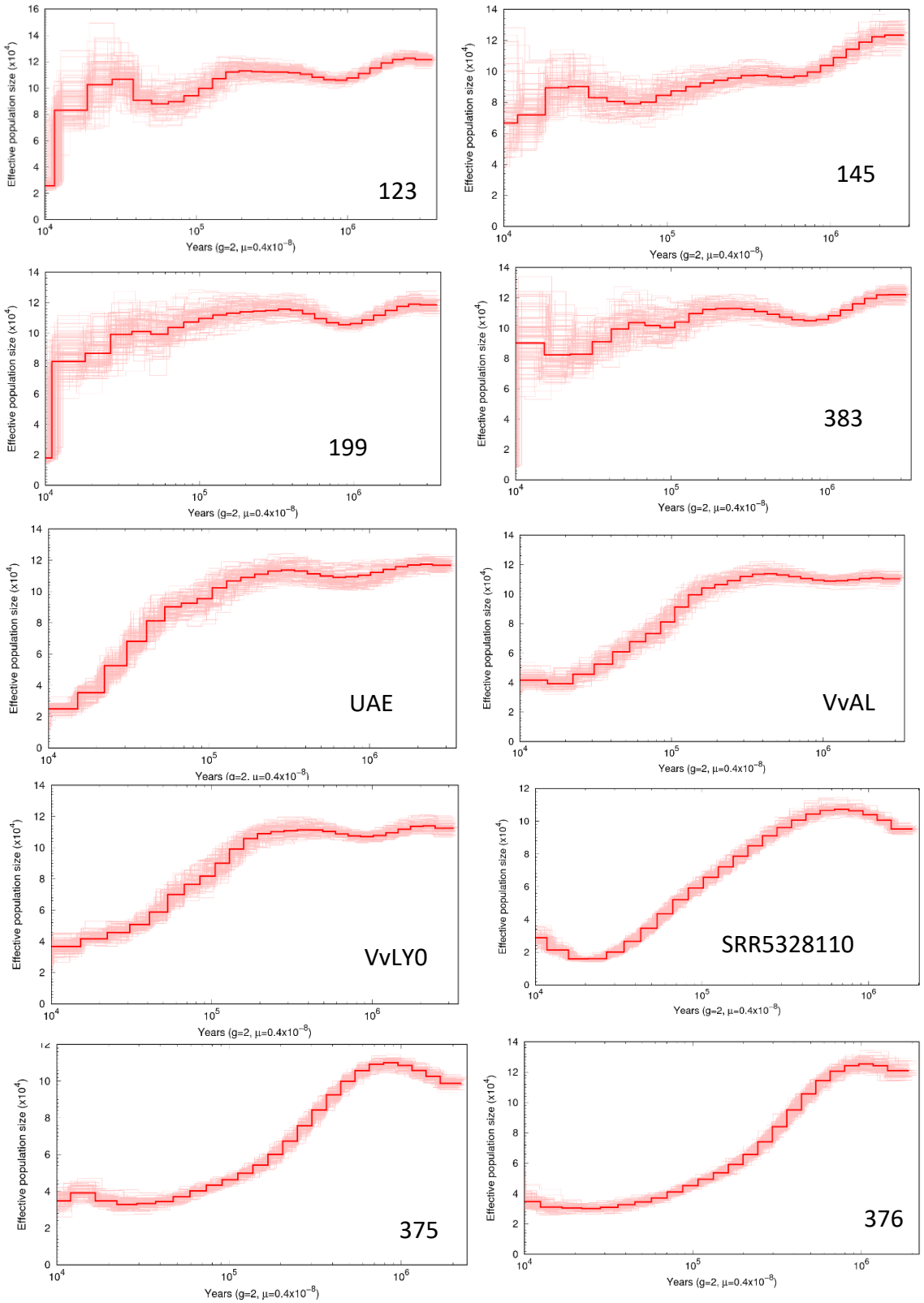


Figure 5.7: PSMC results with 100 bootstrap replicates for *V. vulpes* and *V. rueppellii*, shown separately for each sample. Samples 375 and 376 are *V. rueppellii* and all others are *V. vulpes.* Thick lines represent the median N_e, and thin light lines correspond to the 100 bootstrap replicates. For details on samples see table 5.1.

5.3.2 Mitogenome

The number of assembled whole mitogenome reads and the length of the produced whole mitogenome from the four approaches are shown in table 5.3. Sequencing depth of coverage along the mitogenome was high, with averages per individual ranging from ca. 400 to 19,000 (Table 5.3 and Appendix 5.5). The DNA sequences from the four bioinformatic methods could be annotated as expected to the coding and non-coding regions of the *V. vulpes* mitochondrial reference genome (Arnason et al. 2006), yielding an overall identical organization, number and length of annotated regions: 13 protein-coding, two rRNA and 22 tRNA genes, and the D-loop. A 711 bp portion of the D-loop (positions: 16103 to 16813 on *V. vulpes* mitochondrial reference genome from Arnason et al., 2006) showed a poor alignment with numerous indels and highly fluctuating sequencing coverage (Appendix 5.5), likely reflecting the failure of short-read sequencing data to properly reconstruct the tandem repeat region. This 711 bp region was therefore trimmed from the alignment, leaving a 16,102 bp alignment for downstream phylogenetic analysis.

Table 5.3: Results from four bioinformatic approaches to obtain the whole mitogenomes of *V. vulpes* and *V. rueppellii*

Sample	Assembly method	No. of input read pairs*	No. of assembled mito-genome reads (Average coverage)	Mitogenome length (bp)
123	NOVOPlasty	147,942,476	251,748 (2,410x)	16576
	MITObim	147,942,476	51,105 (n.d)	17862
	Reference-based (Ploidy 1 &2)	147,358,791	588,285 (5,240x)	17055
145	NOVOPlasty	115,044,935	1,402,414 (13,756x)	16542
	MITObim	115,044,935	335,041 (n.d)	17252
	Reference-based (Ploidy 1 &2)	114,056,103	1,318,800 (11,582x)	16815
199	NOVOPlasty	179,180,687	433,370 (3,963x)	16615
	MITObim	179,180,687	109,322 (n.d)	18800
	Reference-based (Ploidy 1 &2)	178,198,379	1,018,327 (9,054x)	16814
383	NOVOPlasty	137,985,996	548,798 (5391x)	16545
	MITObim	137,985,996	74,277 (n.d)	17749
	Reference-based (Ploidy 1 &2)	137,397,128	1,177,041 (10,491x)	16814
UAE2	NOVOPlasty	135,225,980	440,868 (4,181x)	16609
	MITObim	135,225,980	89,485 (n.d)	17836
	Reference-based (Ploidy 1 &2)	134,619,399	816,029 (7,235x)	16814
VvAL09	NOVOPlasty	171,520,840	79,432 (787x)	16607
	MITObim	171,520,840	52,712 (n.d)	17879
	Reference-based (Ploidy 1 &2)	170,594,892	210,668 (1,840x)	16814

VvLY02	NOVOPlasty	151,732,762	1,095,864 (10,798x)	16594
	MITObim	151,732,762	347,799 (n.d)	17203
	Reference-based (Ploidy 1 &2)	150864803	2,143,262 (19,038x)	16814
SRR5280494	NOVOPlasty	64,005,039	166,560 (1,140x)	16569
	MITObim	64,005,039	30,018 (n.d)	17442
	Reference-based (Ploidy 1 &2)	61,965,677	155,259 (908x)	16812
SRR5280501	NOVOPlasty	60,364,521	64,402 (417x)	16435
	MITObim	60,364,521	2631 (n.d)	16927
	Reference-based (Ploidy 1 &2)	59,124,082	58,596 (347x)	16814
375	NOVOPlasty	218,591,573	418,834 (4,176x)	16521
	MITObim	218,591,573	361,475 (n.d)	20611
	Reference-based (Ploidy 1 &2)	216,537,628	855,917 (7,401x)	16813
376	NOVOPlasty	148,382,476	673,370 (7,201x)	16518
	MITObim	148,382,476	332,217 (n.d)	18735
	Reference-based (Ploidy 1 &2)	146,792,335	999,342 (8,656x)	16812

* raw (NOVOPlasty and MITObim), trimmed (reference-based mapping, ploidy 1&2, respectively)

Phylogenetic tree

The obtained maximum likelihood tree recovered the same clades (Holarctic, Nearctic, Palearctic, Africa 1 and Africa 2) as identified previously based on short mtDNA sequences (chapter 2), with high support for all branches except some terminal branches. The tree grouped *V. rueppellii* inside the diversity of *V. vulpes* in the Palearctic clade, with a high support (bootstrap value, BV: 99), confirming the paraphyly of the latter (Fig. 5.8). The differences in results between the four bioinformatic approaches only affected 10 sites, out of which four were within the tandem repeat region. Most ambiguous positions were only from two individuals, SRR5280494 and SRR5280501 (see Appendix 5.6). However, the sequences from the four tested assembly approaches (A, B, C and D) clustered together for each individual.

I obtained a similar high support for all the main clades that had been defined from cytochrome b and D-loop sequences in chapter 2, Fig. 2.2. This included BV=99 for the Palearctic, and BV=100 for the Nearctic, Holarctic, Africa 1 and Africa 2 clades. Furthermore, I obtained a high support (BV=96) for the two African clades to cluster with the joint Holarctic/Nearctic clades, compared with the previously obtained low support in chapter 2 from cytochrome b and D-loop data (Bayesian posterior probability, BPP: p=0.82; chapter 2). Within the Palearctic clade, *V. rueppellii* clustered into two subclades, corresponding to

subclade 1 and subclade 2, and with a higher support (BV=98) for the two clustering together, as a sister lineage of Palearctic clade *V. vulpes*, than in chapter 2. Each of these subclades received BV=100 support.

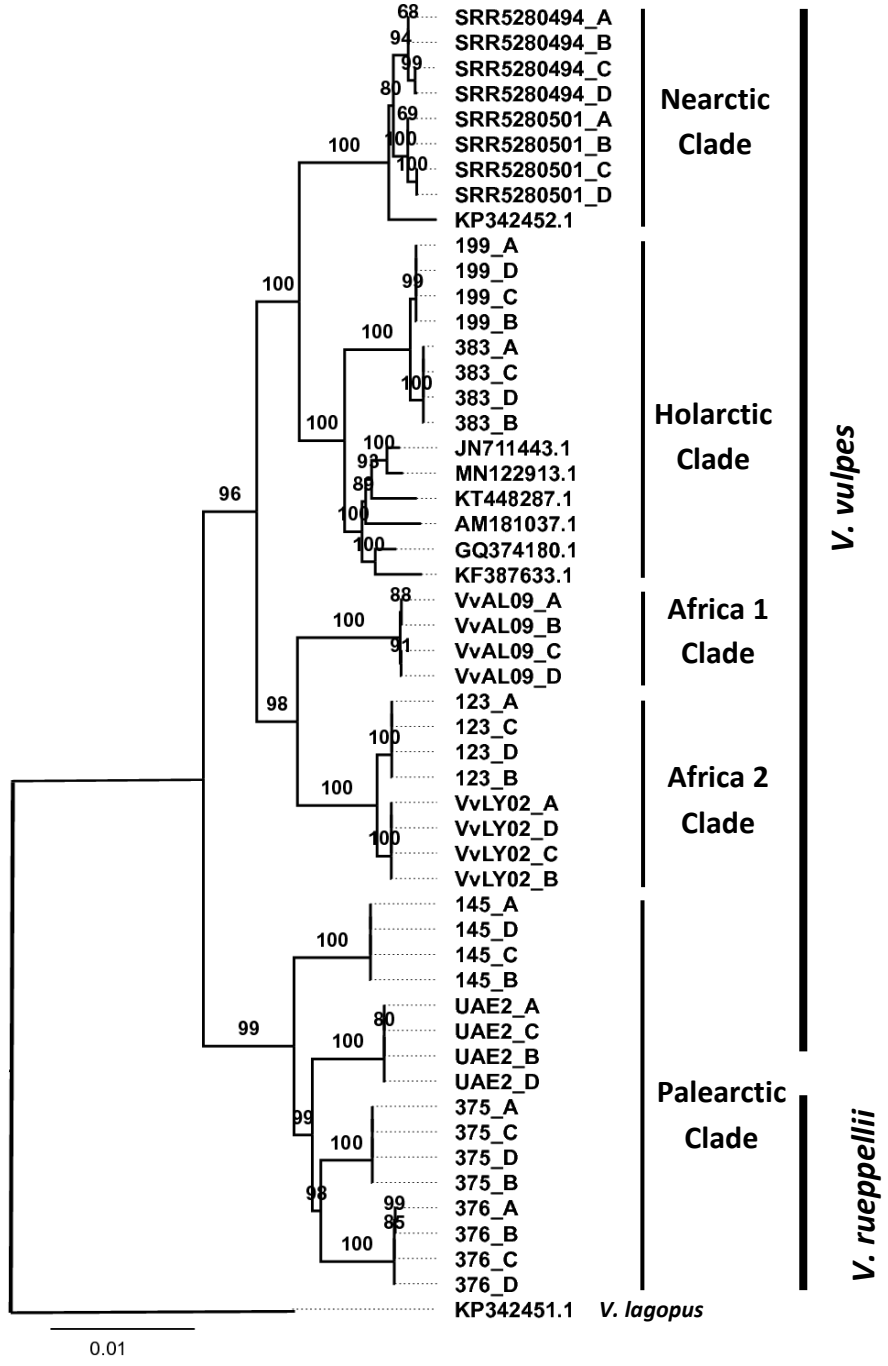


Figure 5.8: Maximum likelihood tree conducted by IQ-TREE based on an alignment of on 16,114 bp with 1000 bootstrap replicates and *V. lagopus* as an outgroup. Sample names are followed by a letter for each assembly approach (A= MITObim, B= NovoPlasty, C= reference-based-ploidy_1 and D= reference-based-ploidy_2). Accession numbers that are not followed by a letter are from GenBank. Numbers on branches are (ultrafast) bootstrap values, and scale bar shows nucleotide substitutions per site. See table 5.2 for details on samples.

5.4 Discussion

This thesis presents the first two *V. rueppellii* genomes sequenced to date, along with the first 'wild' *V. vulpes* genomes (n=7). These data complement the only previously sequenced red fox genome to date, which comprised individuals from the Russian captive breeding program (Kukekova et al., 2018). The obtained mitogenome results are also the first phylogeny that received high support for all main mtDNA clades (Holarctic, Nearctic, Africa 1 & 2, Palearctic), and for how these clades group together. Altogether, these results provide a range of novel insights into the evolutionary history of both species.

5.4.1 Comparison of WGR and ddRAD-seq signals regarding genomic differentiation and genetic structure of *V. rueppellii* and *V. vulpes*

Analyses of both WGR (this chapter) and ddRAD-seq (chapter 4) data clearly separated *V. vulpes* from *V. rueppellii* in PCA, which was also found for Admixture results at K=2-6, as well as in all obtained TreeMix phylogenies in both chapters. In addition, the ddRAD-seq data revealed a geographic gradient of genetic structuring among *V. vulpes* populations, which was less clear from the WGR data, likely due to the much smaller sample size of the latter (including only single representative individuals from a subset of populations). WGR of more samples will be needed to investigate this in detail. Based on the current WGR sampling, two groups could be identified within *V. vulpes*: one group in northwest Africa (Algeria and Libya), and another group in northeast Africa-Arabia (Egypt and UAE), with individual 123 from the Western Mediterranean Coastal Desert (WMCD) showing affinity to both groups. The WMCD population was also found to be admixed in the ddRAD-Seq data, showing affinity to the Nile, Algeria, Libya and UAE populations (chapter 4). The two nuclear genomic datasets therefore appear to reveal broadly concordant biogeographic signals.

Weak signals of interspecific gene flow were recovered by TreeMix for both ddRAD-seq (chapter 4, Fig. 4.5) and WGR data (Fig. 5.5). TreeMix 'migration edges' pointing to the tips of the graph are usually interpreted as evidence of secondary gene flow (e.g., Richards et al., 2018). Therefore, the gene flow from the Algerian *V. vulpes* population to western desert *V.*

rueppellii (chapter 4, Fig. 4.5) likely reflects recent admixture. This signal may be reflected in the admixture results, which showed a *V. rueppellii* individual in the western desert population (WDesert) comprising approximately equal proportions of ancestry in both species, and the PCoA results where the same individual was placed approximately halfway between the two species (chapter 4, Fig. 4.2). This individual is possibly a first generation (F1) hybrid, although at time of tissue sampling nothing unusual was noted about this fox.

Another noteworthy WGR gene flow signal from TreeMix originated from an ancestral branch of *V. rueppellii*, leading to North African *V. vulpes* (Fig. 5.5). This could indicate an ancient introgression event, pre-dating the diversification of *V. vulpes* gene pools in North Africa. Unfortunately, I did not sequence the admixed individual from ddRAD-seq for WGR, precluding a comparison of signals for the two methods. The finding of weak ancient signals of gene flow in the WGR data highlight the power of dense genomic sampling for detection of signals that can be missed by more sparse genomic SNPs from ddRAD-seq approach.

Further comparisons between WGR and ddRAD-seq results from this thesis are complicated by differences in sampling and sample size between the two datasets. The patterns of genetic diversity were somewhat different between WGR and ddRAD-seq: in ddRAD-seq, *V. rueppellii* displayed a lower heterozygosity and nucleotide diversity than *V. vulpes* (chapter 4, Table 4.2), while WGR showed more similar nucleotide diversity for the two species. This could be explained by the ability of WGR to characterize variability across the entire genome, while ddRAD-seq might cover only a biased subset of the genome – along with possible issues with data filtering and missing data (Arnold et al. 2013). However, sample size is another factor that should be considered, as the nucleotide diversity was calculated in WGR for individuals, while in ddRAD-seq it was determined for populations. In conclusion, evidence for *V. rueppellii* having lower genomic diversity than *V. vulpes* remains unclear, and more individuals need to be sequenced to determine variability in this species more accurately.

The broad similarity in evolutionary signals among ddRAD-seq and WGR highlight the power of ddRAD-seq as RRS approach in genomic and biogeographic analyses within species, and also between closely related species. Given the much lower cost of ddRAD-seq than WGR per individual (in the present thesis <£30 for ddRAD-Seq, and ca. £450-500 for WGR), this highlights advantages of ddRAD-seq, allowing researchers to sequence a larger number of

samples (Andrews et al. 2016). Moreover, prior genomic information for the taxa under study is not an essential requirement for ddRAD-seq (Andrews et al. 2016; Barría et al. 2018), although access to a closely related reference genome will provide more robust and unbiased results (Wright et al. 2019). ddRAD-seq and other RRS approaches are suitable for many applications where fine-scale genomic resolution is not required, such as paternity testing (Thrasher et al. 2018), estimating genome-wide hybrid ancestry (Toews et al. 2018; Walsh et al. 2020), genomic diversity (Nyinondi et al. 2020), and population structure (Lavretsky et al. 2019). However, analysis of genomic patterns selection and quantification of genomic divergence landscapes (Szarmach et al. 2021), as well as demographic analysis (Miller et al. 2012; Kozma et al. 2016; Nadachowska-Brzyska et al. 2016; Yi et al. 2020; Sarabia et al. 2021) will benefit more from the WGR analysis (Szarmach et al. 2021). Therefore, both ddRAD-seq and WGR approaches are used to answer different evolutionary questions (Szarmach et al. 2021).

5.4.2 Biogeography and Demographic history of the two species

Besides their overall clear genomic distinction, the two focal species showed different PSMC results (Figs. 5.6& 5.7), suggesting independent long-term demographic trajectories. Most remarkably different was the PSMC pattern between 1.1 Mya and 750 kya, where *V. vulpes* showed a decline in the N_e, while *V. rueppellii* experienced an increase. This timing coincides with a period of increasing aridity of the Sahara around 1.44 ± 0.2 Mya (Trauth et al. 2009), during which desert-adapted species expanded their ranges (Tamar et al. 2018; Moutinho et al. 2020). Mesic North African and Middle Eastern species showed an opposite pattern, expanding during humid periods and contracting during the dry ones (Cosson et al. 2005; Iyengar et al. 2007; Lerp et al. 2011; Husemann et al. 2014; Leite et al. 2015; Bertola et al. 2016; Dinis et al. 2019; Sarabia et al. 2021). Hence, the observed demographic patterns suggest expansion of arid-adapted *V. rueppellii* and contraction of mesic *V. vulpes* during dry periods of the mid-Pleistocene. A study based on mtDNA by Leite et al. (2015) proposed that *V. vulpes* and *V. rueppellii* diverged during the mid-Pleistocene at around 1.2 Mya (confidence interval: 0.8-1.7 Mya), consistent with my finding of the two species showing different demographic trends since approximately this speciation time estimate. The mid-Pleistocene

has been described as a driver of speciation for many taxa in the Sahara (deMenocal 2004). For example, this period encompasses the emergence of African golden wolves (Sarabia et al. 2021), the appearance of several clades of rodents (Nicolas et al. 2008 and 2009; Ndiaye et al. 2012) and the formation of new haplogroups of scimitar-horned oryx (Iyengar et al. 2007).

The PSMC peak in N_e for *V. vulpes* individuals 145 (West Nile population, Egypt) and 123 (Western Mediterranean Coastal Desert) at ca. 50 - 20 kya could have been caused by admixture: Admixture results of WGR data from 145 showed affinity to individual 383 from the Nile region, while individual 123 showed affinity to 383 (Nile) (ddRAD-seq, population: Nile; WGR: 383) and also to Northwest African populations (Algerian VvAlg09 and Libyan VvLY02). Interestingly, this signal received some support by Admixture and TreeMix analyses of ddRAD-seq data (chapter 4, Figs. 4.3& 4.5): Admixture suggested a mixed genomic ancestry for the WMCD population, mirrored by gene flow signals from Libya to WMCD in TreeMix. For the Western Desert *V. vulpes* population, ddRAD-seq data showed some admixture with the Nile population, but also signals of admixture with UAE for some values of K. These findings are consistent with geological evidence, which suggest a corridor of mesic habitats during the Quaternary between the Nile Valley and the Kharga depression (where individual 145 was collected) via a tributary of the Qena River, connecting to that river near Toshka (Said 1990; Issawi and McCauley 1992; Maxwell et al. 2010). Absence of distinct barriers for semi-arid and mesic habitats along the North African coast may explain the gene flow between Northwest Africa (Algeria and Libya) to the Mediterranean coast of Egypt. It should however be noted that the precise timing of the PSMC signals remains putative, since a non-fox mutation rate was used here.

5.4.3 Whole mitogenome versus short mtDNA

Many previous studies have revealed the power of whole mitogenome sequences for improving phylogenetic and geographic resolution, compared with the commonly used analysis of single/short mtDNA markers (Laurimäe et al. 2018). Prominent examples to illustrate this are from primates (Finstermeier et al. 2013; Pozzi et al. 2014), bears (Keis et al.

2013; Anijalg et al. 2018), wolves, (Koepfli et al. 2015; Koblmüller et al. 2016), and squirrels (Hawkins et al. 2016).

Consistent with this, the whole mitogenome provided a much improved resolution of the phylogenetic relationships of *V. vulpes and V. rueppellii*, strengthening the support of previously insufficiently supported clades (chapter 2). However, the present mitogenomic analysis recovered the same main lineages as found previously (Holarctic, Nearctic, Palearctic, Africa 1 and Africa 2), illustrating the power of cytochrome b and D-loop sequences for phylogenetic inference in foxes. Some previous studies have highlighted the power of individual mtDNA loci, showing similar topologies as for the whole mitogenome, e.g., Murtskhvaladze et al. (2020) who studied several lizard genera and found that the concatenated cytochrome b and 16S RNA sequences produced a tree topology that was congruent with a tree based on whole mitogenome sequences.

The comparison of the performance of the four assembly/reference mapping approaches used in this chapter to extract whole mitogenomes has to my knowledge did not receive much prior attention in the literature. Only few studies have compared de novo and reference-based mapping but using different software and pipelines than I used. For instance, Machado et al. (2016) used three different assembly strategies: (1) reference-based (using BOWTIE2); (2) de-novo (using ABYSS, SOAPDENOVO2 and VELVET); and (3) baiting and iterative mapping (using MIRA and MITObim) to extract the whole mitogenome of a frog. Only strategy 3 succeeded to retrieve the whole mitogenome. Dierckxsens et al. (2017) found NOVOPlasty to provide higher accuracy than MIRA/MITObim, especially for repetitive regions. The exclusion of the tandem repeat region of the D-loop for analyses in the present thesis may explain the comparable performance of the tested methods in the present chapter.

5.5 Conclusion

I here reported the first WGR data for *V. rueppellii* (n=2) and WGR data for seven wild *V. vulpes* individuals, along with their corresponding whole mitogenomes. WGR largely confirmed the results from ddRAD-seq and detected an ancient gene flow signal from *V. rueppellii* into North

African *V. vulpes,* which was not revealed by ddRAD-seq. Demographic analysis revealed independent fluctuations of effective population size in the two species during the mid-Pleistocene, since approximately the proposed timing of species divergence by Leite et al. (2015). The whole mitogenome phylogeny improved the support for several insufficiently supported clades from analyses of short mtDNA fragments, but overall confirmed the paraphyly of *V. vulpes* and terminology of the main mtDNA clades in both species. More samples are required to assess genomic diversity and detailed gene flow signals between the two species. The newly obtained data will be useful for future investigations of the evolutionary history of the two species.

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Chapter 6: General Discussion

6.1 Main findings of the thesis

This thesis reported the sequencing of the first *V. rueppellii* genomes and mitogenomes (n=2), as well as the first *V. vulpes* genomes and mitogenomes (n=7) from wild (as opposed to captive-bred) individuals. Besides the various evolutionary analyses presented in this PhD thesis, the newly acquired data will be a useful resource for future studies of the phylogeography, evolutionary history and adaptations of the two species.

Uncovering complicated evolutionary relationships between closely related species requires a combined analysis of different molecular markers to ascertain the reasons that are responsible for discordant phylogenetic relationships among different loci in their genomes (Brown et al., 1982; Jiang et al., 2016; Liedigk et al., 2014, 2015; Roos et al., 2011). This thesis represents the analyses of the evolutionary history of *V. vulpes* and *V. rueppellii* based on evidence from different genetic markers and data from more than a single individual per species (Sacks et al. 2018) . Thousands and millions of SNPS generated by ddRAD-seq (chapter 4) and WGR (chapter 5) respectively, suggested strong genomic differentiation of the two species. These two genomic methods also allowed me to detect both recent (ddRAD-seq) and ancient (WGR) signals of hybridization between the two species. In contrast, short mtDNA sequences (chapter 2) and whole mitogenomes (chapter 5) confirmed the clustering of *V. rueppellii* inside the variation of *V. vulpes*, showing mtDNA paraphyly of *V. vulpes*. Furthermore, sequencing the whole mitogenomes provided high support for all main mtDNA clades, establishing their terminology, which we will be useful for future phylogeographic studies in the two species. My thesis also provides the first evidence of population structuring at both nuclear and mtDNA loci within *V. rueppellii* across a large part of its range. Additional details on the broader significance of the obtained findings are included in the discussion sections of chapters 4 and 5.

I used genomics approaches to obtain high-resolution mtDNA and nuDNA data, important for inferring the complex evolutionary history of the two closely related species. Beyond the obtained results, this study will serve as a basis for future studies about the evolution and adaptations of the two species.

6.2 Conservation and taxonomic implications

The success of conservation and management plans of endangered species depends not only on implementation and acceptance, but also critically on having a scientifically accurate basis about ecology and evolution of the species (Keeley et al. 2019; Díaz et al. 2020). Given that species have traditionally been diagnosed based on morphology, it is unsurprising that our understanding of biodiversity has greatly increased with the advent of modern genetic techniques (Sites and Marshall 2003; Sites and Marshall 2004). Both, *V. vulpes* and *V. rueppellii* have been listed as 'least concern' by the International Union for Conservation of Nature (IUCN). For *V. vulpes* this assessment was based on genetic (Statham et al. 2012; Statham et al. 2014) and non-genetic (Harris 1977; Harris and Rayner 1986; Weber et al. 1999; Heydon and Reynolds 2000; Reynolds and Short 2003; Caley et al. 2015) data from North America, Europe, and to a lesser extent from Asia and North Africa (Hoffmann and Sillero-Zubiri 2021), while the status of *V. rueppellii* was evaluated based on non-genetic studies (Mallon et al., 2015).

My thesis has documented a relatively high genetic variability and hence effective population size of *V. vulpes*, consistent with the high adaptability and wide geographic range of the species, including its occurrence in a highly fragmented habitat in the Sahara. This latter fragmentation might impede gene flow between the populations, leading to either a decrease in the genetic diversity which might result in inbreeding and hence extension, or on longer time scales potentially leading to reproductive isolation and emergence of new species. For instance, South Korean *V. vulpes* have experienced a decline due to habitat fragmentation and habitat loss (Yu et al. 2012). Monitoring of such isolated populations will be useful to see how they might respond to effects of continued climate change and expanded human land/water use.

Most analyses presented in this thesis showed a lower variability of *V. rueppellii* compared to *V. vulpes*. Previous studies highlighted some threats on *V. rueppellii*. These include direct persecution by humans (Cunningham, 2009), e.g., due to perceived impact on game species like Houbara Bustard (*Chlamydotis undulata*) and livestock (Murdoch et al. 2007). Another significant threat is the competition in areas of sympatry with *V. vulpes*, facilitated by the large geographic range and high adaptability to different habitat types of *V. vulpes*, and augmented

by new human settlements that can lead to expansion of the latter into *V. rueppellii* habitats (Sillero-Zubiri et al. 2004). Competitive exclusion of *V. rueppellii* by *V. vulpes* has been reported from Oman and the UAE, where the former species has been displaced around settlements by *V. vulpes* (Mallon & Budd, 2011). Also, extensive camera trapping studies have shown a similar process in the Western 'empty quarter' of Saudi Arabia (Barichievy and Wacher pers. obs). Consolidating these previously described threats with the low variability and recent hybridization reported here (chapter 4, ddRAD-seq), *V. rueppellii* is expected to be influenced negatively by competition with *V. vulpes* in sympatric areas. Indeed, detrimental impacts of *V. vulpes* on other sympatric *Vulpes* species have been reported before, e.g., on *V. lagopus* (Tannerfeldt et al. 2002), *V. corsac* and *V. macrotis* (Sillero-Zubiri et al. 2004). The findings of this thesis therefore prompt the monitoring of *V. rueppellii* populations, especially in areas of sympatry. More broadly, *V. rueppellii* requires a long-term integrated conservation plan to properly evaluate its status, to ensure the continued survival of this long-term evolved desert-adapted species.

Moving forward, I recommend increasing study/sampling of both species across their ranges, to expand the available specimen pool for future analyses in genomics research and conservation. It is noteworthy that many areas in North Africa, and in central Asia remain largely unstudied for both species.

6.3 Future directions and limitations

6.3.1 Male-mediated gene flow – Y chromosome

In mammals, many species have male-biased dispersal, with males dispersing more often and/or further than females, so maternally inherited mtDNA may show a more structured view of population structuring than paternally inherited (Y-chromosome) markers, which has been shown for, e.g., bears (Bidon et al. 2014), foxes (Statham et al. 2014), and goats (Pidancier et al. 2006). Although Y-chromosome data have been generated for *V. vulpes*, mainly from North America, no Y-chromosome data have been characterized to date from this species in North Africa and the Middle East, and to my knowledge none exist for *V. rueppellii*. For *V. vulpes*, Y chromosome data would be expected to reveal different patterns of genetic diversity and structuring than those revealed through mtDNA, because males

disperse more widely than females (Harris and Trewhella 1988). Sex-specific effects have been found to influence patterns of genetic diversity in *V. vulpes* (e.g., Sacks et al., 2016). Thirteen Y-chromosome microsatellite markers have already been developed for *V. vulpes* (Statham et al. 2014; Rando et al. 2017), and been used to investigate population history of *V. vulpes* in North America, Great Britain, and Russia. Two of those thirteen microsatellites were used in a wide-range study (which however did not include North Africa), which showed a continental structuring in contrast to mtDNA (Statham et al. 2014). Also, Kasprowicz et al. (2016) reported differences in the frequencies of European mitochondrial and Y-chromosome haplotypes in mid-Atlantic *V. vulpes*. Development of Y-chromosomal SNPs would provide (a) higher resolution and (b) lower incidence of homoplasy signals (which hamper studies of long-term processes) than microsatellite markers (Bidon et al. 2014; Sacks et al., 2021), and the availability of data newly generated in this thesis will be useful for this goal. Analysis of Y-chromosome data from a variety of populations of both species will expand the knowledge of their Y-chromosomal variation and the distribution of their diversity across their ranges, especially in so far non-sampled regions in North Africa and the Middle East.

6.3.2 Desert adaptations, genes of adaptations

Given the findings of this thesis which support the long-term evolutionary distinctness of *V. rueppellii,* studies of this species' adaptations seem warranted – but are so far largely lacking. Besides more detailed work on diet and morphometric/physiological adaptations (see sections below), available genomic data from both species can now be used to look for signals of adaptive evolution in both species (e.g., Hoban et al., 2016). A range of candidate genes are known from previous work on other desert-adapted taxa (Rocha et al. 2021), and it will be interesting to see if these loci are involved in desert adaptation in the two focal fox species, and how alleles at such adaptive genes might show preferential introgression from *V. rueppellii* into *V. vulpes* in sympatric areas, allowing the latter to colonise and survive in the range of the former.

6.3.3 Dietary analysis using different resources

Dietary analysis can help understanding ecological interactions between species. The high dietary opportunism of *V. vulpes* (Szuma 2003) is expected to affect resource partitioning between it and *V. rueppellii*. Thus, investigating the dietary composition of both species will help to determine the extent and severity of their dietary competition. Furthermore, increased knowledge on the diet of both species, especially when in sympatry, will help determine how the two species might respond to future climate change and habitat changes.

Besides direct observation, two methods are commonly applied for dietary analysis: morphological analysis of scat contents, and metabarcoding analysis of faecal/gut content. Morphological identification of prey remnants in scat has been conducted for many mammalian taxa, e.g., African wolves (*Canis lupaster*) and Ethiopian wolves (*C. simensis*), Gutema et al., 2019; grey wolf (*C. lupus*) and dingo (*C. lupus dingo*), Nowak et al., 2011; and grey wolf (*C. lupus)*, Migli et al., 2005. But morphological identification of prey remains can lead to overestimation of relatively undigested prey, where highly digested prey may be missed (Boyer et al., 2015; Brown et al., 2012; Shehzad, McCarthy, et al., 2012). Advances in DNA sequencing technologies have fostered the analysis of environmental samples and identification of the constituents of faecal material (Pompanon et al. 2012). DNA metabarcoding approaches (utilizing high-throughput sequencing, HTS), where DNA mixtures are extracted and sequenced in parallel, have been successfully applied to several faecal dietary studies, e.g., in fish (Berry et al., 2015), carnivores (Hacker et al., 2022), tapirs (Hibert et al., 2013), bats (Ingala et al., 2021), penguins (Murray et al., 2011), primates (Quéméré et al., 2013) and cats (Shehzad et al., 2012).

6.3.4 Morphometric analysis

Morphological variation plays an important role in evolutionary diversification and is of central importance for interpretation of interspecific differences (Cheverud 1996). Several studies conducted at a broad geographic scale of *V. vulpes* showed a variation in the size of the body and craniodental measurements. This trend has been explained by the influence of many factors such as climate (Dayan et al. 1989), latitude (Kolb 1978; Viranta and Kauhala

2011; Yom-Tov et al. 2013), population density (Cavallini 1995), intraspecies competition, habitat productivity and differential food availability (Gortázar et al. 2000; Yom-Tov et al. 2013), phylogenetic distance (Cavallini 1995), genetic diversity (Simonsen et al. 2003), and competition with other canid species (Dayan et al. 1989; Viranta and Kauhala 2011).

The difference in external morphology of *V. vulpes* and *V. rueppellii* has been established, with *V. vulpes* being larger, and with longer hind legs, longer tail, shorter ears and larger skull than *V. rueppellii* (Lariviere and Seddon 2001). However, this view is based on only limited morphometric data from North Africa. Considering habitat diversity and the fragmentation of populations within each species, a relatively high level of intraspecific morphometric variation would be expected, along with possible impacts of introgression.

By taking the effect of the factors mentioned above into consideration, several questions about the morphological characters of the two species in North Africa may be raised. (1) Which geographical and climatic factors influence the geographic variability of craniodental characters in the two species? In the Nearctic, a large number of dental characters in *V. vulpes* showed a geographical gradient related to longitude, whereas in the Palearctic, a gradient related to latitude was found (Szuma 2007). (2) What is the effect of food availability on body and skull size of the two species? This question could be especially relevant in *V. vulpes,* which occupies a range different ecological habitats even within North Africa (e.g., Nile River floodplain versus desert oases). Several studies have reported an increase in skull (Yom-Tov et al. 2003; Yom-Tov et al. 2007) and body (Gortázar et al. 2000) size of *V. vulpes* living in high-productive (agricultural) habitats, compared to those in low-productive (non-agricultural) habitats. (3) Is there any character displacement occurring between the two species in North Africa? Character displacement is defined as “a situation when two species of animals overlap geographically, the differences between them are prominent in the sympatric zone and weakened or lost entirely in the parts of their range outside this zone” (Brown & Wilson, 1956). Dayan et al. (1989) found constant size ratios (1.18-1.21) between carnassial (large upper premolar and lower molar teeth of a carnivore, adapted for shearing flesh) lengths of *V. vulpes* and *V. rueppellii* throughout the Saharo-Arabian region, where they occur in sympatry - different than the pattern reported for *V. vulpes* in allopatric zone. Accordingly, the authors suggested the length of carnassial as a suitable morphological character to study

ecological character displacement between the two species, because it relates directly to the feeding habits. In North Africa, the two species occur in partial sympatry (and in allopatry), thus I would expect to see a clear effect of the character displacement, with both *V. vulpes* and *V. rueppellii* showing constant size ratios of carnassial lengths in sympatry, but weak or no differences of carnassial lengths in allopatry. (4) Are there any modifications in the dentition of the two species across their ranges? Normally *V. vulpes* has a larger size skull and feeds on larger prey than *V. rueppplli*i, so the sharpness of the canine and subsequently the bite force is expected to be stronger in the former than the later. (5) Which ecological factors are responsible for any geographic craniodental variability within each species?

6.3.5 Monitoring studies

Monitoring animals provides information on their abundance, population dynamics, movements and conservation status (Thomas et al. 2020). The methods used to monitor mammals can be classified as direct (direct contact with the animal being monitored; Lyra-Jorge et al., 2008) and indirect (based on presence signs such as foot tracks, radio tracking, non-invasive genetic sampling (NGS) and camera trapping (CT) (Mattioli et al. 2018; Valente et al. 2018).

NGS and CT are used extensively in monitoring programs of mammals (Mattioli et al. 2018). NGS depends on collection of ideally fresh samples (usually hairs or scats) and can provide a set of useful information such as species, gender, kinship, dispersal and hybridization (Wayne and Morin 2004; Goossens and Bruford 2009), but this method is more frequently used in large-scale monitoring projects with large budgets (considering the cost of lab work), or when dealing with species that are not easily recognizable by photo-identification (Mumma et al. 2015; López-Bao et al. 2018). CT is an efficient tool to monitor mammals, as it is non-invasive, cheap and a reliable means to detect large and medium-sized carnivores, particularly elusive and nocturnal species (Pettorelli et al. 2010). CT has been used successfully to assess the absence and presence of carnivores (Moruzzi et al. 2002; Rosellini et al. 2008; Galaverni et al. 2012) as well as their population dynamics (Karanth et al., 2006), and detection of hybrids

(Kilshaw et al. 2016). Also, CT has been used for density estimation in canids, e.g., coyote (Larrucea et al. 2007), maned wolf (Trolle et al. 2007), and red fox (Sarmento et al. 2009).

Use of CT might help to obtain a rough estimate of the abundance and distribution of *V. vulpes and V. rueppellii* in hitherto understudied areas, particularly with ongoing climate change and the expected colonization of new habitats by *V. vulpes*. Also, CT will provide information on ranging behaviour, activity patterns, and patterns of dispersal and migration (Karanth and Nichols 1998; Sarmento et al. 2009) of both species. Furthermore, this method could be helpful to monitor for possible hybridization, a question with increased relevance due to the findings in chapters 4&5 of this thesis. CT could potentially catch interspecific mating events between the two species, or help discovering hybrid forms, along with use of genetic monitoring methods (i.e., NGS and invasive methods). In fact, camera traps have been successful in detection of hybrid wild cats in Scotland (Kilshaw et al. 2016). Finally, CT can be useful for monitoring fitness and detection of signals of inbreeding depression or diseases. The potential role of *V. vulpes* in spreading of diseases as mange (Baker et al. 2000) , rabies (Chautan et al. 2000) and bovine tuberculosis (Martın-Atance et al. 2005) has been recorded in Europe. Thus, a reliable estimate of fox abundance was important for subsequent disease risk analysis (Sarmento et al. 2009). CT can be a viable approach for estimating population size, assuming enough cameras are used, the distance between the cameras matches up with the spatial ecology of the species, and the length of the trapping period allows for enough recaptures (Sarmento et al. 2009). Considering the advantages and disadvantages of NGS and CT, an integrated approach of both methods could be used to monitor the abundance, distribution and dynamics of *V. vulpes* and *V. rueppellii.*

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Appendices

Chapter 2

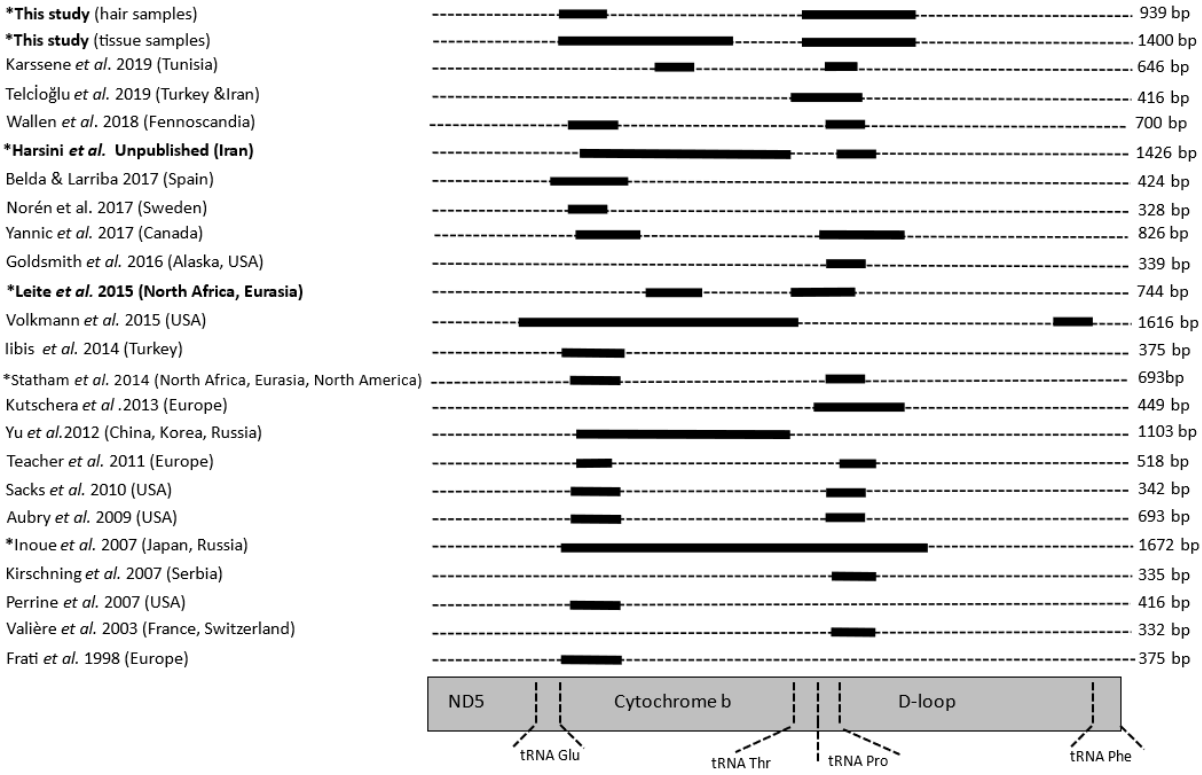
Appendix 2.1

Details of quality control of Sanger sequencing data for chapter 2.

Among the 128 samples newly sequenced as part of this project, ten hair samples did not amplify, and one tissue and one hair sample showed putative signals of heteroplasmy and/or nuclear mitochondrial copies (Numts). These latter two samples showed double peaks in both forward and reverse electropherograms, which was confirmed by re-extraction and independent PCR and sequencing. The double peaks resulted from transition polymorphisms, consistent with (but not strong evidence for) mitochondrial origin and hence heteroplasmy (Nandakumar *et al.,* 2021). Another explanation are nuclear copies of mtDNA (Numts; Sorenson & Fleischer 1996), although we note that we did not see any such signals in the remaining 116 high-quality sequences. Complying with common practice, we therefore excluded these two sequences from the dataset.

Appendix 2.2

Available mtDNA sequences (dark bars) used in previous phylogeographic studies of *V. vulpes* and *V. rueppellii*. Additionally, few near-complete mitogenomes are available on GenBank. Studies in bold are those that contain sequences from both *V. vulpes* and *V. rueppellii*. Asterisks denote studies included in data analysis for the present study.



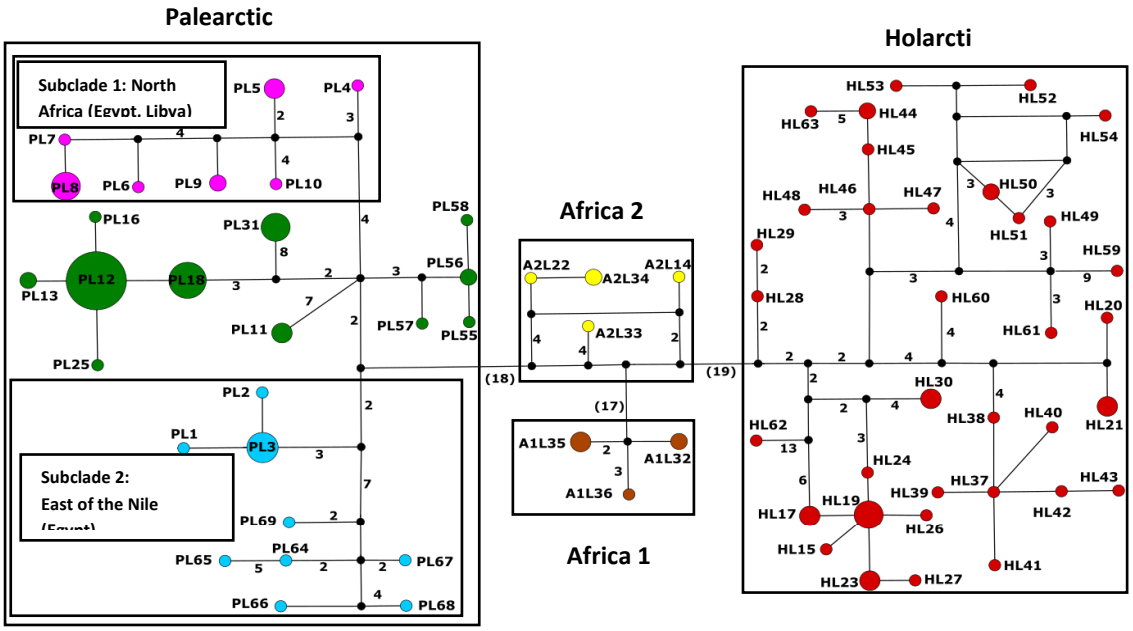
Appendix 2.3

Average number of nucleotide substitution per site between the main mitochondrial clades.

Clade #1	Clade #2	Dxy (%) (183 sequences, 635 bp) Figure 2B	Dxy (%), (145 sequences, 1150 bp) Figure S2
Palearctic	Holarctic	3.6	3.3
Africa 1	Holarctic	4.0	3.2
Africa 2	Holarctic	3.3	2.9
Africa 1	Palearctic	3.9	3.3
Africa 2	Palearctic	3.2	2.9
Africa 1	Africa 2	3.0	2.1

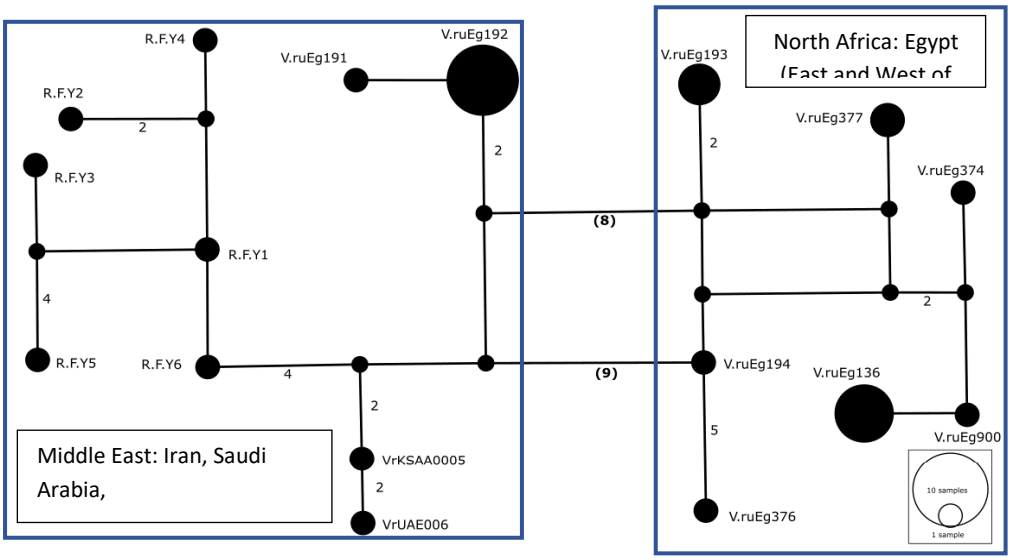
Appendix 2.4

Haplotype network of 145 sequences of *V. vulpes* (115 individuals, 53 haplotypes) and *V. rueppellii* (30 individuals, 16 haplotypes) based on concatenated data of 1150 bp (822 bp cytochrome b and 382 bp D-loop). Numbers denote the number of substitutions along each branch (only values ≥2 are shown). KSA: Kingdom of Saudi Arabia, UAE: United Arab Emirates. See supplementary file 2 for details on haplotypes and samples.



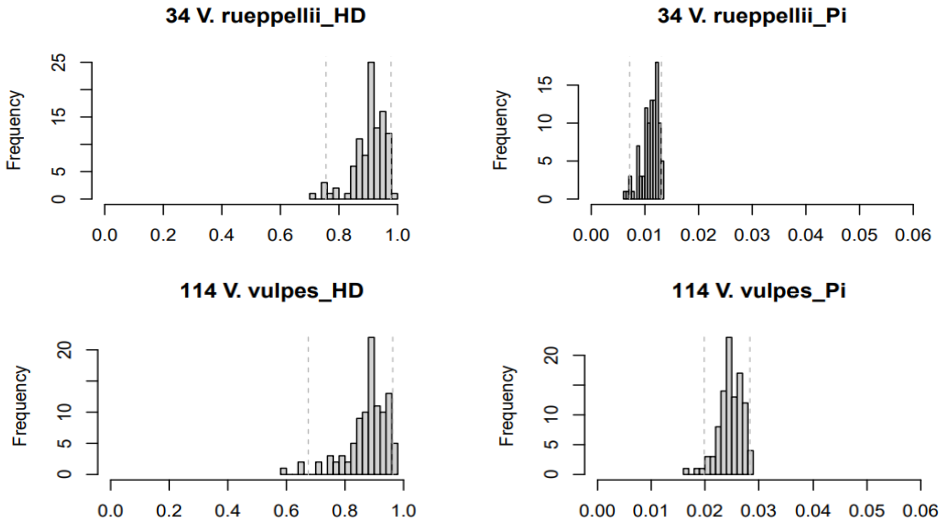
Appendix 2.5

Haplotype network of 33 sequences of *V. rueppellii* (33 individuals, 17 haplotypes based on concatenated data of 688 bp: 361 bp cytochrome b and 327 bp D-loop). Numbers denote the number of substitutions along each branch (only values ≥2 are shown).



Appendix 2.6

Haplotype (HD) and nucleotide diversities (Pi) of 114 *V. vulpes* and 34 *V. rueppellii* (see table 2.2 in chapter 2) based on a resampling bootstrap approach. The two species show a significant nucleotide diversity difference, while the higher haplotype diversity of *V. rueppellii* is non-significant, overlapping with the range values of *V. vulpes*. The two vertical dash lines denote the 95% confidence intervals.



Chapter 4

Appendix 4.1

Samples used in ddRAD-seq analysis, chapter 4.

Sample ID	population	sex	species	coordinates		locality
				y (N) Latitude	x (E) longitude	
370	EDesert	female	*V. ruepellii*	25.62833	34.40694	Wadi om-Khiag, Eastern Desert,Egypt
374	EDesert	female	*V. ruepellii*	25.61528	34.39972	Wadi om-Khiag, Eastern Desert,Egypt
375	EDesert	male	*V. ruepellii*	25.61528	34.39972	Wadi om-Khiag, Eastern Desert,Egypt
136	EDesert	female	*V. ruepellii*	25.1375	33.15222	Wadi-Sakhab, Eastern Desert, Egypt
137	EDesert	female	*V. ruepellii*	25.1375	33.15222	Wadi-Sakhab, Eastern Desert, Egypt
138	EDesert	female	*V. ruepellii*	25.13556	33.14694	Wadi-Sakhab, Eastern Desert, Egypt
139	EDesert	male	*V. ruepellii*	25.13556	33.14694	Wadi-Sakhab, Eastern Desert, Egypt
191	EDesert	male	*V. ruepellii*	26.15167	34.1125	Wadi El-Nakil Eastern Desert, Egypt
192	EDesert	male	*V. ruepellii*	26.145	34.11556	Wadi El-Nakil Eastern Desert, Egypt
900	EDesert	female	*V. ruepellii*	28.43833	32.27389	Wadi El-Tarfa Eastern Desert, Egypt
901	EDesert	male	*V. ruepellii*	26.145	34.11556	Wadi El-Nakil Eastern Desert, Egypt
903	EDesert	female	*V. ruepellii*	26.15167	34.1125	Wadi El-Nakil Eastern Desert, Egypt
904	EDesert	male	*V. ruepellii*	26.145	34.11556	Wadi El Nakil Eastern Desert, Egypt

376	WDesert	male	*V. ruepellii*	25.72639	30.555	kharga Oasis, Western Desert, Egypt
377	WDesert	female	*V. ruepellii*	25.72833	30.55472	kharga Oasis, Western Desert, Egypt
378	WDesert	female	*V. ruepellii*	25.72722	30.55444	kharga Oasis, Western Desert, Egypt
193	WDesert	male	*V. ruepellii*	29.56345	26.50365	Matrouh,Western Desert, Egypt
194	WDesert	female	*V. ruepellii*	29.63036	26.50916	Matrouh,Western Desert, Egypt
VrLY06	WDesert	female	*V. ruepellii*	25.67894444	21.07077778	Tazerbu, Libya
VvAL06	Alg	male	*V. vulpes*	35.352874	1.2489643	Tagdemt forest, Algeria
VvAL07	Alg	female	*V. vulpes*	35.339713	1.2405059	Tagdemt communale, Algeria
VvAL08	Alg	female	*V. vulpes*	35.339713	1.2405059	Tagdemt communale, Algeria
VvAL09	Alg	male	*V. vulpes*	35.339713	1.2405059	Tagdemt communale, Algeria
VvLY01	Lib	male	*V. vulpes*	31.82177778	14.81388889	Misrata, Libya
VvLY02	Lib	male	*V. vulpes*	31.82177778	14.81388889	Misrata, Libya
VvLY03	Lib	female	*V. vulpes*	31.82177778	14.81388889	Misrata, Libya
VvLY04	Lib	female	*V. vulpes*	32.423	12.68986111	Az-Zāwiyah, Libya
VvLY05	Lib	male	*V. vulpes*	32.17419444	12.22555556	Alzintan, Libya
UK1	UK	unknown	*V. vulpes*	51.53833	-3.220278	Vale of Glamorgan, Wales, UK
UK2	UK	unknown	*V. vulpes*	51.53833	-3.220278	Vale of Glamorgan, Wales, UK
UK3	UK	unknown	*V. vulpes*	51.53833	-3.220278	Vale of Glamorgan, Wales, UK
UK4	UK	unknown	*V. vulpes*	51.53833	-3.220278	Vale of Glamorgan, Wales, UK
UK5	UK	unknown	*V. vulpes*	51.53833	-3.220278	Vale of Glamorgan, Wales, UK
Vv567	Port	unknown	*V. vulpes*	38.76667	-9.43333	Portão dos Pocinhos, Pedra Amarela

Vv568	Port	male	*V. vulpes*	39.42022	-8.80386	Alcanede, Santarém
Vv530	Port	male	*V. vulpes*	39.41042	-7.68505	Gafete, Crato
Vv549	Port	male	*V. vulpes*	39.58614	-8.48892	Paialvo, Tomar
Vv540	Port	male	*V. vulpes*	41.37432	-7.61	Torre do Pinhão, Sabrosa
Vv557	Port	male	*V. vulpes*	38.93961	-7.12186	Caia e São Pedro, Elvas
Vv550	Port	male	*V. vulpes*	39.70779	-8.96225	Moita, Alcobaça
UAE1	UAE	male	*V. vulpes*	25.33667	55.52444	Sharjah, UAE
UAE2	UAE	male	*V. vulpes*	25.33667	55.52444	Sharjah, UAE
UAE3	UAE	male	*V. vulpes*	25.33667	55.52444	Sharjah, UAE
UAE4	UAE	male	*V. vulpes*	25.35861	55.54889	Sharjah, UAE
UAE5	UAE	female	*V. vulpes*	25.35861	55.54889	Sharjah, UAE
UAE6	UAE	male	*V. vulpes*	25.35861	55.54889	Sharjah, UAE
170	ENile	female	*V. vulpes*	27.9625	34.38139	Sharm El-sheikh, South Sinai, Egypt
171_2	ENile	male	*V. vulpes*	27.9625	34.38139	Sharm El-sheikh, South Sinai, Egypt
153	ENile	male	*V. vulpes*	25.24639	34.64667	Wadi Dabr-Eastern Desert, Egypt
198	ENile	female	*V. vulpes*	25.255	34.65389	Wadi Dabr-Eastern Desert, Egypt
199	ENile	male	*V. vulpes*	30.9875	32.78889	Rabaa, North Sinai, Egypt
200	ENile	female	*V. vulpes*	30.9875	32.78889	Rabaa, North Sinai, Egypt
196	WMCD	male	*V. vulpes*	31.06951	28.14203	Sidi heneish - El-Dabaa, Matrouh, Western Desert, Egypt
120	WMCD	female	*V. vulpes*	30.96028	28.35278	El Daba Matrouh, Western Desert, Egypt
121	WMCD	female	*V. vulpes*	30.96028	28.35278	El Daba Matrouh, Western Desert, Egypt
122	WMCD	female	*V. vulpes*	30.96028	28.35278	El Daba Matrouh, Western Desert, Egypt
123	WMCD	male	*V. vulpes*	30.96028	28.35278	El Daba Matrouh, Western Desert, Egypt
394	Nile	male	*V. vulpes*	29.38406	30.90356	Monshaat Atifah, Senoures, Faiyum , Egypt

395	Nile	male	*V. vulpes*	29.38406	30.90356	Monshaat Atifah, Senoures, Faiyum , Egypt
397	Nile	male	*V. vulpes*	29.38406	30.90356	Monshaat Atifah, Senoures, Faiyum , Egypt
905	Nile	female	*V. vulpes*	29.57186	30.90503	Kom Oshim, Faiyum, Egypt
906	Nile	male	*V. vulpes*	29.57186	30.90503	Kom Oshim, Faiyum, Egypt
124	Nile	male	*V. vulpes*	30.75139	31.12389	Gharbiya, Nile Delta, Egypt
125	Nile	female	*V. vulpes*	30.75139	31.12389	Gharbiya, Nile Delta, Egypt
398	Nile	male	*V. vulpes*	30.225292	31.101619	Darawah-Ashmon-Monofiya, Nile Delta, Egypt
400	Nile	male	*V. vulpes*	30.225292	31.101619	Darawah-Ashmon-Monofiya, Nile Delta, Egypt
401	Nile	male	*V. vulpes*	30.225292	31.101619	Darawah-Ashmon-Monofiya, Nile Delta, Egypt
402	Nile	male	*V. vulpes*	30.225292	31.101619	Darawah-Ashmon-Monofiya, Nile Delta, Egypt
390	Nile	male	*V. vulpes*	28.4125	30.7475	Matai, Minya, Nile Valley, Egypt
391	Nile	female	*V. vulpes*	28.4125	30.7475	Matai, Minya, Nile Valley, Egypt
387	Nile	male	*V. vulpes*	26.71167	31.47167	Nazet Al Mahazmin, Juhaynah, Sohag ,Nile Valley, Egypt.
383	Nile	male	*V. vulpes*	25.72056	32.67333	Elkarnak El kadem, Luxor, Nile Valley, Egypt
384	Nile	female	*V. vulpes*	25.72056	32.67333	Elkarnak El kadem, Luxor, Nile Valley, Egypt
151	Nile	male	*V. vulpes*	23.355033	32.813525	Khor Abu Stait, West of Lake Nasser, Egypt

152	Nile	female	*V. vulpes*	23.355033	32.813525	Khor Abu Stait, West of Lake Nasser, Egypt
127	Nile	male	*V. vulpes*	23.60639	32.98667	khor Sakr, East of Lake Nasser, Egypt
128	Nile	male	*V. vulpes*	23.64417	32.92139	Wadi Dihmit, East of Lake Nasser, Egypt
129	Nile	female	*V. vulpes*	23.64417	32.92139	Wadi Dihmit, East of Lake Nasser, Egypt
150	Nile	female	*V. vulpes*	23.02421	32.959226	khor absko, East of lake Nasser, Egypt
174	WNile	male	*V. vulpes*	27.067984	27.932581	Well 6, Farafra Oasis, Western Desert, Egypt
175	WNile	male	*V. vulpes*	27.067984	27.932581	Well 5, Farafra Oasis, Western Desert, Egypt
176	WNile	male	*V. vulpes*	27.067984	27.932581	Well 5, Farafra Oasis, Western Desert, Egypt
177	WNile	male	*V. vulpes*	27.067984	27.932581	Well 5, Farafra Oasis, Western Desert, Egypt
380	WNile	male	*V. vulpes*	28.40389	28.89028	Bawiti, Bahariya Oasis, Western Desert, Egypt
382	WNile	male	*V. vulpes*	28.40389	28.89028	Bawiti, Bahariya Oasis, Western Desert, Egypt
172	WNile	female	*V. vulpes*	28.31583	29.07944	Al Hara, Bahariya Oasis, Western Desert, Egypt
173	WNile	female	*V. vulpes*	28.40861	28.89278	Boheyrt El Mamor, Bawiti, Bahariya Oasis, Western Desert, Egypt
147	WNile	male	*V. vulpes*	25.39611	30.53778	Kharga Oasis, Western Desert,Egypt
148	WNile	female	*V. vulpes*	25.39611	30.53778	Kharga Oasis, Western Desert,Egypt

179	WNile	female	*V. vulpes*	24.74344	30.63489	El malkya, Baris, kharga Oasis, Western Desert, Egypt
181	WNile	male	*V. vulpes*	24.71317	30.64554	El malkya, Baris, kharga Oasis, Western Desert, Egypt
182	WNile	female	*V. vulpes*	24.71317	30.64554	El malkya, Baris, kharga Oasis, Western Desert, Egypt
183	WNile	female	*V. vulpes*	24.56882	30.70797	Village 80, Baris, kharga Oasis, Western Desert, Egypt
145	WNile	male	*V. vulpes*	25.54533	29.0472	Dakhla Oasis, Western Desert, Egypt
146	WNile	female	*V. vulpes*	25.54533	29.0472	Dakhla Oasis, Western Desert, Egypt
195	WNile	female	*V. vulpes*	29.17611	25.58667	El Zaytoon, Siwa Oasis, Western Desert, Egypt

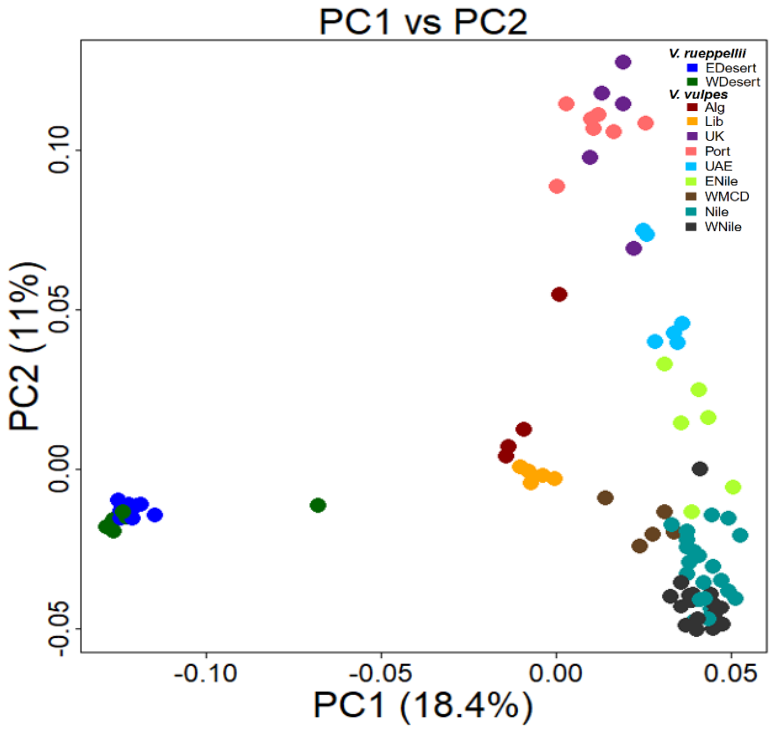
Appendix 4.2

A summary statistic of data used in ddRAD-seq analysis.

Dataset	**Reference genome**	**No of SNPs (raw data)**	**No of SNPs/ excluding stacks with number of SNPs>3**	**Writing single SNPs/Stack**	**No of SNPs/ excluding loci out of Hardy Weinberg Equilibrium (HWE), p<0.05**	**No of SNPs/ Excluding SNPs in Linkage Disequilibrium (LD)**	**No of SNPs after filtering with SambaR Parameters indmiss=0.25, snpmiss=0.1**
Combined dataset	Dog, *Canis lupus familiarise*	105569	72812	43896	39035	No LD filter	34783
Combined dataset	Dog, *C. lupus familiarise*	105569	72812	43896	39035	14615 LD parameters 50 5 0.2	12601
Combined dataset	Dog, *C. lupus familiarise*	105569	72812	43896	39035	5363 LD parameters 50 5 0.1	4503
Combined dataset	Dog, *C. lupus familiarise*	105569	72812	43896	No HWE filter	16714 LD parameters 50 5 0.2	14101
Vv77 dataset	Dog, *C. lupus familiarise*	88091	65333	41269	36055	21657 LD parameters 50 5 0.2	17564
Vr19 dataset	Dog, *C. lupus familiarise*	68490	53066	35602	33010	5804 LD parameters 50 5 0.2	4890

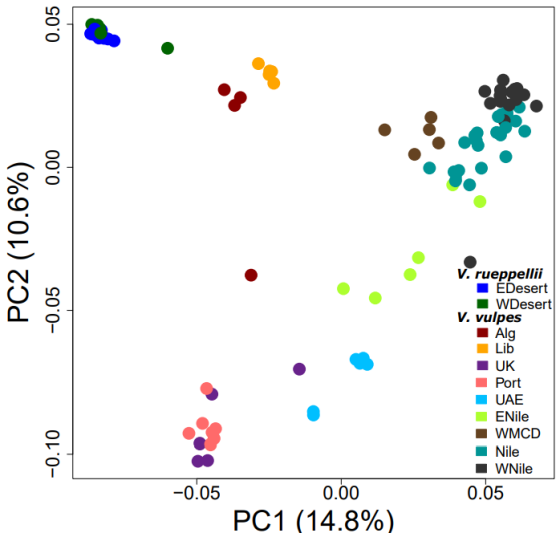
Appendix 4.3

Principal Coordinate Analysis (PCoA) of the *combined dataset*, based on 4,503 SNPs, filtered for LD (r^2 cut-off: 0.1).



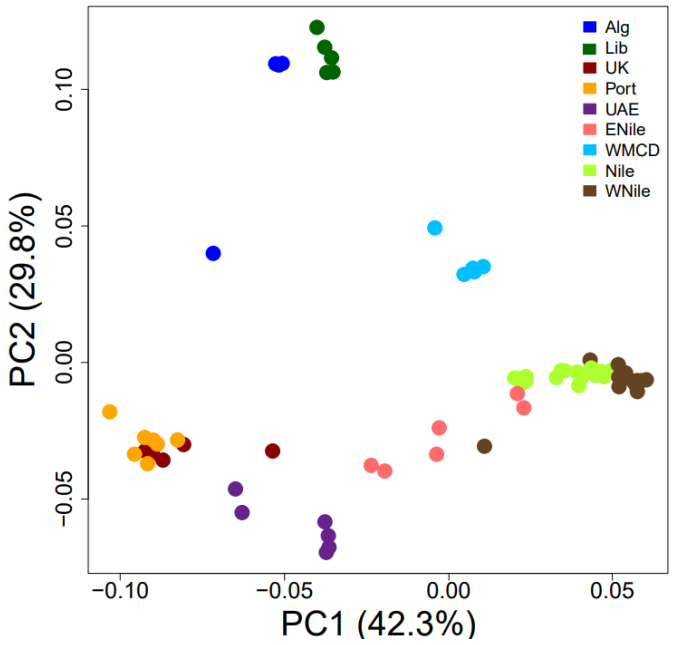
Appendix 4.4

Principal Coordinate Analysis (PCoA) of the *combined dataset* without filtering for HWE based on 14,101 SNPs, filtered for LD (r^2 cut-off: 0.2).



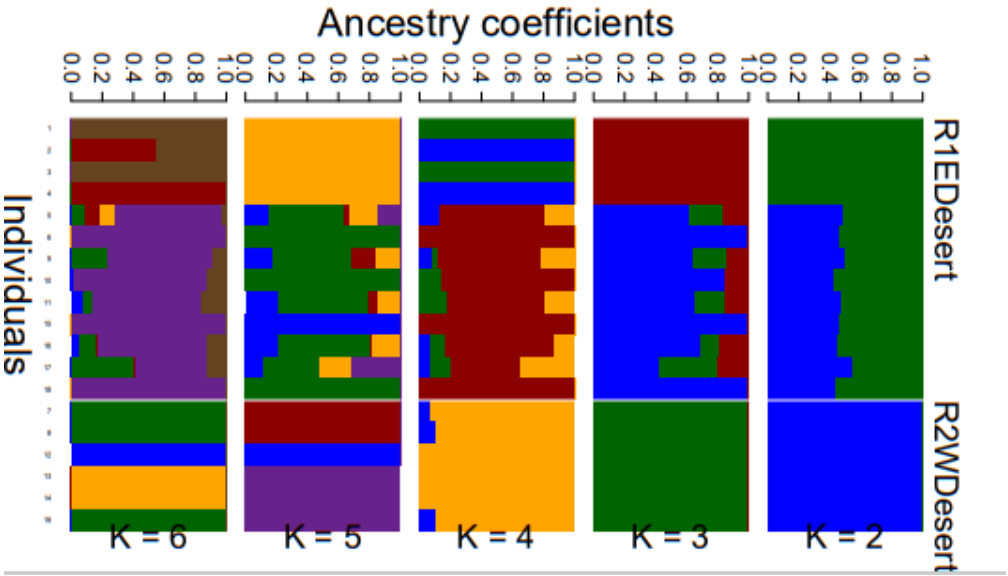
Appendix 4.5

Principal Coordinate Analysis (PCoA) of *V. vulpes* analysed separately (*V.v77* dataset), based on 17,564 SNPs, filtered for LD (r^2 cut-off: 0.2).



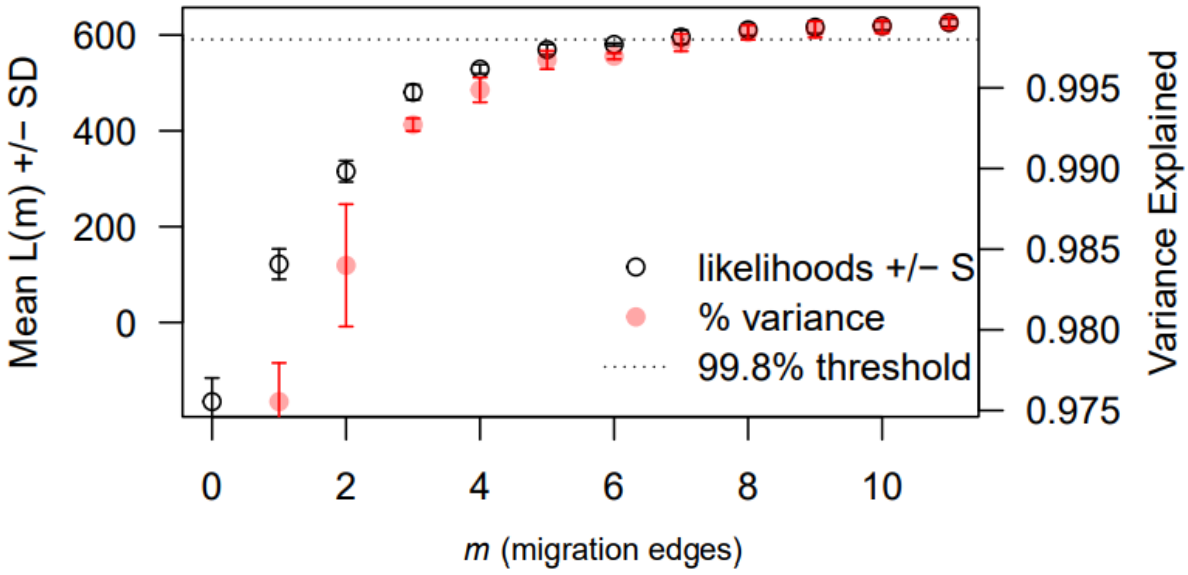
Appendix 4.6

Admixture analysis of *V. rueppellii* (*V.r19 dataset)* at K = 2-6, based on 4,890 SNPs.



Appendix 4.7

The output produced by OptM for the TreeMix results based on 14,485 SNPs (*combined dataset*). A total of 10 iterations were run for each possible number of migration edges, m= 1–11. The mean and standard deviation (SD) for the composite likelihood L(m) (left axis, black circles) and proportion of variance explained (right axis, red "x"s). The dashed line represents the 99.8% threshold that is recommended by Pickrell and Pritchard (2012), suggesting m=7 as the last migration edge to be add.



Appendix 4.8

Admixture *f*3 statistic results based on 14,485 SNPs.

Populations (A; B, C)	***f3*-statistics**	**Standard Error**	**Z**
WDesert;EDesert,Alg	-0.0059009	0.000314504	-18.7625
WDesert;EDesert,Lib	-0.00367472	0.000359284	-10.2279
WDesert;EDesert,WMCD	-0.00263624	0.000368887	-7.14647
WDesert;WNile,EDesert	-0.00191261	0.000375116	-5.09873
WDesert;EDesert,ENile	-0.00179675	0.000352393	-5.09872
WDesert;Nile,EDesert	-0.00194577	0.000384788	-5.05674
WDesert;EDesert,UK	-0.0015182	0.000357505	-4.24664
WDesert;EDesert,Port	-0.00144658	0.000344291	-4.20162
WDesert;EDesert,UAE	-0.00155206	0.000421222	-3.68465

Appendix 4.9

Admixture *f*4 statistic results based on 14,485 SNPs.

A	B	C	D	*f4*-statistics	Standard Error	Z
R1E_Desert	V1Alg	V4Port	V.zerda	0.0699959	0.00127854	54.7467
R1E_Desert	V7WMCD	V.lagopus	V3UK	0.0699627	0.00129925	53.8486
R1E_Desert	R2W_Desert	V2Lib	V7WMCD	0.0578879	0.00113448	51.0259
R1E_Desert	R2W_Desert	V4Port	V.zerda	0.0577052	0.00115008	50.1751
V5UAE	V1Alg	V7WMCD	V4Port	0.0515292	0.00104852	49.1449
V1Alg	V3UK	R2W_Desert	V.zerda	0.0557913	0.00114036	48.9242
R1E_Desert	V1Alg	R2W_Desert	V6E_Nile	0.0572415	0.00119262	47.9965
V.lagopus	V3UK	V5UAE	V2Lib	0.0511377	0.00108128	47.2938
V9W_Nile	V.zerda	V2Lib	V6E_Nile	0.0592451	0.00125828	47.0842
V8Nile	V.lagopus	R1E_Desert	V1Alg	0.0615479	0.00131011	46.9793
R1E_Desert	V1Alg	V5UAE	R2W_Desert	0.037979	0.000816455	46.5169
R1E_Desert	V7WMCD	V.lagopus	V4Port	0.0362369	0.000780482	46.4289
V8Nile	V3UK	V1Alg	V4Port	0.0595953	0.00128455	46.394
V9W_Nile	V3UK	V8Nile	V2Lib	0.0515693	0.00111482	46.2581
V5UAE	V1Alg	V7WMCD	V6E_Nile	0.0534232	0.00116637	45.8028
R1E_Desert	R2W_Desert	V1Alg	V7WMCD	0.0567132	0.00124287	45.631
V.lagopus	V1Alg	V5UAE	V4Port	0.0531095	0.00117315	45.2709
V8Nile	V2Lib	V3UK	V7WMCD	0.0472544	0.00106019	44.5715
V9W_Nile	V.zerda	V2Lib	V7WMCD	0.0649267	0.00146132	44.4303
V5UAE	V.zerda	V2Lib	V7WMCD	0.0327619	0.000739103	44.3265
V9W_Nile	V4Port	V1Alg	V6E_Nile	0.0640872	0.00145601	44.0156
V9W_Nile	R1E_Desert	V7WMCD	V.zerda	0.0652528	0.00148605	43.9102
R1E_Desert	R2W_Desert	V7WMCD	V4Port	0.0628928	0.00144479	43.5308
R1E_Desert	V1Alg	V.lagopus	V3UK	0.0562337	0.00130008	43.2541
V9W_Nile	R1E_Desert	V7WMCD	V4Port	0.0687028	0.00159338	43.1178
V8Nile	V.lagopus	R1E_Desert	V2Lib	0.0575339	0.00133668	43.0422
V9W_Nile	R1E_Desert	V8Nile	R2W_Desert	0.0611644	0.00144411	42.3544
V1Alg	V6E_Nile	V2Lib	R2W_Desert	0.0537053	0.0012725	42.2046
V.lagopus	V3UK	V5UAE	V7WMCD	0.0367168	0.000874186	42.0011
V8Nile	V3UK	V1Alg	V6E_Nile	0.0567452	0.00135399	41.9096
R1E_Desert	V1Alg	V5UAE	V.zerda	0.0390558	0.000935415	41.7524
V1Alg	V6E_Nile	V2Lib	V.zerda	0.0516207	0.00124085	41.601
V9W_Nile	V4Port	V1Alg	V3UK	0.0555555	0.00133568	41.5935
R1E_Desert	V7WMCD	V.lagopus	V5UAE	0.0372604	0.000898032	41.4912
V.lagopus	V3UK	V5UAE	V6E_Nile	0.0384257	0.000932285	41.2167
V8Nile	R2W_Desert	V.lagopus	V2Lib	0.0330978	0.000821363	40.2961
V5UAE	V2Lib	R2W_Desert	V4Port	0.0641118	0.00160622	39.9147
R1E_Desert	V1Alg	V2Lib	R2W_Desert	0.0611184	0.00155253	39.367
R1E_Desert	V.zerda	V3UK	R2W_Desert	0.0557309	0.00142469	39.118
V1Alg	V3UK	V7WMCD	V.zerda	0.0434769	0.00111229	39.0878
V8Nile	R2W_Desert	V1Alg	V3UK	0.0493545	0.00127984	38.563
V1Alg	V2Lib	V7WMCD	V6E_Nile	0.0375156	0.000978815	38.3276
V9W_Nile	V3UK	V8Nile	V1Alg	0.031185	0.000821273	37.9715

V.lagopus	V3UK	R2W_Desert	V6E_Nile	0.0446808	0.00118306	37.7672
V9W_Nile	V.zerda	V5UAE	V2Lib	0.0336175	0.000905722	37.1168
V1Alg	V2Lib	V7WMCD	V4Port	0.0392779	0.0010743	36.5614
R1E_Desert	V3UK	V.zerda	V6E_Nile	0.0437604	0.00120378	36.3526
V1Alg	V6E_Nile	V2Lib	V4Port	0.0589087	0.00165187	35.6619
V9W_Nile	R1E_Desert	V1Alg	V.zerda	0.0558903	0.00156786	35.6475
V9W_Nile	V.lagopus	V8Nile	V7WMCD	0.0312238	0.000885447	35.2633
V.lagopus	V1Alg	V2Lib	V7WMCD	0.0440063	0.00125452	35.0781
V5UAE	V1Alg	R2W_Desert	V6E_Nile	0.0429194	0.00122663	34.9897
V.lagopus	V1Alg	R2W_Desert	V6E_Nile	0.0455824	0.00132039	34.5219
V9W_Nile	V4Port	V3UK	V.zerda	0.0328168	0.000951138	34.5027
V.lagopus	V2Lib	V5UAE	V1Alg	0.0423969	0.00125169	33.8716
V.lagopus	V3UK	V2Lib	R2W_Desert	0.0426424	0.00125947	33.8573
V9W_Nile	R1E_Desert	V.zerda	V6E_Nile	0.0358006	0.001062	33.7105
V8Nile	V2Lib	V1Alg	V4Port	0.0272787	0.000816776	33.398
R1E_Desert	V1Alg	V3UK	V4Port	0.0346013	0.00103707	33.3645
V9W_Nile	R1E_Desert	V.lagopus	V2Lib	0.0276416	0.000829266	33.3325
V8Nile	V3UK	V2Lib	V4Port	0.0400733	0.00121294	33.0381
R1E_Desert	V5UAE	V7WMCD	V4Port	0.0340873	0.00104154	32.7278
V8Nile	R2W_Desert	V.zerda	V6E_Nile	0.0387588	0.00118488	32.711
V9W_Nile	V.zerda	V5UAE	V1Alg	0.037149	0.00113774	32.6515
R1E_Desert	V7WMCD	V.lagopus	V1Alg	0.0321154	0.000985573	32.5855
V.lagopus	V4Port	V5UAE	R2W_Desert	0.0414004	0.0012751	32.4683
V.lagopus	V1Alg	V3UK	V2Lib	0.0498554	0.0015395	32.3842
V8Nile	R1E_Desert	V.lagopus	V2Lib	0.0293641	0.000914793	32.0991
V9W_Nile	V.lagopus	V8Nile	V2Lib	0.0166322	0.00051966	32.0059
V3UK	V6E_Nile	R2W_Desert	V4Port	0.0271124	0.000862148	31.4475
V8Nile	R2W_Desert	R1E_Desert	V.lagopus	0.028572	0.0009099	31.4012
R1E_Desert	V1Alg	V7WMCD	V.zerda	0.0146659	0.000467511	31.3702
V.lagopus	V3UK	V2Lib	V6E_Nile	0.0478258	0.00152662	31.3279
V9W_Nile	V5UAE	R1E_Desert	V2Lib	0.0325859	0.00104295	31.2438
V9W_Nile	V4Port	V3UK	V2Lib	0.0342323	0.00110927	30.8601
V5UAE	V2Lib	V.zerda	V6E_Nile	0.0466028	0.00151167	30.8287
V9W_Nile	V.zerda	V4Port	V6E_Nile	0.0299602	0.000980008	30.5714
V8Nile	V4Port	V1Alg	V3UK	0.028271	0.000925878	30.5343
V8Nile	V3UK	V5UAE	V2Lib	0.0299174	0.000980871	30.5009
R1E_Desert	V4Port	R2W_Desert	V6E_Nile	0.0399188	0.00130969	30.4797
R1E_Desert	V4Port	V2Lib	V.zerda	0.0323524	0.00106286	30.4391
V1Alg	V6E_Nile	V3UK	R2W_Desert	0.0458296	0.00150672	30.4168
V.lagopus	V6E_Nile	V1Alg	V3UK	0.0329715	0.00108483	30.3932
R1E_Desert	V7WMCD	V1Alg	V.zerda	0.0141667	0.000466344	30.3783
V9W_Nile	R1E_Desert	V.lagopus	V7WMCD	0.033266	0.00110127	30.207
V1Alg	V.zerda	V3UK	R2W_Desert	0.0244996	0.000816232	30.0154
V.lagopus	V7WMCD	V1Alg	V3UK	0.0254819	0.000849118	30.0098
V9W_Nile	R1E_Desert	V.lagopus	V3UK	0.0172979	0.000576574	30.0013
V8Nile	V.lagopus	R1E_Desert	V6E_Nile	0.0395507	0.0013191	29.9832
V8Nile	R2W_Desert	V1Alg	V.zerda	0.0286457	0.000961	29.8083
V1Alg	V.zerda	V3UK	V7WMCD	0.0258764	0.000869445	29.762
V9W_Nile	V.lagopus	V8Nile	V6E_Nile	0.0272583	0.000926979	29.4056

V9W_Nile	R1E_Desert	V8Nile	V4Port	0.0374594	0.00127467	29.3875
V8Nile	V4Port	V3UK	V.zerda	0.0269828	0.000918517	29.3765
V8Nile	V3UK	V2Lib	V7WMCD	0.0346144	0.00118129	29.3023
V8Nile	V3UK	V4Port	V.zerda	0.0288514	0.000988251	29.1944
V8Nile	V2Lib	V3UK	V4Port	0.026693	0.000919651	29.0252
V9W_Nile	V.zerda	V5UAE	R2W_Desert	0.0279359	0.000964434	28.9661
V8Nile	V.lagopus	R2W_Desert	V6E_Nile	0.0320175	0.00111197	28.7935
V8Nile	V.lagopus	R2W_Desert	V.zerda	0.0303531	0.00106407	28.5255
V8Nile	V.lagopus	R1E_Desert	V7WMCD	0.0327088	0.00114848	28.4801
V5UAE	V.zerda	R2W_Desert	V4Port	0.0259843	0.000917069	28.3341
R1E_Desert	V3UK	V1Alg	V6E_Nile	0.0247368	0.000876922	28.2086
R1E_Desert	V3UK	V5UAE	V2Lib	0.0337851	0.00120662	27.9998
V9W_Nile	V.lagopus	R1E_Desert	R2W_Desert	0.0149078	0.000534749	27.8781
V.lagopus	V2Lib	V5UAE	R2W_Desert	0.0230977	0.000834327	27.6843
V9W_Nile	V1Alg	V3UK	R2W_Desert	0.0234824	0.000852406	27.5484
R1E_Desert	V5UAE	V2Lib	R2W_Desert	0.0256525	0.000935511	27.4209
V9W_Nile	R1E_Desert	V8Nile	V2Lib	0.0326263	0.00118988	27.4198
V8Nile	V1Alg	V.lagopus	V7WMCD	0.024031	0.000883839	27.1894
V9W_Nile	R1E_Desert	V8Nile	V6E_Nile	0.0385533	0.001422	27.112
V9W_Nile	R1E_Desert	V1Alg	R2W_Desert	0.0274615	0.00101367	27.0911
R1E_Desert	R2W_Desert	V5UAE	V.zerda	0.0270204	0.000998944	27.049
R1E_Desert	V4Port	V3UK	V.zerda	0.033807	0.00125894	26.8535
V1Alg	V3UK	V2Lib	R2W_Desert	0.0131597	0.000490138	26.8491
R1E_Desert	V3UK	V4Port	V.zerda	0.030942	0.00115267	26.8439
V2Lib	V7WMCD	R2W_Desert	V6E_Nile	0.03112	0.00116001	26.8273
V5UAE	V.zerda	V3UK	V2Lib	0.0156131	0.000583946	26.7373
R1E_Desert	V7WMCD	V.lagopus	V6E_Nile	0.0182201	0.000682395	26.7002
V9W_Nile	V1Alg	V2Lib	V7WMCD	0.0230042	0.000861807	26.693
V9W_Nile	R1E_Desert	V8Nile	V3UK	0.0340093	0.00127806	26.6101
R1E_Desert	V7WMCD	V2Lib	R2W_Desert	0.0502392	0.00191383	26.2506
R1E_Desert	V3UK	V2Lib	V6E_Nile	0.0269283	0.00102598	26.2463
V.lagopus	V3UK	R2W_Desert	V4Port	0.0253816	0.000972763	26.0923
V1Alg	V.zerda	V7WMCD	V6E_Nile	0.0253354	0.000973382	26.0282
V5UAE	V2Lib	V1Alg	V6E_Nile	0.0357229	0.00138384	25.8143
V.lagopus	V1Alg	V2Lib	R2W_Desert	0.0251959	0.000977604	25.7731
V9W_Nile	V4Port	V3UK	R2W_Desert	0.0288099	0.00111881	25.7505
V9W_Nile	V.lagopus	V8Nile	V1Alg	0.0143454	0.000557842	25.7159
V8Nile	V.zerda	R1E_Desert	V1Alg	0.0291613	0.00114306	25.5117
R1E_Desert	V6E_Nile	V5UAE	V7WMCD	0.0287766	0.00113181	25.4253
V9W_Nile	V4Port	V2Lib	R2W_Desert	0.030903	0.00122962	25.1321
V8Nile	V3UK	V1Alg	V2Lib	0.0296778	0.00118669	25.009
V5UAE	V7WMCD	R2W_Desert	V6E_Nile	0.0200075	0.00080094	24.98
R1E_Desert	V1Alg	V.lagopus	V4Port	0.0252936	0.00101426	24.9381
R1E_Desert	V5UAE	V3UK	R2W_Desert	0.0308063	0.00123608	24.9226
R1E_Desert	V7WMCD	V.zerda	V6E_Nile	0.0144833	0.000581428	24.9099
V1Alg	V7WMCD	R2W_Desert	V6E_Nile	0.0261399	0.00104963	24.9038
V.lagopus	R2W_Desert	V2Lib	V7WMCD	0.02408	0.000967222	24.8961
V9W_Nile	V4Port	V2Lib	V.zerda	0.0338799	0.00137238	24.687
V1Alg	V6E_Nile	V3UK	V.zerda	0.0246494	0.00100102	24.6244

V5UAE	V1Alg	R2W_Desert	V4Port	0.0304089	0.00124349	24.4546
V5UAE	V2Lib	V1Alg	V7WMCD	0.0255824	0.00104902	24.387
V8Nile	V2Lib	V1Alg	V.zerda	0.0121003	0.000497174	24.3382
V1Alg	V6E_Nile	V3UK	V7WMCD	0.0246847	0.00101438	24.3347
V8Nile	V2Lib	V.lagopus	V1Alg	0.0234231	0.000963371	24.3137
R1E_Desert	V6E_Nile	V5UAE	V.zerda	0.0239258	0.000984752	24.2962
V5UAE	V1Alg	V4Port	V6E_Nile	0.0278408	0.00114676	24.2778
V9W_Nile	V.lagopus	V8Nile	V3UK	0.0101165	0.000417126	24.2529
V.lagopus	V7WMCD	V4Port	V6E_Nile	0.0313416	0.00129359	24.2283
R1E_Desert	V1Alg	R2W_Desert	V7WMCD	0.0263015	0.00109164	24.0936
R1E_Desert	V7WMCD	V2Lib	V.zerda	0.0319868	0.00132953	24.0587
R1E_Desert	V4Port	V.lagopus	R2W_Desert	0.0255175	0.0010611	24.0481
V9W_Nile	R1E_Desert	V.lagopus	R2W_Desert	0.0154868	0.000648995	23.8628
V8Nile	R2W_Desert	V1Alg	V2Lib	0.023831	0.000998855	23.8583
V9W_Nile	R1E_Desert	V8Nile	V5UAE	0.0299209	0.00125804	23.7838
V8Nile	V2Lib	V3UK	V6E_Nile	0.0230423	0.000971358	23.7217
V9W_Nile	V4Port	V2Lib	V6E_Nile	0.0251455	0.00106064	23.7078
R1E_Desert	V1Alg	V2Lib	V.zerda	0.0301784	0.00127893	23.5965
V1Alg	V7WMCD	R2W_Desert	V.zerda	0.0319727	0.00135666	23.5672
V.lagopus	V3UK	V1Alg	R2W_Desert	0.0237244	0.0010098	23.4942
V8Nile	V.lagopus	R1E_Desert	R2W_Desert	0.0295305	0.00126587	23.3283
V.lagopus	V4Port	V1Alg	V3UK	0.0323408	0.00138875	23.2877
V9W_Nile	V.zerda	V3UK	V6E_Nile	0.0244157	0.00105214	23.2058
V9W_Nile	V3UK	V8Nile	V.zerda	0.0235688	0.00101666	23.1825
V.lagopus	V5UAE	V2Lib	R2W_Desert	0.0221663	0.000957108	23.1597
V8Nile	V2Lib	V1Alg	V7WMCD	0.00811543	0.000350572	23.1491
V9W_Nile	V4Port	V2Lib	V7WMCD	0.0242852	0.00104982	23.1327
R1E_Desert	R2W_Desert	V7WMCD	V6E_Nile	0.032208	0.00139278	23.125
V5UAE	V.zerda	V2Lib	R2W_Desert	0.0172378	0.000747511	23.0602
R1E_Desert	R2W_Desert	V.lagopus	V5UAE	0.0315371	0.00136978	23.0234
R1E_Desert	V.lagopus	V5UAE	R2W_Desert	0.0320169	0.00140674	22.7596
R1E_Desert	V3UK	V5UAE	V1Alg	0.0223922	0.000985022	22.7327
V1Alg	V2Lib	R2W_Desert	V7WMCD	0.0295671	0.0013019	22.7109
V.lagopus	V4Port	V5UAE	V1Alg	0.0217235	0.000956884	22.7023
V.lagopus	V1Alg	R2W_Desert	V4Port	0.026028	0.00114693	22.6935
V.lagopus	V4Port	V1Alg	V2Lib	0.0319437	0.00140781	22.6904
R1E_Desert	V5UAE	V2Lib	V6E_Nile	0.0244575	0.00108003	22.6453
R1E_Desert	V4Port	V3UK	V6E_Nile	0.0285289	0.00126062	22.6309
R1E_Desert	V6E_Nile	V3UK	V7WMCD	0.0227771	0.00100826	22.5905
V9W_Nile	R1E_Desert	R2W_Desert	V7WMCD	0.0117959	0.000525341	22.4538
V1Alg	V6E_Nile	V3UK	V2Lib	0.0267693	0.00119366	22.4262
V8Nile	V3UK	V4Port	V6E_Nile	0.0260013	0.00115985	22.4177
V1Alg	V3UK	V4Port	V6E_Nile	0.0252058	0.00112553	22.3946
V.lagopus	V3UK	V2Lib	V4Port	0.0285266	0.00127386	22.3938
V3UK	V2Lib	V4Port	V6E_Nile	0.0209975	0.000939706	22.3447
R1E_Desert	V7WMCD	V3UK	R2W_Desert	0.0337029	0.00150964	22.3252
V5UAE	V7WMCD	V2Lib	V.zerda	0.0214267	0.000964681	22.2112
V8Nile	V.lagopus	R1E_Desert	V4Port	0.0247733	0.00111603	22.1978
V8Nile	V2Lib	V1Alg	V6E_Nile	0.0146483	0.000660263	22.1856

V8Nile	R1E_Desert	V1Alg	R2W_Desert	0.021023	0.000947832	22.18
V9W_Nile	V1Alg	V2Lib	V6E_Nile	0.0222809	0.00100497	22.1707
V8Nile	V3UK	V2Lib	R2W_Desert	0.0274157	0.00123694	22.1642
V.lagopus	V5UAE	V4Port	V6E_Nile	0.0237756	0.00107768	22.0619
R1E_Desert	V6E_Nile	V5UAE	V3UK	0.0230936	0.00105174	21.9576
V5UAE	V2Lib	V1Alg	V4Port	0.0250343	0.0011424	21.9138
V.lagopus	V4Port	V5UAE	V3UK	0.0206871	0.000947605	21.8309
R1E_Desert	V.zerda	V5UAE	R2W_Desert	0.0247908	0.00113673	21.8089
V9W_Nile	R1E_Desert	V5UAE	V7WMCD	0.0301496	0.00138594	21.7538
V9W_Nile	V.zerda	V2Lib	R2W_Desert	0.0302973	0.00139325	21.7458
V9W_Nile	V4Port	V1Alg	V2Lib	0.0298549	0.0013754	21.7063
V9W_Nile	V.lagopus	V5UAE	V4Port	0.0142998	0.000659038	21.698
V9W_Nile	R1E_Desert	R2W_Desert	V4Port	0.0130581	0.000602162	21.6853
R1E_Desert	V1Alg	V5UAE	V7WMCD	0.0137622	0.000635241	21.6645
R1E_Desert	R2W_Desert	V5UAE	V7WMCD	0.0257538	0.00119173	21.6105
V8Nile	V.lagopus	R2W_Desert	V4Port	0.0263391	0.0012202	21.5859
V9W_Nile	V5UAE	V.lagopus	V4Port	0.0200818	0.000933051	21.5227
V9W_Nile	V3UK	V8Nile	R1E_Desert	0.00999525	0.000467344	21.3874
R1E_Desert	V3UK	V1Alg	V.zerda	0.0192669	0.000902775	21.3419
R1E_Desert	R2W_Desert	V2Lib	V.zerda	0.0260284	0.00122081	21.3206
R1E_Desert	V7WMCD	V2Lib	V4Port	0.0329022	0.00154418	21.3072
V9W_Nile	R1E_Desert	R2W_Desert	V.zerda	0.0135241	0.000636954	21.2325
V2Lib	R2W_Desert	V7WMCD	V4Port	0.0221075	0.0010442	21.1717
V9W_Nile	V1Alg	V2Lib	V4Port	0.0174644	0.000828018	21.0919
R1E_Desert	V3UK	V7WMCD	V.zerda	0.0204767	0.000971592	21.0754
R1E_Desert	V1Alg	V.lagopus	V7WMCD	0.0243899	0.00116227	20.9848
R1E_Desert	R2W_Desert	V3UK	V.zerda	0.0272031	0.00130327	20.8729
R1E_Desert	V1Alg	V5UAE	V4Port	0.0127544	0.000611119	20.8705
V5UAE	R2W_Desert	V3UK	V6E_Nile	0.0201723	0.0009799	20.5861
V9W_Nile	V.zerda	V3UK	V7WMCD	0.023992	0.00116893	20.5247
V1Alg	V6E_Nile	R2W_Desert	V7WMCD	0.0254808	0.00124356	20.4902
V1Alg	V3UK	V.zerda	V6E_Nile	0.0121802	0.000596001	20.4366
V.lagopus	V4Port	R2W_Desert	V6E_Nile	0.0133736	0.000654511	20.4329
V8Nile	V4Port	V3UK	V2Lib	0.0168448	0.000825976	20.3938
V9W_Nile	R1E_Desert	V.lagopus	V4Port	0.00735775	0.000362841	20.2781
V9W_Nile	R2W_Desert	V7WMCD	V.zerda	0.0127721	0.000630285	20.2641
R1E_Desert	V7WMCD	V1Alg	R2W_Desert	0.0133346	0.00065887	20.2386
V3UK	V7WMCD	V2Lib	R2W_Desert	0.0195123	0.000972183	20.0706
V5UAE	V3UK	V1Alg	V4Port	0.0231844	0.00115901	20.0036
V8Nile	V5UAE	R1E_Desert	V2Lib	0.0135387	0.000677951	19.97
V.lagopus	V6E_Nile	R2W_Desert	V4Port	0.0225648	0.00113469	19.8863
V8Nile	V2Lib	V.lagopus	V6E_Nile	0.0118631	0.000597339	19.8599
R1E_Desert	V1Alg	V5UAE	V2Lib	0.014265	0.000719913	19.8149
R1E_Desert	V5UAE	V1Alg	V4Port	0.0236787	0.001195	19.8148
V8Nile	V2Lib	V1Alg	R2W_Desert	0.0104535	0.000527664	19.8109
V1Alg	V6E_Nile	V3UK	V4Port	0.0237221	0.00120035	19.7626
V8Nile	V1Alg	R1E_Desert	V7WMCD	0.0186634	0.000945808	19.7327
V8Nile	R1E_Desert	V3UK	R2W_Desert	0.0228889	0.00117311	19.5113
V.lagopus	V2Lib	V1Alg	V4Port	0.0192992	0.000995187	19.3925

V5UAE	V2Lib	V1Alg	V.zerda	0.0231403	0.00119671	19.3366
V9W_Nile	V6E_Nile	R1E_Desert	V2Lib	0.0186742	0.000965787	19.3358
V5UAE	V.zerda	V3UK	R2W_Desert	0.0125895	0.000651661	19.3191
V8Nile	V4Port	V3UK	R2W_Desert	0.0232053	0.0012039	19.2751
V1Alg	V7WMCD	V3UK	V4Port	0.0211802	0.00110345	19.1946
V5UAE	V2Lib	V1Alg	R2W_Desert	0.0229861	0.00120246	19.1158
V1Alg	V3UK	V4Port	V.zerda	0.0187185	0.000982976	19.0427
V8Nile	V2Lib	V.lagopus	V5UAE	0.0122575	0.000644173	19.0283
V1Alg	V3UK	V2Lib	V7WMCD	0.0217186	0.00115015	18.8832
V.lagopus	R2W_Desert	V1Alg	V3UK	0.0195544	0.00103679	18.8605
V9W_Nile	V.lagopus	V2Lib	V7WMCD	0.0132344	0.000703537	18.8112
V5UAE	V1Alg	R2W_Desert	V.zerda	0.020521	0.00109398	18.758
V8Nile	V1Alg	V7WMCD	V4Port	0.0191256	0.00102187	18.7163
V1Alg	V3UK	R2W_Desert	V6E_Nile	0.0116392	0.000624648	18.6333
V9W_Nile	V3UK	V7WMCD	V4Port	0.00769124	0.000413622	18.5948
V8Nile	V.lagopus	R1E_Desert	V5UAE	0.0223367	0.00120198	18.5833
V8Nile	V1Alg	V5UAE	V4Port	0.0151784	0.000818263	18.5495
V8Nile	V1Alg	V2Lib	V.zerda	0.0150212	0.000810206	18.5399
V9W_Nile	V5UAE	V3UK	V7WMCD	0.0177293	0.000956991	18.526
V1Alg	V4Port	V3UK	V7WMCD	0.0228393	0.00123594	18.4793
V.lagopus	V1Alg	V7WMCD	V6E_Nile	0.0175903	0.000956592	18.3885
R1E_Desert	V6E_Nile	V2Lib	V4Port	0.0190403	0.00103811	18.3412
R1E_Desert	V5UAE	V.lagopus	V6E_Nile	0.0202778	0.00110914	18.2825
V.lagopus	V3UK	V7WMCD	V4Port	0.0136191	0.000745988	18.2564
V8Nile	R2W_Desert	R1E_Desert	V6E_Nile	0.0138369	0.000762269	18.1523
V8Nile	V.zerda	R1E_Desert	V6E_Nile	0.018415	0.00101892	18.073
V8Nile	V.zerda	R1E_Desert	R2W_Desert	0.0232837	0.00128848	18.0707
V1Alg	V4Port	V2Lib	V6E_Nile	0.0218296	0.00120855	18.0626
V8Nile	V1Alg	V2Lib	V7WMCD	0.0154156	0.000857308	17.9814
V8Nile	V.zerda	V7WMCD	V4Port	0.015052	0.000838693	17.947
V8Nile	V2Lib	V.zerda	V6E_Nile	0.011041	0.000616498	17.9092
V9W_Nile	R1E_Desert	V8Nile	V.zerda	0.0237635	0.00132753	17.9006
V8Nile	V3UK	V2Lib	V.zerda	0.0230134	0.00128945	17.8475
V5UAE	V1Alg	V3UK	V2Lib	0.0231431	0.00130314	17.7595
V1Alg	V3UK	R2W_Desert	V4Port	0.0130161	0.000735127	17.7059
V3UK	V6E_Nile	V2Lib	V4Port	0.00950142	0.000537064	17.6914
V8Nile	R2W_Desert	V.lagopus	V4Port	0.0100361	0.000567716	17.6781
V3UK	R2W_Desert	V4Port	V.zerda	0.0188328	0.00106613	17.6646
V.lagopus	V6E_Nile	V3UK	V2Lib	0.0169601	0.000961957	17.6308
V8Nile	V1Alg	V3UK	V6E_Nile	0.0162377	0.000926635	17.5233
R1E_Desert	V2Lib	V.lagopus	V7WMCD	0.00569719	0.000325577	17.4988
V8Nile	V.zerda	R1E_Desert	V2Lib	0.0178593	0.00102742	17.3827
V5UAE	V.zerda	V1Alg	V7WMCD	0.0132495	0.000772177	17.1586
V8Nile	R2W_Desert	V7WMCD	V.zerda	0.0149278	0.00087056	17.1474
R1E_Desert	V3UK	V1Alg	R2W_Desert	0.0168321	0.000983691	17.1112
V8Nile	V.zerda	V2Lib	V7WMCD	0.0138794	0.000815705	17.0152
R1E_Desert	V7WMCD	V5UAE	V2Lib	0.0182525	0.00107562	16.9693
V9W_Nile	R1E_Desert	V.lagopus	V1Alg	0.00957389	0.000565977	16.9157
V9W_Nile	V.lagopus	V5UAE	V6E_Nile	0.00778421	0.000460958	16.887

V5UAE	V.zerda	V7WMCD	V6E_Nile	0.0195424	0.00115822	16.8728
R2W_Desert	V4Port	V7WMCD	V.zerda	0.0178123	0.00105766	16.8412
V9W_Nile	R1E_Desert	V2Lib	V.zerda	0.0134268	0.000798944	16.8057
V3UK	V4Port	R2W_Desert	V6E_Nile	0.0122347	0.000730188	16.7556
V1Alg	R2W_Desert	V2Lib	V4Port	0.0165134	0.000986914	16.7324
V3UK	R2W_Desert	V2Lib	V7WMCD	0.00395513	0.000236913	16.6944
V8Nile	V4Port	V2Lib	V7WMCD	0.0190083	0.00113988	16.6756
V9W_Nile	R2W_Desert	R1E_Desert	V2Lib	0.0136959	0.000821796	16.6658
V3UK	V7WMCD	V.zerda	V6E_Nile	0.00398828	0.00023955	16.6491
V8Nile	V.zerda	V3UK	V7WMCD	0.0143069	0.000861691	16.6033
V8Nile	V.lagopus	V5UAE	V2Lib	0.0132117	0.000795749	16.6028
V9W_Nile	R1E_Desert	R2W_Desert	V6E_Nile	0.011785	0.000711765	16.5575
V3UK	V.zerda	V2Lib	V4Port	0.0194787	0.00118358	16.4574
V5UAE	V.zerda	V4Port	V6E_Nile	0.0106051	0.000650167	16.3113
V8Nile	V.zerda	V5UAE	V2Lib	0.0146492	0.000898169	16.3101
V8Nile	V6E_Nile	V5UAE	V3UK	0.0141174	0.000870723	16.2135
V.lagopus	V4Port	V5UAE	V2Lib	0.0148325	0.000916004	16.1927
V8Nile	V1Alg	V.lagopus	R2W_Desert	0.0132961	0.00082117	16.1917
V8Nile	V.zerda	R1E_Desert	V7WMCD	0.0113021	0.000698074	16.1903
V8Nile	R2W_Desert	V3UK	V.zerda	0.0170872	0.00105687	16.1678
V5UAE	V2Lib	V1Alg	V3UK	0.0234184	0.00144986	16.1522
V5UAE	R2W_Desert	V1Alg	V.zerda	0.020605	0.00127807	16.1219
R1E_Desert	V7WMCD	V5UAE	V.zerda	0.0177299	0.00110462	16.0507
V9W_Nile	V3UK	V7WMCD	V6E_Nile	0.00657067	0.00040945	16.0476
V.lagopus	V3UK	V7WMCD	V6E_Nile	0.0113352	0.000711014	15.9423
V8Nile	V6E_Nile	V3UK	V2Lib	0.0106465	0.000667984	15.9383
V8Nile	V7WMCD	R1E_Desert	V2Lib	0.0158565	0.00100047	15.8491
V8Nile	V5UAE	V3UK	V4Port	0.0126128	0.000797725	15.8109
V9W_Nile	R1E_Desert	V8Nile	V1Alg	0.0226865	0.001436	15.7984
V8Nile	V5UAE	V7WMCD	V6E_Nile	0.0139051	0.00088482	15.7152
V9W_Nile	V3UK	R2W_Desert	V7WMCD	0.00804747	0.000513139	15.6828
V9W_Nile	V.lagopus	R2W_Desert	V6E_Nile	0.0106963	0.000684209	15.6331
V8Nile	R2W_Desert	V1Alg	V7WMCD	0.0154239	0.000986738	15.6312
V9W_Nile	V5UAE	V3UK	V6E_Nile	0.0155145	0.000997182	15.5583
V8Nile	R2W_Desert	V.lagopus	V3UK	0.00897819	0.000579335	15.4974
V9W_Nile	V5UAE	R1E_Desert	V7WMCD	0.0124665	0.000805005	15.4862
V8Nile	V2Lib	R2W_Desert	V.zerda	0.0126304	0.000825007	15.3094
V9W_Nile	V5UAE	V1Alg	V3UK	0.0106163	0.00069438	15.2888
V5UAE	V6E_Nile	V3UK	V4Port	0.0154422	0.00101605	15.1982
R1E_Desert	V7WMCD	V4Port	V6E_Nile	0.0183793	0.00121274	15.1552
R1E_Desert	V7WMCD	V3UK	V6E_Nile	0.017606	0.00116427	15.122
V9W_Nile	V.lagopus	V1Alg	V6E_Nile	0.0106468	0.000705971	15.081
R1E_Desert	V3UK	V1Alg	V4Port	0.0128184	0.000856149	14.9722
R1E_Desert	V4Port	V.lagopus	V5UAE	0.0191069	0.0012763	14.9705
R1E_Desert	V7WMCD	V5UAE	V6E_Nile	0.0165363	0.00110471	14.969
V9W_Nile	V7WMCD	V8Nile	V1Alg	0.00987081	0.000659735	14.9618
R1E_Desert	V.lagopus	V2Lib	R2W_Desert	0.0108194	0.000724131	14.9412
R1E_Desert	V4Port	V1Alg	V.zerda	0.0202561	0.0013565	14.9326
R1E_Desert	V7WMCD	V5UAE	V4Port	0.0176474	0.00118393	14.9057

V8Nile	V4Port	V5UAE	V6E_Nile	0.0101381	0.000683973	14.8223
V8Nile	R2W_Desert	R1E_Desert	V5UAE	0.0120748	0.000816536	14.7878
V9W_Nile	V.lagopus	R1E_Desert	V6E_Nile	0.00992284	0.000675873	14.6815
V8Nile	V.lagopus	V7WMCD	V4Port	0.0192341	0.00131115	14.6697
V8Nile	V.lagopus	V5UAE	V6E_Nile	0.0170402	0.0011637	14.6432
V1Alg	R2W_Desert	V.zerda	V6E_Nile	0.00945269	0.000646521	14.6209
V9W_Nile	R2W_Desert	V5UAE	V2Lib	0.0105204	0.000719804	14.6157
V5UAE	V.zerda	V2Lib	V6E_Nile	0.0117785	0.000809526	14.5499
V8Nile	V.lagopus	V1Alg	R2W_Desert	0.0147774	0.00102075	14.4771
V9W_Nile	R2W_Desert	R1E_Desert	V7WMCD	0.0147728	0.00103711	14.2442
V5UAE	V4Port	V3UK	R2W_Desert	0.0175267	0.00123568	14.1839
V8Nile	V.lagopus	V2Lib	R2W_Desert	0.0172141	0.00122057	14.1033
V8Nile	R2W_Desert	V.lagopus	V7WMCD	0.0124073	0.000880119	14.0973
V9W_Nile	V.lagopus	R1E_Desert	V3UK	0.00956228	0.000679459	14.0734
V9W_Nile	R1E_Desert	V3UK	V.zerda	0.0137306	0.000976939	14.0547
R1E_Desert	V3UK	V.lagopus	R2W_Desert	0.00434884	0.000309449	14.0535
V9W_Nile	V7WMCD	V8Nile	V2Lib	0.0113371	0.000809644	14.0026
V8Nile	V6E_Nile	V5UAE	R2W_Desert	0.00950582	0.000703909	13.5043
V8Nile	V7WMCD	V5UAE	V.zerda	0.0103436	0.00076778	13.4721
V8Nile	R2W_Desert	V.lagopus	V1Alg	0.0107376	0.000798608	13.4454
V9W_Nile	V3UK	R1E_Desert	V6E_Nile	0.00722385	0.000537825	13.4316
V5UAE	R2W_Desert	V7WMCD	V4Port	0.0104603	0.000785974	13.3087
V9W_Nile	V2Lib	V8Nile	V6E_Nile	0.00513782	0.00038649	13.2935
R1E_Desert	V7WMCD	V1Alg	V2Lib	0.00848375	0.000638895	13.2788
V5UAE	V.zerda	V2Lib	V4Port	0.0121569	0.000916555	13.2637
V9W_Nile	R2W_Desert	V1Alg	V2Lib	0.00753844	0.000568886	13.2512
V9W_Nile	V5UAE	V2Lib	V.zerda	0.00663766	0.000503125	13.1929
R1E_Desert	V5UAE	V7WMCD	V6E_Nile	0.00962973	0.000733567	13.1273
R1E_Desert	V1Alg	V7WMCD	V6E_Nile	0.00470027	0.000358568	13.1085
V5UAE	V.zerda	R2W_Desert	V7WMCD	0.00883564	0.000675277	13.0845
V1Alg	V2Lib	V7WMCD	V.zerda	0.00884473	0.000677974	13.0458
V9W_Nile	R1E_Desert	V2Lib	R2W_Desert	0.0108923	0.000838418	12.9915
V.lagopus	V5UAE	V1Alg	R2W_Desert	0.0112159	0.000865781	12.9547
R1E_Desert	V1Alg	V3UK	V7WMCD	0.0102114	0.000789906	12.9274
R1E_Desert	V7WMCD	V1Alg	V3UK	0.00455126	0.000356248	12.7755
V8Nile	R1E_Desert	V.lagopus	V4Port	0.00617611	0.00048741	12.6713
V8Nile	V6E_Nile	R2W_Desert	V7WMCD	0.0116655	0.000930895	12.5315
V8Nile	V2Lib	V1Alg	V3UK	0.00815306	0.000650615	12.5313
R1E_Desert	V4Port	R2W_Desert	V7WMCD	0.00954777	0.000764005	12.497
R1E_Desert	V1Alg	V7WMCD	V4Port	0.00887746	0.000711073	12.4846
V8Nile	R1E_Desert	V.lagopus	R2W_Desert	0.0110855	0.000890441	12.4495
V9W_Nile	R2W_Desert	V3UK	V7WMCD	0.00910779	0.000734722	12.3962
V9W_Nile	R2W_Desert	V2Lib	V6E_Nile	0.0088142	0.000716143	12.3079
V9W_Nile	V6E_Nile	V8Nile	V3UK	0.00827276	0.000673884	12.2762
V5UAE	V.zerda	V3UK	V6E_Nile	0.0067775	0.000553512	12.2445
V9W_Nile	R2W_Desert	R1E_Desert	V1Alg	0.0102458	0.000839261	12.2082
V9W_Nile	R2W_Desert	R1E_Desert	V6E_Nile	0.0113228	0.000940807	12.0352
V.lagopus	V5UAE	V1Alg	V3UK	0.0102916	0.000858614	11.9863
V9W_Nile	V2Lib	V.lagopus	V5UAE	0.00541627	0.000452341	11.9739

V9W_Nile	V5UAE	R2W_Desert	V7WMCD	0.0119894	0.00101334	11.8315
V9W_Nile	R1E_Desert	V3UK	R2W_Desert	0.011196	0.000950542	11.7785
V9W_Nile	V.zerda	V7WMCD	V4Port	0.00920183	0.000782535	11.759
V9W_Nile	V2Lib	V1Alg	V6E_Nile	0.00579318	0.000494336	11.7191
V.lagopus	V5UAE	V3UK	R2W_Desert	0.0101672	0.000867591	11.7189
V8Nile	V7WMCD	V.zerda	V6E_Nile	0.0121547	0.00103756	11.7147
V.lagopus	V4Port	R2W_Desert	V7WMCD	0.00819015	0.000700846	11.6861
V9W_Nile	V3UK	V.lagopus	V7WMCD	0.00585551	0.000501092	11.6855
V8Nile	V.zerda	V1Alg	V7WMCD	0.00912325	0.000781839	11.669
R1E_Desert	V5UAE	V3UK	V.zerda	0.0105285	0.000902724	11.663
V9W_Nile	V1Alg	V7WMCD	V4Port	0.0122673	0.00105449	11.6334
V9W_Nile	V5UAE	V1Alg	V7WMCD	0.010664	0.000923498	11.5474
V9W_Nile	R2W_Desert	V1Alg	V7WMCD	0.00842049	0.000730047	11.5342
V9W_Nile	R1E_Desert	V5UAE	V6E_Nile	0.00833911	0.000731783	11.3956
V5UAE	V1Alg	V3UK	V6E_Nile	0.0111934	0.000982286	11.3953
V8Nile	V.lagopus	V5UAE	V7WMCD	0.00992725	0.000876827	11.3218
V8Nile	V3UK	V7WMCD	V.zerda	0.0126575	0.00111908	11.3106
R1E_Desert	V3UK	V5UAE	V4Port	0.011393	0.0010081	11.3015
V8Nile	V1Alg	V.lagopus	V6E_Nile	0.00856973	0.000760468	11.269
V5UAE	V1Alg	V3UK	R2W_Desert	0.0115433	0.00102653	11.245
V8Nile	R2W_Desert	R1E_Desert	V7WMCD	0.0102089	0.000910864	11.2079
V9W_Nile	V4Port	V8Nile	V.zerda	0.00853166	0.000762268	11.1925
V5UAE	V4Port	V3UK	V.zerda	0.0110916	0.000992621	11.174
R1E_Desert	V2Lib	V.zerda	V6E_Nile	0.00347101	0.000310979	11.1616
V9W_Nile	V.lagopus	R2W_Desert	V4Port	0.00840955	0.000754889	11.1401
R1E_Desert	V6E_Nile	V7WMCD	V.zerda	0.00973636	0.000874022	11.1397
R1E_Desert	V7WMCD	V.lagopus	V2Lib	0.00902177	0.000809885	11.1396
V9W_Nile	V6E_Nile	V8Nile	R1E_Desert	0.00855174	0.000771989	11.0776
V9W_Nile	V.zerda	R1E_Desert	V3UK	0.00813214	0.000734755	11.0678
V2Lib	V7WMCD	V4Port	V6E_Nile	0.0100287	0.000909026	11.0323
V9W_Nile	V6E_Nile	V8Nile	V4Port	0.010075	0.000919183	10.9608
V.lagopus	V1Alg	V5UAE	V7WMCD	0.00910326	0.000837176	10.8738
V9W_Nile	V.lagopus	V8Nile	R2W_Desert	0.00598539	0.000553508	10.8136
R1E_Desert	V3UK	V5UAE	V6E_Nile	0.00984914	0.000920951	10.6945
R1E_Desert	V4Port	V2Lib	V6E_Nile	0.0120963	0.00113294	10.677
R1E_Desert	V3UK	R2W_Desert	V.zerda	0.00215652	0.000202501	10.6494
V2Lib	V7WMCD	R2W_Desert	V.zerda	0.0122844	0.0011604	10.5863
V9W_Nile	V.lagopus	V5UAE	V7WMCD	0.00706988	0.00066876	10.5716
V9W_Nile	V5UAE	V.lagopus	V3UK	0.00946551	0.00089539	10.5714
V1Alg	V3UK	V2Lib	V.zerda	0.00794848	0.000753384	10.5504
V9W_Nile	V7WMCD	V3UK	V2Lib	0.00772661	0.000733913	10.528
V8Nile	V7WMCD	V5UAE	V3UK	0.00753398	0.000717274	10.5036
V9W_Nile	V5UAE	R1E_Desert	V4Port	0.00818148	0.000780303	10.485
V5UAE	V3UK	R2W_Desert	V4Port	0.0127368	0.00121493	10.4836
V5UAE	V3UK	V1Alg	V6E_Nile	0.0123045	0.00117425	10.4786
V9W_Nile	V5UAE	V1Alg	V2Lib	0.00763605	0.000733264	10.4138
R1E_Desert	V5UAE	V4Port	V.zerda	0.00634877	0.000616217	10.3028
V8Nile	V2Lib	V5UAE	V1Alg	0.0127814	0.00124465	10.2691
V5UAE	V6E_Nile	V3UK	V7WMCD	0.0114148	0.00111324	10.2537

V9W_Nile	V7WMCD	V5UAE	V2Lib	0.00894697	0.000878184	10.188
V5UAE	V6E_Nile	V3UK	R2W_Desert	0.0124948	0.00122977	10.1603
V9W_Nile	V.zerda	V8Nile	V1Alg	0.00962552	0.000948259	10.1507
V9W_Nile	R2W_Desert	V5UAE	V1Alg	0.00787081	0.000778297	10.1129
V5UAE	V7WMCD	R2W_Desert	V4Port	0.00172895	0.000171211	10.0984
V9W_Nile	V3UK	R1E_Desert	V2Lib	0.00491984	0.000489694	10.0468
V9W_Nile	V6E_Nile	R1E_Desert	V5UAE	0.0094026	0.000952832	9.86806
V9W_Nile	V1Alg	V5UAE	R2W_Desert	0.0077159	0.000783693	9.84557
V9W_Nile	V.lagopus	V1Alg	V2Lib	0.00836003	0.000850745	9.82672
V8Nile	R2W_Desert	R1E_Desert	V1Alg	0.00551032	0.00056232	9.79927
V8Nile	V4Port	V2Lib	V.zerda	0.00797457	0.000815692	9.77644
V9W_Nile	V.lagopus	R1E_Desert	V2Lib	0.00727554	0.000748509	9.72004
V9W_Nile	V2Lib	R1E_Desert	V3UK	0.00636267	0.000654665	9.71897
V9W_Nile	V2Lib	V5UAE	V1Alg	0.00539326	0.000556502	9.69137
V9W_Nile	V7WMCD	V5UAE	V1Alg	0.00748065	0.00077819	9.61289
V8Nile	R1E_Desert	V7WMCD	V.zerda	0.00931109	0.000978535	9.51534
V9W_Nile	R1E_Desert	V1Alg	V7WMCD	0.0089867	0.000945282	9.5069
V8Nile	V5UAE	R1E_Desert	V1Alg	0.0092037	0.000969712	9.49118
V9W_Nile	V1Alg	V5UAE	V7WMCD	0.00562313	0.000595406	9.44419
V9W_Nile	V5UAE	V2Lib	R2W_Desert	0.00864988	0.000917349	9.42921
V9W_Nile	V3UK	R2W_Desert	V4Port	0.00574346	0.000610259	9.41152
V3UK	V6E_Nile	R2W_Desert	V7WMCD	0.00611491	0.000651183	9.39046
R1E_Desert	V.lagopus	R2W_Desert	V7WMCD	0.00645425	0.000687709	9.38515
V5UAE	V6E_Nile	V3UK	V.zerda	0.010186	0.00108734	9.36785
R1E_Desert	V3UK	V.lagopus	V1Alg	0.00212266	0.000227199	9.34273
V8Nile	R2W_Desert	R1E_Desert	V3UK	0.00788152	0.000844706	9.33048
V9W_Nile	V.lagopus	V3UK	R2W_Desert	0.00671875	0.000720934	9.31951
V9W_Nile	V7WMCD	V.lagopus	V2Lib	0.00865533	0.000929685	9.30996
V8Nile	R1E_Desert	V.lagopus	V6E_Nile	0.00793934	0.000855355	9.28193
V.lagopus	V3UK	V5UAE	V4Port	0.0084953	0.00092277	9.20629
V9W_Nile	V7WMCD	R1E_Desert	V1Alg	0.00716607	0.000782202	9.1614
V5UAE	V3UK	V7WMCD	V.zerda	0.00520312	0.000569195	9.1412
V9W_Nile	V7WMCD	V8Nile	V4Port	0.00365393	0.000401601	9.09841
V9W_Nile	V.zerda	R1E_Desert	V7WMCD	0.00778256	0.000862958	9.01846
V.lagopus	V6E_Nile	V1Alg	V4Port	0.0104066	0.00116586	8.92618
V9W_Nile	V7WMCD	R1E_Desert	V2Lib	0.00863239	0.000968702	8.9113
V9W_Nile	R2W_Desert	V7WMCD	V4Port	0.00546943	0.000616678	8.86918
V9W_Nile	V5UAE	R2W_Desert	V4Port	0.0080924	0.000918027	8.81499
V9W_Nile	V4Port	V8Nile	R2W_Desert	0.00767134	0.000876881	8.74843
V9W_Nile	V6E_Nile	R2W_Desert	V4Port	0.00508163	0.000581746	8.73513
R1E_Desert	V7WMCD	R2W_Desert	V4Port	0.00568299	0.000659335	8.61928
V1Alg	V3UK	V2Lib	V4Port	0.00416374	0.000484614	8.59186
V9W_Nile	V1Alg	V5UAE	V4Port	0.00739278	0.000861473	8.58155
V8Nile	R2W_Desert	V.lagopus	V5UAE	0.00452582	0.000527552	8.57891
V9W_Nile	V4Port	R2W_Desert	V7WMCD	0.00727844	0.000850156	8.5613
V9W_Nile	V2Lib	V3UK	V6E_Nile	0.00455255	0.000535241	8.50561
R1E_Desert	V2Lib	V1Alg	V6E_Nile	0.00243253	0.000287517	8.46047
V9W_Nile	R2W_Desert	R1E_Desert	V3UK	0.00615742	0.000729816	8.43694
R1E_Desert	V7WMCD	V1Alg	V4Port	0.00514496	0.000610231	8.43118

V9W_Nile	V7WMCD	V.lagopus	V1Alg	0.00718901	0.000852846	8.42944
V9W_Nile	V4Port	V.lagopus	V3UK	0.0076162	0.000905999	8.4064
V8Nile	R1E_Desert	V.zerda	V6E_Nile	0.0017621	0.000209673	8.40405
V9W_Nile	V3UK	V5UAE	V1Alg	0.00452986	0.000539111	8.40245
V9W_Nile	V2Lib	R1E_Desert	V5UAE	0.00615546	0.000733684	8.3898
V9W_Nile	V5UAE	V8Nile	R1E_Desert	0.00405392	0.000489294	8.28526
V9W_Nile	V5UAE	R2W_Desert	V6E_Nile	0.0076153	0.000921265	8.26613
V9W_Nile	V6E_Nile	V.lagopus	V4Port	0.0061506	0.000744746	8.25865
R1E_Desert	V5UAE	V3UK	V2Lib	0.00712759	0.000863075	8.25836
V8Nile	R2W_Desert	V3UK	V6E_Nile	0.00697417	0.000851918	8.18643
V9W_Nile	R2W_Desert	V3UK	V6E_Nile	0.00645816	0.000788923	8.18605
V9W_Nile	V7WMCD	V8Nile	V3UK	0.00361052	0.000441679	8.17452
V9W_Nile	V6E_Nile	V2Lib	V4Port	0.00752908	0.000922115	8.16501
V9W_Nile	V.lagopus	V8Nile	R1E_Desert	0.00591881	0.00072535	8.15994
V9W_Nile	V.lagopus	V7WMCD	V4Port	0.00593587	0.000729292	8.13922
V9W_Nile	R1E_Desert	V.lagopus	V5UAE	0.00426189	0.000525761	8.10612
V8Nile	V1Alg	V.zerda	V6E_Nile	0.00408072	0.000508298	8.02821
V3UK	V4Port	V2Lib	V6E_Nile	0.00703068	0.000875813	8.0276
V.lagopus	V5UAE	R2W_Desert	V4Port	0.00689093	0.000861506	7.99871
V8Nile	V2Lib	V.lagopus	R2W_Desert	0.00659789	0.000825171	7.99578
V9W_Nile	V3UK	R1E_Desert	V4Port	0.00379927	0.000475852	7.98415
V9W_Nile	V3UK	V5UAE	V2Lib	0.0027714	0.000347208	7.98196
V9W_Nile	V4Port	R2W_Desert	V6E_Nile	0.00938383	0.0011812	7.94431
V9W_Nile	V2Lib	V.lagopus	R2W_Desert	0.00341516	0.000430621	7.93078
V8Nile	V6E_Nile	V5UAE	V.zerda	0.00291674	0.000368857	7.90752
V5UAE	V4Port	R2W_Desert	V7WMCD	0.00541356	0.000688686	7.86071
V9W_Nile	V6E_Nile	V2Lib	R2W_Desert	0.00572684	0.000728702	7.85896
V9W_Nile	V3UK	V.lagopus	V4Port	0.00504177	0.000641557	7.85864
V8Nile	R1E_Desert	V.lagopus	V1Alg	0.00824146	0.00105583	7.80564
V9W_Nile	V6E_Nile	V.lagopus	V5UAE	0.00795284	0.0010203	7.79459
V9W_Nile	V1Alg	V5UAE	V2Lib	0.00690105	0.000891687	7.73932
R1E_Desert	V5UAE	V3UK	V6E_Nile	0.00515377	0.000666544	7.73207
R1E_Desert	V1Alg	V3UK	R2W_Desert	0.00578846	0.000750119	7.71672
V9W_Nile	V5UAE	V8Nile	V7WMCD	0.00715927	0.000929188	7.70486
V9W_Nile	R2W_Desert	R1E_Desert	V.zerda	0.00886281	0.00115162	7.69595
V5UAE	V.zerda	R2W_Desert	V6E_Nile	0.00581201	0.000758089	7.66666
V.lagopus	V7WMCD	V5UAE	V3UK	0.00587705	0.000770501	7.62757
V3UK	V2Lib	V4Port	V.zerda	0.0061198	0.000804927	7.60293
V5UAE	V4Port	V1Alg	R2W_Desert	0.00397861	0.000524411	7.58681
V8Nile	V4Port	V5UAE	V7WMCD	0.00617208	0.000813958	7.58279
V9W_Nile	V3UK	R2W_Desert	V6E_Nile	0.00462289	0.000610631	7.57068
V2Lib	V7WMCD	R2W_Desert	V4Port	0.00761561	0.00100662	7.56555
V9W_Nile	V3UK	R1E_Desert	V7WMCD	0.00391513	0.000522903	7.4873
V9W_Nile	R1E_Desert	V2Lib	V6E_Nile	0.00508774	0.000685364	7.42341
V8Nile	V4Port	R1E_Desert	V.zerda	0.00583794	0.000790396	7.38609
R1E_Desert	V.lagopus	R2W_Desert	V6E_Nile	0.00518763	0.000703222	7.37695
V9W_Nile	R2W_Desert	V.lagopus	V1Alg	0.0072344	0.000982921	7.36011
V9W_Nile	R2W_Desert	V8Nile	V7WMCD	0.00555833	0.000756304	7.34932
V2Lib	V.zerda	R2W_Desert	V4Port	0.00825056	0.00112321	7.34549

V9W_Nile	V2Lib	V3UK	V7WMCD	0.00379487	0.000518039	7.32545
V9W_Nile	V3UK	V.lagopus	V5UAE	0.0039212	0.000535684	7.31998
V9W_Nile	R1E_Desert	V5UAE	V4Port	0.00580454	0.000795146	7.29997
V8Nile	V.lagopus	V4Port	V6E_Nile	0.0068419	0.000939572	7.28193
R1E_Desert	V3UK	V5UAE	R2W_Desert	0.00644851	0.000886768	7.27192
V1Alg	V4Port	V7WMCD	V6E_Nile	0.00713949	0.000982831	7.2642
V.lagopus	V1Alg	V4Port	V6E_Nile	0.00584918	0.000807136	7.24684
V9W_Nile	V4Port	V8Nile	R1E_Desert	0.00873445	0.00120686	7.23734
V8Nile	V4Port	V.lagopus	V6E_Nile	0.00545889	0.00075432	7.23683
V8Nile	V.zerda	R2W_Desert	V4Port	0.00549275	0.000759584	7.23126
V9W_Nile	V2Lib	V1Alg	V3UK	0.00379207	0.00052463	7.22808
V7WMCD	V4Port	V.zerda	V6E_Nile	0.00633024	0.000882756	7.171
V1Alg	V7WMCD	V4Port	V6E_Nile	0.00732328	0.00102146	7.16939
V9W_Nile	R2W_Desert	V1Alg	V6E_Nile	0.00577086	0.000807798	7.14394
V5UAE	V.zerda	V3UK	V7WMCD	0.00356288	0.000500228	7.12251
V9W_Nile	V7WMCD	V8Nile	V6E_Nile	0.00221993	0.000312799	7.09699
V5UAE	V4Port	V.zerda	V6E_Nile	0.00453609	0.000640977	7.07684
V8Nile	R1E_Desert	V.lagopus	V3UK	0.00475527	0.000673037	7.0654
V8Nile	R2W_Desert	V5UAE	V3UK	0.00744696	0.00105643	7.04915
V8Nile	V7WMCD	V5UAE	V4Port	0.00309586	0.000440063	7.03504
V5UAE	R2W_Desert	V.zerda	V6E_Nile	0.00508092	0.000722848	7.02902
V3UK	V.zerda	V2Lib	R2W_Desert	0.00460105	0.000657679	6.99589
V8Nile	V7WMCD	R1E_Desert	V5UAE	0.0055129	0.000793703	6.9458
R1E_Desert	V7WMCD	R2W_Desert	V.zerda	0.00485087	0.000698438	6.94531
V9W_Nile	V5UAE	V8Nile	V6E_Nile	0.00745394	0.00107377	6.94182
V3UK	V2Lib	V7WMCD	V.zerda	0.00338651	0.000488506	6.93239
V.lagopus	V4Port	V1Alg	R2W_Desert	0.00487826	0.000708394	6.88636
R1E_Desert	V2Lib	V1Alg	V4Port	0.00159305	0.000231788	6.87286
V3UK	V6E_Nile	V7WMCD	V4Port	0.00561601	0.000817589	6.86899
V8Nile	V2Lib	V.lagopus	V3UK	0.00425984	0.000621477	6.85438
R1E_Desert	V6E_Nile	V7WMCD	V4Port	0.00599956	0.000878257	6.83121
R1E_Desert	V2Lib	V1Alg	R2W_Desert	0.00131449	0.000192493	6.82878
V8Nile	V2Lib	V3UK	R2W_Desert	0.00385559	0.000564982	6.82427
R1E_Desert	V2Lib	R2W_Desert	V.zerda	0.00445449	0.000653392	6.81749
V9W_Nile	V3UK	V5UAE	V4Port	0.00265554	0.000393476	6.74893
V1Alg	V3UK	V2Lib	V6E_Nile	0.00431502	0.000643438	6.70619
V1Alg	V2Lib	V3UK	V7WMCD	0.00521127	0.000794667	6.5578
V9W_Nile	V.lagopus	V1Alg	V7WMCD	0.00413115	0.000630662	6.5505
V8Nile	R2W_Desert	V2Lib	V.zerda	0.00531082	0.000811999	6.54043
R1E_Desert	V1Alg	V2Lib	V7WMCD	0.00488473	0.000748767	6.5237
V9W_Nile	V2Lib	V.zerda	V6E_Nile	0.00280649	0.000431974	6.4969
V8Nile	V4Port	V5UAE	V3UK	0.00636055	0.000996873	6.3805
V8Nile	R2W_Desert	V7WMCD	V4Port	0.00481473	0.000762379	6.31541
V1Alg	V4Port	V3UK	R2W_Desert	0.0061297	0.000973338	6.29761
V9W_Nile	R2W_Desert	V1Alg	V.zerda	0.00408841	0.000652524	6.26553
V9W_Nile	V2Lib	V.lagopus	V1Alg	0.00265748	0.000425786	6.24134
V9W_Nile	R1E_Desert	V5UAE	R2W_Desert	0.00444014	0.000715484	6.20579
V9W_Nile	V.zerda	R1E_Desert	V1Alg	0.00566844	0.000918992	6.1681
V8Nile	R1E_Desert	V7WMCD	V4Port	0.00621179	0.0010099	6.15088

V.lagopus	V3UK	V5UAE	R2W_Desert	0.0051834	0.000843043	6.14844
V9W_Nile	R1E_Desert	V3UK	V6E_Nile	0.00539144	0.000879101	6.1329
V8Nile	V4Port	V1Alg	V7WMCD	0.00368957	0.000602812	6.12059
V8Nile	R2W_Desert	R1E_Desert	V.zerda	0.00445238	0.000727478	6.12029
V.lagopus	V7WMCD	V5UAE	R2W_Desert	0.00585971	0.000959277	6.10846
V8Nile	V1Alg	V4Port	V6E_Nile	0.00360732	0.000599521	6.01701
R1E_Desert	V3UK	R2W_Desert	V4Port	0.00111804	0.000186295	6.00148
R1E_Desert	V2Lib	V1Alg	V7WMCD	0.00164641	0.000274933	5.98843
V.lagopus	V3UK	V4Port	V6E_Nile	0.00480223	0.000803824	5.97423
V9W_Nile	V5UAE	R1E_Desert	V6E_Nile	0.00437409	0.000736089	5.94233
V9W_Nile	V1Alg	V2Lib	V.zerda	0.00601797	0.00101511	5.9284
V.lagopus	V6E_Nile	V2Lib	R2W_Desert	0.00414786	0.000705991	5.87523
V9W_Nile	V3UK	V8Nile	V6E_Nile	0.00375022	0.000640933	5.85118
V9W_Nile	V.lagopus	V1Alg	R2W_Desert	0.00419773	0.000718642	5.8412
V9W_Nile	V.zerda	V5UAE	V6E_Nile	0.00554455	0.000951975	5.82426
V9W_Nile	V1Alg	V2Lib	R2W_Desert	0.00553978	0.000954431	5.80427
V8Nile	V6E_Nile	V.lagopus	V4Port	0.00413059	0.000713267	5.79109
V3UK	R2W_Desert	V.zerda	V6E_Nile	0.00687873	0.00119127	5.7743
V8Nile	V.lagopus	V5UAE	V3UK	0.00401406	0.000695565	5.77093
R1E_Desert	V.lagopus	R2W_Desert	V4Port	0.00500493	0.000868805	5.7607
V8Nile	V2Lib	V5UAE	V6E_Nile	0.00479565	0.000836276	5.73453
V9W_Nile	V1Alg	V.zerda	V6E_Nile	0.00481651	0.000842447	5.71729
V.lagopus	V1Alg	V3UK	V4Port	0.00622101	0.00108845	5.71549
V9W_Nile	R2W_Desert	V.lagopus	V7WMCD	0.00345003	0.000606717	5.68639
R1E_Desert	V3UK	V.lagopus	V2Lib	0.00108418	0.000191062	5.67451
V8Nile	V1Alg	R1E_Desert	V5UAE	0.00435642	0.000768383	5.6696
V9W_Nile	V8Nile	V.zerda	V6E_Nile	0.00215096	0.000380288	5.65612
V5UAE	R2W_Desert	V7WMCD	V6E_Nile	0.00500102	0.000892533	5.60318
V9W_Nile	V7WMCD	V8Nile	R2W_Desert	0.00342837	0.000614385	5.58016
V9W_Nile	R2W_Desert	V5UAE	V6E_Nile	0.00395793	0.000709573	5.5779
V5UAE	V4Port	V2Lib	V6E_Nile	0.00657792	0.00119202	5.5183
V9W_Nile	V1Alg	V8Nile	V3UK	0.00304441	0.000554638	5.489
R1E_Desert	V1Alg	V2Lib	V4Port	0.00538752	0.000984629	5.47162
V5UAE	R2W_Desert	V3UK	V2Lib	0.00464828	0.000859927	5.40544
V5UAE	V.zerda	V1Alg	V4Port	0.00500805	0.000929223	5.3895
V9W_Nile	V2Lib	V5UAE	R2W_Desert	0.00258677	0.000482616	5.3599
V.lagopus	V7WMCD	V5UAE	V2Lib	0.00453467	0.000852496	5.31928
V9W_Nile	R2W_Desert	V.lagopus	V5UAE	0.00483304	0.000910944	5.30553
V8Nile	V4Port	V2Lib	R2W_Desert	0.00419704	0.000791078	5.30548
V8Nile	V3UK	V.lagopus	V2Lib	0.00554139	0.00104854	5.28486
V1Alg	V2Lib	V3UK	V.zerda	0.0050676	0.000962323	5.266
V9W_Nile	V.lagopus	R2W_Desert	V7WMCD	0.00418067	0.00079829	5.23704
V9W_Nile	V8Nile	V.lagopus	V6E_Nile	0.00237558	0.0004546	5.22565
V9W_Nile	V1Alg	V8Nile	V5UAE	0.00239112	0.000462155	5.17384
V9W_Nile	V8Nile	R2W_Desert	V6E_Nile	0.00189571	0.000366495	5.17255
V5UAE	R2W_Desert	V3UK	V7WMCD	0.00302363	0.000586546	5.15497
V9W_Nile	V4Port	V8Nile	V7WMCD	0.00400697	0.000785036	5.10418
V9W_Nile	V1Alg	V8Nile	V.lagopus	0.00376227	0.00073928	5.0891
V9W_Nile	V2Lib	V1Alg	V4Port	0.00303439	0.000596392	5.08791

V9W_Nile	V7WMCD	V8Nile	V5UAE	0.00239015	0.000471479	5.06948
V8Nile	V.zerda	R1E_Desert	V5UAE	0.00542446	0.00107675	5.03782
V9W_Nile	V.lagopus	R1E_Desert	V7WMCD	0.00340722	0.000678053	5.025
V1Alg	V2Lib	V3UK	V6E_Nile	0.00423177	0.000844566	5.01059
V9W_Nile	V3UK	V7WMCD	V.zerda	0.00264947	0.000529285	5.00575
V8Nile	R1E_Desert	V5UAE	V3UK	0.00485872	0.000971202	5.00279
V9W_Nile	V2Lib	R1E_Desert	R2W_Desert	0.00397155	0.000796655	4.98528
R1E_Desert	V2Lib	V1Alg	V3UK	0.00134835	0.000270948	4.97642
V8Nile	V4Port	V1Alg	V2Lib	0.00285008	0.000575305	4.95404
V9W_Nile	R2W_Desert	V8Nile	V2Lib	0.00264963	0.000539435	4.91186
V8Nile	V6E_Nile	V5UAE	V4Port	0.00377382	0.000771295	4.89284
V9W_Nile	R2W_Desert	V5UAE	V3UK	0.00321775	0.000660165	4.87415
V9W_Nile	V8Nile	R1E_Desert	V6E_Nile	0.00192887	0.000396758	4.86157
V9W_Nile	V3UK	V8Nile	R2W_Desert	0.00204082	0.000420346	4.85508
V9W_Nile	V3UK	V5UAE	V7WMCD	0.00201278	0.000414661	4.85404
V9W_Nile	V6E_Nile	V.lagopus	V3UK	0.00069047	0.000142271	4.85319
V8Nile	V.lagopus	V5UAE	V4Port	0.00404966	0.00083908	4.82631
V.lagopus	V4Port	V7WMCD	V6E_Nile	0.00305425	0.00063556	4.80561
V3UK	V6E_Nile	V4Port	V.zerda	0.00388541	0.000810045	4.79654
V9W_Nile	V.zerda	R1E_Desert	V2Lib	0.0041667	0.000868862	4.79558
R1E_Desert	V2Lib	V1Alg	V.zerda	0.00124288	0.000259316	4.7929
V9W_Nile	V6E_Nile	V8Nile	V.zerda	0.00281263	0.000590291	4.76483
V9W_Nile	V5UAE	V1Alg	V6E_Nile	0.00402633	0.000848142	4.74724
V8Nile	V3UK	V.zerda	V6E_Nile	0.00365076	0.000772779	4.72419
V9W_Nile	R2W_Desert	V5UAE	V7WMCD	0.00342266	0.000734876	4.65747
V8Nile	V7WMCD	V1Alg	V2Lib	0.0034842	0.000748291	4.65622
R1E_Desert	V2Lib	V.lagopus	V6E_Nile	0.00430746	0.000926565	4.64884
V8Nile	V4Port	V7WMCD	V6E_Nile	0.00433501	0.000938945	4.61689
V9W_Nile	V3UK	V5UAE	V6E_Nile	0.00194778	0.000424115	4.59257
V9W_Nile	V4Port	V5UAE	R2W_Desert	0.00297694	0.000648624	4.58962
V9W_Nile	V.lagopus	R1E_Desert	V4Port	0.00304666	0.000679996	4.4804
V9W_Nile	V3UK	V.lagopus	V6E_Nile	0.00194038	0.000438136	4.42872
V9W_Nile	V7WMCD	V8Nile	R1E_Desert	0.00270474	0.000610789	4.42827
V8Nile	V1Alg	V7WMCD	V.zerda	0.00371003	0.000839787	4.41783
V9W_Nile	V4Port	V.lagopus	V7WMCD	0.00580197	0.00131562	4.41006
V9W_Nile	V8Nile	R2W_Desert	V7WMCD	0.0017166	0.000389298	4.40947
V5UAE	V.zerda	V1Alg	V3UK	0.00382759	0.000875738	4.3707
V9W_Nile	V.lagopus	V8Nile	V4Port	0.00233233	0.000533906	4.36842
V8Nile	R1E_Desert	V2Lib	V7WMCD	0.00362801	0.000830885	4.36644
V8Nile	V6E_Nile	R1E_Desert	V2Lib	0.00325861	0.000746839	4.36321
V9W_Nile	V7WMCD	V8Nile	V.zerda	0.00234318	0.00053763	4.35835
V9W_Nile	V8Nile	R1E_Desert	V7WMCD	0.00174975	0.000403787	4.33336
V8Nile	V.lagopus	V2Lib	V6E_Nile	0.00400239	0.000925055	4.32665
V8Nile	V7WMCD	R1E_Desert	V1Alg	0.00370177	0.000856385	4.32255
V.lagopus	V1Alg	V3UK	V7WMCD	0.00427304	0.000989467	4.31853
V8Nile	R2W_Desert	V.lagopus	V6E_Nile	0.00196448	0.000455955	4.30851
V9W_Nile	V8Nile	V.lagopus	V5UAE	0.00225429	0.000527835	4.27082
V9W_Nile	V8Nile	V.lagopus	V7WMCD	0.00219646	0.000514945	4.26544
V8Nile	V5UAE	V2Lib	V6E_Nile	0.0039475	0.000929496	4.24692

V9W_Nile	V2Lib	V8Nile	V5UAE	0.0019438	0.000459904	4.22654
V8Nile	V4Port	V2Lib	V6E_Nile	0.00377752	0.000895963	4.21616
R1E_Desert	V1Alg	V2Lib	V6E_Nile	0.00387689	0.000923386	4.19856
V9W_Nile	V7WMCD	R1E_Desert	R2W_Desert	0.000723625	0.00017345	4.17195
V9W_Nile	V.zerda	R1E_Desert	V6E_Nile	0.00381712	0.00091587	4.16775
V5UAE	V3UK	V4Port	V6E_Nile	0.00180249	0.000433308	4.15983
V9W_Nile	V.lagopus	V5UAE	V3UK	0.00358649	0.000868097	4.13143
R1E_Desert	V4Port	V3UK	R2W_Desert	0.00382344	0.000928792	4.11657
V5UAE	V3UK	V2Lib	V7WMCD	0.00235997	0.000573936	4.11191
V5UAE	V7WMCD	V2Lib	R2W_Desert	0.00314814	0.000769906	4.089
V9W_Nile	V8Nile	V.lagopus	V3UK	0.00195139	0.000480019	4.06524
V9W_Nile	V6E_Nile	V8Nile	V7WMCD	0.00192956	0.0004761	4.05284
V2Lib	R2W_Desert	V7WMCD	V6E_Nile	0.00304718	0.00075195	4.05237
R1E_Desert	V3UK	V4Port	V6E_Nile	0.00401372	0.000990696	4.05142
V8Nile	V5UAE	V.lagopus	V7WMCD	0.00379472	0.000936858	4.05048
V9W_Nile	V.zerda	V5UAE	V7WMCD	0.00352025	0.000872227	4.03594
V9W_Nile	V4Port	V1Alg	V.zerda	0.00369657	0.000925468	3.99427
V9W_Nile	V7WMCD	V8Nile	V.lagopus	0.00268179	0.000674183	3.97784
V8Nile	V1Alg	R1E_Desert	V6E_Nile	0.00320205	0.00080652	3.97021
V9W_Nile	V4Port	V8Nile	V3UK	0.00507008	0.00128408	3.94842
V8Nile	V6E_Nile	V.lagopus	V5UAE	0.00312361	0.000805251	3.87905
V9W_Nile	V6E_Nile	R1E_Desert	V4Port	0.00283829	0.000737805	3.84694
V9W_Nile	V1Alg	V8Nile	V6E_Nile	0.0026004	0.000680745	3.81993
V1Alg	V2Lib	V3UK	V4Port	0.00369077	0.000975805	3.78228
V9W_Nile	V.zerda	V8Nile	V6E_Nile	0.00365727	0.000967738	3.7792
V1Alg	V4Port	V7WMCD	V.zerda	0.00365224	0.00096747	3.77504
V9W_Nile	V1Alg	V8Nile	V7WMCD	0.00274815	0.000731451	3.75712
V9W_Nile	R1E_Desert	V5UAE	V2Lib	0.00253457	0.000677624	3.74038
V7WMCD	V6E_Nile	V4Port	V.zerda	0.00348618	0.000939313	3.71142
V9W_Nile	V8Nile	R1E_Desert	V3UK	0.00150468	0.000411428	3.65721
V9W_Nile	V8Nile	R1E_Desert	V5UAE	0.00180758	0.000500294	3.61303
V9W_Nile	V2Lib	V5UAE	V.zerda	0.00219925	0.000614826	3.57704
V9W_Nile	V8Nile	V.lagopus	V4Port	0.0015885	0.000454836	3.49248
V8Nile	V4Port	R1E_Desert	V2Lib	0.00298785	0.000856147	3.48989
V9W_Nile	V6E_Nile	V5UAE	R2W_Desert	0.00180224	0.000520229	3.46432
V1Alg	V4Port	V2Lib	V7WMCD	0.00264246	0.000765892	3.45017
V9W_Nile	V7WMCD	V1Alg	V2Lib	0.00146632	0.000427559	3.42952
V5UAE	V3UK	V7WMCD	V4Port	0.00257041	0.000759106	3.3861
V8Nile	V.zerda	V1Alg	V6E_Nile	0.0030114	0.000892242	3.3751
V9W_Nile	V6E_Nile	V8Nile	V1Alg	0.00198269	0.000591148	3.35397
V8Nile	V5UAE	V3UK	V6E_Nile	0.00196624	0.000589696	3.33433
V8Nile	R1E_Desert	R2W_Desert	V.zerda	0.00142083	0.00042818	3.31831
V5UAE	R2W_Desert	V1Alg	V3UK	0.00345626	0.00104495	3.30759
V8Nile	R1E_Desert	V5UAE	V6E_Nile	0.00342914	0.00104609	3.27805
R1E_Desert	V2Lib	V.lagopus	V5UAE	0.00280591	0.000860014	3.26263
V8Nile	V7WMCD	V1Alg	V3UK	0.00222197	0.00068507	3.24343
V5UAE	V4Port	R2W_Desert	V6E_Nile	0.00201293	0.00062116	3.2406
V1Alg	V7WMCD	V3UK	V6E_Nile	0.0021199	0.000655444	3.23429
V8Nile	R1E_Desert	R2W_Desert	V6E_Nile	0.00318407	0.000987469	3.22447

V9W_Nile	R2W_Desert	V4Port	V6E_Nile	0.00170624	0.00053533	3.18728
R1E_Desert	V3UK	V2Lib	V7WMCD	0.00243479	0.000770452	3.1602
V9W_Nile	V8Nile	V2Lib	V7WMCD	0.00107021	0.000343653	3.11421
V9W_Nile	V6E_Nile	V8Nile	V5UAE	0.00152326	0.000495975	3.07124
V3UK	V6E_Nile	V7WMCD	V.zerda	0.00314527	0.00102953	3.05505
V9W_Nile	V5UAE	V2Lib	V6E_Nile	0.0022148	0.000726333	3.0493
V9W_Nile	V5UAE	V1Alg	V4Port	0.00201411	0.000661083	3.04669
V9W_Nile	V.lagopus	V2Lib	V6E_Nile	0.00252103	0.000836403	3.01413
V9W_Nile	V2Lib	V4Port	V6E_Nile	-0.00134294	0.000442049	-3.038
V8Nile	V5UAE	V3UK	V.zerda	-0.00150617	0.000492187	-3.06017
V9W_Nile	V.zerda	V8Nile	V5UAE	-0.0020243	0.000661223	-3.06144
V8Nile	V2Lib	R1E_Desert	V5UAE	-0.00254801	0.000832237	-3.06164
V.lagopus	V5UAE	V2Lib	V7WMCD	-0.00302675	0.000988499	-3.06197
V.lagopus	V4Port	V5UAE	V7WMCD	-0.00204413	0.000662948	-3.08339
V3UK	V.zerda	R2W_Desert	V4Port	-0.00273329	0.000884892	-3.08884
V9W_Nile	V7WMCD	V3UK	V6E_Nile	-0.00139059	0.000448382	-3.10134
V8Nile	R1E_Desert	V1Alg	V.zerda	-0.00309661	0.000994307	-3.11435
V8Nile	V5UAE	R1E_Desert	V3UK	-0.00195269	0.000618899	-3.1551
V9W_Nile	V7WMCD	V4Port	V6E_Nile	-0.001434	0.000449256	-3.19194
R1E_Desert	V2Lib	V.lagopus	V1Alg	-0.00292025	0.000912226	-3.20123
V9W_Nile	V8Nile	V4Port	V.zerda	-0.00136388	0.000425596	-3.20463
V9W_Nile	V2Lib	V3UK	R2W_Desert	-0.00159839	0.000495502	-3.22581
V8Nile	V6E_Nile	V5UAE	V1Alg	-0.00172826	0.000528037	-3.27299
V.lagopus	V2Lib	V5UAE	V7WMCD	-0.00228389	0.000697502	-3.27439
V8Nile	R2W_Desert	V5UAE	V4Port	-0.00266609	0.000806015	-3.30775
V5UAE	V3UK	V4Port	V.zerda	-0.00217612	0.000657593	-3.30922
V9W_Nile	V6E_Nile	R1E_Desert	V3UK	-0.00224334	0.000674861	-3.32414
V8Nile	V7WMCD	V.lagopus	V2Lib	-0.00242872	0.000727909	-3.33657
V.lagopus	V3UK	V5UAE	V1Alg	-0.0033119	0.000988721	-3.34968
V8Nile	V5UAE	R1E_Desert	V4Port	-0.00217851	0.000642525	-3.39055
V5UAE	V6E_Nile	V3UK	V2Lib	-0.00278483	0.000817276	-3.40746
R1E_Desert	V7WMCD	V.lagopus	R2W_Desert	-0.00333878	0.000978549	-3.41197
V8Nile	V.lagopus	V5UAE	R2W_Desert	-0.0023557	0.000690242	-3.41286
V8Nile	V2Lib	R1E_Desert	V3UK	-0.00278522	0.000807802	-3.4479
V9W_Nile	V6E_Nile	V1Alg	V2Lib	-0.00212216	0.000615024	-3.45054
R1E_Desert	V3UK	V2Lib	V.zerda	-0.00219151	0.000628925	-3.48453
V9W_Nile	V4Port	V.zerda	V6E_Nile	-0.00358186	0.00102028	-3.51066
V3UK	V.zerda	V4Port	V6E_Nile	-0.00374825	0.00106705	-3.51271
V9W_Nile	V1Alg	R2W_Desert	V7WMCD	-0.00353147	0.000984065	-3.58865
V9W_Nile	V3UK	V4Port	V6E_Nile	-0.00230401	0.000641763	-3.59013
V1Alg	V4Port	V3UK	V2Lib	-0.00300007	0.000831224	-3.60922
V9W_Nile	V6E_Nile	V3UK	R2W_Desert	-0.00254593	0.000703759	-3.61761
V9W_Nile	R2W_Desert	V1Alg	V3UK	-0.00209996	0.000578374	-3.6308
V8Nile	V6E_Nile	R1E_Desert	V7WMCD	-0.00245339	0.000675199	-3.63359
V5UAE	V7WMCD	V1Alg	V3UK	-0.00284406	0.000782164	-3.63614
R1E_Desert	V5UAE	V7WMCD	V.zerda	-0.00328096	0.000891527	-3.68016
V8Nile	V5UAE	V7WMCD	V4Port	-0.00333741	0.000904195	-3.69103
V1Alg	V6E_Nile	R2W_Desert	V.zerda	-0.00348725	0.000942293	-3.70081
V1Alg	V2Lib	V4Port	V6E_Nile	-0.00363346	0.000978608	-3.71288

V8Nile	V1Alg	V5UAE	V2Lib	-0.00233805	0.000627515	-3.72589
V8Nile	R1E_Desert	V2Lib	R2W_Desert	-0.0030993	0.000830958	-3.72979
V9W_Nile	V8Nile	V5UAE	R2W_Desert	-0.00177442	0.00047472	-3.73782
V.lagopus	V1Alg	V5UAE	V2Lib	-0.00325407	0.000870245	-3.73926
V9W_Nile	V.lagopus	V.zerda	V6E_Nile	-0.00360354	0.000962021	-3.7458
V9W_Nile	V8Nile	V3UK	R2W_Desert	-0.00147152	0.000392525	-3.74886
V8Nile	V1Alg	V3UK	V4Port	-0.00288793	0.000764221	-3.77892
V5UAE	V3UK	V7WMCD	V6E_Nile	-0.00196568	0.000514771	-3.81855
V5UAE	V7WMCD	V2Lib	V4Port	-0.0031821	0.000823587	-3.86371
V9W_Nile	V4Port	V1Alg	R2W_Desert	-0.00391962	0.00101391	-3.86586
V9W_Nile	R2W_Desert	V5UAE	V.zerda	-0.00225168	0.000582304	-3.86685
V9W_Nile	V8Nile	V5UAE	V.zerda	-0.00202966	0.000522893	-3.8816
V5UAE	R2W_Desert	V4Port	V6E_Nile	-0.00383462	0.00097228	-3.94395
V9W_Nile	V8Nile	V3UK	V.zerda	-0.00172677	0.000430411	-4.0119
V9W_Nile	V1Alg	V4Port	V6E_Nile	-0.003666	0.000909962	-4.02874
V8Nile	V6E_Nile	V2Lib	V4Port	-0.00246543	0.000611282	-4.03321
V5UAE	V7WMCD	V1Alg	R2W_Desert	-0.00318046	0.00078615	-4.04561
V8Nile	V7WMCD	V1Alg	V4Port	-0.00221615	0.00054431	-4.07148
V9W_Nile	V2Lib	V1Alg	V.zerda	-0.00235888	0.000576492	-4.09178
V5UAE	V6E_Nile	V4Port	V.zerda	-0.00525621	0.00128303	-4.0967
V9W_Nile	R2W_Desert	V8Nile	R1E_Desert	-0.00213567	0.000515918	-4.13954
V9W_Nile	V6E_Nile	V1Alg	V3UK	-0.00241368	0.000582363	-4.14463
R1E_Desert	V1Alg	V3UK	V2Lib	-0.00442297	0.00106115	-4.16809
V9W_Nile	V2Lib	R1E_Desert	V6E_Nile	-0.00361452	0.000865607	-4.17571
V8Nile	R1E_Desert	V2Lib	V.zerda	-0.00380077	0.000906523	-4.19269
V8Nile	V5UAE	V.lagopus	V3UK	-0.00362437	0.000853346	-4.24725
V9W_Nile	V2Lib	V8Nile	V1Alg	-0.00200111	0.000467727	-4.27838
V.lagopus	V7WMCD	V5UAE	V6E_Nile	-0.00357563	0.000835379	-4.28025
V9W_Nile	V6E_Nile	V8Nile	R2W_Desert	-0.00181478	0.000422494	-4.29538
V9W_Nile	V2Lib	V1Alg	V7WMCD	-0.00210343	0.000488774	-4.30348
R1E_Desert	V3UK	V.lagopus	V6E_Nile	-0.00083948	0.000194757	-4.31043
V9W_Nile	V6E_Nile	V3UK	V2Lib	-0.0022742	0.000525472	-4.32793
V9W_Nile	V1Alg	V8Nile	V.zerda	-0.00331826	0.000749613	-4.42664
V9W_Nile	V4Port	V8Nile	V5UAE	-0.00366437	0.000826359	-4.43435
V9W_Nile	V6E_Nile	V3UK	V.zerda	-0.00283744	0.000637316	-4.45217
V8Nile	V1Alg	V5UAE	R2W_Desert	-0.00292556	0.000651795	-4.48847
R1E_Desert	V3UK	R2W_Desert	V7WMCD	-0.00140171	0.000311734	-4.49651
V9W_Nile	V5UAE	V7WMCD	V.zerda	-0.00432097	0.000956841	-4.51588
R1E_Desert	V.lagopus	V1Alg	R2W_Desert	-0.00436514	0.000965205	-4.5225
V9W_Nile	V3UK	V.lagopus	V2Lib	-0.00193431	0.000426354	-4.53687
V9W_Nile	V.zerda	V1Alg	V7WMCD	-0.00396544	0.000873647	-4.53895
V8Nile	V5UAE	V.lagopus	V2Lib	-0.00315265	0.000693559	-4.54561
V8Nile	V.zerda	R2W_Desert	V7WMCD	-0.00363051	0.000797948	-4.5498
V3UK	R2W_Desert	V7WMCD	V4Port	-0.00439417	0.000964852	-4.55424
R1E_Desert	V2Lib	V.lagopus	V3UK	-0.00288144	0.000626111	-4.60212
V9W_Nile	V.lagopus	R1E_Desert	V1Alg	-0.0036721	0.000794911	-4.61951
V8Nile	V2Lib	V5UAE	V.zerda	-0.00362903	0.000780647	-4.64874
V8Nile	V1Alg	V2Lib	R2W_Desert	-0.00410445	0.000881569	-4.65585
V.lagopus	V6E_Nile	V1Alg	V7WMCD	-0.00378763	0.000810194	-4.67497

V9W_Nile	V2Lib	R2W_Desert	V4Port	-0.00324714	0.000692266	-4.6906
R1E_Desert	V3UK	R2W_Desert	V6E_Nile	-0.00129624	0.000276081	-4.69516
V9W_Nile	V8Nile	V7WMCD	V.zerda	-0.00197184	0.000419283	-4.70288
V9W_Nile	V3UK	V8Nile	V7WMCD	-0.00563362	0.00117779	-4.78321
R1E_Desert	V4Port	V3UK	V2Lib	-0.00309986	0.000642215	-4.82682
V9W_Nile	R2W_Desert	V3UK	V.zerda	-0.00366434	0.000754925	-4.85391
V9W_Nile	V.zerda	V1Alg	R2W_Desert	-0.00464254	0.000947541	-4.89956
V8Nile	V6E_Nile	V3UK	R2W_Desert	-0.00347242	0.00070255	-4.94259
V.lagopus	V1Alg	V5UAE	R2W_Desert	-0.00398809	0.000805115	-4.95344
V2Lib	V.zerda	V7WMCD	V6E_Nile	-0.00466876	0.000940247	-4.96546
V9W_Nile	V5UAE	V4Port	V6E_Nile	-0.00461565	0.00092691	-4.97961
V9W_Nile	V4Port	V5UAE	V.zerda	-0.0057575	0.00114353	-5.03485
R1E_Desert	V5UAE	V1Alg	R2W_Desert	-0.0041797	0.000829512	-5.03874
R1E_Desert	V4Port	V2Lib	V7WMCD	-0.00455448	0.00088607	-5.14009
V2Lib	V7WMCD	V.zerda	V6E_Nile	-0.00479776	0.00093122	-5.15212
R1E_Desert	V6E_Nile	V3UK	R2W_Desert	-0.00488549	0.000947845	-5.15431
V5UAE	V7WMCD	V.zerda	V6E_Nile	-0.00460129	0.000891411	-5.16181
R1E_Desert	V2Lib	R2W_Desert	V7WMCD	-0.00437315	0.000841976	-5.19392
V1Alg	V2Lib	V4Port	V.zerda	-0.00378474	0.000727801	-5.20024
V1Alg	R2W_Desert	V2Lib	V.zerda	-0.00176227	0.000338136	-5.21172
V9W_Nile	V2Lib	V8Nile	V7WMCD	-0.00205624	0.000392491	-5.23895
V2Lib	V.zerda	R2W_Desert	V7WMCD	-0.00520338	0.000993094	-5.23956
V9W_Nile	R1E_Desert	V5UAE	V.zerda	-0.00389896	0.000740432	-5.26579
V5UAE	V4Port	V1Alg	V.zerda	-0.00284315	0.000539471	-5.27026
V.lagopus	V7WMCD	V5UAE	V4Port	-0.00390603	0.000735597	-5.31002
R1E_Desert	V6E_Nile	V3UK	V.zerda	-0.00405337	0.000761438	-5.32331
V8Nile	V7WMCD	R1E_Desert	V6E_Nile	-0.0044273	0.000830774	-5.32913
V8Nile	R1E_Desert	V3UK	V.zerda	-0.00568308	0.00106408	-5.34084
V8Nile	V1Alg	V3UK	R2W_Desert	-0.00374767	0.000700356	-5.35108
V8Nile	V6E_Nile	V5UAE	V2Lib	-0.00395023	0.000735332	-5.37204
R1E_Desert	V2Lib	V7WMCD	V6E_Nile	-0.00136785	0.000253604	-5.39365
R1E_Desert	V2Lib	R2W_Desert	V4Port	-0.00425881	0.000782203	-5.44464
V9W_Nile	V.zerda	R2W_Desert	V7WMCD	-0.00531886	0.000973482	-5.46375
R1E_Desert	V2Lib	R2W_Desert	V6E_Nile	-0.00380787	0.000695707	-5.47338
V9W_Nile	V4Port	V8Nile	V6E_Nile	-0.00452469	0.000823809	-5.4924
V9W_Nile	V6E_Nile	V8Nile	V.lagopus	-0.00222107	0.00040401	-5.49757
R1E_Desert	V4Port	R2W_Desert	V.zerda	-0.00504087	0.000916766	-5.49854
V8Nile	V.zerda	V2Lib	R2W_Desert	-0.00352437	0.00064055	-5.50211
V8Nile	V7WMCD	V3UK	V6E_Nile	-0.00423985	0.000769139	-5.51246
V9W_Nile	V.lagopus	R1E_Desert	V5UAE	-0.00473755	0.000854838	-5.54205
V9W_Nile	V5UAE	V.zerda	V6E_Nile	-0.00298022	0.000530646	-5.6162
V8Nile	V1Alg	V2Lib	V4Port	-0.00414208	0.000737047	-5.61984
V8Nile	V1Alg	V7WMCD	V6E_Nile	-0.00429747	0.000759025	-5.66183
V8Nile	V.zerda	V5UAE	V1Alg	-0.00401419	0.000701692	-5.72072
V8Nile	R2W_Desert	R1E_Desert	V2Lib	-0.00256134	0.000445792	-5.74559
V9W_Nile	V4Port	R1E_Desert	V.zerda	-0.00597138	0.00103762	-5.75487
V8Nile	V3UK	R2W_Desert	V6E_Nile	-0.00724003	0.0012515	-5.78507
V.lagopus	V5UAE	V3UK	V2Lib	-0.00441112	0.000760181	-5.80273
V9W_Nile	V6E_Nile	V1Alg	V4Port	-0.00310415	0.000533644	-5.81689

V9W_Nile	V1Alg	V.lagopus	V6E_Nile	-0.00449192	0.00076711	-5.85564
V8Nile	V5UAE	R2W_Desert	V4Port	-0.00441921	0.000754643	-5.85603
R1E_Desert	V5UAE	V.lagopus	V7WMCD	-0.00340088	0.000574915	-5.91546
V9W_Nile	R2W_Desert	V1Alg	V4Port	-0.00435164	0.000734251	-5.92664
V8Nile	V7WMCD	V.lagopus	V5UAE	-0.00369095	0.000622481	-5.92941
V8Nile	V2Lib	R1E_Desert	V4Port	-0.00419484	0.000703956	-5.95894
V8Nile	V5UAE	V3UK	R2W_Desert	-0.00431487	0.000723553	-5.96345
V9W_Nile	V5UAE	V.lagopus	V6E_Nile	-0.00483043	0.00080917	-5.96961
V9W_Nile	V2Lib	V1Alg	R2W_Desert	-0.00233132	0.00039048	-5.97041
V9W_Nile	V2Lib	R2W_Desert	V6E_Nile	-0.00273579	0.000456114	-5.99803
R1E_Desert	V5UAE	V.zerda	V6E_Nile	-0.0053747	0.000887516	-6.05589
V8Nile	V.zerda	V5UAE	V7WMCD	-0.00361132	0.000595552	-6.06382
V8Nile	V6E_Nile	V.lagopus	V7WMCD	-0.00346547	0.000569999	-6.07978
V9W_Nile	V2Lib	V7WMCD	V6E_Nile	-0.00319401	0.000521696	-6.12236
V8Nile	R1E_Desert	V.lagopus	V5UAE	-0.00268263	0.000437782	-6.12777
V9W_Nile	V.lagopus	V2Lib	R2W_Desert	-0.00365306	0.000596001	-6.12929
V3UK	V7WMCD	V4Port	V6E_Nile	-0.0066327	0.00107885	-6.14792
V9W_Nile	V1Alg	V3UK	V4Port	-0.00486746	0.000786805	-6.18637
V8Nile	V3UK	V5UAE	V7WMCD	-0.0044023	0.000709548	-6.20437
V8Nile	V.zerda	V2Lib	V6E_Nile	-0.004784	0.00076983	-6.21436
R1E_Desert	V2Lib	V7WMCD	V4Port	-0.00103848	0.000167004	-6.21829
V8Nile	V4Port	V.lagopus	V3UK	-0.00719865	0.00115399	-6.23805
V9W_Nile	V4Port	V5UAE	V2Lib	-0.00661782	0.00105925	-6.24765
V9W_Nile	V.zerda	V8Nile	V4Port	-0.00596824	0.000953231	-6.26106
V9W_Nile	V2Lib	R1E_Desert	V1Alg	-0.00340731	0.000538971	-6.32189
V5UAE	V4Port	V2Lib	V7WMCD	-0.00643508	0.00101705	-6.32717
R1E_Desert	V3UK	V2Lib	V4Port	-0.00620523	0.000977673	-6.34694
V9W_Nile	V1Alg	V4Port	V.zerda	-0.00414419	0.000652703	-6.34929
V5UAE	V7WMCD	V1Alg	V4Port	-0.00490941	0.00076824	-6.39046
V9W_Nile	V1Alg	V5UAE	V.zerda	-0.00525936	0.000817737	-6.43161
V8Nile	V.zerda	V5UAE	V6E_Nile	-0.00448901	0.000696102	-6.44878
V5UAE	R2W_Desert	V1Alg	V4Port	-0.00537938	0.000832978	-6.458
R2W_Desert	V4Port	V.zerda	V6E_Nile	-0.00174206	0.000269734	-6.45844
R1E_Desert	V1Alg	V3UK	V.zerda	-0.00417719	0.000645701	-6.46923
V.lagopus	V1Alg	V7WMCD	V4Port	-0.00170891	0.000262681	-6.50564
V8Nile	V5UAE	V3UK	V2Lib	-0.00462978	0.000708816	-6.53171
V9W_Nile	V5UAE	V7WMCD	V4Port	-0.00716637	0.00109572	-6.54032
V8Nile	V.lagopus	V3UK	V7WMCD	-0.00793553	0.0012109	-6.55343
V9W_Nile	V.lagopus	V4Port	V6E_Nile	-0.00422888	0.00064331	-6.57363
R1E_Desert	V5UAE	V4Port	V6E_Nile	-0.00405378	0.000613087	-6.61208
V8Nile	V5UAE	V.lagopus	V4Port	-0.00332532	0.000502744	-6.61433
V8Nile	R2W_Desert	V5UAE	V1Alg	-0.00797692	0.00120485	-6.62068
R1E_Desert	V2Lib	V.lagopus	R2W_Desert	-0.00433434	0.000636157	-6.81332
R1E_Desert	V4Port	V.lagopus	V2Lib	-0.00485355	0.000706798	-6.86695
R1E_Desert	V3UK	V.lagopus	V5UAE	-0.00118966	0.000173056	-6.87439
V9W_Nile	V2Lib	V.lagopus	V6E_Nile	-0.00248034	0.000360787	-6.8748
V3UK	R2W_Desert	V7WMCD	V6E_Nile	-0.00520403	0.000751556	-6.92434
V5UAE	V4Port	V3UK	V2Lib	-0.00826761	0.00119132	-6.9399
R1E_Desert	V7WMCD	R2W_Desert	V6E_Nile	-0.00393249	0.000566506	-6.94166

V8Nile	V4Port	V7WMCD	V.zerda	-0.00736663	0.00104673	-7.03775
V9W_Nile	V3UK	V5UAE	R2W_Desert	-0.00342458	0.000483196	-7.08734
V8Nile	V2Lib	R1E_Desert	V6E_Nile	-0.00649526	0.000912109	-7.12114
V8Nile	V1Alg	R1E_Desert	R2W_Desert	-0.00489828	0.000684684	-7.15407
V3UK	V7WMCD	V2Lib	V6E_Nile	-0.00545927	0.000762132	-7.16316
V9W_Nile	V6E_Nile	V5UAE	V3UK	-0.00726237	0.00101232	-7.17396
V8Nile	V7WMCD	V.lagopus	V1Alg	-0.00443812	0.000618194	-7.17918
V3UK	R2W_Desert	V2Lib	V.zerda	-0.00553464	0.000770207	-7.18592
V8Nile	V.zerda	R1E_Desert	V4Port	-0.00711298	0.000987738	-7.20129
V1Alg	V6E_Nile	R2W_Desert	V4Port	-0.00648732	0.000900181	-7.20669
V8Nile	V7WMCD	V4Port	V6E_Nile	-0.00550208	0.000755653	-7.28123
V8Nile	V1Alg	V5UAE	V3UK	-0.00394724	0.000541502	-7.28943
V8Nile	V1Alg	V5UAE	V6E_Nile	-0.00398487	0.000545629	-7.30326
V8Nile	R1E_Desert	V1Alg	V6E_Nile	-0.00754899	0.00102812	-7.34252
V9W_Nile	R2W_Desert	V4Port	V.zerda	-0.0058	0.000781977	-7.4171
V9W_Nile	V6E_Nile	V3UK	V7WMCD	-0.00546013	0.000735134	-7.42739
V8Nile	V5UAE	V1Alg	V6E_Nile	-0.00694737	0.000932068	-7.45372
V5UAE	V7WMCD	V2Lib	V6E_Nile	-0.00793737	0.00105568	-7.51875
V3UK	V6E_Nile	V2Lib	V7WMCD	-0.00333592	0.000443667	-7.51898
R1E_Desert	V6E_Nile	V2Lib	V7WMCD	-0.0037368	0.000496189	-7.53101
V9W_Nile	V2Lib	V5UAE	V4Port	-0.00395169	0.000522847	-7.55803
V9W_Nile	V1Alg	V.lagopus	V2Lib	-0.00568157	0.000751249	-7.56283
V8Nile	V3UK	V.lagopus	V4Port	-0.00607341	0.000801944	-7.57336
V8Nile	R2W_Desert	V5UAE	V6E_Nile	-0.00748083	0.000986944	-7.57979
V5UAE	V4Port	V1Alg	V3UK	-0.00340063	0.000448093	-7.58913
V3UK	V7WMCD	V2Lib	V.zerda	-0.00647201	0.000852553	-7.59132
R1E_Desert	V6E_Nile	V5UAE	V2Lib	-0.00818965	0.00107111	-7.64592
V8Nile	V5UAE	V2Lib	R2W_Desert	-0.00712004	0.000927067	-7.68017
V8Nile	V1Alg	V5UAE	V.zerda	-0.0071854	0.00092791	-7.74364
V9W_Nile	R2W_Desert	V8Nile	V3UK	-0.00465307	0.000599505	-7.76151
V.lagopus	V5UAE	V2Lib	V6E_Nile	-0.00743788	0.00095373	-7.79872
V9W_Nile	V3UK	V1Alg	V7WMCD	-0.00455789	0.000582784	-7.82089
V9W_Nile	V2Lib	V8Nile	V3UK	-0.00275879	0.000351945	-7.8387
V8Nile	V7WMCD	R2W_Desert	V6E_Nile	-0.00752316	0.000959631	-7.83964
V8Nile	V4Port	V.zerda	V6E_Nile	-0.00861309	0.00109448	-7.86956
R1E_Desert	V4Port	V3UK	V7WMCD	-0.00527806	0.000670652	-7.87005
V9W_Nile	R2W_Desert	V8Nile	V.lagopus	-0.00616457	0.000782313	-7.87992
V8Nile	V5UAE	V1Alg	V4Port	-0.00424655	0.000537129	-7.90601
V9W_Nile	V7WMCD	V2Lib	R2W_Desert	-0.00790876	0.000992713	-7.96682
V.lagopus	V6E_Nile	V2Lib	V4Port	-0.00545511	0.000682005	-7.99864
V8Nile	V1Alg	R2W_Desert	V7WMCD	-0.00536723	0.000670803	-8.00119
V9W_Nile	V7WMCD	V1Alg	R2W_Desert	-0.00644244	0.000797439	-8.07891
V9W_Nile	R2W_Desert	V5UAE	V4Port	-0.00438735	0.000541528	-8.10179
V8Nile	V5UAE	V1Alg	V7WMCD	-0.00741909	0.00091281	-8.12775
V5UAE	V.zerda	V1Alg	V6E_Nile	-0.00704222	0.000866322	-8.12887
V8Nile	V7WMCD	V1Alg	V6E_Nile	-0.00531201	0.000652588	-8.13991
V8Nile	V.zerda	V4Port	V6E_Nile	-0.00564338	0.00068972	-8.18213
V8Nile	V6E_Nile	R1E_Desert	V3UK	-0.00680288	0.000829885	-8.19738
V2Lib	V.zerda	V7WMCD	V4Port	-0.00728799	0.000888627	-8.20141

V9W_Nile	V2Lib	V5UAE	V3UK	-0.00356417	0.00043431	-8.20652
V5UAE	V.zerda	V1Alg	R2W_Desert	-0.00321463	0.000390936	-8.2229
V8Nile	V.zerda	V5UAE	V3UK	-0.00530114	0.000643699	-8.23545
V8Nile	V1Alg	R2W_Desert	V.zerda	-0.00536768	0.000646399	-8.30397
V9W_Nile	R2W_Desert	V.lagopus	V4Port	-0.00993979	0.00119463	-8.32038
V8Nile	V4Port	V.lagopus	V7WMCD	-0.00576301	0.000689456	-8.35879
V9W_Nile	R1E_Desert	V4Port	V6E_Nile	-0.00929041	0.00110909	-8.37664
V3UK	V.zerda	R2W_Desert	V7WMCD	-0.0083493	0.000996074	-8.38221
V9W_Nile	V5UAE	V7WMCD	V6E_Nile	-0.00746105	0.0008882	-8.40019
V9W_Nile	V1Alg	V7WMCD	V6E_Nile	-0.00662593	0.000788471	-8.40351
V.lagopus	V3UK	R2W_Desert	V7WMCD	-0.0070118	0.000833723	-8.41023
V8Nile	V1Alg	V3UK	V2Lib	-0.0080075	0.000950151	-8.42761
V8Nile	V6E_Nile	V7WMCD	V4Port	-0.00570035	0.000672997	-8.47009
R1E_Desert	V3UK	V.lagopus	V4Port	-0.0024859	0.00029213	-8.50957
V9W_Nile	V6E_Nile	V5UAE	V4Port	-0.00809231	0.000948649	-8.53036
V9W_Nile	V3UK	V1Alg	V6E_Nile	-0.00565042	0.000659498	-8.56776
V8Nile	R2W_Desert	V5UAE	V2Lib	-0.00964026	0.00112061	-8.6027
V8Nile	R1E_Desert	V3UK	V6E_Nile	-0.00824442	0.000955588	-8.62759
R1E_Desert	V2Lib	V7WMCD	V.zerda	-0.00187796	0.000217301	-8.64222
V9W_Nile	R2W_Desert	V2Lib	V7WMCD	-0.00609359	0.000703017	-8.66777
V9W_Nile	R2W_Desert	V8Nile	V.zerda	-0.00669984	0.000771416	-8.68511
R1E_Desert	V1Alg	V.lagopus	V6E_Nile	-0.0093077	0.00106945	-8.70325
R1E_Desert	V4Port	V2Lib	R2W_Desert	-0.00827288	0.000949635	-8.71164
V9W_Nile	R2W_Desert	V2Lib	V.zerda	-0.00648731	0.000744167	-8.71755
V5UAE	V4Port	V1Alg	V7WMCD	-0.00812476	0.000927125	-8.7634
V9W_Nile	V5UAE	V3UK	V4Port	-0.00907956	0.00103595	-8.76449
V8Nile	V1Alg	V2Lib	V6E_Nile	-0.00840192	0.000958624	-8.76456
V9W_Nile	V4Port	V5UAE	V7WMCD	-0.00959477	0.00109423	-8.76848
V8Nile	V6E_Nile	V5UAE	V7WMCD	-0.00616638	0.000696937	-8.84783
V8Nile	V.zerda	V3UK	R2W_Desert	-0.00607095	0.000677125	-8.96578
V9W_Nile	V3UK	V1Alg	V2Lib	-0.00567846	0.000632766	-8.97403
V5UAE	R2W_Desert	V1Alg	V7WMCD	-0.00821565	0.00091061	-9.02214
V9W_Nile	V1Alg	V8Nile	R2W_Desert	-0.00600771	0.000663632	-9.05278
V1Alg	V.zerda	V7WMCD	V4Port	-0.00732422	0.000806684	-9.07942
V9W_Nile	V5UAE	V4Port	V.zerda	-0.00969728	0.00106046	-9.14442
V3UK	V.zerda	V2Lib	V6E_Nile	-0.00763366	0.000828114	-9.21813
V9W_Nile	V6E_Nile	V4Port	V.zerda	-0.00629007	0.000682346	-9.21831
V1Alg	V2Lib	R2W_Desert	V4Port	-0.00786523	0.00084527	-9.30499
V.lagopus	V3UK	V1Alg	V6E_Nile	-0.00811413	0.000867694	-9.35137
V5UAE	R2W_Desert	V1Alg	V6E_Nile	-0.008594	0.000916449	-9.3775
R1E_Desert	V2Lib	V5UAE	V7WMCD	-0.00222813	0.000234695	-9.49376
V1Alg	V.zerda	V3UK	V4Port	-0.00870105	0.000908056	-9.58207
V9W_Nile	V3UK	V.zerda	V6E_Nile	-0.0050357	0.000525177	-9.58857
V8Nile	V6E_Nile	R1E_Desert	V5UAE	-0.00658908	0.000686793	-9.59398
V3UK	V7WMCD	R2W_Desert	V4Port	-0.00722133	0.000751087	-9.61451
V.lagopus	V7WMCD	V3UK	R2W_Desert	-0.00839443	0.000871474	-9.63245
V8Nile	R1E_Desert	V3UK	V7WMCD	-0.00877313	0.000907952	-9.66255
V9W_Nile	V7WMCD	V1Alg	V3UK	-0.00626029	0.00064767	-9.66587
V9W_Nile	V1Alg	R2W_Desert	V.zerda	-0.00718874	0.000730198	-9.84492

V9W_Nile	V5UAE	V8Nile	V1Alg	-0.0112203	0.00113536	-9.88261
V9W_Nile	V5UAE	V3UK	R2W_Desert	-0.0116188	0.00117402	-9.8966
V9W_Nile	V5UAE	V3UK	V.zerda	-0.0112944	0.0011385	-9.92037
V8Nile	V5UAE	R2W_Desert	V7WMCD	-0.00863585	0.000869106	-9.93648
V8Nile	V7WMCD	V5UAE	R2W_Desert	-0.00772405	0.000777189	-9.93846
V2Lib	V4Port	R2W_Desert	V6E_Nile	-0.0115475	0.00115932	-9.96055
V.lagopus	V1Alg	R2W_Desert	V7WMCD	-0.00826112	0.000828716	-9.96859
R1E_Desert	V5UAE	V2Lib	V4Port	-0.00843473	0.000845483	-9.97622
V8Nile	V.zerda	R2W_Desert	V6E_Nile	-0.00715488	0.000716561	-9.98502
V8Nile	V3UK	R2W_Desert	V7WMCD	-0.0108691	0.00108694	-9.9997
V9W_Nile	V6E_Nile	V5UAE	V7WMCD	-0.00814545	0.000810933	-10.0445
R1E_Desert	V1Alg	V.lagopus	V2Lib	-0.00829986	0.000826162	-10.0463
R1E_Desert	V2Lib	V5UAE	V1Alg	-0.00352438	0.000350628	-10.0516
V9W_Nile	V7WMCD	V1Alg	V.zerda	-0.00752763	0.000743286	-10.1275
R1E_Desert	V3UK	V7WMCD	V6E_Nile	-0.00989441	0.000967736	-10.2243
V.lagopus	V2Lib	V7WMCD	V6E_Nile	-0.0087408	0.000854618	-10.2277
V5UAE	V1Alg	V4Port	V.zerda	-0.00988796	0.000965362	-10.2427
V8Nile	V3UK	V.lagopus	V6E_Nile	-0.00972416	0.00094838	-10.2534
V8Nile	V2Lib	R2W_Desert	V4Port	-0.00653289	0.000634142	-10.3019
V5UAE	V7WMCD	V1Alg	V.zerda	-0.00935657	0.000904471	-10.3448
R1E_Desert	R2W_Desert	V3UK	V7WMCD	-0.00937007	0.000902966	-10.377
V8Nile	V5UAE	V2Lib	V7WMCD	-0.00468835	0.000451385	-10.3866
V8Nile	R1E_Desert	V5UAE	V4Port	-0.00701371	0.000670497	-10.4605
V1Alg	V2Lib	R2W_Desert	V.zerda	-0.00801651	0.000766221	-10.4624
V9W_Nile	V4Port	R1E_Desert	V5UAE	-0.00972159	0.000926142	-10.4969
V8Nile	R2W_Desert	V4Port	V.zerda	-0.0101131	0.000963114	-10.5004
V.lagopus	V7WMCD	V2Lib	R2W_Desert	-0.00943534	0.000898193	-10.5048
V8Nile	V.zerda	R1E_Desert	V3UK	-0.0129906	0.00123088	-10.5539
V9W_Nile	V7WMCD	V2Lib	V.zerda	-0.00899395	0.000849752	-10.5842
V9W_Nile	V7WMCD	V2Lib	V6E_Nile	-0.0091172	0.000852953	-10.689
V8Nile	V6E_Nile	R2W_Desert	V4Port	-0.005577	0.000521601	-10.6921
V3UK	R2W_Desert	V4Port	V6E_Nile	-0.00827958	0.000773004	-10.7109
V3UK	R2W_Desert	V2Lib	V4Port	-0.00929179	0.00086653	-10.723
V.lagopus	V6E_Nile	V3UK	R2W_Desert	-0.00756202	0.000702911	-10.7581
V5UAE	R2W_Desert	V4Port	V.zerda	-0.00902664	0.000837276	-10.781
V8Nile	V7WMCD	R1E_Desert	R2W_Desert	-0.00879621	0.000815452	-10.7869
V9W_Nile	V3UK	V2Lib	V7WMCD	-0.00734578	0.000672327	-10.9259
V9W_Nile	V7WMCD	V1Alg	V6E_Nile	-0.00765087	0.000699606	-10.936
V9W_Nile	V.lagopus	V3UK	V6E_Nile	-0.0084266	0.00077	-10.9436
V.lagopus	V2Lib	V7WMCD	V4Port	-0.00929569	0.000846292	-10.984
R1E_Desert	V6E_Nile	V5UAE	V1Alg	-0.00878336	0.000799296	-10.9889
V9W_Nile	V2Lib	V5UAE	V6E_Nile	-0.00615095	0.000557399	-11.0351
V8Nile	V6E_Nile	V1Alg	V2Lib	-0.00638221	0.000577941	-11.043
V8Nile	V6E_Nile	V3UK	V.zerda	-0.00659602	0.000596165	-11.0641
V8Nile	V1Alg	V.lagopus	V2Lib	-0.00665418	0.000600594	-11.0793
R1E_Desert	R2W_Desert	V5UAE	V2Lib	-0.00955277	0.00086171	-11.0858
R1E_Desert	R2W_Desert	V3UK	V2Lib	-0.00930341	0.000839139	-11.0869
V1Alg	V.zerda	V4Port	V6E_Nile	-0.00747551	0.000673745	-11.0955
V9W_Nile	V7WMCD	V1Alg	V4Port	-0.00621688	0.000559651	-11.1085

V5UAE	V6E_Nile	R2W_Desert	V4Port	-0.0129709	0.00116715	-11.1132
V.lagopus	V5UAE	V7WMCD	V4Port	-0.0105031	0.000944521	-11.12
V.lagopus	V3UK	V1Alg	V4Port	-0.00866902	0.000776598	-11.1628
V8Nile	V3UK	R1E_Desert	V4Port	-0.0116148	0.00103935	-11.175
V2Lib	V4Port	V.zerda	V6E_Nile	-0.0124134	0.00110728	-11.2107
R1E_Desert	V1Alg	V.lagopus	R2W_Desert	-0.00805408	0.000715834	-11.2513
R1E_Desert	V1Alg	V.lagopus	V5UAE	-0.00906192	0.000805281	-11.2531
V.lagopus	R2W_Desert	V5UAE	V1Alg	-0.00947509	0.000837214	-11.3174
V5UAE	V3UK	V2Lib	V6E_Nile	-0.0106886	0.000944296	-11.3192
V8Nile	V6E_Nile	R1E_Desert	V4Port	-0.00971963	0.000857885	-11.3297
V9W_Nile	V7WMCD	V2Lib	V4Port	-0.0076832	0.000676295	-11.3607
V9W_Nile	V5UAE	V8Nile	V2Lib	-0.011515	0.00100901	-11.4121
V8Nile	V6E_Nile	V7WMCD	V.zerda	-0.00926224	0.00081047	-11.4282
V1Alg	V.zerda	V3UK	V2Lib	-0.00885234	0.00077457	-11.4287
V5UAE	V2Lib	V7WMCD	V.zerda	-0.01088	0.000949155	-11.4628
V9W_Nile	V3UK	V1Alg	V4Port	-0.00697608	0.000607393	-11.4853
V.lagopus	V7WMCD	V2Lib	V4Port	-0.00872483	0.000758472	-11.5032
V8Nile	V.zerda	V5UAE	V4Port	-0.00938187	0.00081474	-11.5152
V9W_Nile	V1Alg	V3UK	V2Lib	-0.00968397	0.000836461	-11.5773
V8Nile	R1E_Desert	V1Alg	V7WMCD	-0.0101103	0.000872596	-11.5865
V.lagopus	V6E_Nile	R2W_Desert	V7WMCD	-0.0117139	0.00101094	-11.5871
V9W_Nile	R2W_Desert	V8Nile	V5UAE	-0.00934947	0.000805409	-11.6084
V9W_Nile	V2Lib	V8Nile	V.lagopus	-0.00655366	0.000564004	-11.6199
V8Nile	V4Port	V.lagopus	V5UAE	-0.0116009	0.000993539	-11.6764
V3UK	V7WMCD	V2Lib	V4Port	-0.00968663	0.000825774	-11.7304
V8Nile	V2Lib	R2W_Desert	V6E_Nile	-0.0107927	0.000914455	-11.8024
V9W_Nile	V2Lib	V8Nile	R1E_Desert	-0.00556529	0.000468931	-11.868
V8Nile	V.zerda	V5UAE	R2W_Desert	-0.0097241	0.000818551	-11.8796
R1E_Desert	V6E_Nile	V5UAE	R2W_Desert	-0.00933834	0.000784772	-11.8994
V8Nile	V1Alg	V5UAE	V7WMCD	-0.00824471	0.000688669	-11.972
V5UAE	V7WMCD	V3UK	R2W_Desert	-0.01062	0.000885342	-11.9954
V9W_Nile	V5UAE	V8Nile	V.zerda	-0.011782	0.000981713	-12.0015
V9W_Nile	V3UK	V2Lib	R2W_Desert	-0.00733971	0.000611453	-12.0037
V.lagopus	V1Alg	V2Lib	V6E_Nile	-0.0083592	0.000693539	-12.053
V3UK	V.zerda	V7WMCD	V4Port	-0.0103666	0.000859815	-12.0568
V.lagopus	R2W_Desert	V3UK	V2Lib	-0.0102091	0.000846515	-12.0602
V9W_Nile	V.lagopus	V3UK	V7WMCD	-0.0109476	0.000905451	-12.0908
V8Nile	V5UAE	V2Lib	V4Port	-0.0113666	0.0009383	-12.114
R1E_Desert	V5UAE	V1Alg	V7WMCD	-0.0104085	0.000857414	-12.1395
V9W_Nile	V1Alg	R2W_Desert	V6E_Nile	-0.00921304	0.000757566	-12.1614
V9W_Nile	V.lagopus	V5UAE	V1Alg	-0.00651562	0.000534434	-12.1916
V9W_Nile	V6E_Nile	V7WMCD	V4Port	-0.0103665	0.000850167	-12.1935
V9W_Nile	V2Lib	V5UAE	V7WMCD	-0.0058955	0.000478667	-12.3165
V5UAE	V1Alg	V3UK	V7WMCD	-0.0108665	0.000877271	-12.3867
V9W_Nile	V1Alg	R1E_Desert	V5UAE	-0.0127333	0.00102785	-12.3883
V9W_Nile	V1Alg	V8Nile	V4Port	-0.00737887	0.000594712	-12.4075
V8Nile	V5UAE	V1Alg	V2Lib	-0.00757187	0.000610126	-12.4103
V5UAE	V7WMCD	V3UK	V.zerda	-0.0109241	0.000877869	-12.4439
R1E_Desert	V4Port	V.lagopus	V6E_Nile	-0.0132455	0.00106304	-12.46

V.lagopus	V1Alg	V3UK	R2W_Desert	-0.00983727	0.000787834	-12.4865
V5UAE	V3UK	V2Lib	V4Port	-0.0125826	0.00100408	-12.5315
V5UAE	V1Alg	V2Lib	V6E_Nile	-0.0119496	0.000951757	-12.5553
V8Nile	R2W_Desert	R1E_Desert	V4Port	-0.0104429	0.000831524	-12.5587
V.lagopus	V7WMCD	V3UK	V6E_Nile	-0.0081103	0.000645241	-12.5694
V9W_Nile	V1Alg	V5UAE	V6E_Nile	-0.0132701	0.00105337	-12.5978
V8Nile	R1E_Desert	R2W_Desert	V4Port	-0.0074379	0.000589555	-12.6161
V8Nile	V7WMCD	V1Alg	R2W_Desert	-0.00812907	0.000644278	-12.6173
V8Nile	V7WMCD	V.lagopus	V6E_Nile	-0.00591292	0.000468593	-12.6185
V8Nile	V.zerda	V3UK	V6E_Nile	-0.0101517	0.000801465	-12.6664
R1E_Desert	V4Port	V1Alg	R2W_Desert	-0.00942208	0.000742863	-12.6835
V9W_Nile	V1Alg	V8Nile	V2Lib	-0.00672557	0.000528436	-12.7273
V8Nile	V4Port	V5UAE	V.zerda	-0.0112572	0.000882093	-12.7619
V.lagopus	V7WMCD	V1Alg	V4Port	-0.00976574	0.000764709	-12.7705
V9W_Nile	V1Alg	R1E_Desert	V6E_Nile	-0.013157	0.00102238	-12.8689
V9W_Nile	V6E_Nile	V5UAE	V1Alg	-0.00634321	0.000492597	-12.8771
R1E_Desert	R2W_Desert	V2Lib	V4Port	-0.0104781	0.000809155	-12.9494
V.lagopus	V6E_Nile	V2Lib	V7WMCD	-0.0079355	0.000612288	-12.9604
V8Nile	V5UAE	R1E_Desert	V7WMCD	-0.00910757	0.0006984	-13.0406
V9W_Nile	R2W_Desert	V8Nile	V1Alg	-0.0101225	0.000775859	-13.0468
V8Nile	V.lagopus	V3UK	R2W_Desert	-0.0091976	0.000703925	-13.0662
V.lagopus	V1Alg	V2Lib	V4Port	-0.00909321	0.000694552	-13.0922
V3UK	V.zerda	V2Lib	V7WMCD	-0.0109696	0.000836047	-13.1208
V8Nile	V6E_Nile	V.lagopus	V2Lib	-0.00904247	0.000686916	-13.1639
V8Nile	V5UAE	V4Port	V6E_Nile	-0.00759606	0.000575531	-13.1984
V5UAE	V4Port	V1Alg	V2Lib	-0.00737924	0.000558398	-13.215
R1E_Desert	V2Lib	V3UK	V.zerda	-0.00222618	0.000167179	-13.3162
V9W_Nile	V6E_Nile	V5UAE	V.zerda	-0.0067495	0.000506441	-13.3273
V.lagopus	V7WMCD	V3UK	V2Lib	-0.0084407	0.000631075	-13.3751
V8Nile	V1Alg	R1E_Desert	V2Lib	-0.008979	0.000669363	-13.4143
V3UK	V2Lib	V7WMCD	V4Port	-0.00945083	0.000703343	-13.437
R1E_Desert	V5UAE	V2Lib	V.zerda	-0.00920755	0.000677572	-13.589
R1E_Desert	R2W_Desert	V5UAE	V1Alg	-0.00948611	0.000692545	-13.6975
V8Nile	V5UAE	V3UK	V7WMCD	-0.00809525	0.00059027	-13.7145
V5UAE	V4Port	V7WMCD	V.zerda	-0.0137236	0.00099766	-13.7558
R1E_Desert	V1Alg	R2W_Desert	V.zerda	-0.00956471	0.00069454	-13.7713
V8Nile	R1E_Desert	V.lagopus	V7WMCD	-0.010622	0.000770715	-13.782
R1E_Desert	R2W_Desert	V2Lib	V6E_Nile	-0.0105448	0.000762737	-13.8249
V3UK	V.zerda	V7WMCD	V6E_Nile	-0.0148777	0.00107134	-13.887
V9W_Nile	V2Lib	V8Nile	R2W_Desert	-0.0059528	0.000427448	-13.9264
V9W_Nile	V3UK	V4Port	V.zerda	-0.00928009	0.000659315	-14.0754
V8Nile	R1E_Desert	V2Lib	V6E_Nile	-0.0118724	0.000835759	-14.2056
V8Nile	V1Alg	V.lagopus	V5UAE	-0.0107349	0.000752314	-14.2692
R1E_Desert	V5UAE	V1Alg	V6E_Nile	-0.0111814	0.000775268	-14.4226
V8Nile	V3UK	R1E_Desert	V2Lib	-0.0164104	0.00113356	-14.4769
V8Nile	V4Port	R2W_Desert	V6E_Nile	-0.014072	0.00096877	-14.5256
V9W_Nile	V6E_Nile	V5UAE	V2Lib	-0.00856428	0.000588277	-14.5582
V9W_Nile	V3UK	V5UAE	V.zerda	-0.00795443	0.000546054	-14.5671
V9W_Nile	V1Alg	V5UAE	V3UK	-0.0115005	0.000785333	-14.6441

V9W_Nile	V5UAE	V8Nile	R2W_Desert	-0.0146203	0.000995599	-14.6849
V9W_Nile	V5UAE	V.lagopus	V7WMCD	-0.0124457	0.000845193	-14.7253
R1E_Desert	V1Alg	R2W_Desert	V4Port	-0.00981048	0.000665668	-14.7378
V5UAE	V1Alg	V3UK	V.zerda	-0.0192155	0.00130088	-14.7712
R1E_Desert	V4Port	V5UAE	R2W_Desert	-0.00837792	0.00056653	-14.7881
V1Alg	V4Port	V3UK	V6E_Nile	-0.0180663	0.00121907	-14.8197
V8Nile	V4Port	R1E_Desert	V7WMCD	-0.0112219	0.000755626	-14.8511
V.lagopus	V5UAE	V2Lib	V4Port	-0.0176051	0.00118464	-14.8611
R1E_Desert	V6E_Nile	V4Port	V.zerda	-0.00993205	0.000665966	-14.9137
V5UAE	V4Port	V7WMCD	V6E_Nile	-0.0152796	0.00101759	-15.0155
V8Nile	V7WMCD	V1Alg	V.zerda	-0.0112249	0.000747312	-15.0204
V.lagopus	V6E_Nile	V1Alg	R2W_Desert	-0.0141943	0.000942712	-15.0568
V9W_Nile	V.lagopus	V5UAE	R2W_Desert	-0.0120131	0.000792718	-15.1543
R1E_Desert	V4Port	V1Alg	V2Lib	-0.0135509	0.000891427	-15.2014
R1E_Desert	V6E_Nile	V5UAE	V4Port	-0.00961548	0.000632378	-15.2053
V9W_Nile	V5UAE	V2Lib	V4Port	-0.01519	0.000998839	-15.2077
V9W_Nile	V2Lib	V8Nile	V.zerda	-0.00815206	0.000536016	-15.2086
V8Nile	V6E_Nile	V1Alg	V3UK	-0.0131119	0.000858205	-15.2783
V5UAE	V7WMCD	V1Alg	V2Lib	-0.0120392	0.000784117	-15.3538
V8Nile	R1E_Desert	V5UAE	V7WMCD	-0.00807166	0.00052503	-15.3737
V8Nile	V7WMCD	V2Lib	R2W_Desert	-0.0145835	0.000946567	-15.4067
V5UAE	R2W_Desert	V2Lib	V.zerda	-0.0155241	0.00100562	-15.4373
V9W_Nile	V3UK	V2Lib	V4Port	-0.00798247	0.000514525	-15.5142
V8Nile	V1Alg	R2W_Desert	V4Port	-0.0154613	0.000994769	-15.5426
R1E_Desert	V2Lib	V5UAE	V.zerda	-0.00326466	0.000209789	-15.5617
V1Alg	V7WMCD	V2Lib	V6E_Nile	-0.0138569	0.000889294	-15.5819
V3UK	R2W_Desert	V7WMCD	V.zerda	-0.0116155	0.000743939	-15.6135
R1E_Desert	V3UK	V7WMCD	V4Port	-0.0194422	0.00124493	-15.6171
V8Nile	V4Port	V3UK	V7WMCD	-0.0150347	0.000960436	-15.654
V8Nile	V5UAE	V1Alg	V3UK	-0.0089349	0.000567933	-15.7323
V8Nile	V4Port	R1E_Desert	V6E_Nile	-0.0170598	0.00108163	-15.7723
V8Nile	V3UK	R1E_Desert	V6E_Nile	-0.0152656	0.000967599	-15.7767
R1E_Desert	V1Alg	V.zerda	V6E_Nile	-0.00996565	0.000628552	-15.8549
V9W_Nile	R2W_Desert	R1E_Desert	V5UAE	-0.0237804	0.00149564	-15.8998
R1E_Desert	V5UAE	V2Lib	V7WMCD	-0.0104026	0.000650793	-15.9844
V8Nile	V.zerda	V1Alg	R2W_Desert	-0.0126476	0.00079024	-16.0048
V.lagopus	V5UAE	V7WMCD	V6E_Nile	-0.0129835	0.000808856	-16.0517
R1E_Desert	V2Lib	V3UK	R2W_Desert	-0.00575056	0.000356226	-16.143
V3UK	R2W_Desert	V2Lib	V6E_Nile	-0.0148264	0.00091409	-16.2199
V9W_Nile	R1E_Desert	V2Lib	V7WMCD	-0.0220701	0.00135762	-16.2564
V1Alg	V4Port	V.zerda	V6E_Nile	-0.0156999	0.000965749	-16.2567
R1E_Desert	V4Port	V.lagopus	V1Alg	-0.0144013	0.000878271	-16.3973
V9W_Nile	R2W_Desert	V8Nile	V6E_Nile	-0.0073027	0.000440405	-16.5818
R1E_Desert	V4Port	V1Alg	V3UK	-0.0166508	0.0010016	-16.6243
R1E_Desert	R2W_Desert	V7WMCD	V.zerda	-0.0107527	0.000646581	-16.6301
R1E_Desert	V2Lib	V5UAE	R2W_Desert	-0.00410414	0.000246756	-16.6324
V9W_Nile	V5UAE	V8Nile	V4Port	-0.0158359	0.000950566	-16.6595
V8Nile	V2Lib	R1E_Desert	R2W_Desert	-0.0168252	0.00100769	-16.6968
V1Alg	V2Lib	R2W_Desert	V6E_Nile	-0.00899601	0.000538458	-16.707

R1E_Desert	V7WMCD	V3UK	V.zerda	-0.017337	0.00103458	-16.7575
V8Nile	V6E_Nile	V1Alg	V4Port	-0.0141189	0.000841078	-16.7867
V2Lib	V4Port	V7WMCD	V.zerda	-0.0188356	0.00111976	-16.8211
R1E_Desert	V2Lib	V3UK	V7WMCD	-0.0043827	0.000259535	-16.8868
V1Alg	V4Port	R2W_Desert	V.zerda	-0.0191871	0.00113571	-16.8943
R1E_Desert	V2Lib	V.lagopus	V4Port	-0.0190978	0.00112442	-16.9846
V5UAE	V2Lib	V3UK	V6E_Nile	-0.0236168	0.00139036	-16.9861
V9W_Nile	R1E_Desert	V.lagopus	V6E_Nile	-0.0200897	0.00117802	-17.0538
V8Nile	V7WMCD	V2Lib	V.zerda	-0.0158457	0.000928017	-17.0748
V5UAE	V4Port	V1Alg	V6E_Nile	-0.0192164	0.00111507	-17.2333
V5UAE	V1Alg	V2Lib	V7WMCD	-0.0188657	0.00109205	-17.2755
V8Nile	V5UAE	V2Lib	V.zerda	-0.0160549	0.000929164	-17.2789
V8Nile	V5UAE	V7WMCD	V.zerda	-0.0105128	0.000604591	-17.3883
R1E_Desert	V5UAE	V1Alg	V3UK	-0.0138094	0.000790874	-17.461
R1E_Desert	V2Lib	V4Port	V.zerda	-0.00445431	0.000253215	-17.591
V1Alg	V7WMCD	V2Lib	V.zerda	-0.0190603	0.00108343	-17.5925
R1E_Desert	V4Port	V.lagopus	V7WMCD	-0.0147001	0.000833088	-17.6454
V3UK	V7WMCD	V4Port	V.zerda	-0.0136749	0.000774551	-17.6553
V2Lib	V4Port	R2W_Desert	V7WMCD	-0.0170821	0.000966891	-17.6671
V8Nile	V4Port	V3UK	V6E_Nile	-0.0192317	0.00108557	-17.7157
V8Nile	V7WMCD	V2Lib	V4Port	-0.0180677	0.00101853	-17.739
V9W_Nile	R2W_Desert	R1E_Desert	V.lagopus	-0.0248574	0.00139763	-17.7853
V8Nile	V5UAE	V1Alg	R2W_Desert	-0.0122602	0.000684772	-17.9041
V1Alg	R2W_Desert	V7WMCD	V6E_Nile	-0.0182757	0.00102003	-17.9168
V9W_Nile	V2Lib	V8Nile	V4Port	-0.00789661	0.000439836	-17.9535
V5UAE	V1Alg	V2Lib	R2W_Desert	-0.0234929	0.00130557	-17.9944
V5UAE	V7WMCD	V1Alg	V6E_Nile	-0.0137681	0.000763364	-18.0361
V3UK	V2Lib	R2W_Desert	V4Port	-0.0200817	0.0011041	-18.1883
V.lagopus	V4Port	V5UAE	V6E_Nile	-0.0207133	0.00113776	-18.2054
V5UAE	V.zerda	V7WMCD	V4Port	-0.0223985	0.0012226	-18.3204
V5UAE	V3UK	V.zerda	V6E_Nile	-0.0239666	0.00130765	-18.328
V5UAE	R2W_Desert	V2Lib	V4Port	-0.0209833	0.00114199	-18.3744
V.lagopus	V6E_Nile	V7WMCD	V4Port	-0.0187555	0.00101896	-18.4065
V5UAE	V6E_Nile	V1Alg	V3UK	-0.0222936	0.00120891	-18.4411
V8Nile	V6E_Nile	V1Alg	V7WMCD	-0.0172425	0.000931387	-18.5128
V8Nile	V6E_Nile	V.lagopus	V3UK	-0.0119592	0.000645617	-18.5237
V8Nile	V2Lib	V5UAE	V3UK	-0.0218974	0.0011811	-18.5399
R1E_Desert	V3UK	V2Lib	R2W_Desert	-0.0190236	0.00102399	-18.5779
V1Alg	V6E_Nile	V2Lib	V7WMCD	-0.026936	0.00144885	-18.5913
V8Nile	V5UAE	V4Port	V.zerda	-0.011012	0.000591799	-18.6077
V8Nile	R2W_Desert	V2Lib	V6E_Nile	-0.0216716	0.00116423	-18.6146
R1E_Desert	V5UAE	R2W_Desert	V4Port	-0.019479	0.00104631	-18.6168
V5UAE	V6E_Nile	V2Lib	V7WMCD	-0.0170374	0.000913914	-18.6422
V8Nile	V7WMCD	V5UAE	V2Lib	-0.0130361	0.000697789	-18.682
V1Alg	V4Port	V2Lib	R2W_Desert	-0.0190761	0.00101638	-18.7686
V5UAE	V6E_Nile	V7WMCD	V.zerda	-0.016671	0.000888101	-18.7715
V5UAE	R2W_Desert	V7WMCD	V.zerda	-0.0120503	0.000638979	-18.8586
R1E_Desert	V5UAE	V1Alg	V.zerda	-0.0145823	0.000769076	-18.9607
V8Nile	R1E_Desert	R2W_Desert	V7WMCD	-0.00885874	0.00046665	-18.9837

V5UAE	V1Alg	V2Lib	V.zerda	-0.0228161	0.00120071	-19.0023
R1E_Desert	V6E_Nile	V3UK	V4Port	-0.0130751	0.000679891	-19.2312
V2Lib	V4Port	V7WMCD	V6E_Nile	-0.0235044	0.0012219	-19.236
V5UAE	V4Port	V3UK	V7WMCD	-0.0147027	0.000762567	-19.2805
V5UAE	V6E_Nile	R2W_Desert	V7WMCD	-0.0199848	0.0010354	-19.3015
V3UK	V2Lib	R2W_Desert	V6E_Nile	-0.017611	0.000910392	-19.3444
V5UAE	V2Lib	V3UK	V7WMCD	-0.0234626	0.00121055	-19.3818
V2Lib	R2W_Desert	V7WMCD	V.zerda	-0.0251773	0.00128914	-19.5303
V.lagopus	V5UAE	R2W_Desert	V7WMCD	-0.0214795	0.00109378	-19.6379
V5UAE	V1Alg	V2Lib	V4Port	-0.0218376	0.00111143	-19.6482
V8Nile	V2Lib	V3UK	V.zerda	-0.0205614	0.00104645	-19.6487
V9W_Nile	V3UK	V8Nile	V.lagopus	-0.0203843	0.00103495	-19.696
V5UAE	R2W_Desert	V2Lib	V6E_Nile	-0.0171487	0.000865569	-19.8121
V.lagopus	V4Port	V3UK	V6E_Nile	-0.019677	0.000991731	-19.841
V9W_Nile	V5UAE	V8Nile	V.lagopus	-0.0168637	0.000847231	-19.9044
R1E_Desert	V6E_Nile	V.lagopus	V7WMCD	-0.0180168	0.0009026	-19.961
V5UAE	V6E_Nile	V7WMCD	V4Port	-0.0182271	0.000912105	-19.9835
V9W_Nile	V.lagopus	V8Nile	V5UAE	-0.0199419	0.000990421	-20.1348
V3UK	V2Lib	V7WMCD	V6E_Nile	-0.0128373	0.000637239	-20.1453
V9W_Nile	V.zerda	V3UK	R2W_Desert	-0.0313092	0.00155076	-20.1895
V8Nile	R1E_Desert	V4Port	V6E_Nile	-0.0223602	0.00110251	-20.2812
R1E_Desert	V5UAE	R2W_Desert	V6E_Nile	-0.0228798	0.0011267	-20.307
V9W_Nile	R2W_Desert	V8Nile	V4Port	-0.0122582	0.000602235	-20.3545
V8Nile	V7WMCD	V5UAE	V1Alg	-0.0099402	0.000487264	-20.4
V9W_Nile	V2Lib	R2W_Desert	V.zerda	-0.00976998	0.000478249	-20.4286
V9W_Nile	V4Port	V1Alg	V7WMCD	-0.0312704	0.00152217	-20.5433
R1E_Desert	V6E_Nile	V3UK	V2Lib	-0.0136688	0.000663644	-20.5966
V9W_Nile	V.zerda	V8Nile	R2W_Desert	-0.0250038	0.00120522	-20.7463
V2Lib	V6E_Nile	V7WMCD	V4Port	-0.0210913	0.00101517	-20.7762
V1Alg	V4Port	R2W_Desert	V6E_Nile	-0.0221872	0.00106344	-20.8636
V5UAE	V2Lib	R2W_Desert	V6E_Nile	-0.0283889	0.00135921	-20.8863
V9W_Nile	R1E_Desert	V1Alg	V6E_Nile	-0.0223738	0.00106956	-20.9187
V8Nile	R1E_Desert	V4Port	V.zerda	-0.0192609	0.000920363	-20.9275
V9W_Nile	V.zerda	R1E_Desert	V4Port	-0.0234412	0.00111281	-21.0648
V.lagopus	V5UAE	R2W_Desert	V6E_Nile	-0.0205551	0.000971072	-21.1674
V9W_Nile	V1Alg	V8Nile	R1E_Desert	-0.0188932	0.000889796	-21.2332
V9W_Nile	R1E_Desert	V8Nile	V.lagopus	-0.0312435	0.00146788	-21.2848
V9W_Nile	V4Port	R1E_Desert	V6E_Nile	-0.0239065	0.00110374	-21.6597
V9W_Nile	V4Port	V8Nile	V.lagopus	-0.0257006	0.00118319	-21.7214
V9W_Nile	V5UAE	V3UK	V2Lib	-0.0268088	0.00123226	-21.7558
V8Nile	V6E_Nile	V4Port	V.zerda	-0.0129782	0.000594947	-21.8141
R1E_Desert	R2W_Desert	V.lagopus	V7WMCD	-0.0263509	0.00120301	-21.904
R1E_Desert	V4Port	V.zerda	V6E_Nile	-0.0178	0.000810815	-21.9532
V1Alg	V7WMCD	V2Lib	V4Port	-0.0211449	0.000954989	-22.1415
V8Nile	V.lagopus	R1E_Desert	V3UK	-0.0311948	0.00140854	-22.147
V8Nile	V3UK	V1Alg	V7WMCD	-0.0307439	0.00138782	-22.1527
V3UK	V.zerda	R2W_Desert	V6E_Nile	-0.0155706	0.000701768	-22.1877
V5UAE	V3UK	R2W_Desert	V6E_Nile	-0.0236884	0.00106689	-22.2032
V8Nile	V2Lib	V4Port	V6E_Nile	-0.0255264	0.00114542	-22.2856

V8Nile	V3UK	V7WMCD	V6E_Nile	-0.0240795	0.00108022	-22.2913
V.lagopus	V4Port	V3UK	V7WMCD	-0.0265679	0.00118961	-22.3332
V8Nile	R1E_Desert	V5UAE	V2Lib	-0.0206904	0.00092352	-22.4039
V9W_Nile	R2W_Desert	V.zerda	V6E_Nile	-0.0310148	0.00138178	-22.4456
V8Nile	R1E_Desert	V5UAE	R2W_Desert	-0.0241196	0.00107214	-22.4967
V1Alg	R2W_Desert	V3UK	V4Port	-0.0313294	0.0013924	-22.5003
R1E_Desert	R2W_Desert	V.lagopus	V6E_Nile	-0.0251762	0.00111582	-22.5629
R1E_Desert	V.zerda	R2W_Desert	V6E_Nile	-0.023313	0.00103192	-22.5919
V5UAE	V7WMCD	V3UK	V4Port	-0.0211226	0.000933556	-22.626
V8Nile	V4Port	V5UAE	V2Lib	-0.0213953	0.000945513	-22.6282
V9W_Nile	V5UAE	R1E_Desert	V3UK	-0.0244044	0.00107656	-22.6688
V9W_Nile	V.zerda	V5UAE	V4Port	-0.0254275	0.00111329	-22.8401
R2W_Desert	V6E_Nile	V4Port	V.zerda	-0.0277701	0.00121406	-22.8737
V9W_Nile	V4Port	V7WMCD	V6E_Nile	-0.026561	0.00116022	-22.893
R1E_Desert	V6E_Nile	V1Alg	V3UK	-0.0220702	0.000963038	-22.9172
R1E_Desert	V7WMCD	V3UK	V2Lib	-0.0326332	0.00141968	-22.9863
V.lagopus	V2Lib	R2W_Desert	V7WMCD	-0.0250521	0.00108791	-23.0278
V.lagopus	V2Lib	R2W_Desert	V6E_Nile	-0.0254333	0.00110365	-23.0446
V5UAE	V7WMCD	V3UK	V2Lib	-0.0182785	0.000791429	-23.0956
V5UAE	V7WMCD	V3UK	V6E_Nile	-0.0246088	0.0010652	-23.1024
R1E_Desert	R2W_Desert	V.lagopus	V1Alg	-0.0306848	0.00132655	-23.1313
V.lagopus	V5UAE	V1Alg	V7WMCD	-0.0251931	0.0010852	-23.2152
V7WMCD	V.zerda	V4Port	V6E_Nile	-0.0214247	0.000921452	-23.2511
R1E_Desert	V6E_Nile	V1Alg	V.zerda	-0.0229023	0.000984161	-23.2709
V5UAE	V2Lib	V7WMCD	V6E_Nile	-0.0235342	0.00100696	-23.3715
R1E_Desert	V7WMCD	V5UAE	R2W_Desert	-0.0325093	0.00137905	-23.5736
R2W_Desert	V4Port	V7WMCD	V6E_Nile	-0.0258221	0.00109448	-23.593
V8Nile	V1Alg	R2W_Desert	V6E_Nile	-0.0199503	0.000845427	-23.5979
V.lagopus	V4Port	V3UK	V2Lib	-0.0227312	0.000961523	-23.6409
V8Nile	V.lagopus	V1Alg	V7WMCD	-0.024001	0.00101499	-23.6467
V8Nile	V6E_Nile	V3UK	V7WMCD	-0.0100615	0.000423306	-23.7688
V.lagopus	V2Lib	V1Alg	V6E_Nile	-0.0233432	0.000982046	-23.77
R1E_Desert	V7WMCD	V5UAE	V3UK	-0.0325918	0.00137021	-23.7859
V2Lib	R2W_Desert	V.zerda	V6E_Nile	-0.0282245	0.00118601	-23.7978
V8Nile	V7WMCD	V3UK	V2Lib	-0.0233797	0.000979742	-23.8631
R1E_Desert	R2W_Desert	V1Alg	V.zerda	-0.0249015	0.00104133	-23.9133
V.lagopus	V4Port	V3UK	R2W_Desert	-0.0308983	0.0012915	-23.9244
V8Nile	V2Lib	R1E_Desert	V1Alg	-0.0191633	0.00079666	-24.0545
R1E_Desert	V5UAE	V.lagopus	R2W_Desert	-0.0236587	0.000981195	-24.1121
V9W_Nile	V.zerda	R1E_Desert	R2W_Desert	-0.0230916	0.000957418	-24.1186
V5UAE	V4Port	V3UK	V6E_Nile	-0.0257943	0.00106926	-24.1235
R1E_Desert	V3UK	V5UAE	V.zerda	-0.023936	0.000991668	-24.1371
R1E_Desert	V.zerda	R2W_Desert	V4Port	-0.0242168	0.00100062	-24.2017
V8Nile	V.lagopus	V5UAE	V1Alg	-0.0280034	0.00115673	-24.2092
V8Nile	R2W_Desert	V1Alg	V4Port	-0.0233349	0.000962452	-24.2453
V9W_Nile	V4Port	V5UAE	V6E_Nile	-0.0352954	0.00145511	-24.2561
R1E_Desert	V6E_Nile	V1Alg	R2W_Desert	-0.0217536	0.000896802	-24.2568
V9W_Nile	V3UK	V8Nile	V4Port	-0.0295401	0.00121644	-24.2841
V.lagopus	V6E_Nile	V5UAE	V1Alg	-0.033137	0.00136379	-24.2978

R1E_Desert	V4Port	V5UAE	V1Alg	-0.0324803	0.00133295	-24.3672
R1E_Desert	V6E_Nile	R2W_Desert	V4Port	-0.0327023	0.00134145	-24.3784
R1E_Desert	V7WMCD	V4Port	V.zerda	-0.0318599	0.00130399	-24.4327
R1E_Desert	V6E_Nile	R2W_Desert	V.zerda	-0.0337258	0.0013754	-24.5207
V9W_Nile	V4Port	V8Nile	V2Lib	-0.0302253	0.00122733	-24.627
V8Nile	V7WMCD	V2Lib	V6E_Nile	-0.0202838	0.0008212	-24.7002
V8Nile	V4Port	V1Alg	V6E_Nile	-0.0245814	0.00099444	-24.7189
V9W_Nile	V4Port	V.lagopus	V5UAE	-0.0274884	0.00110781	-24.8132
V9W_Nile	V4Port	V3UK	V7WMCD	-0.0323185	0.00130243	-24.8139
R1E_Desert	V4Port	V5UAE	V.zerda	-0.0332791	0.00133794	-24.8735
V8Nile	V4Port	V1Alg	V.zerda	-0.0270673	0.00108618	-24.9197
R2W_Desert	V6E_Nile	V7WMCD	V.zerda	-0.026938	0.00107855	-24.9761
R1E_Desert	V.zerda	R2W_Desert	V7WMCD	-0.0291015	0.00116418	-24.9975
R2W_Desert	V6E_Nile	V7WMCD	V4Port	-0.0261939	0.00104762	-25.0032
V2Lib	V.zerda	R2W_Desert	V6E_Nile	-0.0334279	0.00133481	-25.0431
V8Nile	R1E_Desert	V5UAE	V.zerda	-0.0230616	0.000916569	-25.1608
V5UAE	V2Lib	V4Port	V.zerda	-0.0215686	0.000857041	-25.1663
R2W_Desert	V7WMCD	V.zerda	V6E_Nile	-0.0320431	0.00126974	-25.2359
R1E_Desert	R2W_Desert	V1Alg	V2Lib	-0.0313558	0.00124198	-25.2466
V1Alg	R2W_Desert	V2Lib	V6E_Nile	-0.0262618	0.00103544	-25.3629
V8Nile	V6E_Nile	V2Lib	R2W_Desert	-0.020708	0.000814872	-25.4126
V8Nile	V.lagopus	V.zerda	V6E_Nile	-0.0264377	0.00103897	-25.4459
V1Alg	R2W_Desert	V3UK	V2Lib	-0.0261182	0.00102523	-25.4753
V1Alg	R2W_Desert	V3UK	V.zerda	-0.0270977	0.00106299	-25.4919
V5UAE	V7WMCD	V4Port	V.zerda	-0.023188	0.000908155	-25.533
V1Alg	V7WMCD	V3UK	V2Lib	-0.0261046	0.00102165	-25.5514
V.lagopus	V4Port	V2Lib	R2W_Desert	-0.0502926	0.001961	-25.6465
V5UAE	V6E_Nile	V1Alg	V4Port	-0.0324796	0.00125887	-25.8005
R1E_Desert	V6E_Nile	V1Alg	V4Port	-0.0277531	0.00107275	-25.8711
R1E_Desert	V5UAE	V.lagopus	V1Alg	-0.0300074	0.00115791	-25.9153
V8Nile	V6E_Nile	V2Lib	V.zerda	-0.0236248	0.000911465	-25.9195
V9W_Nile	R2W_Desert	V.lagopus	V6E_Nile	-0.0351032	0.00135223	-25.9595
V.lagopus	R2W_Desert	V1Alg	V4Port	-0.0244518	0.000940151	-26.0084
V.lagopus	V2Lib	R2W_Desert	V4Port	-0.0270905	0.00103738	-26.1143
V9W_Nile	V4Port	R1E_Desert	V2Lib	-0.0332904	0.00127424	-26.1257
V.lagopus	V6E_Nile	V3UK	V7WMCD	-0.0272773	0.00104234	-26.1694
R1E_Desert	V3UK	V.lagopus	V7WMCD	-0.0244935	0.000931897	-26.2835
V1Alg	R2W_Desert	V4Port	V6E_Nile	-0.0243559	0.000923815	-26.3644
V5UAE	R2W_Desert	V3UK	V.zerda	-0.0221568	0.000838351	-26.429
V9W_Nile	V5UAE	V8Nile	V3UK	-0.0209176	0.0007897	-26.488
V9W_Nile	V.zerda	V8Nile	V7WMCD	-0.0289478	0.00108587	-26.6585
V8Nile	V3UK	V5UAE	V6E_Nile	-0.0284818	0.00106652	-26.7054
V8Nile	V3UK	R1E_Desert	V7WMCD	-0.0327665	0.00122168	-26.8208
V5UAE	V3UK	V1Alg	V7WMCD	-0.036271	0.00135105	-26.8465
V3UK	V2Lib	V.zerda	V6E_Nile	-0.023227	0.000862507	-26.9296
R1E_Desert	V5UAE	R2W_Desert	V7WMCD	-0.0248537	0.000920959	-26.9867
V8Nile	R2W_Desert	V4Port	V6E_Nile	-0.0313118	0.00115968	-27.0004
V8Nile	V4Port	V5UAE	R2W_Desert	-0.03824	0.00140138	-27.2873
R1E_Desert	R2W_Desert	V.zerda	V6E_Nile	-0.0261682	0.000956749	-27.3511

V.lagopus	V6E_Nile	V5UAE	V4Port	-0.0254645	0.000924176	-27.5538
V.lagopus	V4Port	V2Lib	V6E_Nile	-0.0313651	0.00113583	-27.6143
R1E_Desert	V4Port	V.lagopus	V3UK	-0.0369069	0.00133611	-27.6227
V2Lib	V6E_Nile	R2W_Desert	V4Port	-0.0303831	0.00109916	-27.6421
V1Alg	R2W_Desert	V2Lib	V7WMCD	-0.0276387	0.000988113	-27.9711
V9W_Nile	V1Alg	V.lagopus	V4Port	-0.0276515	0.000984478	-28.0875
V.lagopus	V6E_Nile	V5UAE	R2W_Desert	-0.0286023	0.00101194	-28.2648
V9W_Nile	V.zerda	R2W_Desert	V4Port	-0.0287601	0.000999723	-28.7681
V8Nile	V.lagopus	V3UK	V6E_Nile	-0.0343732	0.00119392	-28.7901
V1Alg	V3UK	V7WMCD	V4Port	-0.0409057	0.00140571	-29.0996
V9W_Nile	R1E_Desert	V3UK	V7WMCD	-0.0313605	0.00107437	-29.1897
R1E_Desert	V3UK	V5UAE	V7WMCD	-0.0303711	0.00103267	-29.4101
V9W_Nile	V1Alg	R1E_Desert	V2Lib	-0.0381608	0.00129514	-29.4647
V8Nile	V.lagopus	V2Lib	V7WMCD	-0.0412151	0.00138806	-29.6927
V8Nile	V2Lib	V5UAE	V7WMCD	-0.0383078	0.00128195	-29.8824
V9W_Nile	V1Alg	V3UK	V6E_Nile	-0.0271484	0.000903326	-30.0538
V9W_Nile	R2W_Desert	V.lagopus	V3UK	-0.0337202	0.00111826	-30.1542
V9W_Nile	V4Port	R1E_Desert	V7WMCD	-0.0442909	0.00145062	-30.5325
V9W_Nile	V.zerda	V8Nile	V3UK	-0.0309721	0.001012	-30.6048
V8Nile	V4Port	V.lagopus	V2Lib	-0.0356804	0.00116539	-30.6167
V9W_Nile	V4Port	V.lagopus	V6E_Nile	-0.0478727	0.00155513	-30.7838
V9W_Nile	R1E_Desert	V5UAE	V3UK	-0.0475512	0.00153826	-30.9123
V5UAE	V1Alg	V.zerda	V6E_Nile	-0.0423586	0.00136488	-31.0347
V1Alg	V.zerda	V2Lib	V4Port	-0.0478428	0.00151027	-31.6783
V.lagopus	V5UAE	V1Alg	V6E_Nile	-0.0296042	0.000927277	-31.926
V5UAE	R2W_Desert	V2Lib	V7WMCD	-0.029199	0.000914092	-31.9431
V9W_Nile	V1Alg	V.lagopus	R2W_Desert	-0.0346294	0.00108277	-31.9822
V8Nile	R2W_Desert	V1Alg	V6E_Nile	-0.0440437	0.00137611	-32.0059
V.lagopus	V7WMCD	V1Alg	R2W_Desert	-0.0500971	0.00156248	-32.0627
V9W_Nile	V1Alg	V.lagopus	V5UAE	-0.0321435	0.000999549	-32.158
V1Alg	V4Port	V2Lib	V.zerda	-0.0438345	0.00136145	-32.1969
V3UK	V2Lib	R2W_Desert	V7WMCD	-0.0304483	0.000943543	-32.2702
V1Alg	V4Port	V3UK	V.zerda	-0.046477	0.00143777	-32.3258
V.lagopus	V5UAE	V3UK	V7WMCD	-0.0367591	0.00113669	-32.3387
V1Alg	V7WMCD	V2Lib	R2W_Desert	-0.0472848	0.0014584	-32.4223
R1E_Desert	R2W_Desert	V1Alg	V6E_Nile	-0.0365065	0.00111939	-32.613
V8Nile	R2W_Desert	V5UAE	V.zerda	-0.0520206	0.0015943	-32.6291
V5UAE	V6E_Nile	V1Alg	V7WMCD	-0.0337084	0.00102981	-32.7328
R1E_Desert	V5UAE	V.lagopus	V4Port	-0.0348601	0.00106395	-32.7649
V.lagopus	V5UAE	V3UK	V6E_Nile	-0.0342787	0.00104502	-32.8019
V9W_Nile	V.zerda	R1E_Desert	V5UAE	-0.0433831	0.00131924	-32.885
V9W_Nile	V3UK	V8Nile	V5UAE	-0.0499245	0.00151658	-32.9191
V5UAE	V2Lib	V3UK	V.zerda	-0.047151	0.00143013	-32.9697
V8Nile	R2W_Desert	V5UAE	V7WMCD	-0.0445398	0.00134273	-33.1711
V8Nile	R2W_Desert	V2Lib	V7WMCD	-0.0423803	0.00127573	-33.2204
V9W_Nile	R1E_Desert	V5UAE	V1Alg	-0.0533557	0.00160172	-33.3116
V1Alg	R2W_Desert	V4Port	V.zerda	-0.0349629	0.00104918	-33.324
V2Lib	V6E_Nile	V4Port	V.zerda	-0.0359177	0.00107675	-33.3575
V1Alg	R2W_Desert	V3UK	V7WMCD	-0.0351142	0.00103936	-33.7844

V1Alg	R2W_Desert	V7WMCD	V4Port	-0.0332006	0.000982016	-33.8086
R1E_Desert	V.zerda	V2Lib	R2W_Desert	-0.0343555	0.00100968	-34.026
R1E_Desert	V6E_Nile	V2Lib	V.zerda	-0.0327091	0.000960822	-34.0429
V9W_Nile	R1E_Desert	V4Port	V.zerda	-0.0514501	0.00150316	-34.2281
V9W_Nile	V4Port	V.lagopus	V1Alg	-0.0439531	0.00128374	-34.2382
V8Nile	V2Lib	V5UAE	V4Port	-0.0424588	0.0012328	-34.4411
V1Alg	V.zerda	R2W_Desert	V7WMCD	-0.0436111	0.00125991	-34.6144
V8Nile	R2W_Desert	V3UK	V7WMCD	-0.0594676	0.00171261	-34.7234
V1Alg	V3UK	V7WMCD	V6E_Nile	-0.0656641	0.00187689	-34.9856
V9W_Nile	V4Port	R1E_Desert	V3UK	-0.0536747	0.00153167	-35.0432
V.lagopus	V6E_Nile	V5UAE	V3UK	-0.0367126	0.00104549	-35.1153
V.lagopus	V2Lib	V1Alg	V7WMCD	-0.0323934	0.000922337	-35.121
V8Nile	V4Port	R1E_Desert	V5UAE	-0.0411393	0.00116857	-35.2048
V1Alg	R2W_Desert	V7WMCD	V.zerda	-0.0333519	0.000944639	-35.3065
V.lagopus	V2Lib	V1Alg	R2W_Desert	-0.0341023	0.000948718	-35.9457
V.lagopus	V7WMCD	R2W_Desert	V6E_Nile	-0.0455624	0.00126462	-36.0285
V8Nile	V2Lib	V7WMCD	V6E_Nile	-0.0588692	0.00162893	-36.1399
V.lagopus	V7WMCD	R2W_Desert	V4Port	-0.0452783	0.00124706	-36.3082
V.lagopus	V5UAE	V1Alg	V4Port	-0.0397715	0.00109179	-36.4277
R1E_Desert	V6E_Nile	R2W_Desert	V7WMCD	-0.0517426	0.00141995	-36.4398
V8Nile	V2Lib	V5UAE	R2W_Desert	-0.0460878	0.00126394	-36.4637
V1Alg	V.zerda	R2W_Desert	V4Port	-0.0427752	0.00117194	-36.4995
V8Nile	V3UK	R2W_Desert	V4Port	-0.0533278	0.00145433	-36.6682
V9W_Nile	V.zerda	R2W_Desert	V6E_Nile	-0.048702	0.00131852	-36.9369
R1E_Desert	V5UAE	V.lagopus	V2Lib	-0.0340612	0.00091447	-37.247
R2W_Desert	V7WMCD	V4Port	V6E_Nile	-0.0352972	0.000947121	-37.2679
V5UAE	V6E_Nile	V2Lib	V4Port	-0.0352644	0.000940687	-37.488
V9W_Nile	V4Port	V3UK	V6E_Nile	-0.0621733	0.00165589	-37.5469
V.lagopus	V2Lib	V1Alg	V3UK	-0.0335474	0.000882862	-37.9985
R1E_Desert	R2W_Desert	V1Alg	V4Port	-0.0357209	0.000938708	-38.0533
V.lagopus	V2Lib	V3UK	R2W_Desert	-0.0318385	0.000834805	-38.1389
V.lagopus	R2W_Desert	V1Alg	V7WMCD	-0.0335551	0.000878927	-38.1773
V2Lib	R2W_Desert	V4Port	V.zerda	-0.0506581	0.0013241	-38.2587
R1E_Desert	V5UAE	V.lagopus	V3UK	-0.0332884	0.000870041	-38.2607
V1Alg	V.zerda	V2Lib	R2W_Desert	-0.0426316	0.00110753	-38.4924
R1E_Desert	V6E_Nile	V2Lib	R2W_Desert	-0.0310919	0.000803578	-38.6919
V.lagopus	V6E_Nile	V5UAE	V2Lib	-0.037043	0.000950459	-38.9739
R1E_Desert	R2W_Desert	V1Alg	V3UK	-0.0356543	0.000914282	-38.997
R1E_Desert	V6E_Nile	V1Alg	V7WMCD	-0.0316856	0.000812115	-39.0162
V9W_Nile	R2W_Desert	V.lagopus	V2Lib	-0.0638698	0.00162837	-39.2231
V1Alg	V.zerda	R2W_Desert	V6E_Nile	-0.0441521	0.0011123	-39.6943
V.lagopus	V6E_Nile	V5UAE	V7WMCD	-0.0352476	0.000887897	-39.6979
R1E_Desert	V6E_Nile	V.lagopus	V3UK	-0.061479	0.0015392	-39.9423
V8Nile	V3UK	V.lagopus	V7WMCD	-0.0436037	0.00109073	-39.9766
R1E_Desert	V.zerda	V1Alg	R2W_Desert	-0.0335245	0.000837778	-40.0159
V1Alg	V7WMCD	V3UK	R2W_Desert	-0.0515854	0.00128408	-40.1732
V5UAE	V3UK	R2W_Desert	V7WMCD	-0.0518073	0.0012874	-40.242
V9W_Nile	V4Port	V5UAE	V3UK	-0.0651503	0.00161074	-40.4475
R1E_Desert	V6E_Nile	V.lagopus	V2Lib	-0.055796	0.00135999	-41.0268

V9W_Nile	R1E_Desert	V8Nile	V7WMCD	-0.055007	0.00132251	-41.5927
V5UAE	V7WMCD	V4Port	V6E_Nile	-0.0320467	0.000768949	-41.6759
R1E_Desert	V.lagopus	V3UK	R2W_Desert	-0.05533	0.00132484	-41.7634
V9W_Nile	V4Port	V8Nile	V1Alg	-0.0600802	0.00143409	-41.8943
V8Nile	R1E_Desert	V5UAE	V1Alg	-0.0311333	0.000741991	-41.9591
V8Nile	V.lagopus	R2W_Desert	V7WMCD	-0.0559681	0.00131863	-42.4441
V9W_Nile	V1Alg	R1E_Desert	R2W_Desert	-0.0684581	0.00160705	-42.5985
V9W_Nile	R2W_Desert	R1E_Desert	V4Port	-0.05393	0.00126394	-42.6681
V9W_Nile	V4Port	V7WMCD	V.zerda	-0.0564158	0.00131605	-42.8675
R1E_Desert	V6E_Nile	V.lagopus	V1Alg	-0.0566281	0.0013194	-42.9195
V8Nile	V4Port	R2W_Desert	V7WMCD	-0.0653583	0.00151614	-43.1084
R1E_Desert	V4Port	V5UAE	V2Lib	-0.0527581	0.00122	-43.2442
V.lagopus	R2W_Desert	V1Alg	V6E_Nile	-0.0342891	0.000792639	-43.2594
V9W_Nile	V.zerda	V5UAE	V3UK	-0.0557248	0.00128305	-43.4317
V1Alg	V7WMCD	V.zerda	V6E_Nile	-0.0727656	0.00165789	-43.8905
V8Nile	V3UK	V5UAE	V4Port	-0.0581596	0.0013247	-43.9041
V8Nile	V4Port	R1E_Desert	V3UK	-0.0708172	0.00161148	-43.9455
V.lagopus	V7WMCD	V1Alg	V2Lib	-0.0536727	0.00122004	-43.9924
R1E_Desert	V6E_Nile	V.lagopus	V5UAE	-0.0648178	0.00146302	-44.304
R1E_Desert	V4Port	V7WMCD	V.zerda	-0.0632866	0.00142651	-44.3645
V1Alg	V.zerda	V2Lib	V7WMCD	-0.0514763	0.00115768	-44.4651
R2W_Desert	V7WMCD	V4Port	V.zerda	-0.0360312	0.000809342	-44.5191
V1Alg	V.zerda	V2Lib	V6E_Nile	-0.0516276	0.0011433	-45.1566
V9W_Nile	V.zerda	V1Alg	V6E_Nile	-0.0511657	0.00112909	-45.316
V.lagopus	V2Lib	V3UK	V7WMCD	-0.0516926	0.00113648	-45.4848
R1E_Desert	V4Port	V5UAE	V7WMCD	-0.0569378	0.0012474	-45.6453
V5UAE	V2Lib	V3UK	R2W_Desert	-0.0749918	0.0016405	-45.7129
R1E_Desert	V6E_Nile	V.lagopus	V4Port	-0.0654115	0.00142655	-45.8528
R1E_Desert	R2W_Desert	V.lagopus	V2Lib	-0.0564386	0.00122228	-46.175
V9W_Nile	V.zerda	V8Nile	R1E_Desert	-0.0553011	0.00118466	-46.6811
R1E_Desert	V6E_Nile	V.lagopus	R2W_Desert	-0.0554794	0.00118682	-46.7462
V9W_Nile	V.zerda	V8Nile	V2Lib	-0.0612694	0.00129383	-47.3551
V.lagopus	R2W_Desert	V2Lib	V6E_Nile	-0.0538435	0.00113147	-47.5873
V8Nile	V3UK	V7WMCD	V4Port	-0.0537573	0.00111121	-48.3772
V5UAE	V2Lib	V7WMCD	V4Port	-0.051375	0.0010546	-48.7149
R1E_Desert	V5UAE	R2W_Desert	V.zerda	-0.0665675	0.00134911	-49.3418
R1E_Desert	V4Port	V5UAE	V6E_Nile	-0.0581328	0.0011757	-49.4452
R1E_Desert	R2W_Desert	V.lagopus	V4Port	-0.067258	0.00135262	-49.7242
R1E_Desert	R2W_Desert	V.lagopus	V3UK	-0.0671913	0.00134772	-49.8555
V8Nile	V.lagopus	V1Alg	V6E_Nile	-0.0535315	0.00106168	-50.4216
R1E_Desert	V4Port	V5UAE	V3UK	-0.056159	0.00111335	-50.4416
R1E_Desert	V4Port	V7WMCD	V6E_Nile	-0.0673403	0.00133238	-50.5414

Chapter 5

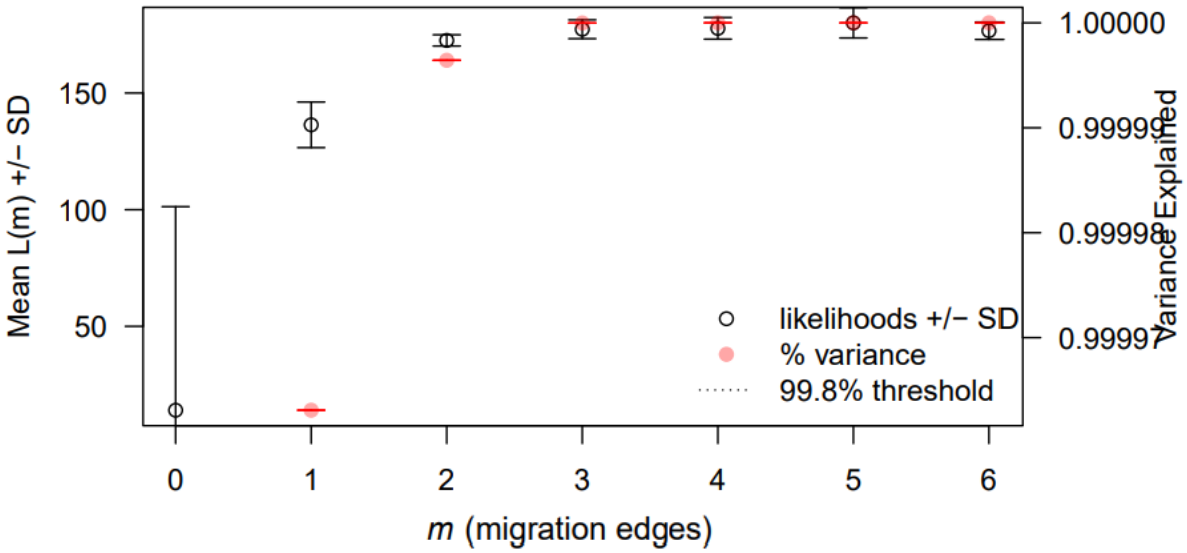
Appendix 5.1

Datasets and the assigned individuals used for whole genome resequencing. More detail on samples is in chapter 5, table 5.1.

Dataset, *allsamples14* Sample ID/SRA number	Dataset, *samples10* Sample ID/SRA number	Species	Reference
123	123	*V. vulpes*	This study
145	145	*V. vulpes*	This study
199	199	*V. vulpes*	This study
383	383	*V. vulpes*	This study
UAE2	UAE2	*V. vulpes*	This study
VvAL09	VvAL09	*V. vulpes*	This study
VvLY02	VvLY02	*V. vulpes*	This study
375	375	*V. rueppellii*	This study
376	376	*V. rueppellii*	This study
SRR5328110	SRR5328110	*V. vulpes*	Kukekova et al., 2018
ERR5417968	-	*V. lagopus*	Hasselgren et al., 2021
ERR5417974	-	*V. lagopus*	Hasselgren et al., 2021
SRR14750349	-	*V. zerda*	Phase One Resequencing for 10,000 Dog Genome Consortium
SRR14750511	-	*V. zerda*	Phase One Resequencing for 10,000 Dog Genome Consortium

Appendix 5.2

The output produced by OptM for the TreeMix results based on 6,570,819 SNPs (dataset, *allsamples14*). A total of 10 iterations were run for each possible number of migration edges, m= 1–6. The mean and standard deviation (SD) for the composite likelihood L(m) (left axis, black circles) and proportion of variance explained (right axis, red "x"s). The 99.8% threshold that is recommended by Pickrell and Pritchard (2012) is not visible here because it reached without adding the migration edges.



Appendix 5.3

The Admixture *f*3 statistic results based on 6,570,819 SNPs.

Populations (A; B, C)	***f3*-statistics**	**Standard Error**	**Z**
V.vulpes_Asia;V.vulpes_NorthAfrica,V.vulpes_Russia	0.00699643	0.000117755	59.4151
V.vulpes_NorthAfrica;V.vulpes_Asia,V.rueppellii	0.00578172	8.29E-05	69.7438
V.vulpes_Asia;V.vulpes_NorthAfrica,V.rueppellii	0.00950944	0.00012114	78.4993
V.vulpes_Asia;V.lagopus,V.vulpes_NorthAfrica	0.00912759	0.000110822	82.3627
V.vulpes_Asia;V.vulpes_NorthAfrica,V.zerda	0.0092068	0.000111353	82.6815
V.vulpes_NorthAfrica;V.vulpes_Asia,V.zerda	0.00608436	7.28E-05	83.59
V.vulpes_NorthAfrica;V.lagopus,V.vulpes_Asia	0.00616357	7.26E-05	84.8534
V.vulpes_Asia;V.vulpes_Russia,V.rueppellii	0.0125435	0.000142801	87.8392
V.vulpes_NorthAfrica;V.vulpes_Asia,V.vulpes_Russia	0.00829472	9.24E-05	89.7674
V.vulpes_Asia;V.lagopus,V.vulpes_Russia	0.0131007	0.000137896	95.0044
V.vulpes_Asia;V.vulpes_Russia,V.zerda	0.0130191	0.000136015	95.7184
V.vulpes_NorthAfrica;V.vulpes_Russia,V.rueppellii	0.0113288	0.000100454	112.776
V.vulpes_NorthAfrica;V.lagopus,V.vulpes_Russia	0.0122679	9.99E-05	122.753

V.vulpes_NorthAfrica;V.vulpes_Russia,V.zerda	0.012107	9.72E-05	124.525
V.vulpes_NorthAfrica;V.lagopus,V.rueppellii	0.0631271	0.000324901	194.297
V.vulpes_NorthAfrica;V.rueppellii,V.zerda	0.0642159	0.000319587	200.934
V.vulpes_Asia;V.lagopus,V.rueppellii	0.066473	0.000299054	222.278
V.vulpes_Asia;V.rueppellii,V.zerda	0.0676409	0.000292667	231.119
V.vulpes_NorthAfrica;V.lagopus,V.zerda	0.0877823	0.000314426	279.183
V.vulpes_Russia;V.vulpes_Asia,V.rueppellii	0.0662631	0.000234817	282.19
V.vulpes_Russia;V.vulpes_NorthAfrica,V.vulpes_Asia	0.0718102	0.000252713	284.157
V.vulpes_Russia;V.lagopus,V.vulpes_Asia	0.0657059	0.000226342	290.295
V.vulpes_Russia;V.vulpes_Asia,V.zerda	0.0657875	0.000226331	290.669
V.vulpes_Russia;V.vulpes_NorthAfrica,V.rueppellii	0.0687761	0.00022972	299.391
V.vulpes_Russia;V.lagopus,V.vulpes_NorthAfrica	0.067837	0.000221347	306.473
V.rueppellii;V.vulpes_NorthAfrica,V.vulpes_Russia	0.142942	0.000465968	306.764
V.vulpes_Russia;V.vulpes_NorthAfrica,V.zerda	0.0679979	0.00022136	307.182
V.vulpes_Asia;V.lagopus,V.zerda	0.0908256	0.000293318	309.649
V.rueppellii;V.vulpes_NorthAfrica,V.vulpes_Asia	0.148489	0.000473894	313.339
V.rueppellii;V.vulpes_Asia,V.vulpes_Russia	0.145455	0.000461268	315.338
V.vulpes_Russia;V.lagopus,V.rueppellii	0.119635	0.000307988	388.442
V.vulpes_Russia;V.rueppellii,V.zerda	0.120885	0.000305201	396.082
V.vulpes_Russia;V.lagopus,V.zerda	0.143512	0.000300782	477.131
V.rueppellii;V.vulpes_Asia,V.zerda	0.0903576	0.000179989	502.018
V.rueppellii;V.vulpes_Russia,V.zerda	0.0908332	0.000180495	503.246
V.rueppellii;V.vulpes_NorthAfrica,V.zerda	0.090055	0.000177473	507.428
V.rueppellii;V.lagopus,V.vulpes_Russia	0.0920828	0.000177909	517.582
V.rueppellii;V.lagopus,V.vulpes_Asia	0.0915256	0.000174988	523.039
V.rueppellii;V.lagopus,V.vulpes_NorthAfrica	0.0911437	0.000172575	528.139
V.lagopus;V.vulpes_NorthAfrica,V.vulpes_Russia	0.15841	0.000297947	531.672
V.lagopus;V.rueppellii,V.zerda	0.0839842	0.000156588	536.339
V.lagopus;V.vulpes_Asia,V.vulpes_Russia	0.160541	0.000291638	550.482
V.lagopus;V.vulpes_Russia,V.zerda	0.0827347	0.000150141	551.046
V.lagopus;V.vulpes_NorthAfrica,V.vulpes_Asia	0.164514	0.000293311	560.887
V.lagopus;V.vulpes_Asia,V.zerda	0.0828163	0.000146131	566.726
V.rueppellii;V.lagopus,V.zerda	0.11471	0.000201429	569.482
V.lagopus;V.vulpes_NorthAfrica,V.zerda	0.0828955	0.000144216	574.802
V.zerda;V.lagopus,V.vulpes_Russia	0.0962989	0.000164068	586.946
V.zerda;V.lagopus,V.vulpes_Asia	0.0962173	0.000160294	600.257
V.zerda;V.lagopus,V.rueppellii	0.0950494	0.00015774	602.568
V.zerda;V.lagopus,V.vulpes_NorthAfrica	0.0961381	0.000158166	607.83
V.zerda;V.vulpes_NorthAfrica,V.vulpes_Russia	0.171813	0.000275992	622.53
V.zerda;V.vulpes_Asia,V.vulpes_Russia	0.174024	0.000274773	633.337
V.zerda;V.vulpes_NorthAfrica,V.vulpes_Asia	0.177836	0.000277272	641.377
V.lagopus;V.vulpes_Russia,V.rueppellii	0.106612	0.000137074	777.766
V.lagopus;V.vulpes_Asia,V.rueppellii	0.107169	0.000136075	787.57
V.lagopus;V.vulpes_NorthAfrica,V.rueppellii	0.107551	0.000132031	814.587
V.zerda;V.vulpes_Russia,V.rueppellii	0.118926	0.000141222	842.121
V.zerda;V.vulpes_NorthAfrica,V.rueppellii	0.119705	0.000138783	862.53
V.zerda;V.vulpes_Asia,V.rueppellii	0.119402	0.000137043	871.274

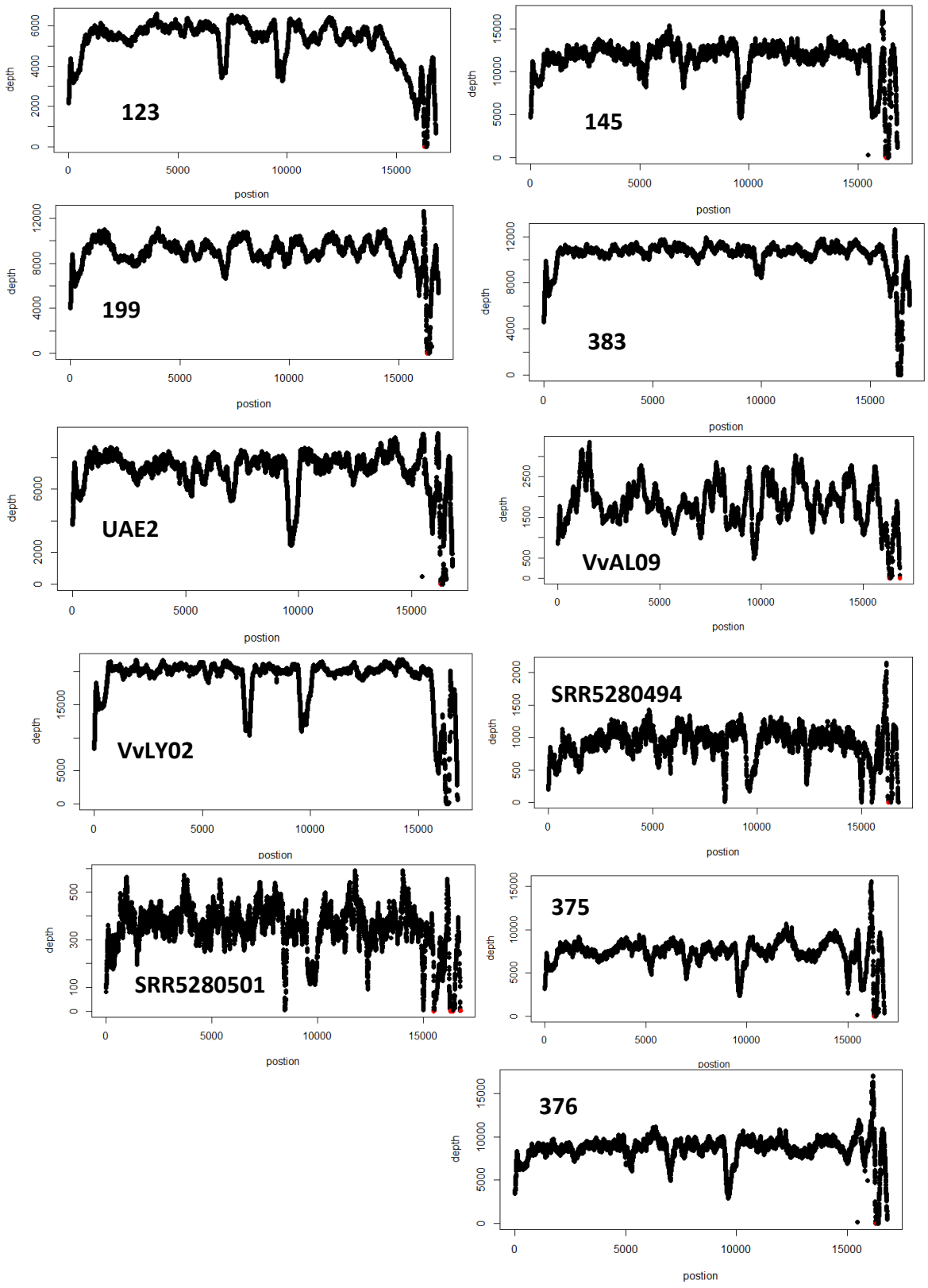
Appendix 5.4

The Admixture *f*4 statistic results based on 6,570,819 SNPs.

Populations ((A, B);(C, D))	*f4*-statistics	Standard Error	Z
V.lagopus,V.vulpes_Asia;V.vulpes_NorthAfrica,V.rueppellii	-0.0573454	0.000339507	-168.908
V.lagopus,V.vulpes_NorthAfrica;V.vulpes_Asia,V.rueppellii	-0.0569636	0.00034357	-165.799
V.lagopus,V.vulpes_Russia;V.vulpes_Asia,V.rueppellii	-0.0539295	0.00033489	-161.036
V.lagopus,V.vulpes_Asia;V.vulpes_Russia,V.rueppellii	-0.0533723	0.000337127	-158.315
V.lagopus,V.vulpes_Russia;V.vulpes_NorthAfrica,V.rueppellii	-0.0517983	0.000338937	-152.826
V.lagopus,V.vulpes_NorthAfrica;V.vulpes_Russia,V.rueppellii	-0.0508593	0.000345386	-147.254
V.lagopus,V.vulpes_NorthAfrica;V.vulpes_Asia,V.vulpes_Russia	-0.00610429	0.000108138	-56.449
V.lagopus,V.vulpes_Asia;V.vulpes_NorthAfrica,V.vulpes_Russia	-0.00397313	0.000113096	-35.1305
V.lagopus,V.rueppellii;V.vulpes_NorthAfrica,V.vulpes_Russia	-0.00093903	6.84E-05	-13.728
V.lagopus,V.rueppellii;V.vulpes_NorthAfrica,V.vulpes_Asia	-0.00038184	4.93E-05	-7.74265
V.lagopus,V.rueppellii;V.vulpes_Asia,V.vulpes_Russia	-0.00055719	7.24E-05	-7.69363
V.lagopus,V.vulpes_Russia;V.vulpes_NorthAfrica,V.vulpes_Asia	0.00213115	8.29E-05	25.706
V.vulpes_NorthAfrica,V.vulpes_Asia;V.vulpes_Russia,V.zerda	-0.00221036	8.41E-05	-26.2873
V.vulpes_NorthAfrica,V.vulpes_Asia;V.rueppellii,V.zerda	0.000302639	4.81E-05	6.29342
V.vulpes_Asia,V.vulpes_Russia;V.rueppellii,V.zerda	0.000475564	6.90E-05	6.88762
V.vulpes_NorthAfrica,V.vulpes_Russia;V.rueppellii,V.zerda	0.000778204	6.56E-05	11.863
V.vulpes_NorthAfrica,V.vulpes_Russia;V.vulpes_Asia,V.zerda	0.0038123	0.000110287	34.567
V.vulpes_NorthAfrica,V.zerda;V.vulpes_Asia,V.vulpes_Russia	0.00602266	0.000105689	56.9849
V.vulpes_NorthAfrica,V.zerda;V.vulpes_Russia,V.rueppellii	0.0521088	0.000339438	153.515
V.vulpes_NorthAfrica,V.rueppellii;V.vulpes_Russia,V.zerda	0.052887	0.000333705	158.484
V.vulpes_Asia,V.zerda;V.vulpes_Russia,V.rueppellii	0.0546218	0.000330454	165.294
V.vulpes_Asia,V.rueppellii;V.vulpes_Russia,V.zerda	0.0550974	0.000328692	167.626
V.vulpes_NorthAfrica,V.zerda;V.vulpes_Asia,V.rueppellii	0.0581315	0.000336828	172.585
V.vulpes_NorthAfrica,V.rueppellii;V.vulpes_Asia,V.zerda	0.0584341	0.000333175	175.386
V.vulpes_NorthAfrica,V.vulpes_Asia;V.vulpes_Russia,V.rueppellii	-0.002513	9.46E-05	-26.5672
V.vulpes_NorthAfrica,V.vulpes_Russia;V.vulpes_Asia,V.rueppellii	0.0030341	0.000116389	26.0685
V.vulpes_NorthAfrica,V.rueppellii;V.vulpes_Asia,V.vulpes_Russia	0.0055471	0.000114471	48.4585

Appendix 5.5

Sequencing depth of coverage across the mitogenomes of eleven individuals of *V. rueppellii* (375 and 376) and *V. vulpes* (all remaining samples). Red dots denote coverage of 10 reads or less. For detail on samples see chapter 5, table 5.2.



Appendix 5.6

Ambiguous positions of the extracted whole mitogenome sequences from the four bioinformatic approaches, chapter 5.

Sample	Gene/marker	Position	de novo		Reference-mapping (GATK)	
			MITObim	NOVOPlasty	Ploidy 1	Ploidy 2
376	tRNA-Phe	19	A	A	G	G
SRR5280494	NADH2	4,069	G	R	G	A
SRR5280494 SRR5280501	COI	5,865	A	A	A	C
UAE2	Cyt b	14,967	T	T	T	C
SRR5280494	Cyt b	15,000	G	G	A	A
SRR5280494 SRR5280501	Cyt b	15,006	C	C	T	T
SRR5280494 SRR5280501	D-loop	15,507	C	C	T	T
SRR5280494 SRR5280501	D-loop	15,509	A	A	G	G
SRR5280494	D-loop	15,546	A	A	C	C
376	D-loop	15,909	G	-	G	-

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