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META-ANALYSIS

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Challenging the European southern refugium hypothesis: Species-specific structures versus general patterns of genetic diversity and differentiation among small mammals

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Abstract

Aim: In this study, we conduct a quantitative meta-analysis to investigate broad patterns of genetic variation throughout large geographical regions in order to elucidate concordant geographical patterns across species and identify common historical processes to better inform the "cryptic refugia" versus the traditional "southern refugia" hypothesis debate.

Location: Europe.

Time period: Late Pleistocene to present day.

Major taxa studied: Small mammals (Rodentia, Eulipotyphla).

Methods: A meta-analysis was performed on large-scale patterns of genetic diversity for 19 species from 59 papers. For each species, haplotype and nucleotide diversity were calculated using the mitochondrial D-loop and compared to the species' range.

Results: No consistent patterns were observed between mitochondrial DNA (mtDNA) diversity indices (nucleotide and haplotype diversity) and any of the indicators of distribution examined [latitude and longitude (max, min, centre, range)]. The patterns of genetic diversity observed in all the 19 species studied appear to be species-specific.

Main conclusions: In contrast to the traditional southern refugial hypotheses, we found no evidence for a consistent south–north post-glacial expansion. Instead individual species appear to respond to climate oscillations in niche-specific ways. This individual nature of each species' phylogeographical history indicates a complex web of post-glacial recolonization dynamics across Europe.

KEYWORDS

colonization, Europe, mammals, molecular diversity, mtDNA, phylogeography, refugia

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1 | INTRODUCTION

The investigation of geographical distributions of genetic lineages and genetic diversity has allowed inferences about post-glacial colonization history in recent decades (the phylogeographical approach: Avise, 2000). There has been a particular focus on Europe, with studies on a wide range of plant and animal organisms (Bilton et al., 1998; Hewitt, 1999, 2004 ; Stewart, Lister, Barnes, & Dalén, 2010; Taberlet, Fumagalli, Wust-Saucy, & Cosson, 1998).

Initially, it was felt the data suggested that the majority of European temperate species recolonized central and northern Europe after the last glaciation from southern refugial areas (Hewitt, 1999, 2004 ; Seddon, Santucci, Reeve, & Hewitt, 2001). The loss of genetic diversity from southern to northern Europe has long been remarked upon (e.g., Hewitt, 1999, 2001, 2004 ; Kryštufek & Griffiths, 2002; Taberlet et al., 1998). European studies led to the identification of three glacial refugia in southern Europe, on the Iberian, Italian and Balkan peninsulas, with three main colonization models dominating latitudinal expansion patterns (Hewitt, 1999). Increasingly however, the existence of refugia outside the traditional southern peninsulae (so-called cryptic northern, or extra-Mediterranean, refugia: Bilton et al., 1998; Stewart & Lister, 2001; Schmitt & Varga, 2012 but see Tzedakis, Emerson, & Hewitt, 2013) has been recognized for a wide range of species including small mammals (e.g., central Europe; Deffontaine et al., 2005; Fink, Excoffier, & Heckel, 2004). Boreal and arctic species may be considered to be in refugia today, in comparison to colder periods of our glacial history when they maintained much wider distributions (Stewart & Dalén, 2008). These conflicting reports indicate that there is discordance between competing hypotheses of European post-glacial biogeographical processes.

The loss of both genetic and species diversity from southern to northern Europe has historically been thought to be a result of expansion from few limited refugia, that is, leading edge allele loss (Hewitt, 1996, 1999; Ibrahim, Nichols, & Hewitt, 1996; Taberlet et al., 1998; Waters, Fraser, & Hewitt, 2013), combined with recurrent losses of divergent populations over multiple glacial cycles (Hewitt, 1996; Hofreiter & Stewart, 2009; Seddon et al., 2001; Stewart, 2009; Stewart et al., 2010). Within species, it is generally expected that more northerly populations will be less genetically diverse than their southern counterparts (Hewitt, 2004; Seddon et al., 2001). Although multiple studies have examined patterns within species, few analyses have compared between species. Still fewer have looked at the potential variation in the type of movement (e.g., step-wise versus phalanx-wise; Habel, Ulrich, & Assmann, 2013), or concerned themselves with the lower latitude limit, or "rear edge," of species ranges (Hampe & Petit, 2005).

High-level "overview" hypotheses and trends can encourage understanding at a macro level and enable testing of multiple scenarios. General patterns may not be obvious when individual taxa and their phylogenetic relationships are examined separately (Taberlet et al., 1998). For this reason, syntheses are necessary and useful to provide a consistent overview—it is important to see both the woods *and* the trees. This paper investigates patterns of genetic diversity by examining various diversity indices across 19 small mammal species to test for trends and commonalities. Small mammals were selected because they comprise many species (Mitchell-Jones et al., 1999; Tougard & Renvoisé, 2008); have limited dispersal capabilities related to their small size, enabling relatively accurate estimation of range sizes; and have not been affected by man to the same extent as many larger mammals (Kryštufek & Griffiths, 2002) (with the exclusion of commensals such as domestic rats and house mice, which were excluded here). Furthermore, these species have been important in relation to the study of ice-age refugia in Europe (Bilton et al., 1998; Taberlet et al., 1998). Small mammals display short generation times and a fast-evolving genome (Martin & Palumbi, 1993; Tougard & Renvoisé, 2008) enabling us to detect the genetic variation of interest. A large number of studies from species retaining similar key characteristics were available to make this synthesis possible. Comparison of these species may provide insight into genetic diversity patterns related to colonization pathways in Europe. Here we investigate the previously hypothesized pathways of colonization through the examination of the mitochondrial DNA (mtDNA) control region of a range of small mammal species, and test for congruence of broad-scale genetic interspecific diversity patterns among those species that may infer shared evolutionary history. Further, we conduct an intraspecific analysis of four species with sufficient numerical and geographical coverage in Europe to enable comparison of their individual phylogeographical histories, and use this to further identify whether they support or contradict the "southern refugia" or "cryptic refugia" hypotheses.

2 | METHODS

The study area was taken at a broad scale to be political Europe. An initial list of potential species was compiled covering small mammal species from the orders Rodentia and Eulipotyphla. mtDNA was selected due in part to its relatively rapid rate of mutation, and its haploid maternal inheritance mechanism, both of which make it useful for the elucidation of population historical and demographical changes (Avise, 1995; Avise et al., 1987; Moritz, 1994). These features have allowed mtDNA to become one of the most frequently used markers in molecular ecology and phylogeographical studies, particularly in mammals (e.g., Centeno-Cuadros, Delibes, & Godoy, 2009; Centeno-Cuadros & Godoy, 2010; Hewitt, 2004). GenBank (www.ncbi.nlm.nih.gov/genbank/), the primary public archive for the deposition of genetic sequences, was searched for mtDNA control region (a.k.a. D-loop) sequences for these species. Where sequences were available, the primary literature and source papers for each study were found through online data searches (e.g., Google Scholar, PubMed). From these papers, frequencies and/or numbers of individuals for each haplotype were obtained (where published), enabling the full reconstruction of the original study data set through manual re-assembly using FASTA files downloaded from GenBank and associated haplotype data. These reconstructed data sets were

subsequently combined for each species to create species-level diversity estimates across the sampled range.

The sequences were aligned using the ClustalW algorithm in MEGA v.6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013) and revised manually. The differences in the lengths and/or sections of the mtDNA control region sequenced for each species were too great to enable analysis of the same mtDNA region in the different species. Within each species, sequences were standardized to the minimum overlapping sequence length available for each species. The total number of haplotypes and variable sites observed and the average number of nucleotide differences per sequence were calculated and reported.

Haplotype and nucleotide diversity are frequently used as measures of diversity in population genetics studies (Egeland & Salas, 2008; Goodall-Copestake, Tarling, & Murphy, 2012) and were estimated using DNASP v. 5.10.01 (Librado & Rozas, 2009). The numbers of sequences available per species varied markedly. In an attempt to compensate for this, standardized haplotype and nucleotide diversity estimates were calculated for each species. Standardized haplotype diversity was calculated for each species by random subsampling from the available individuals to match the smallest sample size encountered. Standardized nucleotide diversity was calculated for each species by random subsampling from the available unique haplotypes to match the smallest number of unique haplotypes encountered.

To investigate direction of range expansion, mtDNA diversity indices were related to distribution. Distribution data were downloaded from the International Union for Conservation of Nature (IUCN) terrestrial mammals database (https://maps.iucnredlist.org, date of download 27/05/2014). The ranges of our selected species were clipped to the extent of political Europe using country outlines from the Global Administrative Areas database [(http://)www.gadm.org, downloaded 13/05/2014] and maximum and minimum latitude and longitude were calculated for each species. The clipped extents of occurrence were then transformed using the Lambert Azimuthal Equal Area projection for Europe and area (in km^2) was calculated for each. These variables were then used to test for relationships with absolute and standardized haplotype and nucleotide diversity estimates both across and within species using correlations (Spearman's and Pearson's) and regressions (linear, quadratic and multiple) carried out in the SPSS v. 20 statistical environment (IBM Corp. 2011).

Haplotype sampling coverage (i.e., what proportion of the population/genetic diversity is represented by our samples) was estimated using the methods of Dixon (2006), based on the number of haplotypes and individuals sampled using the Stirling probability distribution and Bayes theorem to obtain a posterior distribution of the total number of haplotypes in the population including those not yet observed. Haplotype accumulation curves were also produced using the R package "spider" (R Core Team, 2017) (Brown et al., 2012).

For a subset of four species, population genetic diversity and distributional trends were investigated. These investigations were limited to those species that had 75% or higher haplotype sampling completeness (according to Dixon's measure, Dixon, 2006), had large enough distributions to display evidence of post-Last Glacial Maximum (post-LGM) expansion and a minimum of 100 sampled individuals spread reasonably well across their distribution [*Cricetus cricetus*, *Erinaceus europaeus*, *Microtus arvalis*, *Clethrionomys (= Myodes) glareolus*]. Individuals of these species were pooled into populations (minimum of five individuals per population, Centeno-Cuadros et al., 2009) based upon the reported study sites in the respective papers, and the aforementioned diversity estimates were again calculated. Where this was not possible (location information not provided, or fewer than five individuals per population), individuals were pooled for a country-level estimate. Sampling site information was reported in a number of different ways in the literature (site names/nearest town, latitude and longitude/grid references) and so all were converted to latitudes and longitudes in decimal degrees for analysis. Where only regional location data were provided, central latitude and longitude values for the region were used for the within-species analysis.

3 | RESULTS

3.1 | Between-species investigation

The data collection was ceased in May 2015 to allow the meta-analysis to proceed. A total of 113 species were investigated for sequences deposited in GenBank (searched using species name, with D-loop or control region as keywords). Fifty-four species were found to have GenBank data from 123 studies. Authors were contacted for studies in which the required information was not presented in the paper or the supporting materials, or for which a published paper could not be found (22% of studies). Of those contacted 61% agreed to provide the information; however, many of the authors of the older studies were no longer contactable.

Difficulty was met in the re-assembly of data from the primary literature. Similar to Goodall-Copestake et al. (2012), we found a wide range in the behaviours of those depositing sequences in public archives. Of those that did deposit their sequences, some deposited sequences for all individuals examined, some deposited only each unique haplotype encountered, and others deposited only a single haplotype with notes on the edits needed to reconstruct all of the haplotypes from the study (a time-consuming process). Of those that did deposit sequences in GenBank, many papers did not report the frequencies/numbers of individuals for each haplotype, thus making reconstruction impossible and necessitating their exclusion from the study, greatly reducing the number of species/studies that could be included, and highlighting the need for detailed data archiving. Only one study (Tougard, Renvoisé, Petitjean, & Quéré, 2008) had to be excluded from the between-species analysis due to the segments of the mtDNA control region analysed not overlapping with those of the other studies examined. These sequences were retained for the within-species population-level diversity estimates and comparisons. This process resulted in a final count of 19 species with complete/reconstructed useable data from 59 papers (see Table 1).

TABLE 1 Distributional area and primary habitat type of the study species

Order	Family	Species	Common name	Distributional area (10^6 km^2)	Habitat
Eulipotyphla	Erinaceidae	*Erinaceus concolor*	Eastern European hedgehog	1.35	Urban, suburban and agricultural areas
Eulipotyphla	Erinaceidae	*Erinaceus europaeus*	Western European hedgehog	3.81	Man-made habitats, agricultural land, deciduous woodland, and grasslands
Eulipotyphla	Erinaceidae	*Erinaceus roumanicus*	Northern white-breasted hedgehog	6.03	Man-made habitats farmland, scrubby and shrubby habitats
Eulipotyphla	Soricidae	*Crocidura russula*	Greater white-toothed shrew	1.93	Shrubland, open habitats, forest edges with abundant vegetation, cultivated fields, urban areas
Eulipotyphla	Soricidae	*Sorex antinorii*	Valais shrew	0.29	Areas with dense vegetation cover
Eulipotyphla	Soricidae	*Sorex minutus*	Eurasian pygmy shrew	14.29	Damp areas with dense vegetation; swamps, grasslands, heaths, sand dunes, woodland edge, rocky areas, shrubland, and montane forests
Rodentia	Cricetidae	*Arvicola sapidus*	Southern water vole	0.88	Hydrophilic vegetation and shorelines
Rodentia	Cricetidae	*Chionomys nivalis*	European snow vole	0.89	Open, rocky areas, typically above the tree line
Rodentia	Cricetidae	*Cricetus cricetus*	Common hamster	6.36	Man-made habitats, fertile steppe and grassland
Rodentia	Cricetidae	*Lemmus lemmus*	Norway lemming	0.76	Alpine and subarctic habitats, for example, peat bogs, dwarf shrub heaths, and sparsely vegetated slopes and ridges
Rodentia	Cricetidae	*Microtus guentheri*	Günther's vole	0.64	Dry grasslands with sparse vegetation on well-drained soil (natural and man-made)
Rodentia	Cricetidae	*Microtus arvalis*	Common vole	6.89	Open habitats including meadows, steppe, and agricultural areas
Rodentia	Cricetidae	*Microtus thomasi*	Thomas's pine vole	0.11	Open areas with deep soil; meadows, high mountain pastures and arable farmland
Rodentia	Cricetidae	*Clethrionomys (Myodes) glareolus*	Bank vole	8.68	All woodland, preferring densely vegetated clearings, woodland edge, and riverbanks. Also found in scrub, parkland, and hedges
Rodentia	Muridae	*Apodemus mystacinus*	Eastern broad-toothed field mouse	0.80	Forest with rocky areas; sparse cover of grass or shrubs
Rodentia	Muridae	*Mus macedonicus*	Macedonian mouse	1.39	Cultivated land, road verges, sand dunes, shrubland, and densely vegetated riverbanks. Absent from dense forests, and avoids urban areas. Restricted to areas with > 400 mm of rain per annum
Rodentia	Muridae	*Mus spicilegus*	Mound-building mouse	0.83	Natural steppe grasslands, pastures, open woodland and clearings
Rodentia	Sciuridae	*Sciurus vulgaris*	Eurasian red squirrel	19.9	Lowland to subalpine coniferous forests and deciduous woods, along with parks and gardens
Rodentia	Sciuridae	*Spermophilus suslicus*	Speckled ground squirrel	0.8	Open areas with short grass; steppes, pastures, road verges and occasionally cultivated

Note. Distributional area refers to the area occupied by each species as obtained from the IUCN database to the nearest 10^6 km^2, which does not take patchiness into account. Habitat is the primary vegetation cover type preference for each species as obtained from IUCN species profiles (www.iucnredlist.org). GenBank accession numbers and source papers are available in Supporting Information Appendix S1.

The minimum number of individuals sampled for a single species was 21 for the eastern broad-toothed field mouse (*Apodemus mystacinus*; sampled from Turkey, Syria, Greece, Bulgaria). This figure is close to the recommended minimum sample size of 25 from Goodall-Copestake et al. (2012) for the cytochrome c oxidase subunit I (COX1) mtDNA gene. We realize that this recommendation was for population level rather than species sampling; however, we would highlight that the cox1 gene is a more conserved region of the mtDNA. This, coupled with the limitations of the available data, led us to accept 21 individuals as the lowest acceptable sample size for this analysis, and thus 21 individuals were randomly selected for each species to calculate the standardized haplotype diversity. The lowest number of haplotypes observed was six haplotypes in 55 individuals of the northern white-breasted hedgehog (*Erinaceus roumanicus*; Czech Republic, Slovak Republic, Poland); accordingly, six randomly selected unique haplotypes were selected from each species for calculating standardized nucleotide diversity values.

Haplotype diversity measures ranged from 0.32 to 0.99 and standardized haplotype diversities from 0.35 to 0.99 (Table 2). Nucleotide diversity measures ranged from 0.002 to 0.048 and standardized nucleotide diversity from 0.006 to 0.069 (Table 2). Although the number of haplotypes increased significantly (R^2 = 0.744, p < 0.0001) with area, as is expected, no relationship was evident between area and any of the measures of genetic diversity used.

Diversity measures can be influenced by under-sampling (Goodall-Copestake et al., 2012); haplotype diversity generally increases with sample size (Pereira, Cunha, & Amorim, 2004). Haplotype sampling coverage estimates (i.e., the proportion of the population genetic diversity represented by our samples) ranged from 19 to 100% complete with the majority (15 out of 19) estimated at over 80% complete (Supporting Information Table S2.1, Appendix S2). However, caution must also be employed when interpreting the level of sampling completeness: a report of 90% coverage of the haplotypes that occur in one region may only be a fraction of the haplotypes that occur within the species' whole range. As such, completeness values of 100% are unlikely to indicate that every haplotype that exists has been sampled but instead indicate a reasonable sample of the population. Estimates of haplotype sampling completeness (Dixon's measure: Dixon, 2006) were well supported by the haplotype accumulation curves (Supporting Information Figure S2.1, Appendix S2), another method for estimating sampling coverage.

Species mtDNA diversity indices (outlined in Table 2) were not found to consistently correlate with any of the indicators of distribution examined [latitude and longitude (max, min, centre, range), and area (Figure 1)], either when tested individually or in multiple regressions.

3.2 | Within-species investigation

For a subset of four species, within-species diversity and population distributional trends were investigated. These investigations were limited to those species that had 75% or higher haplotype sampling completeness, had large enough distributions to display evidence of post-LGM expansion and a minimum of 100 sampled individuals spread reasonably well across their distribution (*C. cricetus*, *E. europaeus*, *M. arvalis*, *C. glareolus*). No consistent patterns were observed between any diversity index and distribution (Supporting Information Appendix S2): patterns of diversity varied in species-specific ways (Figure 2).

4 | DISCUSSION

Overall, the patterns of genetic diversity observed appear species-specific as individual species responded to climate oscillations in niche-specific ways. Each of the taxa that have been sufficiently studied to allow within-species analysis illustrate complex and divergent phylogeographical histories. It has been recognized that most species are not likely to "track" their preferred habitat back to the refugial areas as climate changes (Dalén et al., 2007; Lagerholm et al., 2014). As such, for each glaciation that occurred, a substantial proportion of each species' lineage was lost, and with that its genetic heritage, complicating the interpretation of colonization patterns. The replacement of one phylogroup with another in line with climate oscillations has also been observed (Brace et al., 2012, 2016 ; Martínková et al., 2013; Palkopoulou et al., 2016; Searle et al., 2009) indicating that present-day genetic diversity alone can be insufficient to explain historical patterns (Hofreiter & Stewart, 2009; Searle et al., 2009).

The direction of range expansions can be inferred by genetic diversity gradients (leading-edge founder events, Ibrahim et al., 1996; White, Perkins, Heckel, & Searle, 2013). Many widespread European species dispersed into Europe via western Asia through repeated waves of east–west colonization throughout the Quaternary, thus indicating a decrease in species richness from east to west (an oceanic–continental gradient: Fløjgaard, Normand, Skov, & Svenning, 2011; Stewart et al., 2010). Conversely, many species recolonized after the LGM from the south, creating a south–north genetic diversity gradient (Hewitt, 1996, 1999, 2000). Importantly, this meta-analysis indicates that common genetic signals are missing and instead it is species-specific responses that are of primary importance for reconstructing post-glacial recolonization patterns.

4.1 | Within-species analysis

All four species (*C. cricetus*, *E. europaeus*, *M. arvalis*, *C. glareolus*) for which intraspecific investigation was possible highlight a species-specific response to climate change, as each provides its own complex colonization history different to each of the others. Interestingly, these species also highlight the presence of "northern" refugia—a term often used to cover all refugia outside of the traditional "southern" refugia of the European Iberian, Italian and Balkan peninsulas, but that actually refer to a number of regions, such as the Carpathians and areas of central Europe—often around Germany—and are increasingly being viewed not as traditional "refugia" but instead as a continuum of fractured microhabitats (Bhagwat & Willis, 2008).

TABLE 2 Genetic diversity of the species studied: Species diversity measures; normal and standardized haplotype (HaplDiv, StdHaplDiv) and nucleotide (NuclDiv, StdNuclDiv) diversity measures for the between-species analysis (see Methods)

Species	Individuals included	Haplotypes reported	Haplotypes observed	% Sampling complete	Sequence length	Variable sites	AvgeNucDiff (k)	HaplDiv (*SD*)	StdHaplDiv (*SD*)	NuclDiv (*SD*)	StdNuclDiv (*SD*)
Apodemus mystacinus	21	21	19	19	1,009	136	45.48	0.99 (0.018)	0.99 (0.018)	0.045 (0.009)	0.065 (0.012)
Arvicola sapidus	240	87	76	96	203	61	9.47	0.97 (0.005)	0.98 (0.023)	0.047 (0.002)	0.058 (0.007)
Chionomys nivalis	584	19	10	100	804	88	24.67	0.76 (0.012)	0.64 (0.095)	0.031 (0.001)	0.004 (0.013)
Cricetus cricetus	397	48	27	100	337	19	2.80	0.83 (0.009)	0.86 (0.047)	0.008 (0.0002)	0.011 (0.002)
Crocidura russula	388	38	18	100	323	33	2.05	0.63 (0.02)	0.48 (0.121)	0.006 (0.004)	0.013 (0.003)
Erinaceus concolor	63	32	17	100	380	23	6.96	0.91 (0.019)	0.86 (0.048)	0.018 (0.002)	0.02 (0.069)
Erinaceus europaeus	386	66	56	100	400	47	9.23	0.95 (0.005)	0.96 (0.026)	0.023 (0.001)	0.031 (0.005)
Erinaceus roumanicus	55	7	6	100	405	6	0.81	0.32 (0.078)	0.35 (0.131)	0.002 (0.001)	0.006 (0.001)
Lemmus lemmus	104	24	17	100	165	17	1.31	0.8 (0.037)	0.61 (0.116)	0.008 (0.001)	0.018 (0.003)
Microtus guentheri	33	-	26	52	387	50	8.62	0.98 (0.014)	0.99 (0.019)	0.022 (0.002)	0.025 (0.005)
Microtus arvalis	677	114	84	100	273	59	4.65	0.88 (0.01)	0.95 (0.031)	0.017 (0.001)	0.037 (0.005)
Microtus thomasi	169	15	84	81	539	96	8.32	0.98 (0.004)	0.99 (0.018)	0.015 (0.001)	0.025 (0.004)
Mus macedonicus	71	39	52	49	730	66	6.39	0.99 (0.006)	0.99 (0.022)	0.009 (0.001)	0.011 (0.002)
Mus spicilegus	36	21	13	100	429	16	4.13	0.88 (0.034)	0.9 (0.047)	0.01 (0.001)	0.01 (0.002)
Clethrionomys (Myodes) glareolus	1,078	104	113	100	258	61	3.73	0.86 (0.007)	0.82 (0.06)	0.014 (0.001)	0.036 (0.012)
Sciurus vulgaris	799	225	155	100	225	81	4.74	0.95 (0.003)	0.93 (0.033)	0.021 (0.001)	0.034 (0.006)
Sorex antinorii	275	40	82	97	331	41	4.79	0.96 (0.005)	0.98 (0.023)	0.014 (0.0003)	0.017 (0.002)
Sorex minutus	250	158	134	76	516	228	21.01	0.98 (0.003)	0.995 (0.016)	0.041 (0.001)	0.056 (0.008)
Spermophilus suslicus	75	17	38	81	310	58	14.98	0.95 (0.013)	0.94 (0.036)	0.048 (0.002)	0.069 (0.01)

Note. Haplotypes reported refers to the summed number of haplotypes across studies. Haplotypes observed are the number of haplotypes observed when studies were pooled by species. Sequence length refers to the minimum overlapping sequence length available for each species. AvgeNucDiff refers to the average number of nucleotide differences.

Evidence from several taxa and multiple studies have suggested the existence of cold-adapted species outside of the Mediterranean peninsulae during glacial periods (e.g., Bilton et al., 1998; Stewart et al., 2010). These glacials in fact represented periods of expansion for cold-adapted species in comparison to modern climate (Hofreiter & Stewart, 2009; Stewart et al., 2010). Taxa such as Norway lemming *Lemmus lemmus*, collared lemming *Dicrostonyx torquatus*, Arctic fox *Alopex lagopus*, rock ptarmigan *Lagopus muta* and mountain avens *Dryas octopetala* were found in mid-latitude Europe during glacial periods (Stewart et al., 2010 and references therein). Some temperate taxa (especially small mammals) may also have been located further north than often suggested (Stewart & Lister, 2001). Furthermore, studies have indicated the presence of a wide range of flora (primarily small-seeded, wind dispersed, coniferous trees) in more northerly locations than previously thought possible (Bhagwat & Willis, 2008; Stewart & Lister, 2001), highlighting that central Europe may have been much more habitable during these periods than previously hypothesized.

4.1.1 | Cricetus cricetus

Neumann, Jansman, Kayser, Maak, and Gattermann (2004) and Neumann et al. (2005) revealed the separation of two distinct lineages of *C. cricetus* in Europe, one labelled "North" and one "Pannonian" (based on multiple mtDNA markers and microsatellites). Here, we found that fringe populations of the "North" lineage demonstrate lower diversity than those from the core area centred around Germany (Figure 2a), where the "Central" and "West" clades of the Northern lineage are to be found (Banaszek, Jadwiszczak, Ratkiewicz, & Ziomek, 2009). Although of a similarly high diversity level (Figure 2a), the Czech Republic population shares affinities with the southern "Pannonian" region (Neumann et al., 2004, 2005). Polish populations present a complex pattern with mixed signals from both Russia and Pannonia but with generally low diversity levels reflective of their status as a fringe population, similar to those of the low diversity "West" clade of the North lineage, despite their central location (Banaszek & Ziomek, 2011; Banaszek et al., 2009; Banaszek, Jadwiszczak, Ratkiewicz, Ziomek, & Neumann, 2010). Unfortunately, haplotype information was not available from Neumann et al. (2005); as such we were unable to include their Hungarian samples in this meta-analysis and so the picture presented here is somewhat incomplete in relation to the "Pannonian" lineage. The same main lineages identified above are also identified using cytochrome *b*, 16S ribosomal (r)RNA and microsatellite data (Banaszek et al., 2010; Neumann et al., 2005); however, Neumann et al. (2005) concluded that the contemporary phylogenetic structure observed is due to recolonization from eastern refugia.

4.1.2 | Clethrionomys [= Myodes] glareolus

Despite the availability of over 1,000 mtDNA control region sequences sampled from individuals of *C. glareolus* from multiple studies (see Table 1 and Supporting Information Appendix S2), use of these sequences has primarily been restricted to investigating the associated *Puumala* virus (Dekonenko et al., 2003; Johansson et al., 2008; Razzauti, Plyusnina, Niemimaa, Henttonen, & Plyusnin, 2012) or the effects of Chernobyl radiation (Matson, Rodgers, Chesser, & Baker, 2000; Meeks et al., 2007; Meeks, Chesser, Rodgers, Gaschak, & Baker, 2009; Wickliffe et al., 2006) on *C. glareolus*. Figure 2b indicates diversity values generally reflective of the aforementioned complexity; however, regionally restricted sampling does not enable accurate phylogeographical inferences to be made using the control region sequences available here. Of note however, even with this low distributional coverage, is the considerable variation in diversity values in Austria and the Ukraine which would seem to indicate an underlying complexity in colonization pattern.

Investigations into colonization patterns of *C. glareolus* have also been carried out using cytochrome *b* data (Colangelo, Aloise, Franchini, Annesi, & Amori, 2012; Deffontaine et al., 2005, 2009; Kotlík et al., 2006), and mitogenome sequencing (Filipi, Marková, Searle, & Kotlík, 2015). These studies have revealed perhaps the most complex phylogeographical pattern of any small mammal studied to date, with up to seven proposed well-defined clades, including some with subclades (Deffontaine et al., 2009; Filipi et al., 2015). Earlier papers provided evidence of multiple refugia outside of the traditional "southern" areas (Deffontaine et al., 2005, 2009; Kotlík et al., 2006); however, full mitogenome analysis (Filipi et al., 2015) has allowed the resolution of both "northern" and "southern" refugia. It appears that post-glacial expansion was likely via multiple routes, from multiple refugia, with the traditional Mediterranean peninsular refugia maintaining long-term intraspecific populations throughout the Pleistocene but without necessarily contributing to the post-glacial recolonization of mainland Europe (Bilton et al., 1998; Colangelo et al., 2012; Deffontaine et al., 2005; Filipi et al., 2015). Kotlík et al. (2006) provided evidence of a Carpathian refugium and subsequent expansion, pre-dating the LGM, supported by fossil evidence, along with plant pollen and macrofossils indicating the presence of suitable habitat. Surprisingly, mitogenomic phylogeny has grouped parts of Britain, Sweden and Norway within the "Carpathian" lineage, which may be due to a two-phase colonization, with the "Western" clade replacing the "Carpathian" clade throughout parts of central Europe and Britain (Filipi et al., 2015). Multiple colonization events are evident throughout the species' range, resulting in geographically disjointed lineages that arose from multiple northern refugia.

4.1.3 | Erinaceus europaeus

The European colonization of *E. europaeus* is perhaps one of the best characterized, and has been held up as a prime example of recolonization from southern refugial areas (Hewitt, 1999, 2000). Three *E. europaeus* clades have been identified, a western group stemming from the Iberian peninsula up through France to the Netherlands, UK and Ireland, a central European group recolonizing from Italy up into Austria, Switzerland, Germany, the Netherlands, Scandinavia and Estonia, and a regionally restricted Sicilian group (Bolfíková & Hulva, 2012; Seddon et al., 2001). These groups are strongly separated

using mtDNA (D-loop, cytochrome *b*) but not discriminated using nuclear microsatellites (Bolfíková & Hulva, 2012). A contact zone with the sister species *E. roumanicus* exists in central Europe (Poland, the Czech Republic, Austria and Italy); however, reproductive isolation appears to be maintained (Bolfíková & Hulva, 2012).

It has been hypothesized that long-distance colonization can cause diversity loss through serial bottlenecks (Hewitt, 1996); however, with the exception of island populations (e.g., UK and Ireland) or those separated by large bodies of water (e.g., Norway) where only single haplotypes were found, diversity was not found to be consistently lower in northern regions compared to southern "refugial" areas (Figure 2c). Indeed, the highest haplotype diversity values were found in more central European regions (e.g., Germany, France, Switzerland, Netherlands and areas of the Czech Republic all demonstrated higher haplotype diversity than those in Iberia and Italy). This could potentially be due to secondary contact between the two lineages but has also been suggested as possible evidence of a northern refugium for this species (Stewart & Lister, 2001). Seddon et al. (2001) similarly found a distinct group of six mitotypes restricted to southern Germany, northern Switzerland, southern Austria and Poland. Furthermore, the relatively high diversity values across mainland European populations is comparable to levels seen in *M. arvalis* and *C. glareolus*, which are hypothesized to be due to more recent expansion within central Europe. Interestingly, Bhagwat

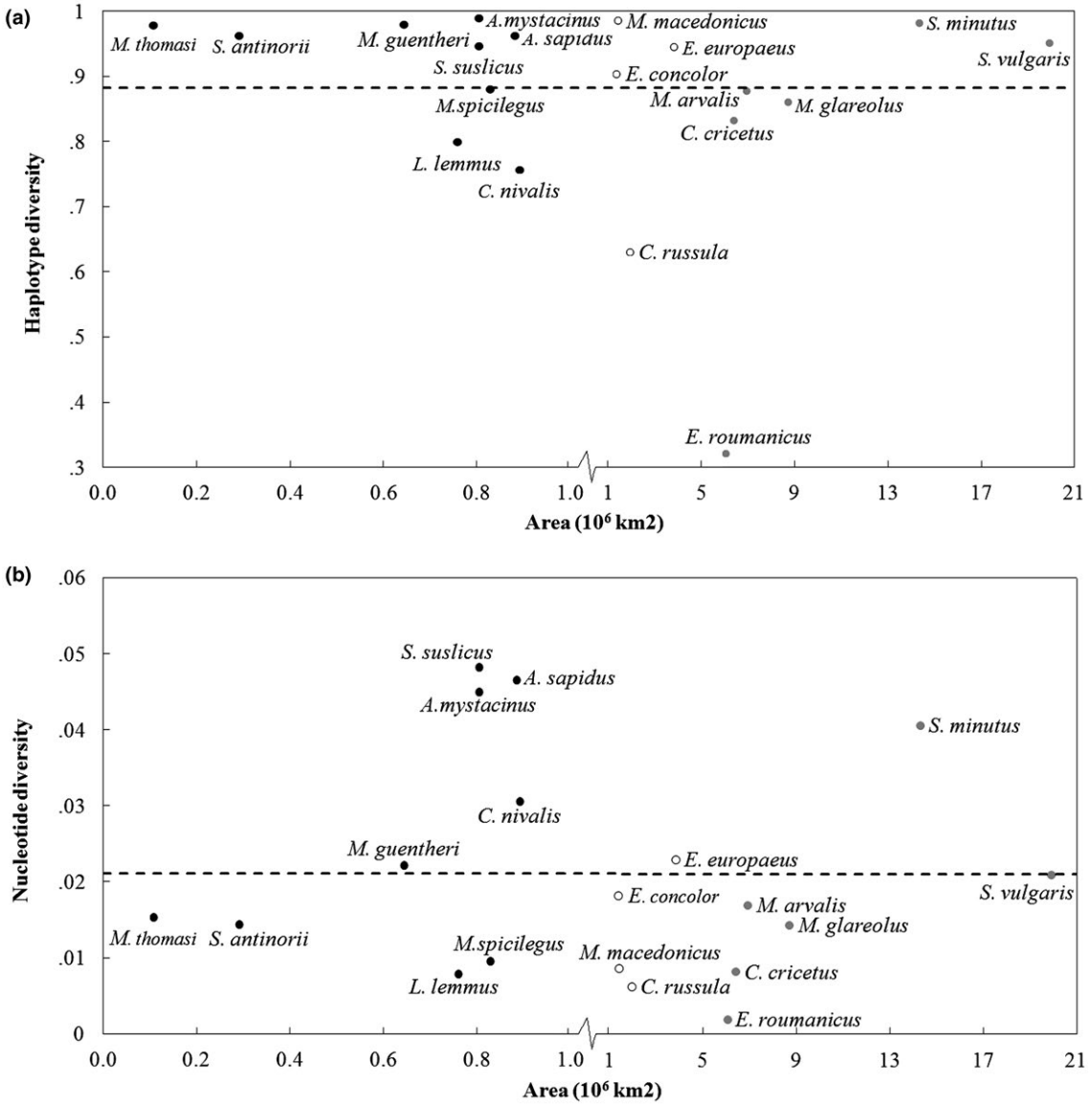

FIGURE 1 Mitochondrial DNA (mtDNA) diversity indices of all 19 small mammal species examined in relation to area for (a) haplotype diversity and (b) nucleotide diversity (see Supporting Information Appendix S2 for standardized estimates). The dotted line indicates the mean diversity value and thus an indication for "high" and "low" diversity for the small mammal species investigated. Distributions were further classed into endemic (black closed circles < 1 million km^2) and widespread areas (open circles between 1–5 million km^2, or grey circles > 5 million km^2). Species mtDNA diversity indices were not found to consistently correlate with area (illustrated here), or any of the indicators of distribution examined. Note: axis break and scale change

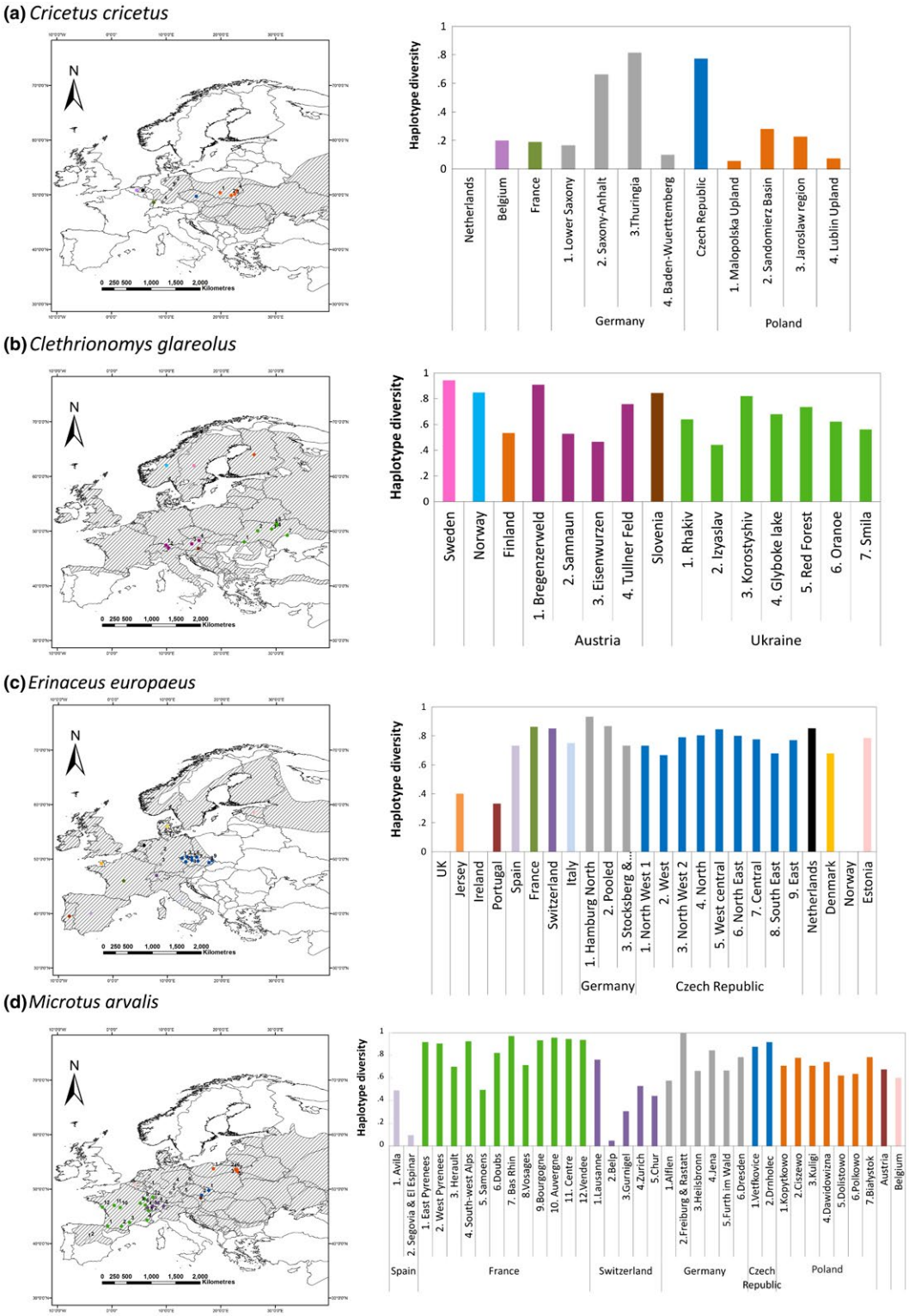

FIGURE 2 Maps and mitochondrial DNA (mtDNA) control region haplotype diversity estimates of *Cricetus cricetus*, *Clethrionomys (Myodes) glareolus*, *Erinaceus europaeus* and *Microtus arvalis* used for within-species population analyses. Where sufficient information was available (minimum of five individuals from a region/location) individuals were grouped into populations within countries and for diversity estimates. Where this was not possible, individuals were pooled for a country-level estimate. Bar charts indicate the diversity level for each population, bar colours correspond to the map markers and shaded areas map the species' known distribution International Union for Conservation of Nature (IUCN), www.iucnredlist.org). "Missing" bars indicate monomorphic populations. No consistent patterns were observed between haplotype diversity and distribution: species diversity patterns varied in species-specific ways [Colour figure can be viewed at wileyonlinelibrary.com]

and Willis (2008) also suggest that the genetic evidence of a southern refugium for this species does not fit with their analysis, which indicated that *E. europaeus* has the life history traits suited to persistence in northern refugia.

4.1.4 | Microtus arvalis

Microtus arvalis is a well-known example of a species that does not conform to the southern refugium hypothesis (Fink et al., 2004; Heckel, Burri, Fink, Desmet, & Excoffier, 2005; Tougard et al., 2008). Multiple genetic markers (cytochrome *b*, control region, microsatellites) indicate that there are at least five main evolutionary lineages of *M. arvalis* in Europe [Western (North and South), Central, Eastern, Italian and Balkan] with further levels of complexity within these lineages (Buzan et al., 2010; Martínková et al., 2013; Stojak, McDevitt, Herman, Searle, & Wójcik, 2015). Furthermore, all estimates of divergence times for these lineages indicate a pre-LGM or LGM (*c.* 18,000 years ago) split (Fink et al., 2004; Heckel et al., 2005; Lischer, Excoffier, & Heckel, 2013; Stojak et al., 2015). *M. arvalis* was not restricted to the classic southern refugia, and instead persisted in multiple northern refugia, likely in patchy favourable habitats (Buzan et al., 2010; Martínková et al., 2013; Stojak et al., 2015; Tougard et al., 2008). The Eastern lineage is hypothesized to have arisen in the northern Balkans and/or the Carpathian basin, with the Central lineage arising north of the Alps (Braaker & Heckel, 2009; Buzan et al., 2010; Beysard & Heckel, 2014; Stojak et al., 2015; Stojak et al., 2016) although more detailed sampling is required to confirm this. Switzerland has been found to present an interesting case of contact and limited admixture (Italian, Western and Central lineages), as a result of comparatively recent Alpine colonization and hybridization (Beysard & Heckel, 2014; Braaker & Heckel, 2009; Sutter, Beysard, & Heckel, 2013).

Although patterns are not immediately evident from Figure 2d, closer inspection of the control region data reveals support for the above reported results. High genetic diversity is evident throughout the range of *M. arvalis*, with geographical partitioning in keeping with the lineages described above. Furthermore, the region with the most variable diversity values is Switzerland, the location identified as a multi-lineage contact zone (Beysard & Heckel, 2014; Braaker & Heckel, 2009; Stojak et al., 2015; Sutter et al., 2013). In contrast to Heckel et al. (2005) we did not detect a significant relationship between haplotype diversity and latitude or longitude.

5 | CONCLUSION

Meta-analyses, such as this, allow the examination and testing of general relationships that enable us to ask ecological questions on a larger scale than usually possible at a single study level (Kaiser et al., 2006). They do however come with a set of caveats that must be highlighted. Firstly, they are subject to publication bias (Kaiser et al., 2006), and importantly for this study, accuracy of species distribution data is a concern (Kryštufek & Griffiths, 2002; Taberlet et al., 1998). Not only do substantial differences in mapping efforts occur between countries, but also ranges such as those extracted from the IUCN (used here) necessarily ignore the fact that realized ranges are mosaics rather than continuous. However, at the continental scale of this analysis and in the absence of fitting to environmental variables, these effects should be minimal.

The results presented here, along with the recognition of the implications of recurrent periods of glaciation (Fink et al., 2004; Stewart et al., 2010), the primarily individualistic nature of retreat (Graham & Grimm, 1990; Huntley, 1991; Stewart 2008, 2009), and evidence of refugia within refugia (Centeno-Cuadros et al., 2009; Colangelo et al., 2012), have highlighted how the acceptance of broad patterns can conceal a deeper complexity. Despite our use of a much tighter taxonomic grouping than Taberlet et al.'s (1998) use of 10 taxa from diverse clades across mammals, amphibians, arthropods and plants, our findings reflect theirs; "congruence on a continental scale may be the exception rather than the rule", and the southern refugia highlighted by many (Hewitt, 1996, 1999, 2000; Seddon et al., 2001; Taberlet et al., 1998) may better represent a "hotspot" of endemism rather than the sole recolonization sources for Europe (Bilton et al., 1998; Kryštufek & Griffiths, 2002; Stewart et al., 2010; Tougard et al., 2008). It seems that perhaps unsurprisingly, distribution was then, and is now, primarily dictated by species' biological traits (tolerance ranges, adaptive capacity, plasticity) in combination with competition rather than simply regional restriction, and that northerly regions were neither as hostile nor barren as previously assumed. Further investigations using a nestedness approach (e.g., Habel et al., 2013) may provide further insight into these patterns, and help to identify the type of movement that led to the recolonization of these species.

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CONFLICT OF INTEREST

The authors confirm that no conflict of interest exists.

DATA ACCESSIBILITY

As detailed in the Methods, all data are sourced from published sources freely available online. Sequences were obtained through GenBank (see Supporting Information Appendix S1 for the source papers and GenBank accession numbers). Area occupied by each species and primary habitat/vegetation preferences were obtained from IUCN species profiles (www.iucnredlist.org).

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BIOSKETCH

Debbi Pedreschi comes from a varied background employing multidisciplinary methods to inform management practices, including through improving phylogeographical understanding. Her interests centre around understanding anthropogenic pressures, ecosystem management, and investigating diversity at a range of levels. The team's research has focused on the examination of genetic variation in a range of species to reconstruct post-glacial geographical range change and contribute to understanding the role of ice-age refugia.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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