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Pan-European phylogeography of the aquatic snail *Theodoxus fluviatilis* (Gastropoda: Neritidae)

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Abstract

Investigating the geographical distribution of genetic lineages within species is critical to our understanding of how species evolve. As many species inhabit large and complex ranges, it is important that phylogeographical research take into account the entire range of widespread species to clarify how myriad extrinsic variables have affected their evolutionary history. Using phylogenetic, nested clade, and mismatch distribution analyses on a portion of the mitochondrial COI gene, I demonstrate that the wide-ranging freshwater snail *Theodoxus fluviatilis* possesses in parallel many of the phylogeographical patterns seen in less widespread freshwater species of Europe. Fragmentary forces play a major part in structuring the range of this species, with 12 of 14 geographically structured nested clades displaying a distribution consistent with fragmentation or restricted dispersal. Certain regions of southern Europe harbour the majority of genetic diversity (total haplotype diversity, $H = 0.87$), particularly Italy ($H = 0.87$) and areas surrounding the Black Sea ($H = 0.81$). Post-Pleistocene range expansion is pronounced, with the majority of northern European populations (95% of sample sites) having arisen from northern Italian individuals that initially colonized northern Germany. Additionally, two highly divergent haplotype lineages present in northern Germany imply that there were at least two postglacial recolonization routes. Estuaries may also provide a means of dispersal given that no genetic differentiation was found between estuarine populations and neighbouring freshwater populations. Taken together, these data reveal a species with a complex genetic history resulting from the fragmentary effects of European geology as well as continuous and discrete range expansion related to their aquatic biology.

Keywords: lineage sorting, mitochondrial DNA, nested clade phylogeographical analysis, Pleistocene glaciation, vicariance

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Introduction

Phylogeography has proven powerful in elucidating patterns of gene flow, hybridization, historical range fragmentation, range expansion, and speciation among many organisms (Avise 2000; Lessios *et al*. 2001; Templeton 2001). However, rarely do phylogeographical analyses take into account the entire range of a widespread species. Most phylogeographical studies restrict their analysis to portions of a range or to a species with restricted distribution. Investigating the entire range of such species is likely to reveal concurrent processes that act differentially to produce intraspecific phylogenetic structure (e.g. Bernatchez 2001). The effects of geographically restricted mechanisms may be among the most important for bringing about speciation as allopatric processes are likely to produce populations that are less likely to interbreed with distant conspecifics (Knowles 2001; Bernardi *et al*. 2003).

Phylogeographical analyses of freshwater organisms have revealed historical patterns of river capture, fluvial range expansion, vicariance, lineage introgression, and long-distance dispersal (Durand *et al*. 1999; Avise 2000; Bernatchez 2001). Species that range in northern continental habitats, where climatic and geographical variation is high, are especially prone to these processes (Bernatchez & Wilson

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1998; Hewitt 2004). This study focuses on one such species, the snail *Theodoxus fluviatilis*, which lives in the lakes, rivers, streams, and estuaries of Europe and southwest Asia.

Theodoxus fluviatilis is the most widely distributed species in this freshwater genus of the gastropod family Neritidae. It is distributed throughout most of Europe, including Scandinavia along the Baltic Sea (Skoog 1971), as well as Anatolia (Yildirim 1999). It ranges as far west as Ireland (Lucey *et al*. 1992) and as far east as the Black Sea and Baltic Sea drainages (Anistratenko *et al*. 1999), although there are large areas within Europe that are uninhabited, particularly in mountain ranges. All members of this genus are sexually reproducing, dioecious snails that lay benthic egg capsules which hatch a single juvenile egg (Fretter & Graham 1962; Orton & Sibly 1990). Individuals survive on hard benthic substrates, typically rocks, in calcium-rich waters (Fretter & Graham 1962). There are several populations of *T. fluviatilis* that live in up to 18‰ salt water in both the Baltic Sea (Bondesen 1941; Bielawski 1960) and the Black Sea (Butenko 2001) and are assigned to the subspecies *T. f. littoralis* and *T. f. euxinus*, respectively.

A continental freshwater distribution can be described as being distributed on habitat islands, with large tracts of land separating aquatic populations that may or may not be connected by linear paths (i.e. rivers and streams). This is particularly true of *T. fluviatilis*, which has a patchy distribution across its range, so it is reasonable to predict that this snail will demonstrate a high degree of phylogeographical structure (Avise 2000).

Vicariance has left an indelible signature on the phylogenies of many freshwater fish clades (Zardoya & Doadrio 1999; Hrbek & Meyer 2003). The geographical results of these vicariant processes also continue to act as barriers to dispersal within fish species (Durand *et al*. 1999; Salzburger *et al*. 2003; Kotlik *et al*. 2004). Due to limited avenues of dispersal, these allopatric distributions may be particularly important for structuring freshwater species with low vagility (Zardoya & Doadrio 1999); thus it can be expected that *T. fluviatilis* also experiences barriers to dispersal as a result of allopatric range structure. It is also possible that Holocene modification of the freshwater environment has resulted in higher rates of gene flow and a more panmictic distribution of haplotypes as canals have connected previously disjunct populations. However, given the recency of major anthropogenic modification (e.g. connecting the North Sea to the Black Sea via the first Main-Danube Canal, *c*. 1845) and the low vagility of *T. fluviatilis* (Fretter & Graham 1962), it is unlikely that much evidence exists for gene flow across the range.

An important aspect of freshwater phylogeography in Europe is Pleistocene climate change. As recently as 13 000 years ago, huge tracts of the range of *T. fluviatilis*, including most of northern Europe and areas surrounding the Alps and Pyrenees, were covered with ice (Hewitt 1999; Haase *et al*. 2003). Dramatic climate and hydrological shifts throughout the Pleistocene are among the most significant factors affecting extant species distributions (Bernatchez 2001; Hewitt 2004). In accord with other research on post-Pleistocene demographical change in European fish and gastropods (Bernatchez & Dodson 1991; Haase *et al*. 2003; Perdices *et al*. 2003), I expect range expansion to have left a significant signature on the genealogy of *T. fluviatilis*. Following glaciation, most European lineages have expanded northwards from southern refugia, particularly in southern peninsulas (Taberlet *et al*. 1998; Hewitt 1999). Which of these regions may have served as refugia for *T. fluviatilis* is elucidated in this study.

Because of its wide distribution in a complex habitat, I expect multiple phylogeographical patterns to be present simultaneously in *T. fluviatilis* — patterns that have been described for freshwater organisms with more limited distributions in Europe. To that end, this study has four particular questions under investigation: (i) What fragmentary forces, if any, have led to geographical associations of lineages within *T. fluviatilis*? (ii) What area(s) of the range of *T. fluviatilis* harbour(s) the majority of intraspecific lineage diversity? (iii) What effects did Pleistocene climate change have on this species? (iv) Do brackish and marine waters act as a barrier to dispersal in this species?

The ecology and distribution of this species also allow the evaluation of several specific hypotheses: (i) If the freshwater habitat of *T. fluviatilis* is, or has historically been, especially disjunct as a result of both geology and climate, then this highly subdivided range should result in numerous geographically restricted, reciprocally monophyletic lineages (Perdices *et al*. 2003). I test the hypothesis that there are geographically isolated clades within *T. fluviatilis* using phylogenetic and nested clade analyses. (ii) Even though anthropogenic forces have lowered barriers to dispersal across major drainages for some organisms (e.g. Bernatchez 2001), I hypothesize that there is relatively little evidence for gene flow across the range of *T. fluviatilis*, in particular across watersheds such as the Main/Rhine and Danube drainages. (iii) Using phylogenetic and population genetic methods, I test whether brackish populations represent a genetically distinct lineage from the majority freshwater populations or if these individuals are simply physiological variants (Kangas & Skoog 1978). (iv) This species is distributed throughout the southern European peninsulas and may have expanded northwards following the last glacial retreat from any number of refugia. I evaluate whether the route(s) of recolonization is (are) similar to those found for many terrestrial organisms, i.e. arising in Iberia and the Balkans but not Italy (Taberlet *et al*. 1998) or if they include areas such as Italy or Black Sea drainages (Durand *et al*. 1999; Kotlik *et al*. 2004).

With the above questions and hypotheses in mind, I apply several different techniques — phylogenetic, nested

clade phylogeographical analysis, genetic diversity, and pairwise mismatch distribution — to attempt to elucidate the phylogeographical history of this widespread European snail.

Materials and methods

Specimen collection and sampling design

The sampling protocol was designed to cover the entire range of *Theodoxus fluviatilis* while sampling densely within certain areas in order to assess the geographical limits of haplotype diversity. The densest sampling occurred in the glacial lakes of northern Germany and in the lakes and rivers of northern Italy. In these two regions, samples were collected from many lakes and rivers within a small geographical area. Additionally, a recently described species, *Theodoxus velox* (Anistratenko *et al*. 1999), was sampled from the lower Dniper River in the Ukraine because a preliminary phylogenetic analysis of the genus indicated that *T. velox* was likely a member of the *T. fluviatilis* lineage (P. Bunje, unpublished). Figure 1 shows the locations from which samples were collected; exact locality data are available from the author. These localities come from collection trips to different geographical regions, noted in Table 1, that represent major watersheds. Only 'Northern Germany' includes two major drainages (see Discussion): localities 065–072 drain to the North Sea, all others to the Baltic Sea. Tissue was dissected for DNA extraction from individuals that were either alive, frozen, or preserved in 96% ethanol.

DNA extraction, amplification and sequencing

Between 15 and 35 mg of foot and/or head tissue was used to extract genomic DNA using the DNeasy Tissue Kit (QIAGEN). Partial sequences of the mitochondrial gene cytochrome *c* oxidase subunit 1 (COI) were amplified by polymerase chain reaction (PCR). Six hundred base pairs (600 bp) of COI were amplified for 297 individuals using the primers F4d, 5′-TACTTTRTATATTATGTTTGGT-3′, and R1d, 5′-TGRTAWARAATDGGRTCWCCHCCVCC-3′. Each 50-μL reaction included 1 μL genomic DNA, 10 pmol of each primer, 3 nmol dNTPs, 5 μL of 10× PCR buffer (Applied Biosystems), 125 nmol magnesium chloride, 1 unit Ampli*Taq* Gold DNA polymerase (Applied Biosystems) and 33.8 μL water. The cycling parameters were an initial cycle of 95° for 10 min followed by 36 cycles of denaturation at 95° for 50 s, annealing at 54° for 1 min, and extension at 72° for 1 min. PCR was completed with a 7-min final extension at 72°. PCR products were purified using QIAquick PCR Purification columns (QIAGEN) or ExoSAP-IT enzyme buffer (USB). Purified PCR products were then sequenced with BigDye version 2.0 from Applied Biosystems using the PCR primers and visualized on an ABI 377. Both strands were sequenced and then analysed using SEQUENCE NAVIGATOR (version 1.0.1, Applied Biosystems) to ensure the accuracy of the final sequence.

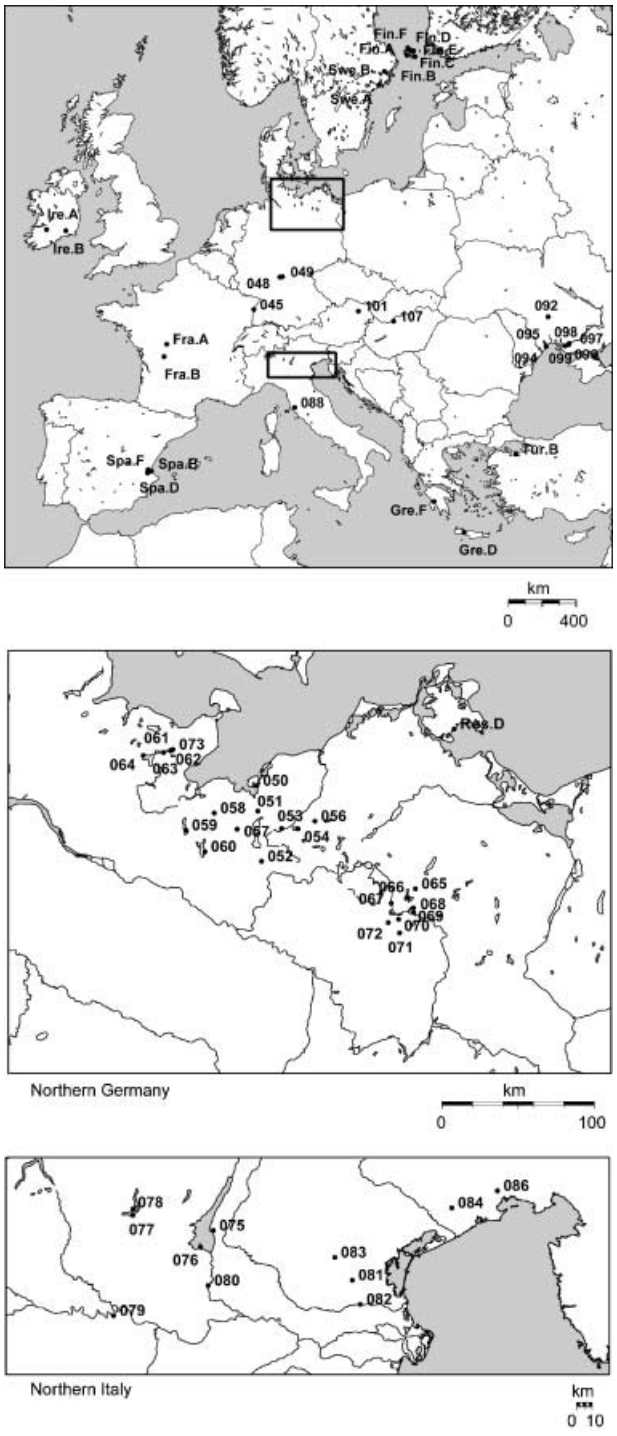

Fig. 1 Collection sites for *Theodoxus fluviatilis*. Specific locality data are available from the author. Collection sites in northern Germany and northern Italy are shown in the inset.

Table 1 Localities from which each haplotype was sampled. Exact locality data for each sample site are available from the author. The number in each cell corresponds to the number of times a haplotype was sampled at each locality. Localities marked with an asterisk are brackish; the site marked with a cross fluctuates between freshwater and brackish water. See Fig. 2 for definitions of the regions

		CG			Northern Germany																								Finland						Sw		Ir	
Haplotype	GenBank #	045	048	049	050*	051	052	053	054	056	057	058	059	060	061	062	063	064	065	066	067	068	069	070	071	072	073	Ros.D*	Fin.A*	Fin.B*	Fin.C*	Fin.D*	Fin.E	Fin.F	Swe.A*	Swe.B	Ire.A	Ire.B
F1	AY765306	7	1	1																																		
F2	AY765307	2																																				
F3	AY765308				8	5	5	5	5		5	3	5		5	4	4	5								5	4		4	1	2	1	1			3	4
F4	AY765309				1																																	
F5	AY765310									4																												
F6	AY765311									1																												
F7	AY765312											1																										
F8	AY765313											1																										
F9	AY765314													5																								
F10	AY765315															1																						
F11	AY765316																1																					
F12	AY765318																		1						1	3			3		2	2	1	3	4	5		
F13	AY765317																		2	3	1	2	1	6	6	2												
F14	AY765343																		2																			
F15	AY765341																			2	4	3	4															
F16	AY765319																																					
F17	AY765320																																					
F18	AY765321																																					
F19	AY765322																																					
F20	AY765323																																					
F21	AY765324																																					
F22	AY765325																																					
F23	AY765326																																					
F24	AY765327																																					
F25	AY765328																																					
F26	AY765329																												1									
F27	AY765330																																	1				

Table 1 *Continued*

Haplotype	GenBank #	Northern Italy											WI	Fr		Spain			Da		Ukraine									Cr	Gr	Tu
		075	076	077	078	079	080	081	082	083	084	086	088	Fra.A	Fra.B	Spa.B	Spa.D	Spa.F	101	107	093*	099†	091	092	098	094*	095*	096	097	Gre.D	Gre.F	Tur.B
F2	AY765307																															
F3	AY765308			3	3						2	4																				
F4	AY765309																															
F5	AY765310																															
F6	AY765311																															
F7	AY765312																															
F8	AY765313																															
F9	AY765314																															
F10	AY765315																															
F11	AY765316																															
F12	AY765318																															
F13	AY765317																															
F14	AY765343																															
F15	AY765341																															
F16	AY765319	3	1				1																									
F17	AY765320	1																														
F18	AY765321	1					1																									
F19	AY765322		1																													
F20	AY765323		1																													
F21	AY765324					4		4	2	3																						
F22	AY765325						1																									
F23	AY765326										1																					
F24	AY765327										1																					
F25	AY765328												8					4														
F26	AY765329																															
F27	AY765330																															
F28	AY765331													5	5																	
F29	AY765332															4	3															
F30	AY765333																				4	5	1	1	1							
F31	AY765336																		4	5		1					2					
F32	AY765340																							1				4	4			
F33	AY765339																								1							
F34	AY765342																								4			1	4			
F35	AY765334																														2	
F36	AY765335																															3
F37	AY765337																									1	1					
F38	AY765338																		1													
F39	AY765344																													3		
F40	AY765345																											1				

Phylogenetic analyses

Gene trees were estimated using phylogenetic analyses implemented in PAUP* (version 4.0b10; Swofford 2000). A maximum-parsimony analysis that included all sampled individuals was performed using default settings and an heuristic search strategy with TBR (tree-bisection–reconnection) branch swapping and 10 random addition-sequence replicates. Additionally, maximum likelihood (ML) was employed using unique haplotypes only. After all duplicate haplotypes were culled, MODELTEST version 3.06 (Posada & Crandall 1998) was used to estimate the best model of nucleotide substitution. Using the chosen model for DNA sequence evolution (HKY + Γ), a likelihood analysis was performed in PAUP* with an heuristic search strategy employing TBR branch swapping and 10 random addition-sequence replicates.

Support for reconstructed branches was estimated by bootstrapping the maximum-parsimony analysis with 1000 bootstrap replicates and saving a maximum of 500 trees per bootstrap replicate. Bootstrap values were calculated for the ML tree using at least 200 bootstrap replicates, the 'fast' heuristic search algorithm, and the same model parameters as used for the ML analysis. Bayesian posterior probabilities were also estimated for reconstructed nodes. Bayesian analysis was performed in MRBAYES version 3.0 (Huelsenbeck & Ronquist 2001) and with the following parameters: the HKY + Γ substitution model, a four-chain (one cold, three heated; T = 0.2) Metropolis-coupled Monte Carlo analysis run for 10^6 generations, trees sampled every 100 generations starting after a burn-in of 50 000 generations. The ML tree was rooted using an outgroup from *Theodoxus danubialis*. A strict consensus of the parsimony trees was left unrooted to allow visual inspection of intraspecific relationships.

Nested clade phylogeographical analysis

In order to describe general phylogeographical patterns within this species, I utilized the nested clade phylogeographical analysis (NCPA) of Templeton *et al*. (1995). The four steps of the NCPA were implemented as recommended by Templeton *et al*. (1995) and employed by other researchers (e.g. Bernatchez 2001). (i) Construction of a haplotype network employed the statistical parsimony procedure implemented in the program TCS version 1.13 (Clement *et al*. 2000). (ii) The networked haplotypes were then nested into hierarchical clades following the nesting rules outlined in Templeton *et al*. (1987) and extended in Templeton & Sing (1993). These rules group the most closely related haplotypes within clades and then group those clades into higher-level clades, at each step increasing the inclusion by one mutational difference. (iii) The statistical test of geographical association among and between haplotypes and nested clades was performed using GEODIS version 2.0 (Posada *et al*. 2000) run with 10 000 permutations. This is done using two statistics that measure the distances within and between nested clades. Distances are calculated from the locality data, with these locations considered continuous variables in a geographical distance matrix. The average distance within clades is the statistic D_c, a metric that summarizes the mean distance of every individual (or nested clade) in that clade from the geographical centre of all individuals (or nested clade) in the clade, thus describing the extent of geographical spread within a clade (Templeton 2001). The second metric, D_n, measures the mean distance of every individual within a nested clade from the geographical centre of all individuals in the next-higher-level clade, thus describing the extent of spread between clades at a given hierarchical (i.e. nesting) level (Templeton *et al*. 1995). At this step, it is possible to assess the probability of geographical association or non-association with phylogenetic structure because the test uses randomized geographical distances to test the probability that the particular geographical distances sampled within a given clade (or haplotype) were sampled from a null distribution of geographical distances (Templeton 1998; Knowles & Maddison 2002). (iv) Finally, the updated inference key of Templeton (2004) is used to infer particular historical processes for clades where significant geographical association or dispersion was found. Because invoking a particular historical process for a unique geographical distribution of lineages is by definition nonstatistical (Knowles & Maddison 2002), it is wise to coordinate the results from the NCPA with those from other phylogeographical techniques (such as cladistic and population genetic methods) to reach a robust conclusion about the geographical history of intraspecific lineages (Avise 2000).

Genetic diversity and mismatch distribution

In order to describe the genetic variation present in *T. fluviatilis*, analyses were carried out in ARLEQUIN version 2.001 (Schneider *et al*. 2000) using standard descriptive methodologies including haplotype (H) and nucleotide (π) diversities (Nei 1987). These analyses were performed for each of four major quadrants of the range in order to identify areas of high genetic diversity. Using the regions from which samples were collected, the range was divided into northwest (central Germany, northern Germany, Ireland, Sweden, and Finland), southwest (northern Italy, western Italy, Spain, and France), southeast (Greece, Crete, and Turkey), and Ponto-Pannonian (the Ukraine and Danube; see Table 1 and Fig. 1). Additionally, corrected pairwise genetic distances between haplotypes were calculated within the clade as a whole and for the four geographical quadrants using the Kimura 2-parameter substitution model.

With regard to hypothesized post-Pleistocene range expansion, I utilize the pairwise mismatch distribution of Rogers & Harpending (1992), which can identify whether a group of related haplotypes is the likely result of recent population expansion (Slatkin & Hudson 1991). This method was used to assess the probability that northern European samples are the result of a single recent range expansion. This hypothesis is derived from the distribution of *T. fluviatilis* in northern Europe, a recently glaciated area. Slatkin & Hudson (1991) have shown that the distribution of pairwise differences between haplotypes in a sample will be a unimodal Poisson-like distribution if the haplotypes are the result of exponential population growth, whereas a multimodal distribution is typical of stable population sizes. Therefore, the samples from northern Europe as well as the three other quadrants of the range and the entire range itself were subjected to mismatch distribution analyses.

Results

A total of 297 individuals were sequenced, resulting in 40 unique haplotypes (including 15 singletons). These sequences have been deposited in GenBank with the accession nos AY765306–AY765345. The sites and geographical regions from which haplotypes were recovered are listed in Table 1.

Phylogenetic analyses

Haplotypes each consisted of 600 bp. Of 52 variable sites, 28 were parsimony informative, one resulted in a nonsynonymous substitution (in haplotypes F1 and F2), and the estimated transition/transversion ratio was 12.0. The parsimony analysis produced 60 most parsimonious trees, 72 steps in length, with a CI of 0.764 and an RI of 0.986. The strict consensus tree is shown in Fig. 2. The likelihood analysis produced a single tree with a score of

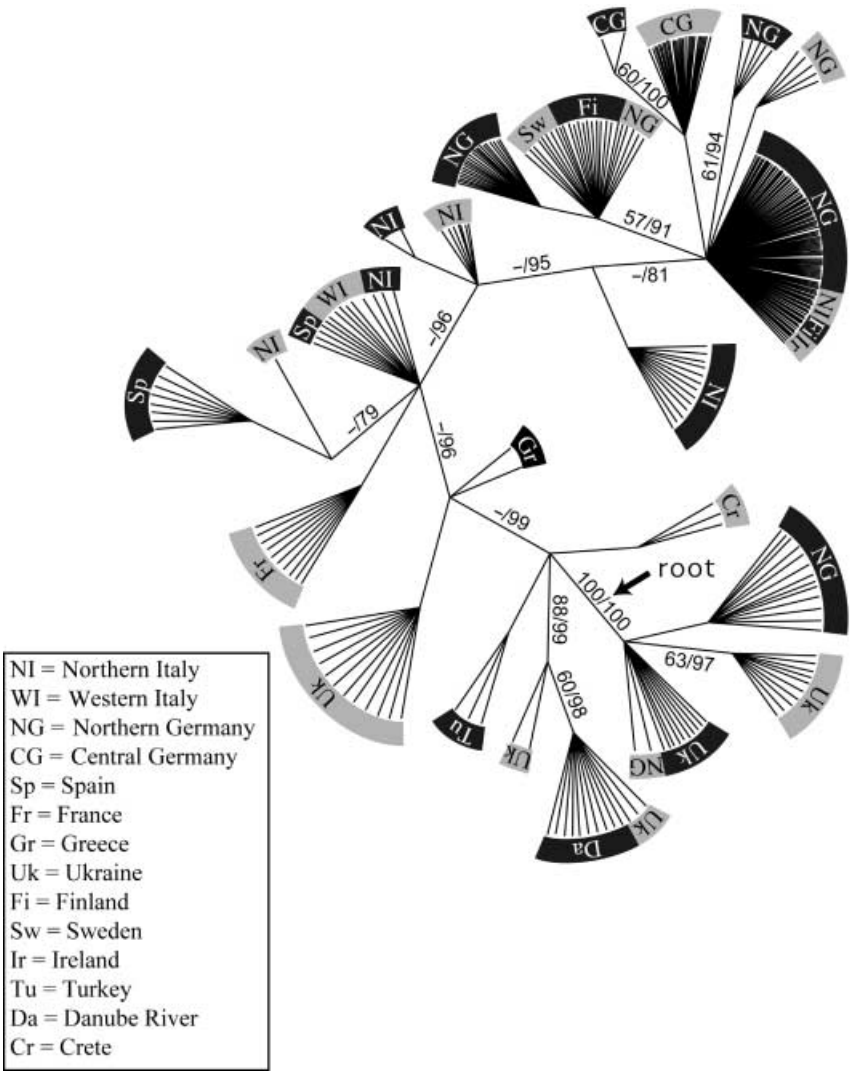

Fig. 2 Strict consensus tree of parsimony analysis for *Theodoxus fluviatilis*. The location of the root as determined in the maximum-likelihood analysis is shown. Regions in which the haplotypes are found are shown, see Table 1 and text for region definitions. Numbers on each branch are the support indices for that branch — bootstrap before the slash and Bayesian posterior probabilities after each slash. A dash indicates less than 50% support for that metric.

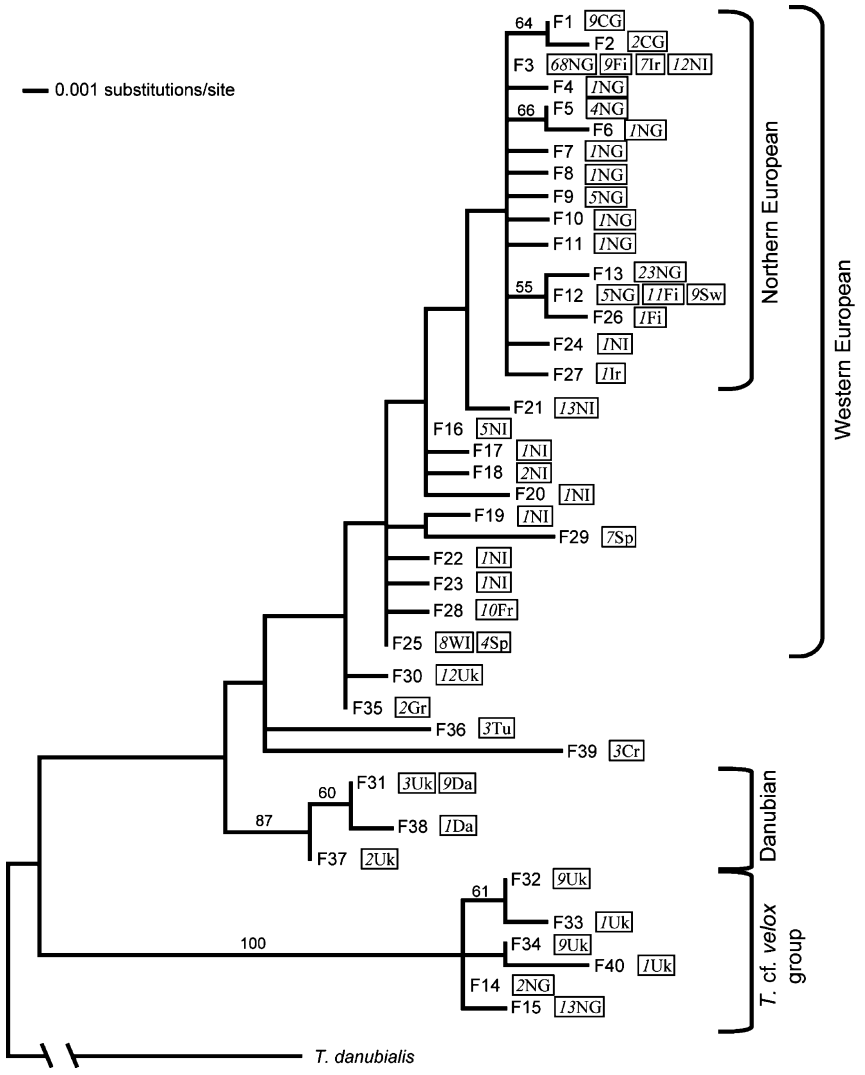

Fig. 3 Maximum-likelihood tree of unique haplotypes found in *Theodoxus fluviatilis*. Bootstrap values are shown for branches that received > 50% support. The tree was rooted with an outgroup (*Theodoxus danubialis*). Geographical frequency of haplotypes is indicated by the italic number before the sampling region. See the legend to Fig. 2 and Table 1 for definitions of geographical codes. Monophyletic groups discussed in the text are shown by the brackets.

1259.1028 (Fig. 3). The parsimony bootstrap percentages and Bayesian posterior probabilities are shown in Fig. 2 and the likelihood bootstrap values in Fig. 3. The largest pairwise difference between haplotypes is 3.6%. Most haplotypes are separated by only one or two nucleotide substitutions and the average pairwise distance is 1.51% ± 0.71% (mean ± SE). This high degree of divergence is reduced to 0.90% ± 0.44% if the divergent '*T*. cf. *velox*' clade is ignored (see Fig. 3).

Geographical structure is evident in both the parsimony and likelihood trees, which contain several monophyletic groups with restricted distributions. The gene trees place all *Theodoxus fluviatilis* from northern Europe in one of two clades. The larger of the two clades (labelled 'Northern European' in Fig. 3) received less than 50% bootstrap support and Bayesian posterior probability of 81%. The basal haplotype, F3, is also found in northern Italy. The smaller clade found in northern Europe includes related haplotypes primarily in the Ukraine (designated the '*T*. cf. *velox*' group). This clade is well supported by both bootstrap values and Bayesian posterior probabilities (100% in all three cases). In northern Germany, they are found in only four populations very near to each other (all in the region of the city of Neustralitz in Mecklenburg-Vorpommern, north of Berlin). These haplotypes are never found in nearby populations across the watershed divide. This watershed, although vertically distinct by only a few metres and now traversed by canals, represents a division between drainages to the Rhin and Havel rivers. The more common haplotypes in northern Germany occur sympatrically with the haplotypes of the *T*. cf. *velox* group. The pattern present in northern Europe is consistent with range expansion from two relictual populations following the most recent glacial retreat (Hewitt 2004).

Another monophyletic group with a restricted distribution is a clade native to the Danube River and nearby drainages of the northwestern Black Sea, including the Dnister River (haplotypes F31, F37, and F38; designated the

'Danubian' group in Fig. 3). This clade comes in contact with other phylogroups in the Ukraine, where a number of independent mitochondrial lineages can be found (Fig. 2). Notably, however, these different lineages appear restricted to different drainages of the Black Sea. In particular, *T.* cf. *velox* was found only in the Dniper River and its estuaries; all other populations were more closely related to Danubian and Anatolian populations.

All haplotypes from western Europe, excluding the *T.* cf. *velox* group, form a monophyletic unit with 96% posterior probability. These haplotypes are in turn related to haplotypes found in Greece (F35) and Ukraine (F30) that together form a distinct clade with 99% posterior probability. Haplotypes from Spain and Italy fall into multiple phylogroups. Northern Italy in particular appears to harbour several lineages (Figs 2 and 3).

All individuals sampled from estuarine habitats in the Baltic Sea shared the same haplotype as individuals sampled from nearby freshwater sites. Only one haplotype was found restricted to brackish populations in the Black Sea (two individuals with F37 from two estuaries near Odessa), and it is separated by no more than two nucleotide substitutions.

Nested clade phylogeographical analysis

The nested clade analysis resulted in five nesting levels (Fig. 4 and Table 2). The final two clades (i.e. at the fourth

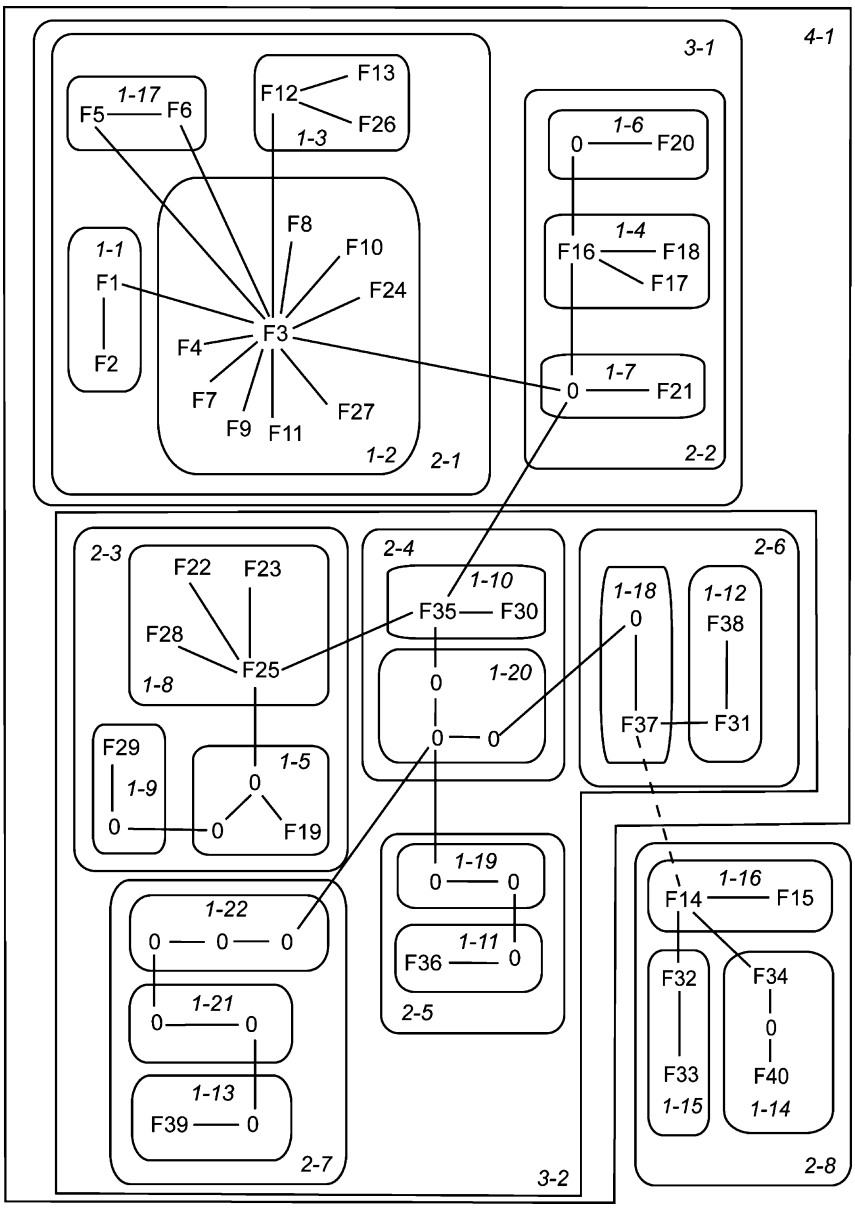

Fig. 4 Haplotype network and nested clades for *Theodoxus fluviatilis*. Names of nested clades are in italics, see Table 2 for phylogeographical inferences of nested clades. The dashed line indicates where the statistical parsimony procedure could not connect two clades; this connection is inferred from the phylogenetic analyses.

Clade	Interior haplotypes/clades	D_c (km)	D_n (km)	Inference chain
1-2				1-2-3-5-15-16-18-No: F/REC/IBD
	F3	425.0	429.6	
	F4	0.0	106.4	
	F7	0.0	77.6	
	F8	0.0	77.6	
	F9	0.0*	51.9*	
	F10	0.0	112.8	
	F11	0.0	111.5	
	F24	0.0	841.5	
	F27	0.0	294.2†	
	I-T	425.0†	189.5	
1-3				1-2-3-5-6: REC/RGF
	F12	225.5*	401.2	
	F13	10.0*	435.3†	
	F26	0.0	436.5	
	I-T	215.9†	–34.2*	
1-8				1-19-20-2-3-5-15-No: PF/LDC
	F22	0.0	532.3	
	F23	0.0	697.2†	
	F25	489.2	578.9†	
	F28	39.3*	379.6*	
	I-T	456.5†	160.1†	
1-10				1-19-20-Yes: INC
	F30	109.5*	305.9*	
	F35	0.0	36.1†	
	I-T	109.5	730.1†	
1-14				1-2-11-17-No: INC
	F40	0.0	3.3	
	F34	3.4	3.4	
	I-T	3.4	0.0	
1-16				1-19-No: AF
	F14	0.0	11.4†	
	F15	7.1	7.2*	
	I-T	–7.1	4.2†	
2-1				1-2-3-4-No: RGF with IBD
	1-1	108.3*	520.3	
	1-2	414.1	402.6	
	1-3	415.3	456.0	
	1-17	0.0*	10.4*	
	I-T	94.6	–94.6	
2-2				1-2-3-4-No: RGF with IBD
	1-4	15.6*	40.4†	
	1-6	0.0	43.9	
	1-7	59.6	68.4†	
	I-T	44.2*	14.7	
2-3				1-2-3-4-9-No: PF
	1-5	0.0	677.1	
	1-8	495.0	529.8†	
	1-9	11.4*	443.2*	
	I-T	463.8†	92.5†	
2-6				1-2-3-4-No: RGF with IBD
	1-12	439.0*	521.1	
	1-18	0.2	489.5	
	I-T	438.8	–31.7	

Table 2 Results of the nested clade analysis of *Theodoxus fluviatilis*. Only those clades that could reject the null hypothesis of no phylogeographical structure are shown. D_c represents the geographical spread within a clade and D_n is the geographical spread between nested clades. I-T is the average distance between interior and tip nodes in a given nested clade. Abbreviations for the phylogeographical mechanism inferred by the nested clade analysis are as follows: IBD, isolation by distance; LDC, long-distance colonization; REC, range expansion/colonization; RGF, restricted dispersal/gene flow; F, fragmentation; PF, past fragmentation; AF, allopatric fragmentation; CRE, contiguous range expansion; INC, inconclusive outcome

Table 2 *Continued*

Clade	Interior haplotypes/clades	D_c (km)	D_n (km)	Inference chain
2-8				1-2-3-5-15-16-18-No: F/REC/IBD
	1-14	3.4*	786.0	
	1-15	80.1*	735.7*	
	1-16	7.7*	797.9†	
	I-T	–34.0	37.1†	
3-1				1-2-3-5-15-16-No: AF
	2-1	416.3*	454.0*	
	2-2	58.9*	821.1†	
	I-T	357.4*	367.1†	
3-2				1-2-3-5-6: REC/RGF
	2-3	508.7*	189.6†	
	2-4	471.3*	82.1	
	2-5	0.0*	992.8	
	2-6	511.2*	819.4*	
	2-7	0.0*	101.4	
	I-T	18.4	11.2	
4-1				1-2-3-4-No: RGF with IBD
	3-1	505.3*	632.0*	
	3-2	59.4†	229.0†	
	I-T	554.1†	597.0†	

*Distances that are significantly ($P < 0.05$) smaller than expected.
†Distances that are significantly ($P < 0.05$) larger than expected.

nesting level), 2-8 and 4-1 (*T.* cf. *velox* vs. the remainder), were separated by 14 mutational steps and could not be connected at the 95% confidence level of the statistical parsimony procedure. Out of the 34 nested clades, 14 could reject the null hypothesis of no phylogeographical structure. Eight out of the 11 clades above the first nesting level showed phylogeographical structure. Inferred reasons for these associations include recent increased gene flow and/or range expansion and past range fragmentation (Table 2).

The NCPA indicates that recent phylogeographical structuring has involved mostly nonallopatric processes (i.e. restricted dispersal, range expansion, isolation by distance, or long-distance dispersal). Of the eight one-step clades for which geographical structure was found (out of 14), only three were consistent with some types of range fragmentation or allopatric distribution (clades 1-2, 1-8, and 1-16). One of three inferences for clade 1-2 is fragmentation. Clade 1-8 includes Italian and French haplotypes, separated by the Alps. Clade 1-16 appears to be the result of allopatry on a very small scale because the two populations that show allopatric fragmentation are from lakes in northern Germany that are separated by at most 18 km.

At the second nesting level, five of eight nested clades showed statistically significant geographical association. All phylogeographical structure appears to result from either restricted gene flow with isolation by distance (clades 2-1, 2-2, 2-6, and perhaps 2-8) or fragmentation (clade 2-3 and perhaps 2-8). There is a phylogroup that inhabits northern Europe (clade 2-1), corresponding broadly to regions glaciated during the Pleistocene, but including some populations to the south of the glacial maximum. A second phylogroup is restricted to northern Italy (clade 2-2), and a third is found throughout southwestern Europe (clade 2-3; present in Italy, France, and Spain). There are several eastern phylogroups (clades 2-4, 2-5, 2-6, and 2-7; distributed in the Ukraine, Austria, Hungary, Greece, and/or Turkey) and one that corresponds to the *T.* cf. *velox* group (clade 2-8). The distributions of these two-step clades are mapped in Fig. 5 according to their sampling localities (see Table 1). The region of greatest overlap of two-step clades is around the northwestern Black Sea, consistent with a role as the ancestral range of *T. fluviatilis*.

At higher nesting levels, fragmentation seems to have played a more significant role. Allopatric fragmentation inferred for clade 3-1 represents a division between samples from north of the Alps (clade 2-1) and samples from south of the Alps (clade 2-2). Within clade 3-2 — which is distributed from France and Spain, through Italy, the Danubian Drainage, and around the Black Sea (including Turkey) — several groups have been subdivided by past fragmentation and several others demonstrate restricted gene flow (Table 2). In sum, there is significant phylogeographical structuring at all levels within *T. fluviatilis*. Many of the phylogroups that display either fragmentation or reduced gene flow correspond to geographical areas that are separated by barriers or uninhabited areas and are represented by older clades. More recent lineages still show the ability to disperse, although gene flow appears limited

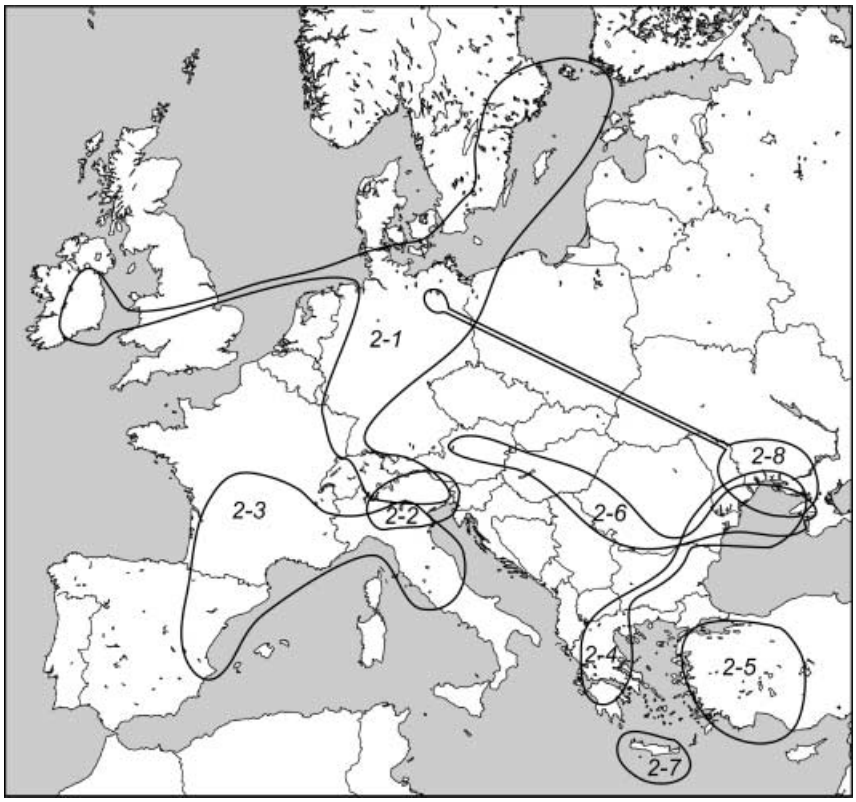

Fig. 5 Map of all two-step clades in *Theodoxus fluviatilis* reconstructed by the statistical parsimony analysis and nesting procedure of the NCPA. The regions in which these clades are distributed are shown. Areas that were not sampled in this study are not included. Clades 2-3, 2-4, 2-5, 2-6, and 2-7 are all nested together in the southern European clade 3-2.

Table 3 Genetic diversity within different regions of the range of *Theodoxus fluviatilis*. The haplotype diversity is equal to the probability that two randomly sampled haplotypes are different and the nucleotide diversity is equal to the probability that two randomly sampled homologous nucleotides are different (Nei 1987). Mean pairwise differences are calculated by averaging all pairwise corrected (Kimura 2-parameter) nucleotide substitutions between unique haplotypes within a sample. The value in parentheses for northern Europe is for all haplotypes excluding those from the *T*. cf. *velox* group. Northern Europe includes samples from Germany, Sweden, Finland, and Ireland. Southwestern Europe includes samples from Italy, Spain, and southern France. The Ponto-Pannonian region includes samples from the Danube River (Austria and Hungary) and around the Black Sea (Ukraine). Southeastern Europe includes samples from Greece (including Crete) and western Turkey

Region	Sample sites	Number of individuals	Number of haplotypes	Haplotype diversity (H)	Nucleotide diversity (π)	Mean pairwise differences
Northern Europe	37	175	17	0.726 ± 0.030	0.0066 ± 0.0037	3.94 ± 1.98
	(37)	(160)	(15)	(0.678 ± 0.034)	(0.0018 ± 0.0013)	(1.05 ± 0.70)
Southwestern Europe	17	67	13	0.870 ± 0.016	0.0049 ± 0.0029	2.95 ± 1.56
Ponto-Pannonian	11	47	8	0.810 ± 0.023	0.0169 ± 0.0088	10.12 ± 4.71
Southeastern Europe	3	8	3	0.750 ± 0.097	0.0116 ± 0.0069	6.86 ± 3.61
Total range	68	297	40	0.871 ± 0.016	0.0116 ± 0.0060	6.93 ± 3.27

across great distances (e.g. peripheral populations in northern Europe), resulting in several young clades that differentiate in contiguous ranges or by long-distance dispersal.

Genetic diversity and pairwise mismatch distribution

The values for haplotype and nucleotide diversity are shown in Table 3 and describe a species with relatively low variation in both the number of haplotypes and patterns of nucleotide substitution when compared to other neritids, i.e. Nei's (1987) $H = 0.961 \pm 0.009$ in *Neritina canalis* and $H = 0.951 \pm 0.015$ in *Clithon spinosa* (P. Bunje, unpublished data). The majority of genetic diversity appears to be located in southern and eastern areas, especially surrounding the Black Sea (Table 3). The high value found for northern Europe is an artefact of the presence of the distantly related *T*. cf. *velox* group (see Table 3). Values for nucleotide

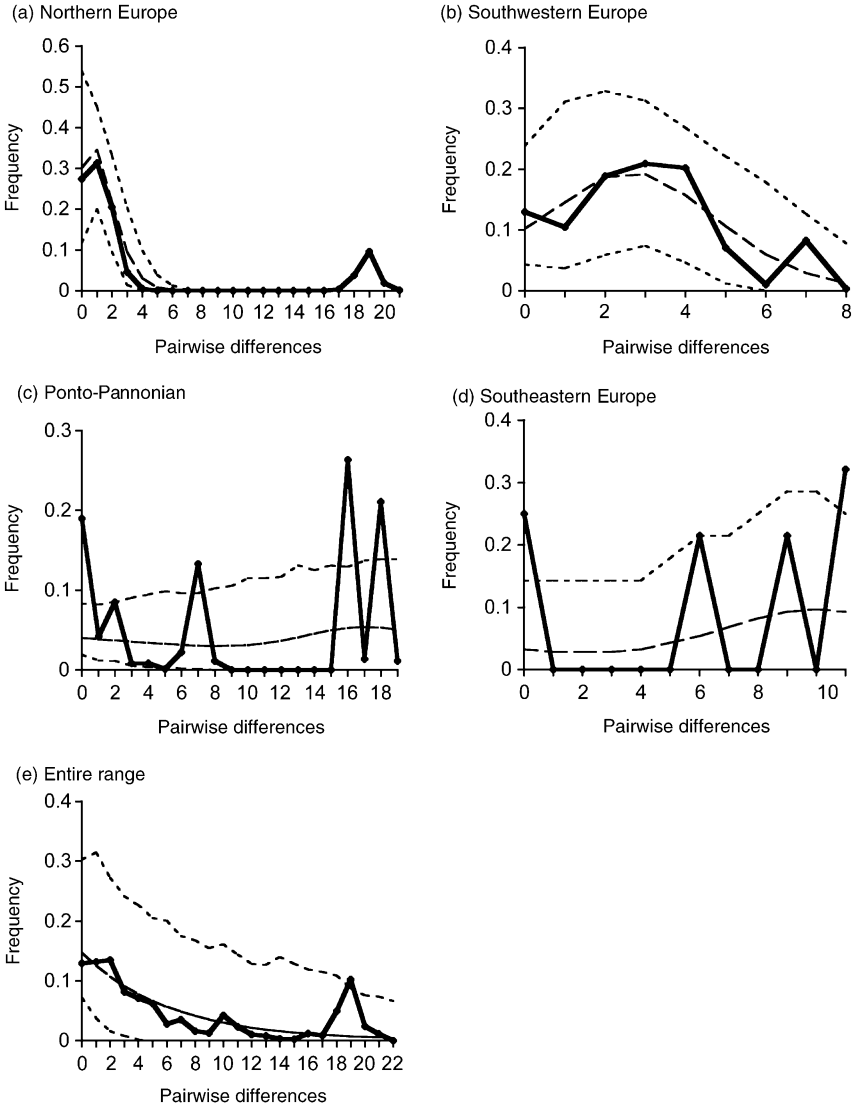

Fig. 6 Pairwise mismatch distribution for *Theodoxus fluviatilis*. Solid lines indicate the observed frequency of pairwise mismatches between haplotypes within each region. Dotted lines represent the lower and upper bounds of the 95% confidence interval and the dashed lines represent the modelled frequency of expected pairwise differences. (a) Samples from northern European sites (Germany, Sweden, Finland, Ireland). (b) Samples from southwestern Europe (Italy, Spain, southern France). (c) Samples from the Ponto-Pannonian region (Austria, Hungary, the Ukraine). (d) Samples from southeastern Europe (Greece, Crete, Turkey). (e) All samples from all localities.

diversity and haplotype diversity are particularly high in the Ponto-Pannonian region, and genetic distances in this area are much higher than for any other part of the range. This may reflect the presence of many basal lineages in eastern Europe as well as the presence of most of the primary phylogroups. Northern Europe houses the least amount of genetic diversity, as expected given recent glaciations.

The pairwise mismatch distribution of northern European haplotypes cannot be statistically distinguished from the model Poisson-like distribution ($P < 0.05$; Fig. 6a). This is consistent with a model of recent exponential growth (Slatkin & Hudson 1991) as would be expected from a recent post-Pleistocene range expansion. The small peak seen at 19 pairwise differences represents the few haplotypes that are most closely related to members of the *T.* cf. *velox* group in the Ukraine. For the species as a whole (Fig. 6e) and all other regions (Fig. 6b–d), the mismatch distribution does not show a unimodal distribution and so cannot be considered to be the result of a single recent population expansion (Rogers & Harpending 1992).

Discussion

Major phylogeographical units

In this section I describe the different phylogeographical groups that can be identified and the hypothesized processes that have produced them. To begin, *Theodoxus fluviatilis* is a species with clear geographical subdivisions, particularly between eastern and western Europe and separating all northern European populations (Figs 2 and 3). Further, the hypothesis that terrestrial barriers to gene flow are promoting geographical associations among

genetic lineages is borne out by the analyses performed here. The NCPA, in particular, indicates that 12 of the 14 clades for which phylogeographical structure was found had distributions consistent with some type of fragmentation or restriction to dispersal (Table 2). Although NCPA inferences cannot always discriminate fragmentation from other mechanisms in one-step clades, for deeper phylogenetic divisions (i.e. higher nesting levels) barriers to dispersal appear particularly important (clade 3-1 is inferred to result from allopatric fragmentation, clade 3-2 is inferred to result from range expansion or restricted gene flow).

There are several large groups within *T. fluviatilis* that are separated by major geographical features of Europe. In particular, the Alpine mountain ranges (including the Alps, Pyranees, Dinaric Alps, and Carpathians) subdivide this species into a northern and southern group. The clade inhabiting northern Europe (haplotype F3 and its descendants) appears to be more recent in origin as it has lower genetic diversity and occupies areas that were heavily glaciated during the Pleistocene (1.33 mean pairwise differences). Within the northern European clade, there is further allopatric structuring where the Alps separate populations in northern Italy that possess the basal haplotype (F3) from the remainder of the clade. The presence of recent allopatric fragmentation supports the notion that barriers to gene flow play a persistent role in shaping the phylogeographical structure of *T. fluviatilis*. This does not, however, preclude the fact that *T. fluviatilis* appears to continue to disperse throughout its range. Particularly in northern Europe, this species appears to be relatively panmictic (haplotype F3 is found in 57% of northern European sites), although this may be due to a lack of resolution in the genetic marker analysed. There is no evidence of recent historical gene flow resulting from anthropogenic modification of habitat, i.e. via the Main-Danube Canal. Indeed, populations in the Danube River are highly distinct (2.1% sequence divergence) from populations of the Main River drainage.

Populations from northern Europe form a distinct clade (excluding the 15 individuals of the *T.* cf. *velox* group), all having arisen from a single ancestral haplotype (F3; see Figs 2 and 3). By contrast, southern Europe is inhabited by a much more heterogenous group of haplotypes and lineages (range-wide haplotype diversity excluding northern European populations is 0.932 ± 0.007), consistent with a longer period of inhabitation. Furthermore, the range is marginally subdivided along east/west lines. Populations from Spain, southern France, and Italy are all closely related (along with the northern clade) to the exclusion of populations from Black Sea drainages (including the Danube of Austria), Turkey, and Greece (96% posterior probability; Fig. 2).

In addition to the haplotype that gave rise to northern European populations, two other major subclades are present in northern Italy (clades 2-2 and 2-3 in Fig. 4). Haplotypes from both subclades are found in close geographical proximity (although never in the same population), so it is reasonable to assume that the separate lineages were present in northern Italy throughout the Pleistocene. The haplotypes that are more closely related to Spanish and French lineages inhabit the southern and western areas sampled in Italy. Specifically, clade 2-3 in Fig. 5 includes haplotype F25 from western Italy site 088 and French and Spanish descendants. The haplotypes related to northern European individuals were most frequently sampled in the sub-Alpine glacial lakes and their outflows, particularly the Po River drainage (clade 2-2 in Fig. 5). The glacial lakes of northern Italy appear to have been colonized by individuals from nearby regions that remained ice free during the Pleistocene. Colonization probably occurred along fluvial paths, as is common for low-vagility aquatic organisms (Taberlet *et al*. 1998; Zardoya & Doadrio 1999).

Spanish populations are separated by three mutational steps from related populations in Italy, indicating some isolation of *T. fluviatilis* on the Iberian Peninsula. The NCPA concluded that this pattern was the result of past fragmentation, perhaps as a result of distribution across the Alps and Pyrenees (clade 1-8; this pattern also holds true one nesting level up in clade 2-3). Because this species is able to tolerate salinity (Bielawski 1960), the distribution could also indicate fragmentation that occurred across the western Mediterranean. Sampling of populations on Corsica or Sardinia, if any exist, would help clarify this situation.

Two of the most distinct lineages in *T. fluviatilis* are from Turkey and Greece (including Crete). In these areas, *T. fluviatilis* is distributed across large geological barriers such as the Balkan mountain ranges and the Black and Mediterranean seas. The high degree of genetic differentiation for these lineages is consistent with this distribution (Crete is separated from its nearest relative by 1.9% sequence divergence, Turkey by 1.3%, but Greece by only 0.2%). Anatolian and Greek populations lie in a basal phylogenetic position amidst other eastern subclades (Fig. 2), supporting the hypothesis that eastern Europe contains the ancestral range of *T. fluviatilis*. More sampling is needed to assess the exact nature of relationships among *T. fluviatilis* populations in southeastern Europe and Anatolia.

One of the most notable features of the phylogeography of *T. fluviatilis* is the disjunct distribution of related haplotypes in the Ukraine and northern Germany. *Theodoxus velox* is a recently described species (Anistratenko *et al*. 1999) from the Dniper delta in the northern Black Sea and nearby river mouths. In addition to identifying individuals thought to be *T. velox* in the Dniper River and its estuary, these analyses also identified related haplotypes from northern Germany, 1400 km away. *T.* cf. *velox* haplotypes are highly divergent (~3% substitution rate) from the rest of *T. fluviatilis* and form a unique clade with high statistical support (100%

bootstrap, 100% Bayesian posterior probability). There are two potential explanations for this pattern that hinge upon the species status of *T. velox*. (i) If *T. velox* is indeed a distinct species (i.e. no intermediate haplotypes are found upon further sampling of northeastern Europe, the individuals from northern Germany are distinct from their cohabitants at many genetic loci, and these mitochondrial haplotypes represent a historically independent organismal lineage; de Queiroz 1998), then the related haplotypes present in northern Germany are probably the result of deep coalescence within sister species (Maddison 1997). Given the lack of morphological differentiation among these snails (Zettler *et al*. 2004) and their close proximity, I find it unlikely that they are separate biological species (*sensu* Mayr 1963). (ii) Alternatively, this may simply be evidence of two old lineages within *T. fluviatilis*, one primarily present in the west of the range, the other more prominent in the east. When the ice sheets receded from northern Europe, these two independent lineages would have then come back into contact in northeastern Europe. In both scenarios, incomplete lineage sorting has led to an interesting pattern of post-Pleistocene range expansion, discussed in more detail below.

Although terrestrial barriers appear to produce geographically restricted clades in *T. fluviatilis*, the same is not true for marine habitats. Only one haplotype was found restricted to brackish populations in the Black Sea, all Baltic haplotypes and the remainder of Black Sea haplotypes are shared between habitats (see Table 1). This confirms the hypothesis that *T. fluviatilis* from brackish habitats are closely related to freshwater populations (Bielawski 1960; Kangas & Skoog 1978). Furthermore, no morphological differentiation was found between brackish and freshwater individuals in a recent analysis (Zettler *et al*. 2004). The fact that this physiological lability is not correlated with morphological and/or genetic differentiation indicates that salinity tolerance represents a phenotypically plastic character in *T. fluviatilis*.

The effects of Pleistocene glaciation

Excepting the *T.* cf. *velox* haplotypes, populations of northern Europe are made up of closely related individuals that have all descended from a few post-Pleistocene colonizers. As would be expected from a group that inhabits an area covered by ice 13 000 years ago, the mismatch distribution of northern European populations is consistent with recent range expansion and population growth (Fig. 6a; Slatkin & Hudson 1991), the product of (re)colonization following the last glacial maximum. The haplotypes found from northern Germany, around the Baltic, and in distant Ireland, do not show fragmentary structuring among them, but are structured instead by the great distances separating them (NCPA infers restricted gene flow with isolation by distance for clade 2-1; Table 2). Except for the populations from central Germany (haplotypes F1 and F2), genotypic diversity in northern Europe is not associated with any particular area (see Table 1). This pattern is consistent with the hypothesis of rapid range expansion following the glacial retreat. Some gene flow is probably occurring, in particular across the Baltic Sea where identical and closely related haplotypes can be found in Germany, Sweden, and Finland. Consequently, the Baltic Sea does not act as a significant barrier to gene flow, and may in fact be facilitating gene flow in contrast to the terrestrial barriers that exist between continental populations.

The phylogenetic relationships of the northern clade indicate that individuals that colonized northern Europe following the glacial retreat probably originated in Italy and possessed the basal, common haplotype F3 (see Table 1 and Fig. 3). This geographical distribution implies that postglacial expansion must have occurred from snails transferred across the Alps, perhaps as a result of avian transport (Wesselingh *et al*. 1999). However, the presence of distantly related *T.* cf. *velox* haplotypes in several populations of northern Germany suggest that there were a minimum of two sources of post-Pleistocene range expansion, as noted above. The *T.* cf. *velox* populations in the Ukraine all come from the Dniper River, which runs northwards into Belarus and near Baltic Sea watersheds. This indicates that a second postglacial colonizing route of northern Europe may have gone through Belarus, Poland, and/or the Baltic States. The Black Sea area is known to have contributed to the postglacial expansion of *Barbus* fishes (Kotlik *et al*. 2004); these data reveal that the area was important for other freshwater organisms as well. The presence of two distinct mitochondrial lineages in northern Germany represents, at the least, the expansion of related *Theodoxus* into formerly glaciated areas of Europe from a minimum of two Pleistocene refugia in the south.

Historical phylogeographical structure

I hypothesize a general scheme of colonization and divergence for *T. fluviatilis* in Fig. 7. This hypothesized history uses the assumption that *T. fluviatilis* began colonizing Europe from the region surrounding Ponto-Pannonian basins. There are several reasons to believe that the ancestral range of *T. fluviatilis* existed in the area of southern Ukraine/Romania/Hungary. These reasons include the basal position of the *T.* cf. *velox* group (Fig. 3), the high haplotype diversity found in this region ($H = 0.810 \pm 0.023$), the presence of most of the major phylogroups in the Ponto-Pannonian region (Fig. 5), and the phylogenetic position of distant populations at the tips of the tree (Figs 2 and 3). In particular, the finding that western Europe contains populations that form a derived monophyletic unit with high support (96% posterior probability) supports a role for the Ponto-Pannonian

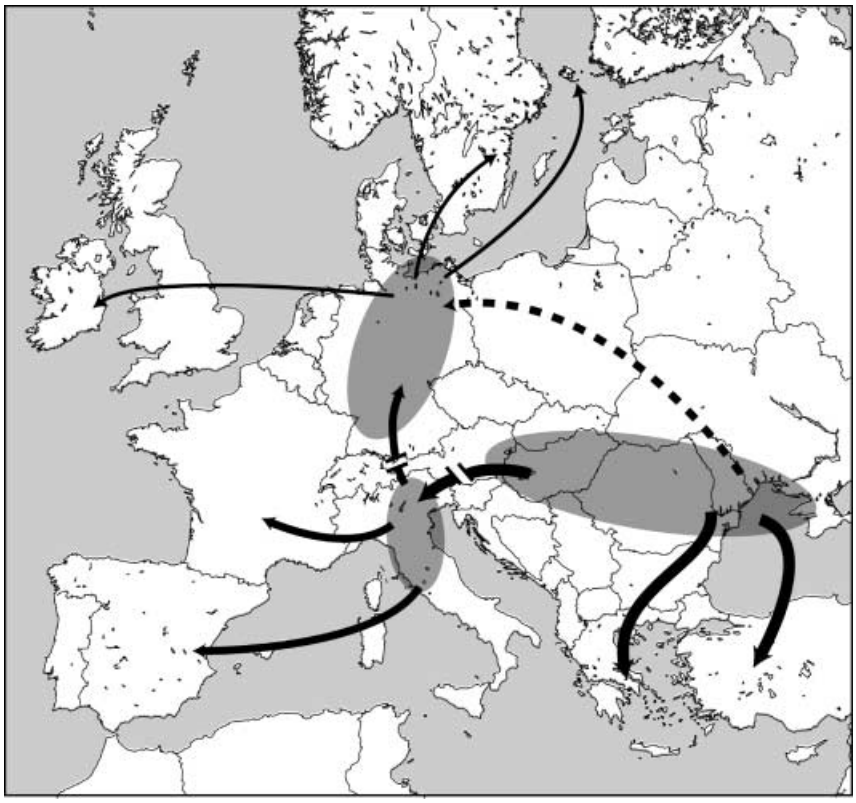

Fig. 7 Hypothesized colonization scheme for *Theodoxus fluviatilis*. The hypothetical ancestral range of this species is in the Ponto-Pannonian region. Dispersal from this area occurred at different times. The thickness of the dispersal lines is indicative of their hypothesized recency: the thicker the line the older the dispersal event. Therefore, the thinnest lines represent hypothesized dispersal in northern Europe occurring during the Holocene. The broken lines represent fragmentation events. The dashed line indicates uncertain dispersal events for individuals from the Ukraine to northern Germany (clade 2-8 in Fig. 4).

region as the cradle of ancestral haplotypes. Following different periods of colonization, range expansion and fragmentation have affected several areas of the distribution of *T. fluviatilis*, resulting in the complex intraspecific phylogenetic relationships that are evidenced today.

In this snail species, two areas appear to have housed the ancestral populations that later colonized northern areas following the glacial retreat: Italy and the Ukraine. This is evidenced by the relationships of northern European haplotypes, derived from the Italian F3 and the Ukrainian *T.* cf. *velox* group. This pattern of ancestry is consistent with research that describes the southern European peninsulas as important regions of glacial refugia and source populations for recolonization (Hewitt 1999, 2004). The Alpine arc that separates all of these peninsulas from the contiguous habitat north of the Alps probably represents at least as strong a phylogeographical force as glaciation does (Durand *et al*. 1999; Bernatchez 2001). However, populations from the Ukraine are not separated by large mountain barriers, indicating that eastern refugia may also represent important sources for recolonization.

The higher haplotype diversity in southern and eastern Europe (Table 3) and the derived phylogenetic position of the northern lineages (Fig. 3), living in areas that were previously glaciated, confirm trends in post-Pleistocene phylogeography seen in many species (Taberlet *et al*. 1998; Hewitt 1999). The pairwise mismatch distribution (Fig. 6) supports the hypothesis that populations to the south of the glacial maximum have been stable for some time. Only the populations inhabiting areas that were glaciated during the Pleistocene (i.e. clade 2-1 in Figs 5 and 6a) show a unimodal mismatch distribution consistent with recent population growth and range expansion (Slatkin & Hudson 1991).

Largely correlated with geography, *T. fluviatilis* has apparently been involved in a complex pattern of colonization and fragmentation associated with its allopatrically distributed habitats and their intricate geological history. This complexity is typical of both terrestrial (Hewitt 1996; Haase *et al*. 2003) and freshwater (Bernatchez & Wilson 1998; Durand *et al*. 1999; Bernatchez 2001; Perdices *et al*. 2003) species in Europe. Notably, this single species shows parallel features of phylogeography that are found in diverse European species with more limited distributions or compared with studies that have focused on only a portion of the range. The finding that Alpine mountain ranges are a significant barrier to dispersal is seen in freshwater animals (Durand *et al*. 1999; Slechtova *et al*. 2004), terrestrial animals (Seddon *et al*. 2002), and plants (van der Velde & Bijlsma 2003). Evidence for postglacial range expansion is pronounced in many organisms, particularly in northern Europe where it is most notable in *T. fluviatilis* (Hewitt 1999; Haase *et al*. 2003; Salzburger *et al*. 2003; Garnier *et al*. 2004). The finding of an Eastern colonization route, likely

from the Black Sea, is noteworthy. However, increased lineage diversity in southern European peninsulas has been reported for several organisms (Hewitt 1996, 2004) and the finding of basal divergence surrounding the Black Sea has been observed in at least one freshwater fish clade (Kotlik *et al*. 2004). In sum, the phylogeographical patterns of this single species encompass many of the patterns and processes seen for European fauna as a whole. The complex geology, heterogenous environments, and strong allopatric boundaries of Europe have led to distinct phylogeographical patterns in many terrestrial groups. It appears that these forces have made at least as important an impact on the divergence and phylogeographical structure of freshwater animals such as *T. fluviatilis*.

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My research interests focus on the processes that drive diversification. I am interested in the interplay of speciation and extinction mechanisms that produce the standing diversity of natural lineages. This research formed a portion of my doctoral research into the diversification of freshwater neritid snails. Currently, I am a postdoctoral researcher investigating the genetic basis for phenotypic diversity in cichlid fish.
