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THE EVOLUTIONARY HISTORY OF BROWN TROUT (*SALMO TRUTTA* L.) INFERRED FROM PHYLOGEOGRAPHIC, NESTED CLADE, AND MISMATCH ANALYSES OF MITOCHONDRIAL DNA VARIATION

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Abstract.—Phylogeographic, nested clade, and mismatch analyses of mitochondrial DNA (mtDNA) variation were used to infer the temporal dynamics of distributional and demographic history of brown trout (*Salmo trutta*). Both new and previously published data were analyzed for 1794 trout from 174 populations. This combined analysis improved our knowledge of the complex evolutionary history of brown trout throughout its native Eurasian and North African range of distribution in many ways. It confirmed the existence of five major evolutionary lineages that evolved in geographic isolation during the Pleistocene and have remained largely allopatric since then. These should be recognized as the basic evolutionarily significant units within brown trout. Finer phylogeographic structuring was also resolved within major lineages. Contrasting temporal juxtaposition of different evolutionary factors and timing of major demographic expansions were observed among lineages. These unique evolutionary histories have been shaped both by the differential latitudinal impact of glaciations on habitat loss and potential for dispersal, as well as climatic impacts and landscape heterogeneity that translated in a longitudinal pattern of genetic diversity and population structuring at more southern latitudes. This study also provided evidence for the role of biological factors in addition to that of physical isolation in limiting introgressive hybridization among major trout lineages.

Key words.—Europe, fish, mismatch, mitochondrial DNA, nested clade, phylogeography, *Salmo*.

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Elucidating the evolutionary history of extant species is an important objective of any research program that seeks to understand population divergence and, ultimately, speciation. This history is also directly relevant to conservation biology because historical contingencies have been largely responsible for creating the most important genetic subdivisions in many, if not most extant taxa (e.g., Zink 1996; Avise et al. 1998; Hewitt 2000). The phylogeographic approach has been used to test the congruence between distributional histories against paleo-environmental settings and determining the chronology of evolutionary diversification (Avise 1998; Moritz and Bermingham 1998). Comparative phylogeography has also emerged as a powerful method to address broader ecological and evolutionary issues.

In northern temperate freshwater fishes, comparative phylogeography revealed predictive trends in phylogeographic structure, genetic diversity, and speciation rates among species inhabiting formerly glaciated and unglaciated regions of North America (Bernatchez and Wilson 1998). Further generalizations of the effect of Pleistocene glaciations on freshwater fish fauna could be gained by comparing phylogeographic data from other regions. Contrasts between Eurasian and North American freshwater fish phylogeographic structure (e.g., Bernatchez et al. 1989) would be particularly informative, given the much less extensive glacial advances in Eurasia and its contrasting landscape with that of North America (Hewitt 2000). However, the phylogeographic structure of the Eurasian fish fauna is still largely unknown (Bernatchez and Dodson 1994; Durand et al. 1999a; Nesbo et al. 1999; Englbrecht et al. 2000).

The brown trout (*Salmo trutta* L.) is the most widely distributed freshwater fish native to the Palearctic region. Its natural range extends from northern Norway and northeastern Russia, southward to the Atlas Mountains of North Africa. From west to east, its range spans from Iceland to the headwaters of Aral Sea affluents in Afghanistan. *Salmo trutta* also exhibits considerable morphological diversity and life-history variation, including specializations for anadromous, fluviatile, and lacustrine modes of life. Large-scale patterns of genetic diversity in brown trout have been studied extensively over the last two decades, using both allozymes (e.g., Ryman 1983; Ferguson 1989; Guyomard 1989; Osinov 1990; García-Marín and Pla 1996; Largiadèr and Scholl 1996) and mitochondrial (mtDNA) analyses (Bernatchez et al. 1992; Giuffra et al. 1994; Hynes et al. 1996; Osinov and Bernatchez 1996; Apostolidis et al. 1997; Hansen and Mensberg 1998; Weiss et al. 2000). Yet, the analysis of brown trout phylogeographic structure is still lacking over important geographic areas, such as North Africa and eastern Europe. Patterns of postglacial recolonizations, finer phylogeographic structure within major trout lineages, and demographic history have not been rigorously assessed or remain controversial (e.g., Hamilton et al. 1989; Osinov and Bernatchez 1996; García-Marín et al. 1999). This ambiguity may be attributed to both limited analytical resolution and statistical treatments.

Previous phylogeographic analyses of brown trout have relied upon the use of haplotype trees and geographic distribution to make biological inference by visual inspection of how geography overlays haplotype relationships. This may not make full use of all historical information contained in gene genealogies. Namely, this does not allow the estimation of the dynamic structure and temporal juxtaposition of different evolutionary factors that are most likely compatible with the patterns of genetic diversity observed in extant populations. The recent development of statistical nested clade analysis of phylogeographic data offers a potentially useful

TABLE 1. Number of populations, sample sizes, and haplotype diversity (h) within major trout evolutionary lineages. For populations, the first number indicates the number of pure populations for a particular lineage and the number in parentheses indicates the number of admixed populations but predominated in relative abundance by the particular lineage.

Lineage	Number of populations	Number of fish	Number of haplotypes			Haplotype diversity (SE)
			Sequence	RFLP	Combined	
Atlantic (AT)	55 (1)	993	8	14	16	0.582 (0.011)
Danubian (DA)	48 (1)	191	14	28	35	0.931 (0.005)
Adriatic (AD)	37 (5)	298	10	11	17	0.851 (0.009)
Mediterranean (ME)	12 (1)	104	3	2	3	0.523 (0.011)
marmoratus (MA)	14 (0)	205	3	2	4	0.511 (0.007)
Total	166 (8)	1791	38	57	75	0.679 (0.008)

framework in that respect (Templeton et al. 1995; Templeton 1998). Yet, the use of this method has been limited thus far (Templeton et al. 1995; Durand et al. 1999b; Nesbo et al. 1999; Turner et al. 2000). The traditional phylogeographic approach is also limited in inferring the dynamics of demographic history. The analysis of mismatch distribution provides a way to estimate the magnitude and age of population growth by statistically comparing the distribution of intrapopulation molecular diversity with that expected under hypotheses of equilibrium (Rogers and Harpening 1992; Rogers 1995; Schneider and Excoffier 1999). However, the use of mismatch distribution has almost entirely been limited to human studies (but see Lavery et al. 1996; Merilä et al. 1997; Petit et al. 1999).

In this study, I performed a genetic diversity analysis of mtDNA and combined phylogeographic, nested clade, and mismatch analyses to infer the temporal dynamics of distributional and demographic history of the brown trout throughout its native geographic range. New results and previously published data were combined to document the overall geographic distribution of major trout evolutionary lineages. I infer the temporal juxtaposition of factors most compatible with the differential patterns of genetic diversity observed among these lineages and discuss these factors against paleoenvironmental settings encountered by brown trout in different parts of its range.

MATERIALS AND METHODS

Samples

This study combines new analyses and previously published results (Appendix). A total of 277 fish representing 92 sampling sites had never been analyzed. Samples previously analyzed for sequence variation of the mtDNA control region (D-loop) (Bernatchez et al. 1992) were reanalyzed using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) to increase the number of character states and allow standardization with previous studies. We also included the results of PCR-RFLP and sequence analyses performed by Giuffra et al. (1994), Bernatchez and Osinov (1995), Osinov and Bernatchez (1996), and Apostolidis et al. (1996a, 1997). The results obtained by Hynes et al. (1996) were standardized by performing the same sequence and PCR-RFLP analyses used for the other fish on representatives of all mtDNA haplotypes identified by these authors using RFLP analyses over the entire mtDNA genome. Sixteen fish from the Mediterranean basin that possessed the mtDNA lineage of Atlantic origin were discarded because they most likely represented the results of stocking, thus potentially blurring historical signals (Guyomard 1989; Bernatchez et al. 1992). Given these exclusions and that several populations and samples overlapped among some of these studies (Appendix), this analysis was based on the genetic information obtained for 1794 trout representing 174 populations (Table 1).

Laboratory Analyses

MtDNA variation was analyzed using both sequencing and RFLP performed on PCR-amplified products. Sequencing was done on the same 310-bp segment (now 313 due to new indels) located at the 5′ end of the control region studied previously in brown trout (Bernatchez et al. 1992; Apostolidis et al. 1997). The primers LN20 (5′-ACC ACT AGC ACC CAA AGC TA-3′) and HN20 (5′-GTG TTA TGC TTT AGT TAA GC-3′), which amplify the whole control region, were used for PCR, whereas primer H2 (5′-CGT TGG TCG GTT CTT AC-3′) was used for sequencing (Bernatchez and Danzmann 1993). Technical procedures of mtDNA purification, amplification, and sequencing are detailed in Bernatchez et al. (1992). RFLP analysis was performed using six restriction enzymes (*Hinf*I, *Hpa*II, *Mbo*I, *Nci*I, *Rsa*I, and *Taq*I) on two adjacent PCR-amplified segments; the complete ND-5/6 region (~2.4 kb), and a second segment (~2.1 kb) comprising the whole cytochrome oxydase *b* gene and the D-loop. Primers, amplifications, restriction digests, and electrophoresis procedures were as described in Bernatchez and Osinov (1995). Distinct single endonuclease patterns were identified by a specific letter in order of appearance and used in combination with sequence variation to define sequence/RFLP composite genotypes.

Genetic Diversity Analyses

The combined information of three previous studies based on a small number of individuals but higher genetic resolution than used here resolved five major mtDNA phylogenetic lineages in brown trout (Fig. 1). Based on the origin of the first haplotypes identified, these were named Atlantic (AT), Danubian (DA), Mediterranean (ME), *marmoratus* (MA), and Adriatic (AD) lineages. Each of these lineages were differentiated by eight to 12 apomorphies and were supported by bootstrap values of >99%. Instead of performing an overall

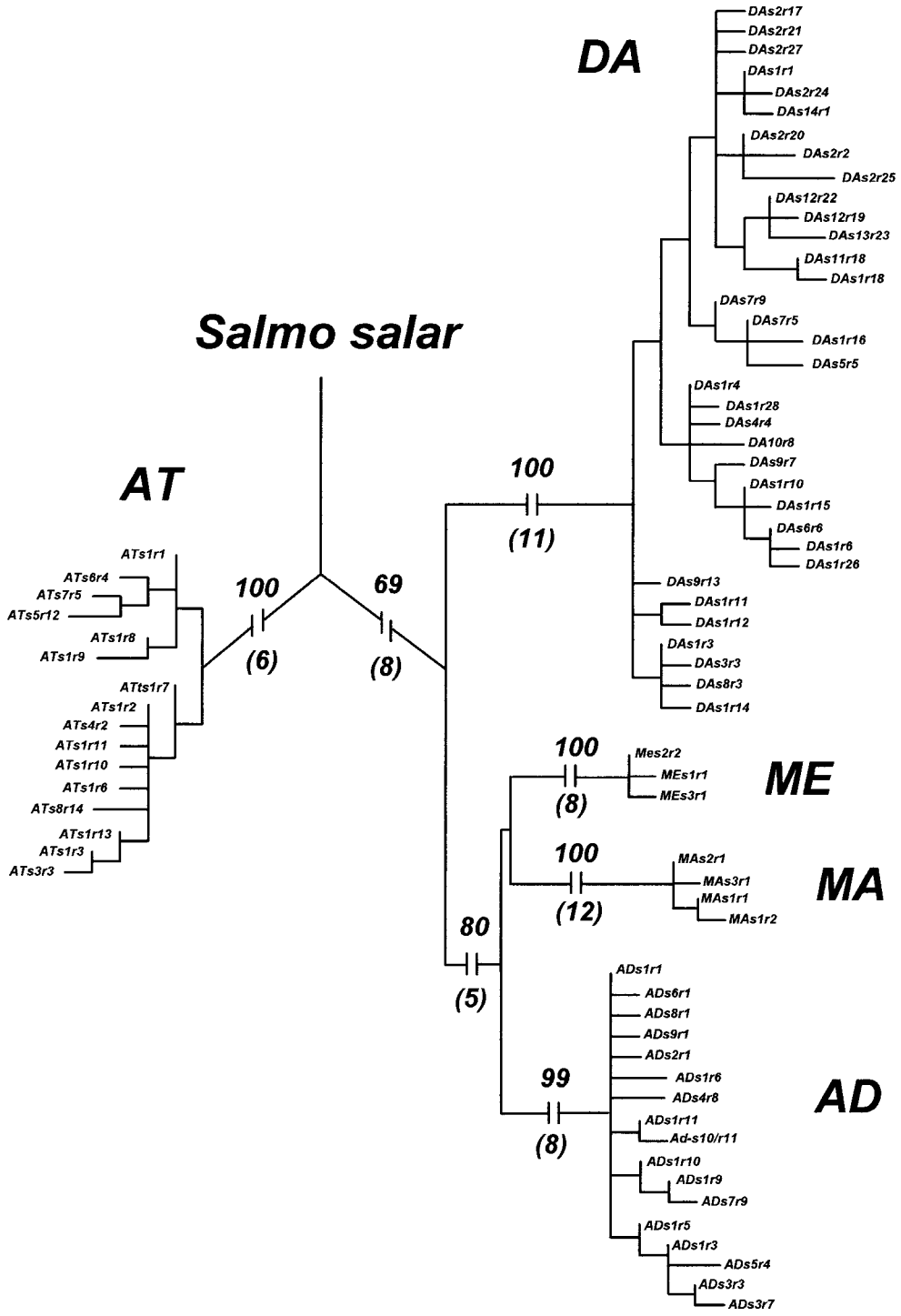

FIG. 1. Consensus tree based on maximum-parsimony analysis relating five major trout mtDNA evolutionary lineages. Relationships among major lineages and their bootstrap values (as a percentage) are derived from the combined information of three studies (Bernatchez et al. 1992; Giuffra et al. 1994; Bernatchez and Osinov 1995) based on sequence analysis of 1250 nucleotides and PCR-RFLP analyses of an estimated 576 additional sites resolved with 19 restriction enzymes. The rooting position of Atlantic salmon (*Salmo salar*) is derived from the sequence analysis of Giuffra et al. (1994). The numbers in parentheses refer to the number of apomorphies unique to each lineage. See Bernatchez and Osinov (1995) for further details. Relationships among haplotypes within each lineage was based on the sequence analysis of a 313-bp segment located at the 5′ end of the control region and PCR-RFLP analysis performed using six restriction enzymes (*Hinf*I, *Hpa*II, *Mbo*I, *Nci*I, *Rsa*I, and *Taq*I) on two adjacent PCR-amplified segments: the complete ND-5/6 region (approximately 2.4 kb) and a second (approximately 2.1 kb), comprising the whole cytochrome oxydase *b* gene and the D-loop. Composite haplotypes are defined in Tables 2 and 3. Branch lengths were scaled using number of mutations, the shortest corresponding to a single mutation.

phylogenetic analysis in the present study, we first assigned haplotypes (with confidence >99%) to these different groups based on their combined composition at 20 synapomorphic positions (13 resolved by RFLP analyses, seven by sequencing) that can unambiguously diagnose haplotypes belonging to any of the five trout lineages (detailed in Bernatchez and Osinov 1995). The phylogenetic relationships among haplotypes within each lineage was first assessed by parsimony analysis, as detailed in Bernatchez and Osinov (1995). A more detailed analysis was then achieved by the nested clade analysis (see below).

Levels of genetic diversity within and among the five major trout lineages were compared using the haplotype diversity and the maximum-likelihood estimation of the average number of nucleotide substitutions per site within and among groups (Nei 1987) using REAP, version 4 (McElroy et al. 1992). A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed using Arlequin, version 2.0 (Schneider et al. 1999) to compare the component of genetic diversity imputable to the variance among the five trout lineages to that observed among populations within each of them. The significance of the variance components associated with the different levels of genetic structure were tested using 1000 permutations.

Mismatch Analysis

A mismatch analysis was performed using Arlequin to compare the demographic history of the five major trout lineages. Because the main objective was to compare the historical demography of the five major trout lineages and population structure has a limited effect on the mismatch distribution (Rogers 1995), pure samples were pooled within each lineage. Following the method of Schneider and Excoffier (1999), we quantified the moment estimators of time to the expansion τ, the mutation parameter before ($\theta_0 = 2\mu N_0$) and following ($\theta_1 = 2\mu N_1$) expansion, expressed in units of mutational time, where N_0 and N_1 are the female effective population sizes before and following an expansion that occurred τ generations ago. These estimators were used to plot the expected distribution of probabilities of observing S differences between two randomly chosen mtDNA haplotypes (Watterson 1975). We also computed the raggedness index of Harpening (1994). The comparison of the sum of square deviation (SSD) between the observed and estimated mismatch distribution was used as a test statistic for the estimated stepwise expansion models (Schneider and Excoffier 1999). Confidence intervals for those parameters were obtained by Monte Carlo simulations of 1000 random samples using the coalescent algorithm of Hudson (1990) as modified by Schneider and Excoffier (1999). We finally used the relationships $\tau = \mu t$ (where μ is the mutation rate and t is the expansion time in generations) to compare the timing of possible demographic expansion among trout lineages.

Nested Clade Analysis

The probability of a parsimonious relationship among haplotypes within each lineage was assessed as described in Templeton et al. (1992), using the program ProbPars kindly provided by A. R. Templeton (Washington University, St. Louis, MO). MtDNA haplotypes differing by up to four mutations had a probability >95% of being connected in a parsimonious manner. Because the number of mutations among adjacent haplotypes never exceeded four mutational steps, we then constructed a minimum spanning network for each trout lineage using Minspnet (Excoffier and Smouse 1994). The resulting networks were then converted into a nested design following the rules of Templeton et al. (1987) and Templeton and Sing (1993). The clade distance, Dc (which measures the geographical range of a particular clade), the nested clade distance, Dn (which measures how a particular clade is geographically distributed relative to other clades in the same higher-level nesting category), and contrasts of these measures between tip and interior clades (Itc [y], Itn [y]) were quantified by first calculating the geographical centers of each clade resolved at all hierarchical levels (Templeton et al. 1995). Secondly, the geographical distances (in kilometer) between individuals and clade centers were calculated as great circle distances. The various distances were recalculated after each of 1000 random permutations of clades and/or haplotypes against sampling locality to statiscally test at $\alpha = 0.05$ for significantly large and small distances and interior-tip contrasts for each clade with respect to the null hypothesis of no geographic association within each of the nested clades. The model of population structure and historical events that was most suitable with the pattern of genetic structure observed within each clade showing statistically geographical associations was identified using the inference key provided as an appendix to Templeton (1998). To reduce bias in parameters estimation and permutations procedures due to large discrepancies among sample sizes (Appendix), those were limited to a maximum of 10 by respecting the observed haplotype frequencies in the total sample (but with a minimum absolute haplotype frequency of $n = 1$). All calculations were performed using Excell (Microsoft, vers. 97) spreadsheets developed by J. E. Stacy (University of Oslo, Norway), as used in Nesbo et al. (1999).

RESULTS

Relationships among Major Trout Lineages and Overall Patterns of Genetic Diversity

Figure 1 illustrates the branching topology among the five major trout lineages resolved from the combined information of previous studies. The salient feature of this tree was the rooting position of the outgroup, Atlantic salmon (*Salmo salar*), which suggested the ancestral divergence of the Atlantic lineage from all others (Giuffra et al. 1994). The combined sequence and RFLP analyses resolved 75 composite haplotypes (Tables 2, 3, Figure 2). These could unambiguously be assigned to either one of the five major trout lineages, based on their composition at 13 RFLP and seven sequence synapomorphic positions, as detailed in Bernatchez and Osinov (1995).

The overall level of haplotype diversity was high ($h = 0.856$), but the extent of nucleotide diversity ($\pi = 0.0133$) was comparable to that observed in other northern temperate freshwater fishes of similar latitudinal distribution (Bernatchez and Wilson 1998). The level of genetic diversity, however, was highly variable among the five trout lineages (Tables 1,

TABLE 2. Variable site positions in the control region among 38 *Salmo trutta* mtDNA genotypes defined by sequence analysis. Dots refer to nucleotide identity with AT-s1 sequence homology and dashes indicate indels. Numbers refer to nucleotide positions in Figure 2. Asterisk indicates sequences reported in Apostolidis et al. (1997) with the original nomenclature given in parentheses.

	Variable sites																												
Genotype	7	13	14	16	22	37	39	41	59	69	86	95	122	126	128	150	157	162	194	199	212	242	250	251	252	259	278	279	280	310
Atlantic (AT)																														
AT-s1	T	G	—	T	A	—	A	T	C	A	T	T	A	T	—	T	—	G	T	G	A	C	G	A	T	T	G	C	T	T
AT-s2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	G	·	·	·	·	·	·
AT-s3	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
AT-s4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C
AT-s5	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·
AT-s6	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
AT-s7	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·
AT-s8	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	A	G	·	·	·	·	·	·
Danubian (DA)																														
DA-s1	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·
DA-s2	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	G	·	·	·	·	·
DA-s3	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	G	·	·	·	·	·
DA-s4	·	A	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	G	G	·	·	·	·	·
DA-s5	·	·	·	C	·	·	·	A	·	·	·	·	G	·	·	·	·	·	·	·	·	·	A	G	G	·	·	·	·	·
DA-s6	·	·	·	C	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·
DA-s7	·	·	·	C	·	·	G	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·
DA-s8	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	—	·	·	·	·	·	·	·	·	G	·	·	·	·	·
DA-s9	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	G	G	·	·	·	·	·
DA-s10	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	G	·	·	·	·	·
DA-s11	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	G	·	·	·	·	·
DA-s12	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	G	G	·	·	·	·	·
DA-s13	·	·	G	C	·	A	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	G	G	·	·	·	·	·
DA-s14 * (J)	·	·	·	C	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	G	·	·	·	·	·
Adriatic (AD)																														
AD-s1	·	·	·	·	·	·	·	C	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
AD-s2	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	C	·	·	·
AD-s3	·	·	·	·	·	·	·	C	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
AD-s4	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	C	·
AD-s5	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	C	·	·	·
AD-s6	·	·	·	·	G	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
AD-s7 * (C)	·	·	·	·	·	·	·	C	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
AD-s8 * (D)	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	C	·	·	·
AD-s9 * (E)	·	·	·	·	·	·	·	—	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
AD-s10 * (F)	·	·	·	·	·	·	·	C	·	G	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	C	·	·	·
Mediterranean (ME)																														
ME-s1	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	A	·	·	C	·	·	·	·	·	·	·	·	·
ME-s2	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	A	·	·	C	·	·	·	·	·	·	·	·	·
ME-s3 * (I)	C	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	A	·	·	C	·	·	·	·	·	·	·	·	·
marmoratus (MA)																														
MA-s1	·	·	·	·	·	·	·	C	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·
MA-s2	·	·	·	·	·	·	·	C	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	A	T	·	·
MA-s3	·	·	·	·	·	·	·	C	·	·	·	·	·	·	A	·	·	·	C	·	·	·	·	·	·	·	A	T	·	·

TABLE 3. Definitions of 75 composite sequence/RFLP (sn/rn) mtDNA haplotypes in *Salmo trutta*: sn refers to sequence definition in Table 1, and rn refers to the composite fragment patterns resolved for restriction enzymes *Hinf*I, *Hpa*II, *Mbo*I, *Nci*I, *Rsa*I, and *Taq*I.

Haplotype	RFLP	Haplotype	RFLP	Haplotype	RFLP
Atlantic[1]		Danubian		Mediterranean	
AT-s1/r1	AAAAAA	DA-s1/r1	CBABBC	ME-s1/r1	DAADCD
AT-s1/r2	BAAAAF	DA-s2/r2	CBCCBC	ME-s2/r2	
AT-s1/r3	BHAEAF	DA-s3/r3	GAAAHC	ME-s3/r1	
AT-s6/r4	QAAAAA	DA-s4/r4	CEAAHC		
AT-s7/r5	KAAAAA	DA-s5/r5	CEAFBC	*Marmoratus*	
AT-s1/r6	BAAGAF	DA-s6/r6	HEAGHC	MA-s1/r1	EACADD
AT-s1/r7	BAAAAA	DA-s9/r7	JEAAHC	MA-s2/r1	EACADD
AT-s1/r8	AAAAJA	Da-s10/r8	CEAAGC	MA-s3/r1	EACADD
AT-s1/r9	BAAAJF	Da-s7/r9	CEAABC	MA-s1/r2	EKCADD
AT-s1/r10	OAAAAF	DA-s1/r10	JEAGHC		
AT-s1/r11	LAAAAF	DA-s1/r11	IAAAHC		
AT-s5/r12	AAAALA	DA-s1/r12	JAAAIC		
AT-s1/r13	BHAAAF	DA-s9/r13	CAAAHC		
AT-s8/r14	BIABAF	DA-s1/r14[3]	NA---C		
AT-s3/r3		DA-s1/r15	CEAGHC		
AT-s4/r2		DA-s1/r16	MEAFBC		
		DA-s1/r15	CEAGHC		
Adriatic		DA-s1/r16	MEAFBC		
AD-s1/r1	CCBACE	DA-s2/r17	FBABBC		
AD-s3/r3	CDBECE	DA-s1/r18	CEAABD		
AD-s5/r4	CDBEEE	DA-s12/r19	CBACBD		
AD-s1/r5	CDBACE	DA-s2/r20	CBACBC		
AD-s1/r6	PCBAME	DA-s2/r21	CBABKC		
AD-s3/r7	CDBECG	DA-s12/r22	CBABBD		
AD-s4/r8	CCBAME	DA-s13/r23	CADABD		
AD-s1/r9[2]	CC---E	DA-s1/r24	CAAABC		
AD-s1/r10[2]	CC---E	DA-s2/r25	CFACFC		
AD-s1/r11[2]	CC---E	DA-s1/r26	HJAGHC		
AD-s1/r3		DA-s2/r27	CBABOC		
AD-s2/r1		DA-s1/r28	CEAAPE		
AD-s6/r1		DA-s7/r5			
AD-s7/r9		DA-s8/r3			
AD-s8/r1		DA-s14/r1			
AD-s9/r1		DA-s1/r4			
AD-s10/r11		Da-s1/r3			
		DA-s1/r6			
		DA-s11/r1			

[1] Correspondance to haplotype definition of Hynes et al. (1996a) : AT-s1/r1 (IX, X), AT-s2/r2 (I, III, VI, VIII, XIII, XIV), AT-s1/r6 (V), AT-s1/r10 (IV), AT-s1/r11 (XI), AT-s1/r14 (XX), AT-s4/r2 (II), AT-s3/r3 (XII).
[2] RFLP patterns described in Apostolidis et al. (1996a): r9, r10, and r11 corresponds to Type 3, 2, 4 of these authors, respectively. Data for *Mbo*I, *Nci*I, *Rsa*I were missing, but these haplotypes had new mutations at other enzymes (either *Alu*I, *Ava*II, *Hae*III) not seen previously.
[3] r14 corresponds to Type 9 of Apostolidis et al. (1996a).

4). The DA lineage was the most genetically diverse, followed by the AD and AT lineages. Both ME and MA lineages exhibited extremely reduced levels of diversity. Assuming that the rooting position of *S. salar* indicates the ancient divergence between two ancestral lineages (AT vs. one from which diverged all others), it is noteworthy that the genetic diversity within the AT lineage one was highly reduced compared to the other. The net nucleotide divergence among the five trout lineages varied between 1.21% and 2.19% (Table 4).

Geographic Distribution of Major Trout Lineages

Contrasting patterns of geographic distribution were observed among the five major trout lineages (Fig. 3A–E), as only eight populations out of 174 (4.6%) with more than one lineage were observed (Appendix). All populations from the Atlantic basin and Morocco were fixed for the AT lineage. The DA lineage was almost exclusively associated with drainages of the Black, Caspian, and Aral Sea basins, as well as the Persian Gulf. The other three lineages (AD, ME, MA) showed little overlap in distribution with the other two and exhibited a differential pattern in their overall geographic distribution within the Mediterranean. The MA lineage was almost strictly associated with the Adriatic basin. The ME lineage was predominantly found in tributaries draining in the western basin, whereas the AD lineage predominated in the eastern part of the Mediterranean basin (Appendix).

Hierarchical Genetic Diversity Analysis

Most of the mtDNA molecular variance observed in native trout populations was imputable to differences among lineages (Table 5). The amount of genetic variance imputable to the among-populations within-lineage component was approximately one order of magnitude less than the among-lineage components (8.942 vs. 0.873). Yet, populations were highly structured within lineages, with an overall Φ_{ST} of

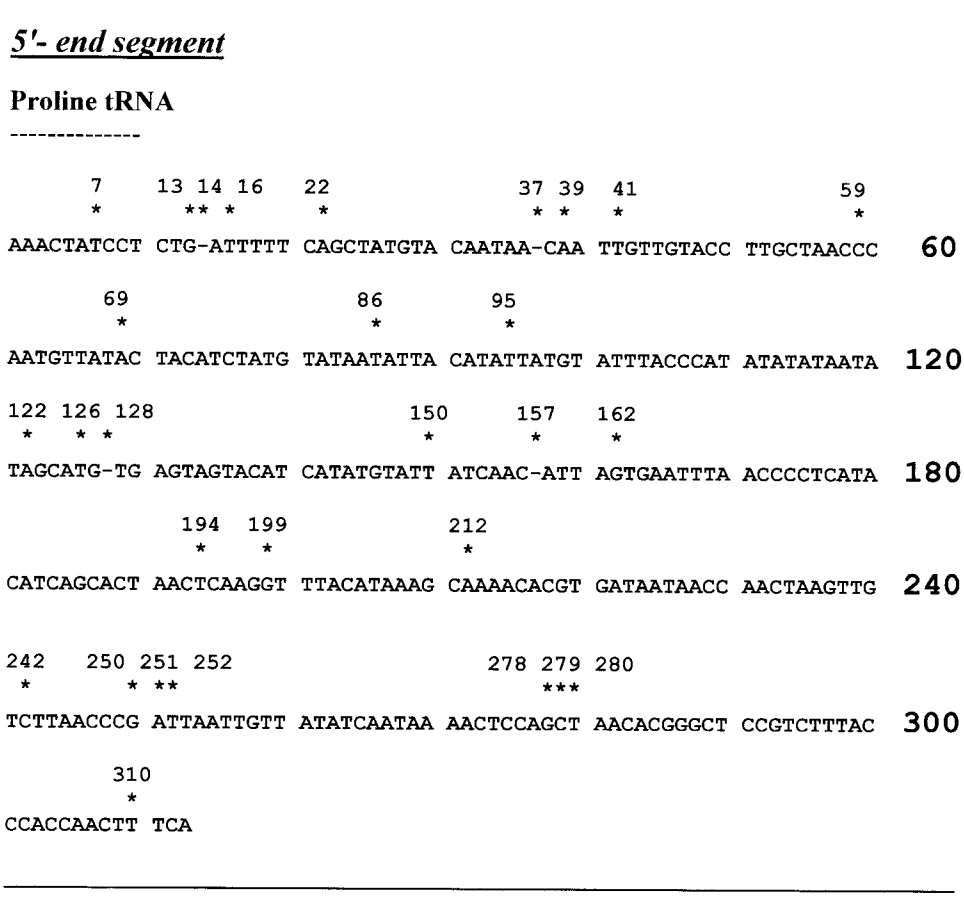

FIG. 2. Sequence of the 5′-end, 313-bp segment of the mtDNA control region; type AT-s1 for *Salmo trutta*. The sequence shown is for the light strand, and includes 10 nucleotides of the proline tRNA gene. Asterisks and numbers above sequence indicate the 30 variable positions among the 38 resolved sequences. The AT-s1 sequence is entered into GeneBank under accession number U18198.

0.776. The amount of molecular variance among populations was highest for the DA and AD lineages, two to three times lower for the AT lineage, and extremely reduced for both the ME and the MA lineages. This also translated into variable Φ_{ST} values depending on lineages.

Mismatch Distribution and Demographic History

The overall mismatch distribution was clearly bimodal, one mode corresponding to the number of differences among major lineages (~20) and the other to differences among individuals within lineages (~2; Fig. 4). The mismatch distribution, however, differed substantially among trout lineages, with the mean number of differences being the lowest in ME (0.53) and MA (0.54) lineages, followed by AT (1.3), AD (2.1), and the highest value observed in DA (4.7) lineage. The mismatch distribution within AT, AD, and DA lineages fitted well the predicted distribution under a model of sudden expansion, but this was refuted for both ME and MA lineages (Table 6). Considering the 95% confidence interval generated by the simulations, the observed number of polymorphic sites values were consistent with simulated sites, meaning that the parameters estimated by the model were sufficiently accurate to account for the observed polymorphism in AT, AD, and DA lineages. The observed values of the age expansion parameter (τ) differed substantially among these three lineages. Confidence intervals on θ_1 values were too large to allow even rough estimates of the magnitude of expansions.

TABLE 4. Average number of nucleotide substitutions per site within (main diagonal) and net nucleotide divergence (below main diagonal) among the five major evolutionary lineages of brown trout.

Lineage	AT	DA	AD	ME	MA
Atlantic (AT)	0.0020				
Danubian (DA)	0.0162	0.0045			
Adriatic (AD)	0.0185	0.0204	0.0018		
Mediterranean (ME)	0.0175	0.0203	0.0121	0.0005	
marmoratus (MA)	0.0188	0.0219	0.0142	0.0146	0.0005

Nested Clade Analysis

Contrasting patterns of nested clade design, both in numbers of haplotypes and levels of clades was observed among the five trout evolutionary lineages (Figs. 5–7). The null hypothesis of no association between the position of haplotypes

in a cladogram with geographic position was rejected for at least several clades at any nesting level for AT, DA, or DA lineages (Tables 7–9). Thus, we identified the likely causes for the geographic association using the inference key of Templeton (1998).

A temporal juxtaposition of past fragmentation at all clade levels best explained the overall genetic structure observed within the AD lineage (Table 7). This pattern was mainly driven by more easterly distributed populations. One-step clades were highly structured geographically in the eastern part of the distribution range (from southern Italy and eastward), whereas a more homogeneous pattern was observed among western populations, where only two clades, largely overlapping in distribution, were represented (Fig. 5B).

The geographic pattern of genetic diversity observed within the AT lineage was best explained by the temporal juxtaposition of past fragmentation and range expansion (Table 8). This was mainly driven by the strong geographic structuring observed among more southern populations (Fig. 6C). However, clade AT1–4 (corresponding to single haplotype ATs1/r1) was widely distributed among northern populations. Although clades AT1–1 and AT1–2 broadly overlapped in distribution among northern populations, a significantly small Dc values was observed for both (Table 8). Namely, there was a tendency for clade AT1–1 to be mainly found in west-central populations, being almost absent from more northern populations (Iceland, northern Scandinavia, northern Russia). A pattern of either contiguous range expansion or dispersal implying gene flow with isolation by distance best explained the geographic pattern observed among single haplotypes within AT1–1 and AT1–2 clades. In contrast, a signal of past fragmentation best explained the geographic distribution of haplotypes within clade AT1–5 found in southern France and northern Spain.

The geographic pattern of genetic diversity observed within the DA lineage was best explained by a complex pattern of temporal juxtaposition of various historical events (Table 9). Past fragmentation best explained the geographic distribution of three-step clades. A strong geographic discontinuity was particularly clear in more easterly distributed populations, where those from the Caspian and Aral Sea basins were typically characterized by clade DA3–3 and those surrounding the Black Sea area by clade DA3–2 (Fig. 7C). Clade DA3–1 generally had a more western distribution, where it overlapped extensively with DA3–2. At the two-step level, different historical events best explained the clade distribution, depending on geographic areas. The distribution of clades within DA3–1 (DA2–1 and DA2–2), which were found in the most western part of the range, was best explained by a history of either range expansion and/or restricted dispersal with gene flow (Table 9). In contrast, the distribution of clades within either DA3–2 or DA3–3 clades better fit a history of fragmentation. Within DA3–2, clade DA2–3 mainly (but not exclusively) characterized populations from the Black Sea area, whereas DA2–4 was confined to the upper reaches of the Danube drainage (Austria and Slovenia, Fig. 7B). Within DA3–3, populations from the Caspian Sea basin were characterized by clade DA2–8, whereas DA2–5, DA2–6, and DA2–7 were largely confined to the Aral Sea basin. A geographic east-west dichotomy between a history of fragmentation and dispersal was also apparent at the one-step and zero-step clade levels (Table 9).

Nested clade analysis was not warranted for either ME or MA lineages, which were characterized by the predominance of single haplotypes with a relatively broad geographic distribution within both lineages (Appendix).

Discussion

The combined use of traditional phylogeography, nested clade, and mismatch analyses of mtDNA diversity revealed complex patterns in the distribution and demography of brown trout. The following discussion treats the species' evolutionary history in hierarchical order to infer a temporal juxtaposition of different paleo-environmental settings throughout its distribution range that is most compatible with the differential patterns of genetic diversity observed among and within major evolutionary lineages.

Choice of a Molecular Clock

Clearly, there are many ambiguities in the application of a molecular clock; consequently, this clock was used in conjunction with estimated phylogenetic and demographic patterns only to provide an approximate time frame to evaluate phylogeographic hypotheses. Estimates in the range of 1–2% sequence divergence per million years were chosen for the following reasons. A previous indirect calibration derived from fossil records led to an average estimate of mtDNA mutation rate of 1% per million years for salmonids (Smith 1992). Correlations reported between phylogeographic patterns and successions of Pleistocene glaciation events have previously been reported in other salmonids when using such clock calibration (e.g., Bernatchez and Dodson 1991; Wilson et al. 1996). In a more formal attempt to calibrate a fish mtDNA molecular clock using physically isolated geminates by the Isthmus of Panama, Donaldson and Wilson (1999) estimated a divergence rate of 1% per million years in the ND 5/6 region and 3.6% per million years for the control region, suggesting an average mutation rate for the whole mitochondrial genome intermediate of these values (the control region represented approximately 40% of the subsampled nucleotides in our study). Finally, the oldest fossils reported for *S. trutta* date from the early Pleistocene (2 million years ago; discussed in Osivov and Bernatchez 1996). Consequent-

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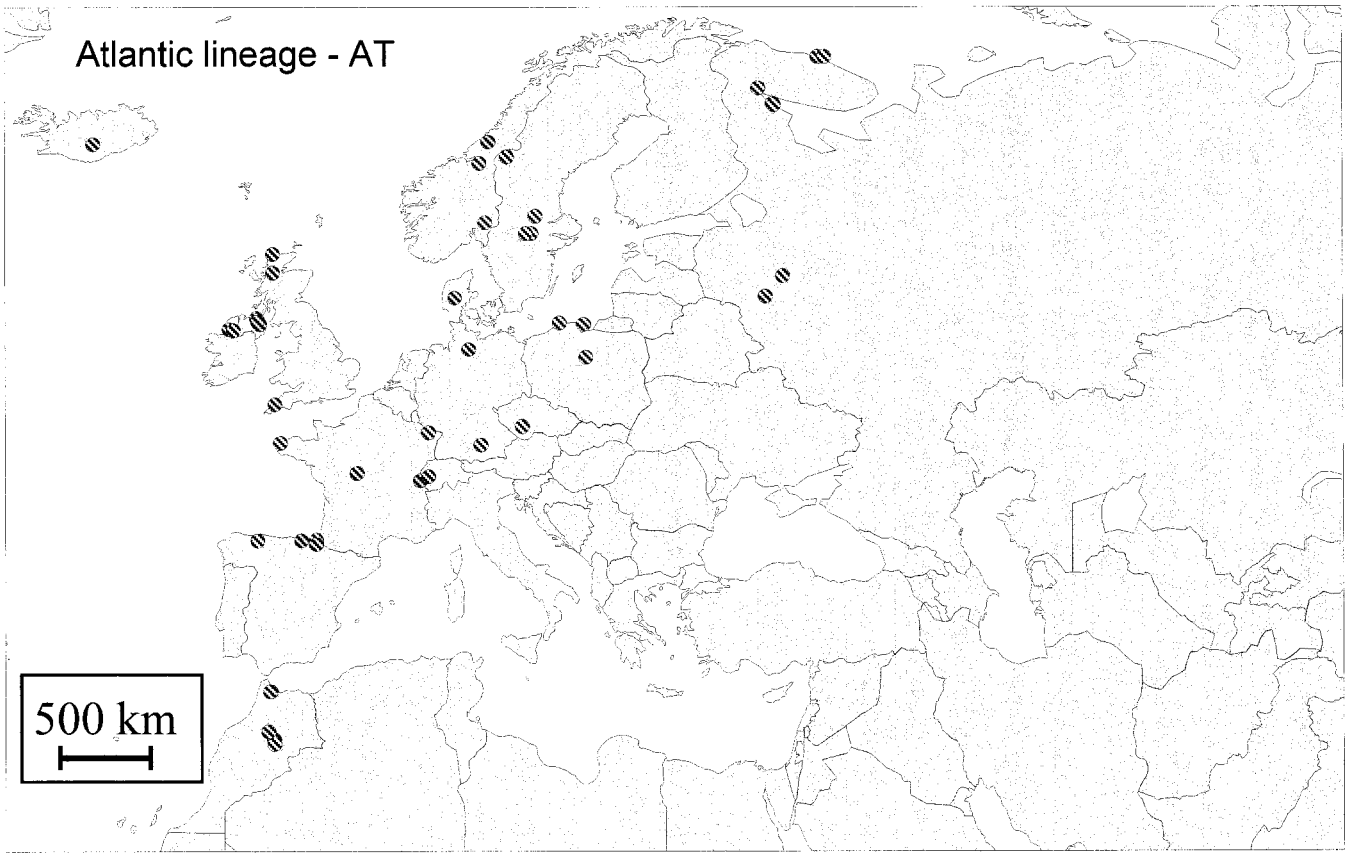
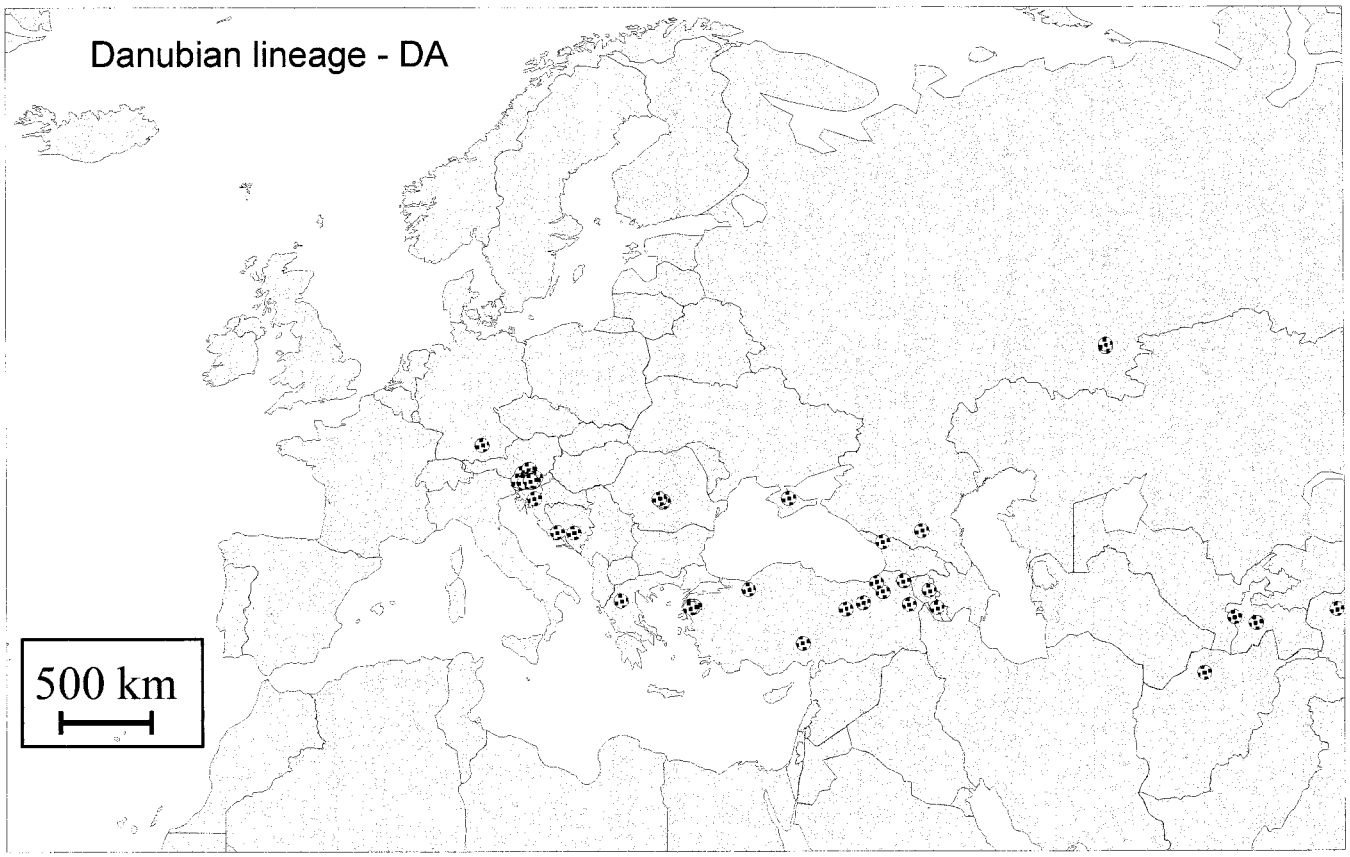
FIG. 3. Geographic distribution of the five major trout mtDNA evolutionary lineages: Atlantic (AT), Danubian (DA), *marmoratus* (MA), Mediterranean (ME), and Adriatic (AD). Symbols were positioned with the exact latitudinal and longitudinal coordinates of each sample using the software MapInfo, and symbols of geographically proximate samples may overlap. A detailed description of lineage distribution among all populations surveyed in provided in the Appendix.





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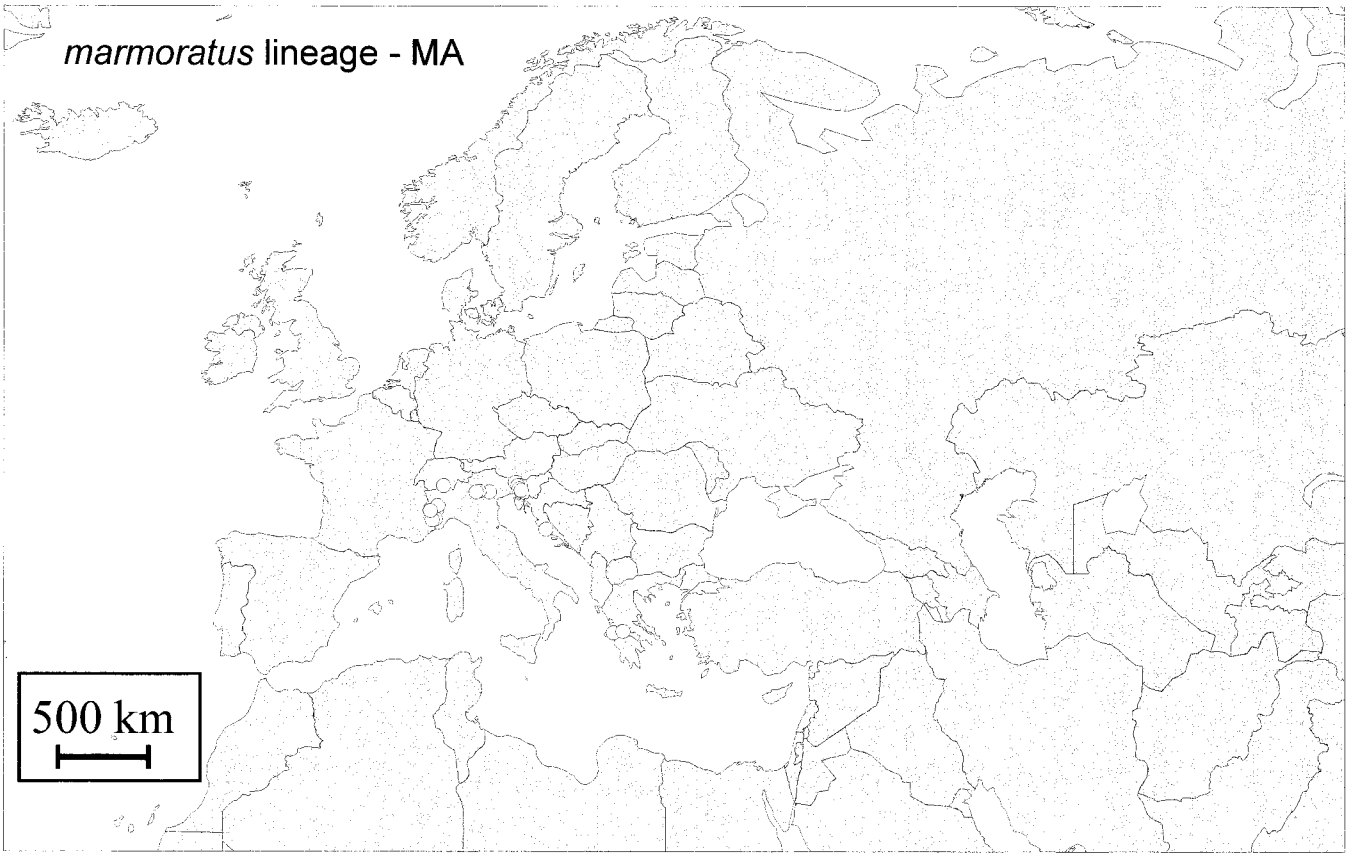

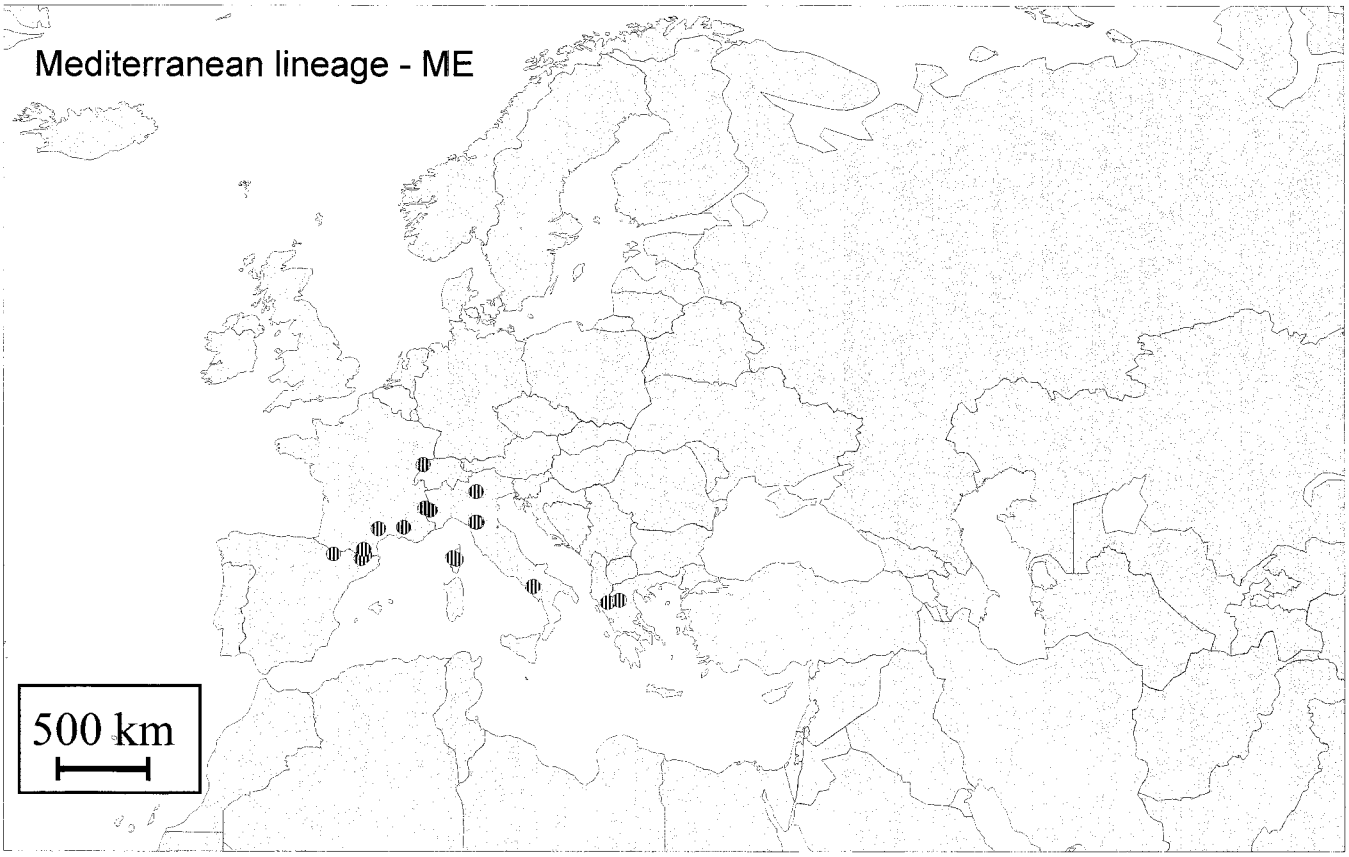

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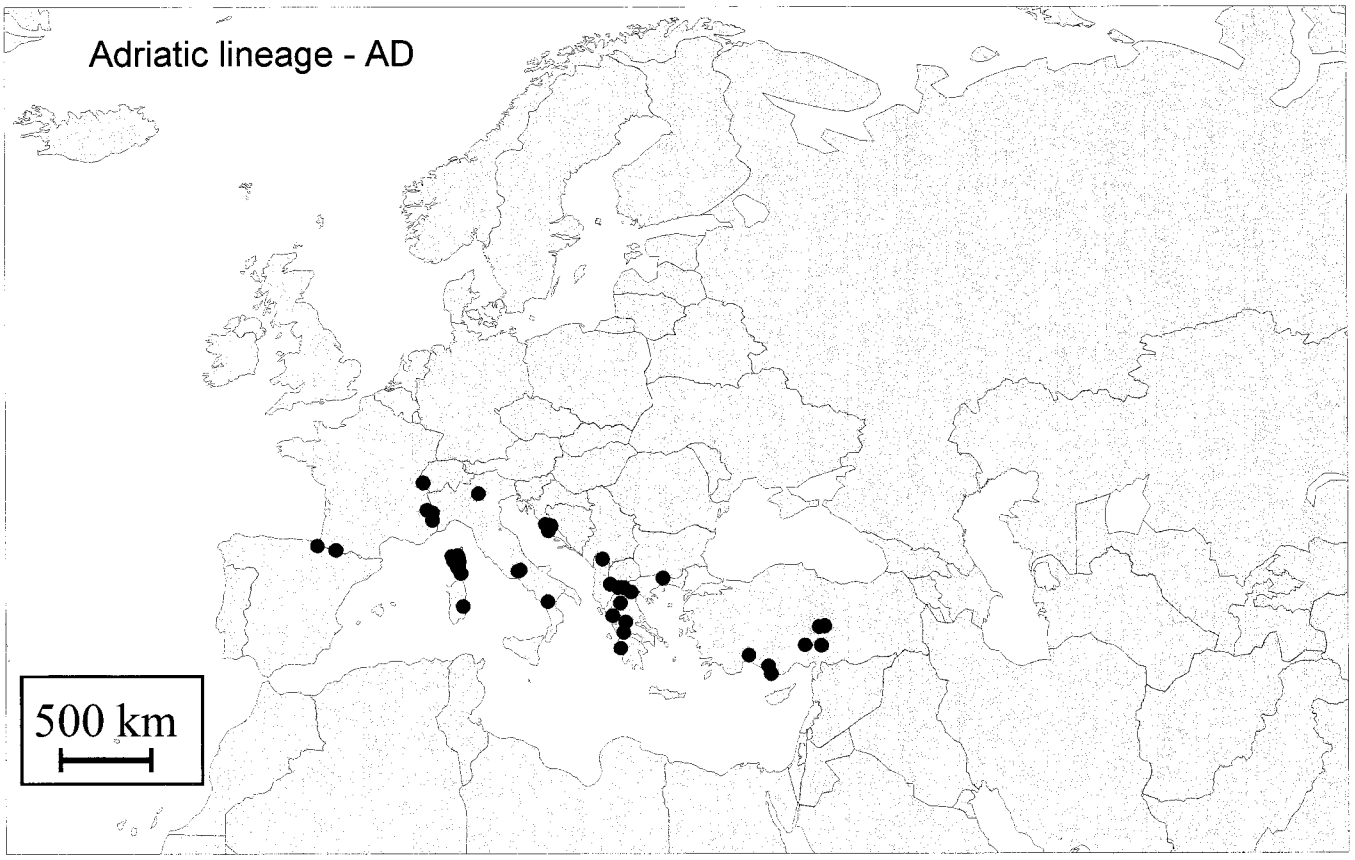


FIG. 3. Continued.

ly, the extent of divergence among the deepest clades in brown trout should fit within this time frame, which is the case if one considers the extent of divergence among the five major trout lineages (1.4–2.0%) and a molecular clock of 1–2% per million years.

Pleistocene Origins of Five Major Evolutionary Lineages in Brown Trout

Extending analyses to its whole distributional range confirmed that the *S. trutta* complex is composed of five major evolutionary lineages and basically ruled out the possibility of additional subdivisions of similarly deep divergence.These lineages most likely identify ancestral populations of trout that evolved independently as a result of ancient allopatric fragmentation. Results of previous studies of nuclear gene variation are also congruent with these lineages. The same populations characterized here by different major mtDNA lineages or with the same geographic distribution were also highly differentiated either by diagnostic alleles or strong allele frequency differences (Guyomard 1989; Bernatchez and Osinov 1995; Giuffra et al. 1996; Apostolidis et al. 1996b). A broad-scale geographic survey of microsatellite loci variation recovered four of the five major mtDNA lineages (Presa 1995). Finally, Berrebi (1995) showed that Corsican trout populations originated from two distinct lineages, most likely corresponding to the ME and AD lineages.

Given their overall extent of divergence, the time frame involved in the lineages' separation would span between 0.5 million and 2 million years. This indicates that major phylogeographic subdivisions in brown trout are associated with the climatic and environmental changes that occurred during Pleistocene glaciations events. The most ancient separation would have involved allopatric fragmentation between the three major drainage subdivisions: the Atlantic (AT lineage),

TABLE 5. Analysis of molecular variance in brown trout using the five major evolutionary lineages as the top level of grouping. Eight populations admixed for different lineages, and populations with sample size <5 were omitted for this analysis. The molecular variance and related Φ-statistics imputable to the among-populations component is provided first for the overall system and then within each lineage. All Φ-statistics values are significant at $\alpha = 0.0001$.

Source of variation	df	Variance components	% variation	ϕ-statistics
Among phylogenetic groups	4	8.942	88.83	0.8883
Among populations within groups	125	0.873	8.67	0.7766
AT	43	0.676	62.42	0.6242
DA	33	1.8731	78.48	0.7848
AD	27	1.203	93.73	0.9373
ME	10	0.237	75.07	0.7507
MA	12	0.251	90.42	0.9042

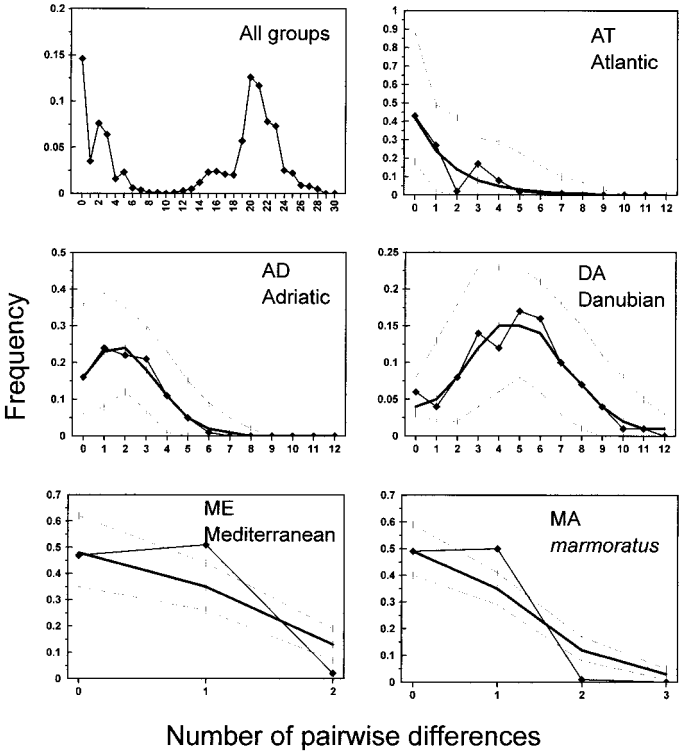


FIG. 4. Frequency distributions of pairwise number of mutational differences between trout individuals observed in all trout mtDNA evolutionary lineages combined (top panel) and separately in the five major trout lineages defined by mitochondrial analysis: Atlantic (AT), Adriatic (AD), Danubian (DA), Mediterranean (ME), and *marmoratus* (MA). Diamonds represent the observed data, the bold curve is the model fitted to the data, and dashed lines delineate the 2.5 and 97.5 percentile values of 1000 simulated samples.

Ponto-Caspian (DA), and Mediterranean followed by subsequent and possibly simultaneous fragmentation within the Mediterranean basin, which led to the divergence of the ME, MA, and AD lineages. The most drastic climatic changes over the last 3 million years occurred approximately 700,000 years ago (Webb and Bartlein 1992; Andersen and Borns 1994); it is also likely that the first large isolation of several large river systems now draining into different basins (e.g., Rhone, Rhine, and Danube) occurred during that period (Villinger 1986). Consequently, it is plausible that the most important genetic subdivisions within the brown trout complex are associated with major climatic changes and basin isolation that occurred in Europe between the early to the upper mid-Pleistocene.

Considering the paleo-environmental settings during the Pleistocene and the overall geographic pattern of distribution and genetic diversity within each of them, it is possible to infer hypothetical centers of origins for the five major trout evolutionary lineages. Given its clear association with the Atlantic basin, the center of origin of AT lineage is obviously associated with drainages of this system. Trout from the Atlantic basin could survive in refuges marginal to ice sheets during the most recent glaciation events (Ferguson and Fleming 1983; Hamilton et al. 1989; Osinov and Bernatchez 1996). Given the much more severe climate, however, and the more important glacial expansion both on land and on the ocean during earlier glacial cycles of the Pleistocene (Webb and Bartlein 1992; Andersen and Borns 1994), it is more likely that the ancestral center of origin of the AT lineage was further south, perhaps in coastal tributaries of the Iberian Peninsula or even North Africa. This is corroborated by the more pronounced pattern of clade diversity and divergence among southern populations (see also Weiss et al. 2000).

Locating the center of origin for the DA lineage is much more problematic, given the less severe direct impacts of habitat loss during glacial advances in the Ponto-Caspian basin and the very complex pattern of expansion, contraction, and interconnection of the Black, Caspian, and Aral Sea basins (Arkhipov et al. 1995). Nevertheless, the localization of early trout fossils in the Caucasus area and the central position of clade 3–2, both geographically and in the DA cladogram (intermediate between 3–1 and 3–3), suggest that the ancestral populations from which all extant populations of the DA lineage originated inhabited from drainages associated with the Black Sea.

The differential pattern of geographic distribution of the three other lineages (ME, MA, and AD) broadly corroborate traditionally recognized Mediterranean refugial areas: a southwest (Ibero-Mediteranean), central (Adriato-Mediterranean or Italian), and an eastern (Balkans/Anatolia) refuge (Keith 1998). The ME lineage was predominantly associated with tributaries draining in the western basin of the Mediterranean Sea, suggesting that it originated from this region. Persat and Berrebi (1990) proposed that a restricted area of southern France (Rousillon region) that was isolated by the Pyrenees to the south and by severe environmental conditions prevailing during glacial advances to the east may have served as a Pleistocene refuge for other fishes. The MA lineage, typical of the phenotypically and ecologically distinct

TABLE 6. Observed (S) and 95% confidence intervals (CI) of simulated number of polymorphic sites, demographic parameters (95% CI), among major trout lineages. τ, θ_0, and θ_1 are the age of expansion and population size before and following expansion, expressed in units of mutational time. $P(SSD_{obs})$ is the probability of observing by chance a less good fit between the observed and the mismatch distribution for a demographic history of the lineage defined by the estimated τ, θ_0, and θ_1 parameters. $P(Rag_{obs})$ is the probability of observing by chance a higher value of the raggedness index than the observed one, under the hypothesis of population expansion.

Lineage	*S*	95% CI	τ	θ_0	θ_1	$P(SSD_{obs})$	Raggedness	$P(Rag_{obs})$
Atlantic (AT)	19	4–21	0.5 (0.0–5.6)	1.36 (0–2.6)	3.8 (0.5–2681)	0.393	0.120	0.590
Adriatic (AD)	19	18–51	2.4 (0.5–5.7)	0.001 (0–1.9)	9.7 (1.5–6792)	0.769	0.022	0.837
Danubian (DA)	37	34–97	5.5 (2.0–8.2)	0.004 (0–3.5)	25.2 (10.4–6491)	0.395	0.015	0.438
Mediterranean (ME)	2	22–42	0.7 (0.3–1.0)	0 (0–0.7)	807 (1–3645)	0.00001	0.235	0.002
marmoratus (MA)	3	51–80	0.7 (0.4–0.89)	0 (0–0.5)	555 (1.6–3444)	0.00001	0.233	0.00001

TABLE 7. Nested geographical analysis of the Adriatic (AD) lineage following the inference key of Templeton (1998). Nested design, haplotype and clade designations are given in Figure 5A. Geographic distribution of clades is provided in Figures 5B and 5C. Following the name of haloptypes or clade number are the clade (Dc) and nested clade (Dn) distances. For those clades containing both interior (bold) and tip nested clades, the average difference between interior versus tip clades for both distance measures is given in the row labelled I-T. A superscript S means that the distance measure was significantly small at the 5% level, and a superscript L means that the distance measure was significantly large at $\alpha = 0.05$. At the bottom of a nested set of clades in which one or more sets of the distance measures was significant is a line indicating the biological inference using the same number and letter codes as those provided in Templeton (1998). Abbreviation: past frag.; past fragmentation.

Haplotypes			One-step clades			Two-step clades		
No	Dc	Dn	No	Dc	Dn	No	Dc	Dn
ADs1r1	620	675						
ADs6r1	0^S	1996^L						
ADs2r1	0	705						
ADs8r1	0^S	761						
ADs9r1	0^S	916						
I-T	620^L	-418^S						
1-2-3-5-15 No: past frag.			**1-1**	757^L	790^L			
ADs1r9	0^S	17^S						
ADs7r9	0^S	176						
ADs1r10	0^S	172						
I-T	0	-158^S						
1-2-3-4-9 No: past frag.			1-2	17^S	598			
ADs1r6	0^S	0	1-3	0^S	524			
ADs4r8	149	0	1-4	149^S	235^S			
ADs1r11	0	0	1-5	0^S	419^S			
ADs10r11	0	0						
			I-T	594^L	312^L			
			1-2-3-4-9 No: past frag.			**2-1**	665^S	670
ADs1r3	563	1152^L						
ADs1r5	0	1200						
ADs3r3	50^S	445^S						
ADs3r7	0	150						
I-T	546^L	616						
1-2-3-5-15 No: past frag.			**1-6**	676	702	2-2	742	746
Ads5r4	0	0	1-7	0^S	1575^L	I-T	−77.6	−76.8
			I-T	676^L	-873^S	1-2-3-4-9 No: past frag.		
			1-2-3-5-15 No: past frag.					

marble trout, was mainly confined to the Pô River basin, but included drainages from Croatia and Slovenia, which could all interconnect during phases of maximal interglacials (Bianco 1990). Previous allozyme studies also support the hypothesis of a North Adriatic origin for the marble trout (Giuffra et al. 1996). Finally, the species' predominance in tributaries of the eastern Mediterranean basin relative to both ME and MA lineages, along with the higher level of clade diversity observed in populations from the Balkans, indicated that the AD lineage most likely originated from the Balkans/Anatolia refuge.

Physical Versus Ecological Constraints to Dispersal and Secondary Intergradation

Given *S. trutta*'s dispersal potential, the variety of habitats it occupies, and the evidence for interconnections among major basins during the Pleistocene, it is remarkable that major evolutionary lineages remained largely allopatric in distribution for hundreds of thousands of generations. For instance, interconnections between the Atlantic and Ponto-Caspian basins have been possible during different glacial phases due to the development of either intermittent fluvial connections or large ice-dam lakes at the ice margins that discharged southward into the Ponto-Caspian basin (Grosswald 1980; Gibbard 1988; Arkhipov et al. 1995). Recent phylogeographic (Durand et al. 1999a; Nesbo et al. 1999a) and traditional biogeographic studies (Banarescu 1990) confirmed that other fishes originating from Ponto-Caspian refuges have indeed recolonized the Atlantic basin following the most recent glaciations. Similarly, fish dispersal between the Ponto-Caspian and the Mediterranean basins was also possible via connections between the Danube and rivers draining to the Mediterranean, increased water discharge from the north that drained into the Black Sea and overflowed to the Mediterranean, or sea level lowering and reduced salinity that allowed fish movements across the Agean Sea basin (Bianco 1990; Economidis and Banarescu 1991; Arkhipov et al. 1995). The identification of a few trout populations characterized by mtDNA lineages not representative of the basin in which they were found confirms that trout could also use such connections. Yet, introgressive hybridization (excluding that due to stocking or contemporary human-induced habitat disturbance) remained relatively limited, as exemplified in studies at nuclear genes (Bernatchez and Osinov 1995; Giuffra et al. 1996).

This indicates that, in addition to physical isolation, biological factors have contributed to limiting dispersal and introgressive hybridization among major trout lineages. One of

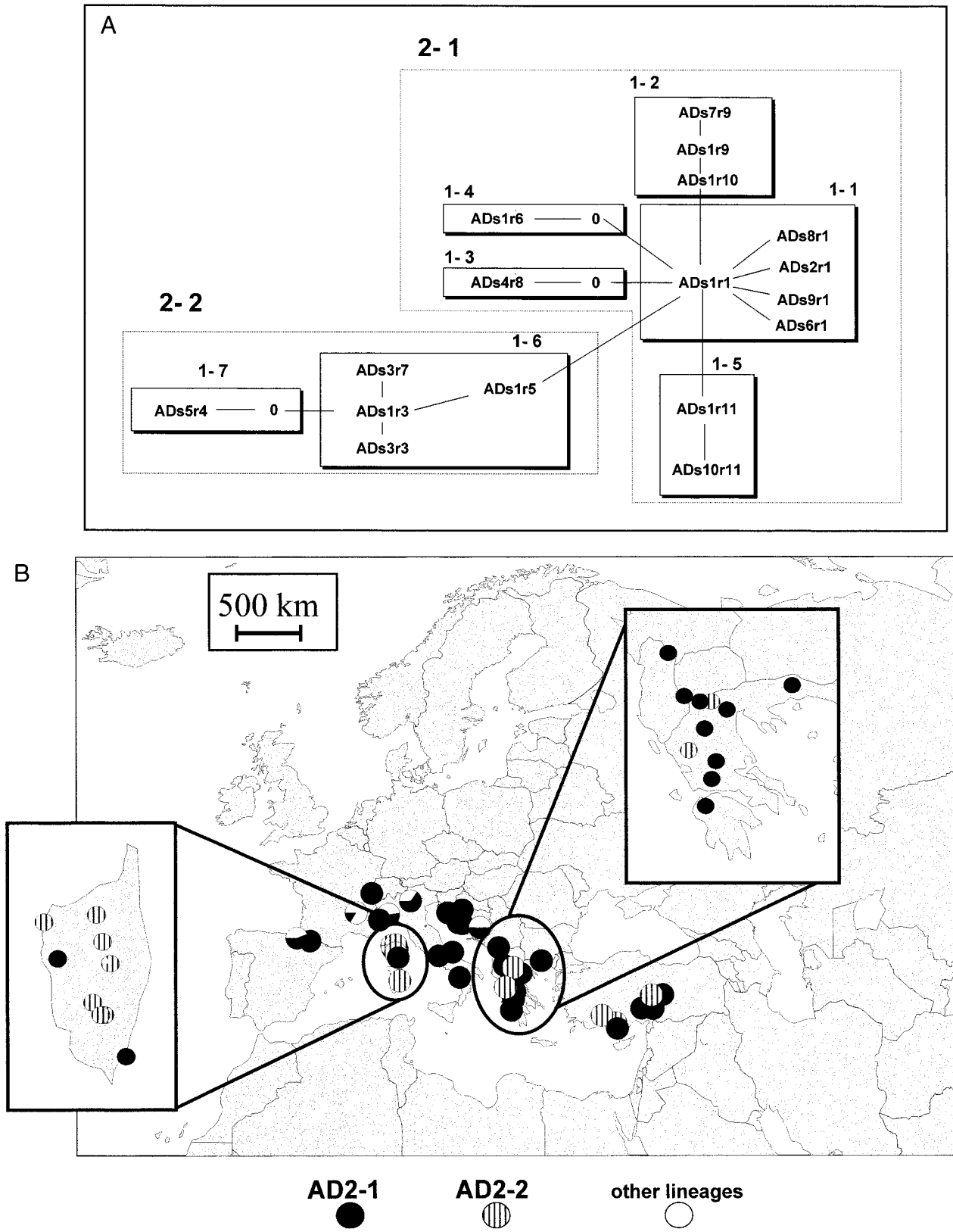


FIG. 5. (A) Minimum spanning network for 17 mitochondrial composite haplotypes resolved in the trout Adriatic lineage. Each line in the network represents a single mutational change. A zero indicates an interior node in the network that was not present in the sample. Composite haplotypes are defined in Tables 2 and 3. Dotted-lined polygons indicate one-step clades nested together into two-step clades, and lined-shaded polygons indicate haplotypes grouped together into one-step clades. (B) Geographic distribution of two-step AD clades.

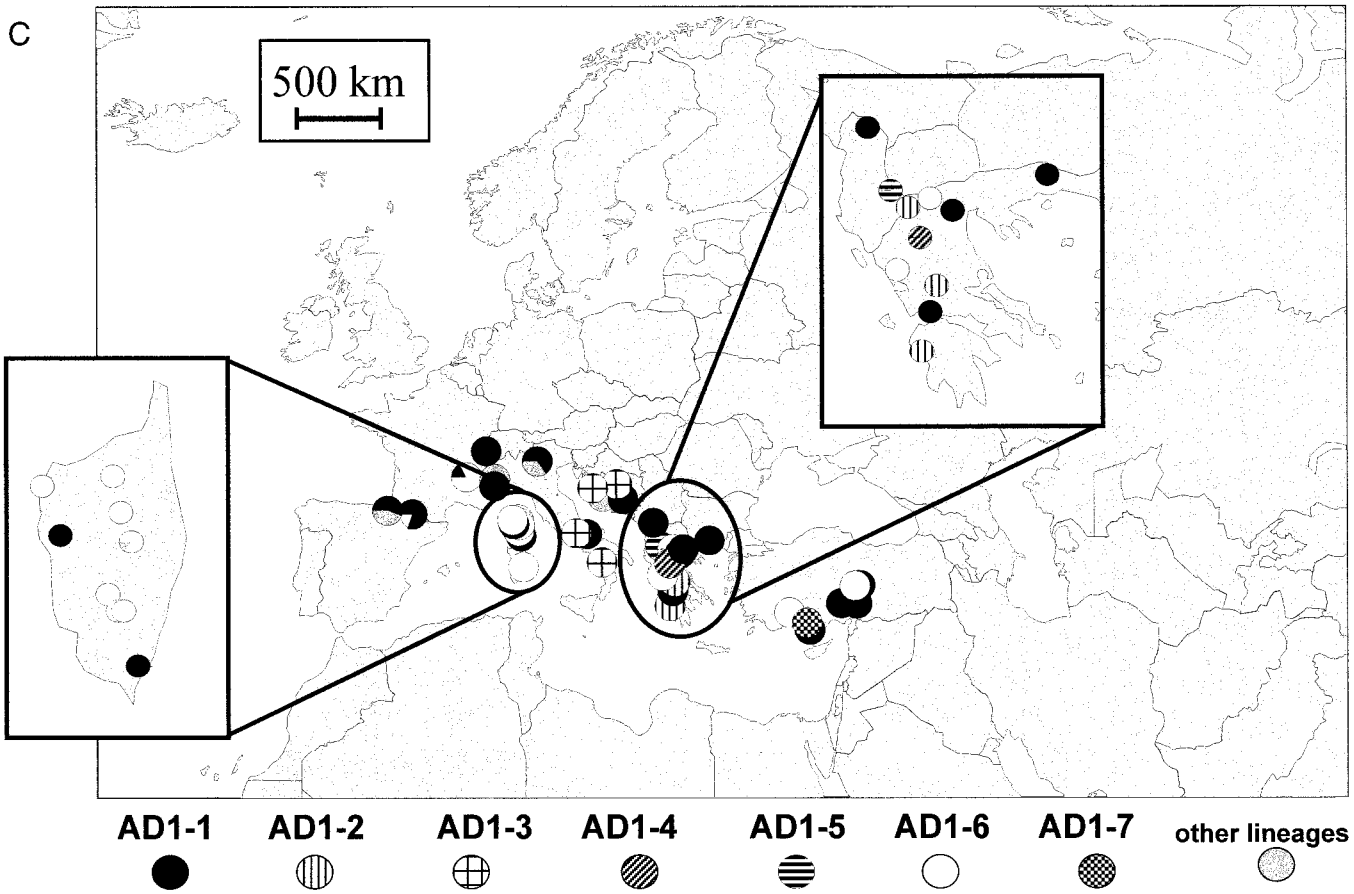


FIG. 5. Continued.

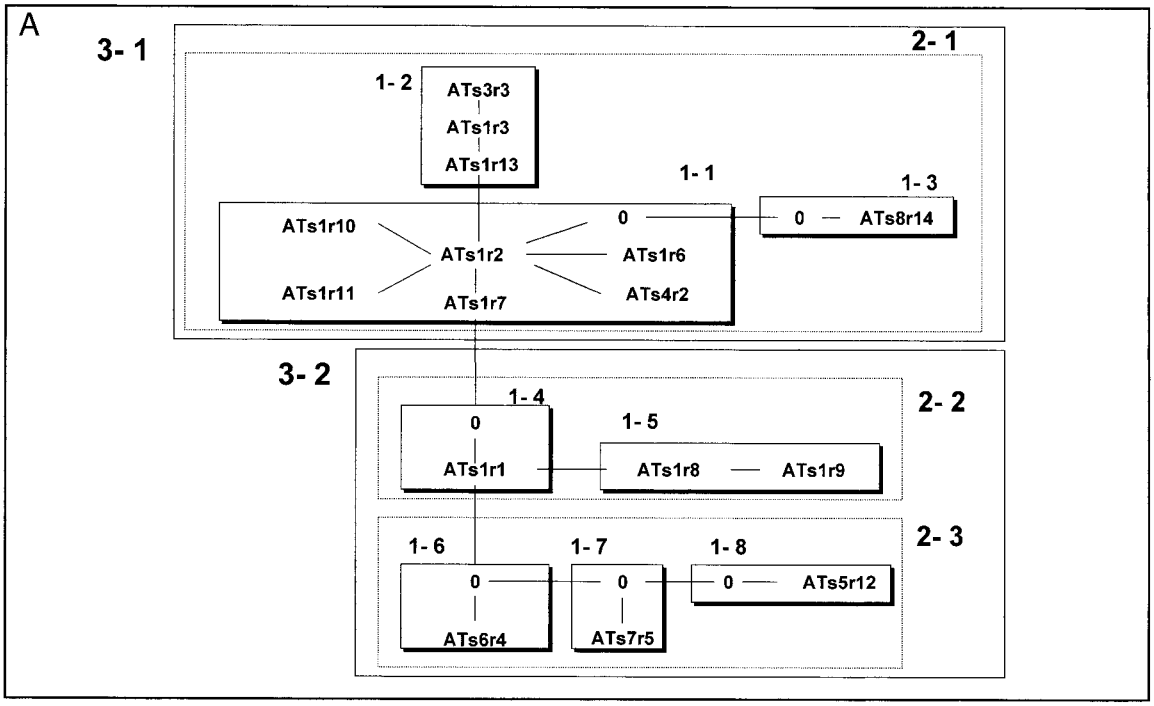
these factors could be the differential dynamics of demographic expansion between pioneer dispersers (presumably those issued from the closest refuge) and later migrants in newly colonized habitats. Simulation studies showed that pioneers would be able to establish and expand rapidly in newly available habitats (Ibrahim et al. 1996). Later migrants would contribute little because they would be entering populations at or near carrying capacity with only limited replacement dynamics. According to this scenario, trout already present in a refuge located in a given basin or geographic area would be the first to expand and occupy available ecological niches, leaving only little probability of establishment for populations that survived in a more remote refugial area. This process would be enhanced if time of geographic isolation and/or differential selection in different environments (in terms of climate, physical environments, and species communities) have been sufficiently important for the accumulation of genetic and ecological differences. For salmonids, it has been shown in lake whitefish (*Coregonus clupeafomis*) that geographic isolation of evolutionary lineages during the last glaciations led to the development of their partial genetic incompatibility, resulting in a much higher embryonic mortality rate of hybrid compared to pure progeny (Lu and Bernatchez 1998). This, along with the potential for occupying distinct trophic niches, apparently explains the maintenance of reproductive isolation between populations of different evolutionary lineages when found in sympatry (Bernatchez et al. 1999; Lu and Bernatchez 1999). Ecological and/or genetic constraints to gene flow among trout lineages have been suggested by studies in which different lineages were found in parapatry (Giuffra et al. 1996; Largiadèr and Scholl 1996; Poteaux et al. 1998).

Mismatch Analysis: Differential Timing of Major Demographic Expansions among Lineages

A model of sudden demographic expansion was statistically supported for the AT, AD, and DA lineages. Because

←

(C) Geographic distribution of one-step AD clades. Other lineages refer to haplotypes not belonging to the Adriatic lineage. Symbols were positioned with the exact latitudinal and longitudinal coordinates of each sample using the software MapInfo, and geographically proximate samples may overlap. A detailed description of single haplotype distribution is provided in the Appendix.


A
3- 1
2- 1
1- 2
ATs3r3
ATs1r3
ATs1r13
1- 1
1- 3
ATs1r10
ATs1r2
ATs1r11
ATs1r7
ATs1r6
ATs4r2
ATs8r14
3- 2
2- 2
1- 4
ATs1r1
1- 5
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1- 6
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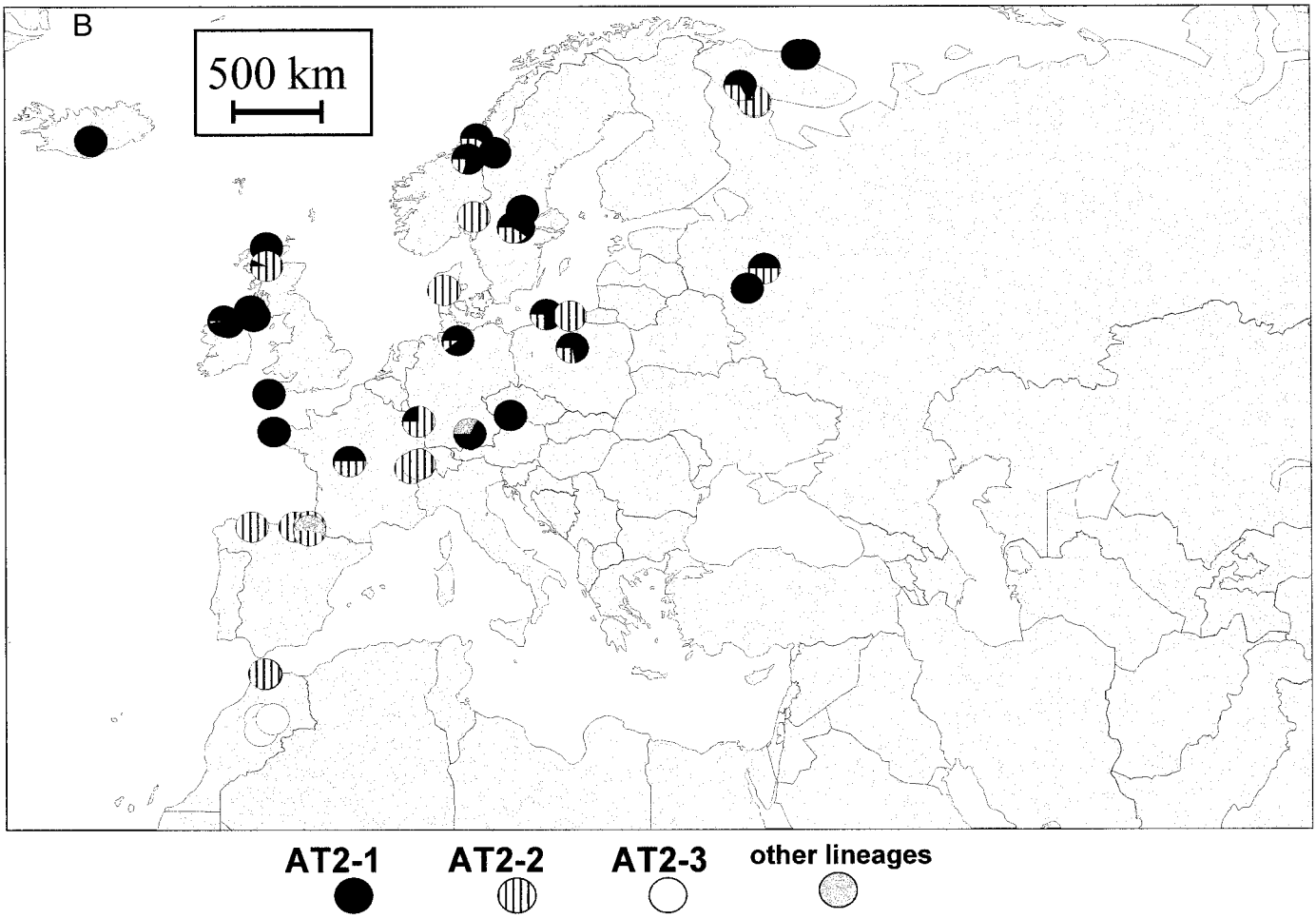
1- 8
ATs6r4
ATs7r5
ATs5r12


B
500 km
AT2-1
AT2-2
AT2-3
other lineages

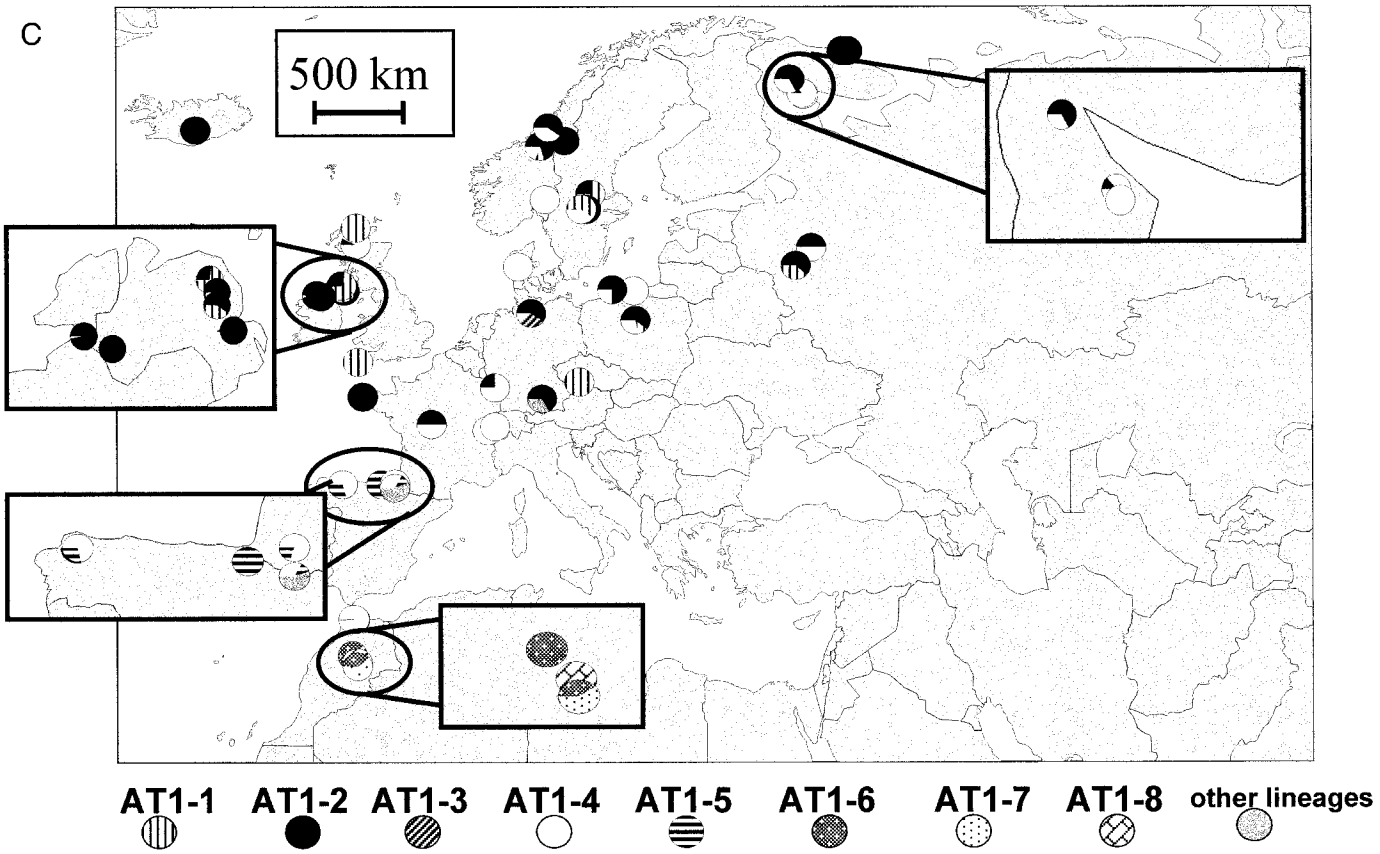


FIG. 6. Continued.

the effects of less important demographic changes may be masked by that of the most important one (Rogers 1995; Lavery et al. 1996), the variable estimates of the age expansion parameter should be interpreted as indicating that the most important demographic expansion of each lineage occurred at different evolutionary times. The most recent demographic expansion was detected within the AT lineage. Because the Atlantic basin was the most directly affected by glaciations in terms of habitat loss, one would predict more important reduction of population abundance during glacial advances in this area, as is generally reported in north temperate fishes differentially affected by glaciations (Bernatchez and Wilson 1998). This was also supported by the more reduced mtDNA diversity in AT relative to both the AD and DA lineages. Although they should be interpreted cautiously given their large 95% CI, absolute estimates obtained by considering the two different mutation rates (1% and 2% per million years) suggested that the time of the most important demographic expansion of the AT lineage (13,400 years ago at 2% per million years, 26,800 years ago BP at 1% per million years) roughly coincided with the onset of the last glacial retreat (approximately 18,000 years ago).

In contrast, the most important demographic expansion that occurred in the DA lineage was much older (mean values = 154,500–309,000 years ago). This is congruent with geological evidence that the geographic range occupied by this lineage was much less directly affected by recent glacial advances compared to the Atlantic basin. This is also reflected by its much higher mtDNA genetic diversity. Several possibilities of large demographic expansion occurred in this region, namely through interconnections that developed among expanding Black, Caspian, and Aral Sea basins. How-

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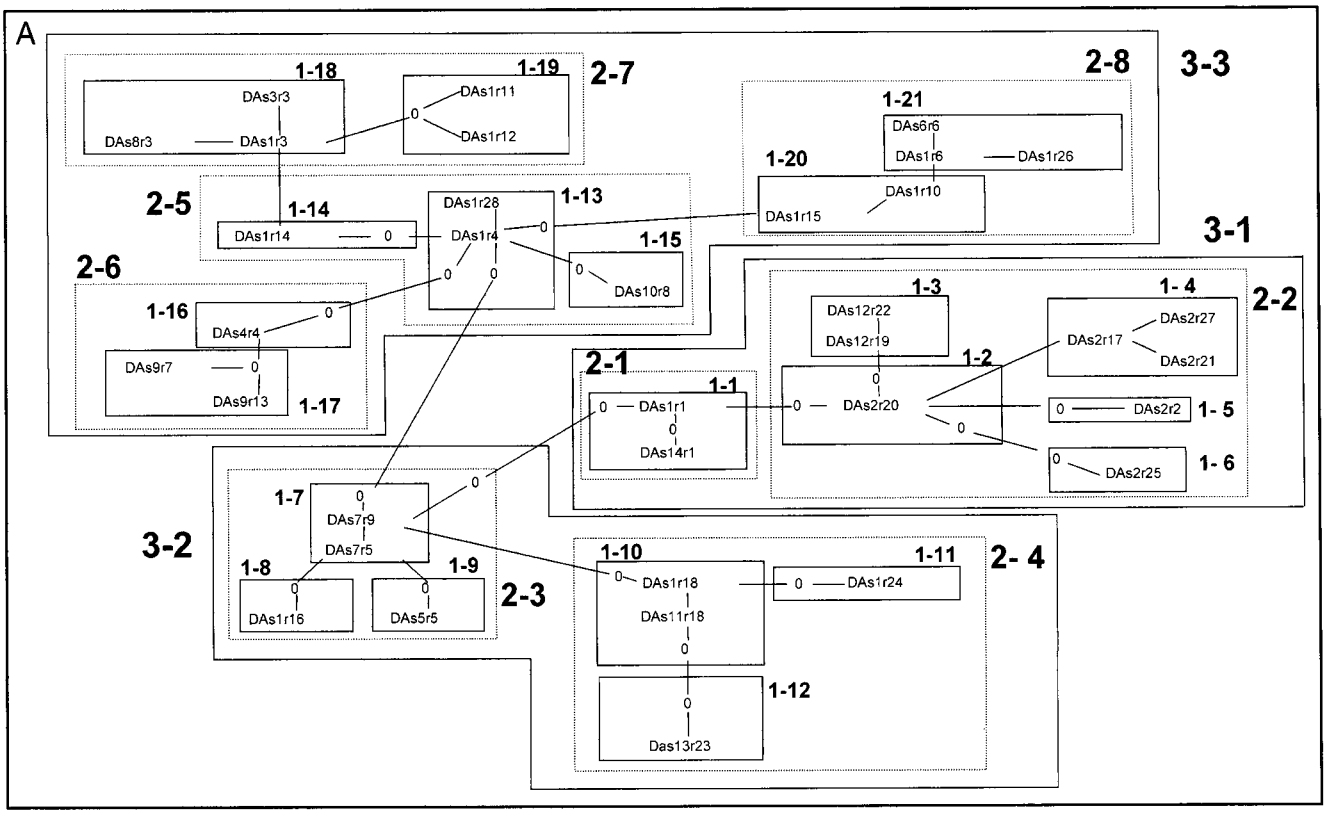
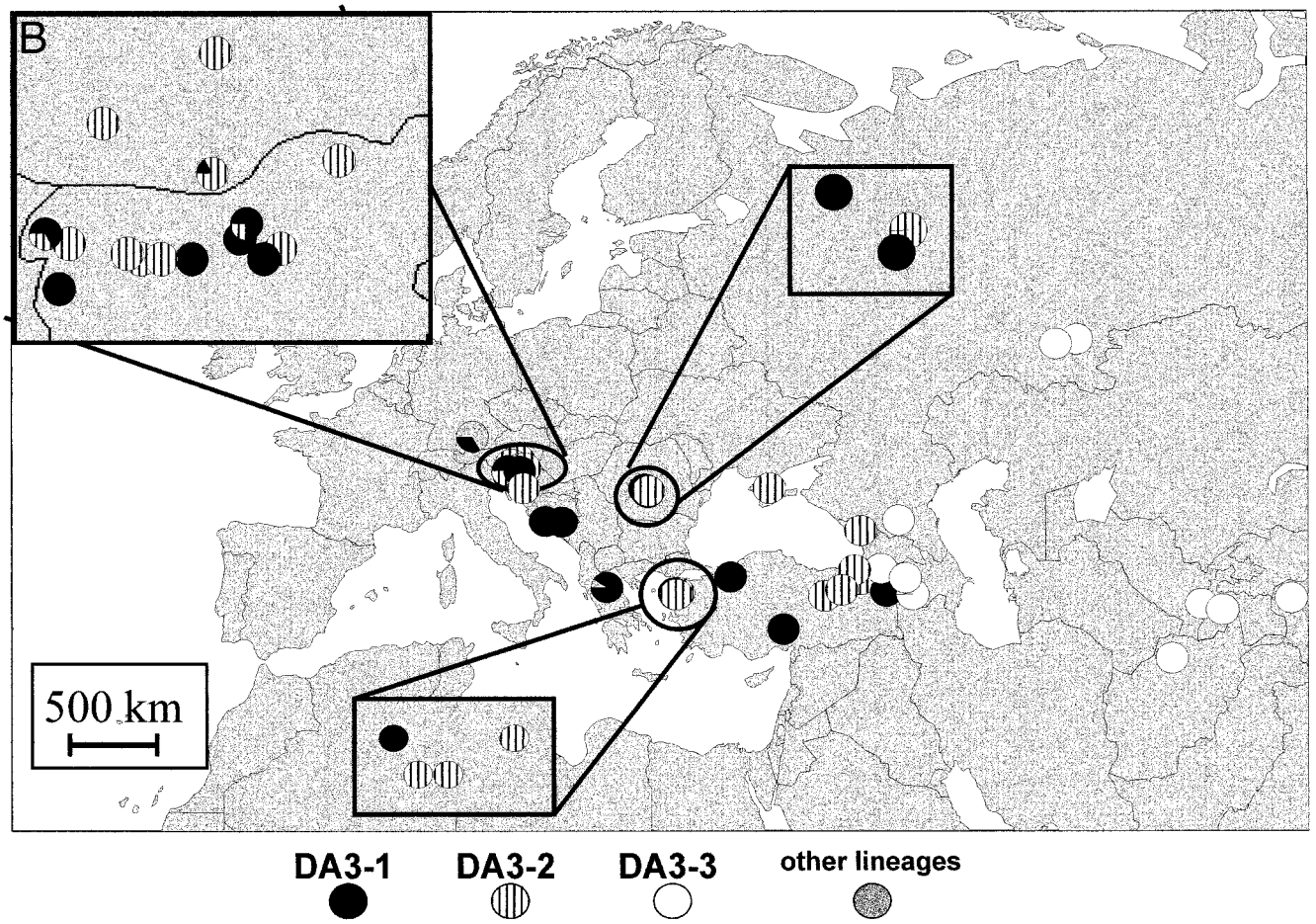
FIG. 6. (A) Minimum spanning network for 16 mitochondrial composite haplotypes resolved in the trout Atlantic lineage. Each line in the network represents a single mutational change. A zero indicates an interior node in the network that was not present in the sample. Composite haplotypes are defined in Tables 2 and 3. Heavier-lined polygons indicate two-step clades nested together into three-step clades, dotted-lined polygons indicate one-step clades nested together into two-step clades, and lined-shaded polygons indicate haplotypes grouped together into one-step clades. (B) Geographic distribution of two-step AT clades. (C) Geographic distribution of one-step AT clades. Other lineages refer to haplotypes not belonging to the Atlantic lineage. A detailed description of single haplotype distribution is provided in the Appendix.


A
1-18
DAs3r3
DAs8r3
DAs1r3
1-19
DAs1r11
DAs1r12
2-7
2-8
3-3
1-21
DAs6r6
DAs1r6
DAs1r26
1-20
DAs1r10
DAs1r15
2-5
1-14
DAs1r14
DAs1r28
1-13
DAs1r4
1-15
DAs10r8
3-1
2-6
1-16
DAs4r4
DAs9r7
DAs9r13
1-17
1-3
DAs12r22
DAs12r19
1-4
DAs2r17
DAs2r27
DAs2r21
2-2
2-1
1-2
1-1
DAs1r1
DAs14r1
DAs2r20
DAs2r2
1- 5
DAs2r25
1- 6
1-7
DAs7r9
DAs7r5
3-2
1-8
DAs1r16
1-9
DAs5r5
2-3
1-10
DAs1r18
DAs11r18
1-11
DAs1r24
2- 4
1-12
Das13r23


B
500 km
DA3-1
DA3-2
DA3-3
other lineages

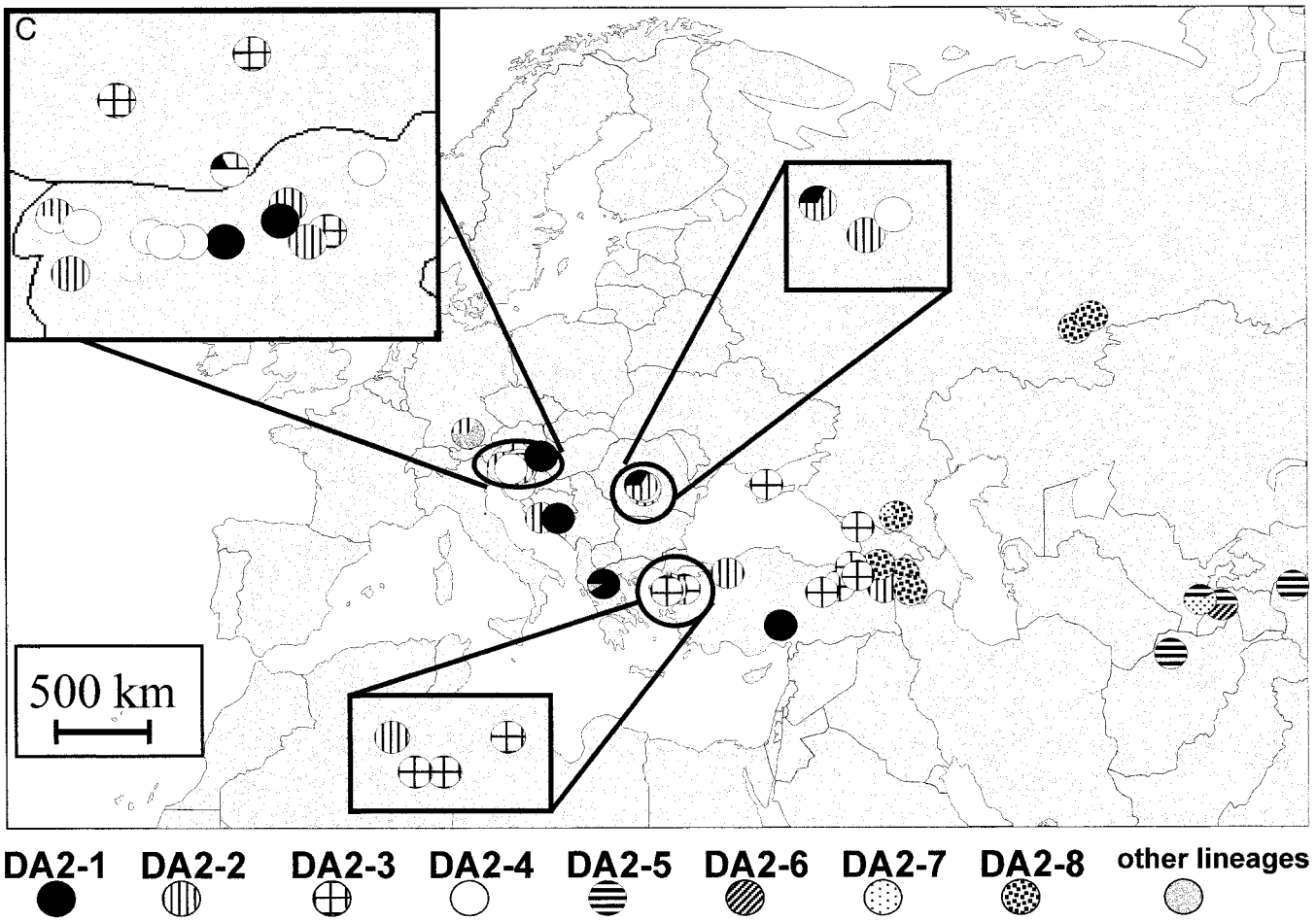


FIG. 7. Continued

ever, the most important interconnections and sea expansion most likely developed approximately 270,000–290,000 years ago (Arkhipov et al. 1995). It is thus plausible that the major demographic expansion within the DA lineage was associated with the opportunities of large-scale dispersal that occurred at that time.

The timing of demographic expansion within the AD lineage (67,300–134,600 years ago) was intermediate between that of the AT and DA lineages. Trout populations from different parts of the Mediterranean basin were likely differentially affected by glacial advances, with habitats of those from the western part of the range being more compressed due to more severe climatic changes and the sea barrier, as compared to more eastern populations (Hewitt 2000). This is reflected by the more important genetic diversity among eastern than western populations. The most important demographic expansion within the AD lineage could thus be associated with westward dispersal that occurred during the early Würm or late Riss glacial period.

A model of sudden demographic expansion was rejected for both the ME and MA lineages. This could reflect a temporally more stable demography and near mutation-drift equilibrium conditions for these lineages (Rogers and Harpening 1992). In such a case, however, an overall high level of genetic diversity, as well as pronounced population structure among physically isolated populations would be expected.

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FIG. 7. (A) Minimum spanning network for 35 mitochondrial composite haplotypes resolved in the trout Danubian evolutionary lineage. Each line in the network represents a single mutational change. A zero indicates an interior node in the network that was not present in the sample. Composite haplotypes are defined in Tables 2 and 3. Heavier-lined polygons indicate two-step clades nested together into three-step clades, dotted-lined polygons indicate one-step clades nested together into two-step clades, and thinned-lined polygons indicate haplotypes grouped together into one-step clades. (B) Geographic distribution of three-step DA clades. (C) Geographic distribution of two-step DA clades. Other lineages refer to haplotypes not belonging to the Atlantic lineage. A detailed description of single haplotype distribution is provided in the Appendix.

TABLE 8. Nested clade analysis of the Atlantic (AT) lineage following the inference key of Templeton (1998). Nested design, haplotype and clade designations are given in Figure 6A. The geographic distribution of clades is provided in Figures 6B and 6C. See Table 7 for further details. Abbreviations: rest. gene flow; restricted gene flow, IBD; isolated by distance., cont. range ext.; contiguous range expansion.

Haplotypes			One-step clades			Two-step clades			Three-step clades		
No	Dc	Dn	No	Dc	Dn	No	Dc	Dn	No	Dc	Dn
ATs1r2	1084^{S}	1083									
ATs1r10	0	942									
ATs4r2	0^{S}	942									
ATs1r6	0^{S}	942									
ATs1r7	0	1678									
I-T	1084^{L}	−43									
1-2-3-4 No: restr. gene flow, IBD			**1-1**	1081^{S}	1085^{S}						
ATs1r3	834^{S}	834^{S}									
ATs3r3	0	867									
ATs1r13	738	778									
I-T	465^{S}	11									
1-2-11-12 No: cont. range exp.			1-2	832^{S}			830^{S}				
ATs8r4	0		1-3	0^{S}	529^{S}						
			I-T	658^{L}	397^{L}						
			1-2-3-5-15 No: past frag.			2-1	1008	1008	3-1	1008^{S}	1082^{S}
ATs1r1	1094	1094	**1-4**	1094^{S}	1091^{S}						
ATs1r8	0	133^{S}									
ATs1r9	256^{S}	106^{S}									
I-T	−256	26.4									
1-2-3-5-15 No: past frag.			1-5	109^{S}	1258						
			I-T	984^{L}	−166						
			1-2-3-5-15 No: past frag.			**2-2**	1110^{S}	1103^{S}			
ATs6r4	44	0	**1-6**	44^{S}	59^{S}						
ATs7r5	0	0	1-7	0^{S}	43^{S}						
ATs5r12	0	0	1-8	0^{S}	29^{S}						
			I-T	44	23						
			1-2-3-5-15 No: past frag.			2-3	47^{S}	2017^{L}			
						I-T	1063^{L}	−913^{S}			
						1-2-3-5-15 No: past frag.			**3-2**	1240	1231
									I-T	232	249
									1-2-3-5-15 No: past frag.		

Clearly, this was not the case, as illustrated by the lineages' extremely reduced diversity and molecular variance imputable to population structuring. Instead, the predominance of single haplotypes found over a wide geographic area is more compatible with nonequilibrium conditions caused by a severe and relatively recent bottleneck. This is also congruent with the evidence for more reduced habitat availability in areas occupied by refugial populations of the ME and MA lineages. Extremely reduced diversity may in turn hamper the detection of demographic signals in the genetic signature of populations (e.g., Lavery et al. 1996). As such, genetic diversity observed for the ME and MA lineages do not follow a typical north-south pattern of glaciation effects on population demography and illustrate that conditions favorable to trout survival may have been more severe in those southern areas than further north during the last glacial advance.

Nested Clade Analysis: Differential Dynamics of Population Structuring among Lineages

The nested clade analysis provided new insights into the resolution of finer evolutionary lineages within major lineages, from which new hypotheses of brown trout evolutionary history can be inferred and contrasted with previous interpretations.

Atlantic lineage: latitudinal contrasts in historical population structuring

The more southern distribution of clade AT3–2, along with its more complex pattern of clade diversity suggest that it represents the ancestral lineage from which lineage AT3–1 derived following isolation in more northern latitudes. Its long history of fragmentation also reflects a more temporally stable population structuring relative to more northern populations, as would be predicted from the glacial history of the different regions. In contrast, the nested clade analysis revealed that northern trout populations were historically subdivided into two ancestral lineages that intermixed extensively since their recent range expansion throughout most of the north Atlantic region following the last glacier retreat. Given their differential pattern of geographic distribution, it is most likely that AT1–1 characterizes a trout lineage that survived in a refuge located in the more northwestern part of the Atlantic range of distribution, whereas lineage AT1–2 identifies a lineage that survived in a northeastern refuge.

In summary, three trout lineages, all from AT origins, were involved in the recolonization process of the North Atlantic basin; one from a southern refuge and more ancient origin (characterized by haplotype ATs1r1 belonging to lineage AT3–2) that first intergraded with an northern ancestral lineage (AT3–1 or AT2–1), then two lineages originating from this admixed group that later evolved in isolation in a west-central and a northeastern refuge.

The origin and postglacial history of brown trout in the North Atlantic basin has been the subject of divergent interpretations. Most researchers agreed on the fact that the North Atlantic was recolonized by different trout evolutionary lineages. However, interpretations varied substantially as to their number, center of origin, and timing of dispersal. The broader geographic coverage in this study, along with the ability of the nested-clade analysis to interpret the temporal juxtaposition of historical processes, partly reconciles previous interpretations. First, these results support the existence of a northwestern refuge, as first proposed by Ferguson and Fleming (1983), then reiterated by Hamilton et al. (1989), Osinov and Bernatchez (1996), and García-Marín et al. (1999). Second, they also support the existence of a northeastern refuge first proposed by Osinov and Bernatchez (1996) and provide evidence for the contribution of a southern refuge, as originally proposed by Hamilton et al. (1989) and later by García-Marín et al. (1999). Unlike, these interpretations, however, the present results implied that northern colonization by this southern group occurred prior to the last glaciation. They also refute the view of a contribution of a Ponto-Caspian lineage (García-Marín et al. 1999) because this would imply the complete disappearance of DA haplotypes in northern Europe, along with all known allozyme alleles private to the DA lineage (see also Weiss et al. 2000). Finally, as previously hypothesized, the present results statistically confirmed that these evolutionary lineages intergraded extensively following the last glacier retreat.

Mediterranean lineages: longitudinal contrasts in historical population structuring

The overall geographic pattern of genetic diversity observed among Mediterranean lineages revealed a longitudinal pattern of reduced population structuring from east to west. The predominance of single haplotypes with a relatively broad geographic distribution within both ME and MA lineages is most compatible with nonequilibrium conditions resulting from recent range expansion, which eventually resulted in partial geographic overlap between these and the AD lineage. An east-west pattern of reduced population structuring was also illustrated within the AD lineage. Thus, the pattern of population structuring among western populations of the AD lineage was more similar to that observed within ME and MA lineages than among eastern populations of this same lineage. This indicated that, in contrast to highly fragmented structure among eastern populations, the observed population structuring among western populations was more compatible with a history of recent range expansion. It is noteworthy that the Balkans, despite its relatively small size, is the area harboring the most diverse phenotypic diversity among trout populations, which may be the outcome of their long-term isolation in different environments compared to other parts of the species range of distribution (Kottelat 1997).

Danubian lineage: complex juxtapositions of various historical events

The geographic area occupied by the DA lineage has been the least investigated previously. The present study provided new insights into the existence of finer phylogeographic subdivisions within this lineage and allowed us to propose hypotheses regarding its evolutionary history. Previous allozyme studies did not show any pattern of major geographic discontinuity in the distribution of genetic diversity (Bernatchez and Osinov 1995). The mtDNA anaylsis of Bernatchez and Osinov (1995) provided slight, but inconclusive evidence for genetic discontinuity among sea basins. The nested clade analysis, however, statistically supported such a structure. These results support the long-standing hypothesis proposed on the basis of morphological variation that populations of each sea basin should be recognized as distinct evolutionary lineages (Berg 1948).

Conclusions

The combined use of traditional phylogeographic, nested-clade, and mismatch analyses of mtDNA diversity proved to be very efficient in improving our knowledge of the complex evolutionary history of brown trout throughout its native range of distribution. This confirmed the existence of five evolutionary lineages that evolved in geographic isolation during the Pleistocene and have remained largely allopatric since then. These should be recognized as the basic evolutionary significant units within brown trout (Bernatchez 1995). In addition to physical isolation, biological factors must have contributed to limiting their dispersal and introgressive hybridization among them. The unique evolutionary histories of each lineage have been shaped both by the the differential latitudinal impact of glaciations on habitat loss and potential for dispersal, as well as climatic impacts and landscape heterogeneity that translated in a longitudinal pattern of genetic diversity and population structuring at more southern latitudes.

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TABLE 9. Nested clade analysis of the Danubian (DA) lineage following the inference key of Templeton (1998). Nested design, haplotype and clade designations are given in Figure. 7A. The geographic distribution of clades is provided in Figures 7B and 7C. Abbreviations: inconcl.; inconclusive, rest. disp-gene fl.; restricted dispersal with gene flow.

Haplotypes			One-step clades			Two-step clades			Three-step clades		
No	Dc	Dn	No	Dc	Dn	No	Dc	Dn	No	Dc	Dn
DAs1r1	448^{S}	527^{S}									
DAs14r1	0	389^{S}									
I-T	448^{S}	138									
1-2-11-12 No: cont. range exp.			**1-1**	481	0	**2-1**	480^{S}	506^{S}			
DAs2r20	0	0	1-2	0^{S}	299^{S}						
DAs12r19	0	154^{S}									
DAs12r22	169^{S}	166^{S}									
I-T	−169^{S}	−12									
1-2-3-4 No: rest. gene flow/IBD			1-3	164^{S}	490^{S}						
DAs2r17	0	230^{S}									
DAs2r21	0	783									
DAs2r17	86	892									
I-T	−43	−607									
1-2-11-17 No: inconcl.			1.4	635^{S}	1054						
DAs2r2	131	0	1-5	131^{S}	763						
DAs2r25	0	0	1-6	0	741						
			I-T	−232	−463						
			1-2-3-5-6-7-8 Yes: rest. gene flow/disp.			2-2	620^{S}	625^{S}			
						I-T	−139	−118			
						1-2-3-5-6 too few clades: range exp/restr. disp-gene fl.			3-1	583^{S}	1326
DAs7r9	900^{S}	939									
DAs7r5	0^{S}	1215									
I-T	900	−275									
1-2-3-5-15 No: past frag.			**1-7**	980^{S}	1015^{S}						
DAs1r16	0	0	1-8	232^{S}	684^{S}						
DAs5r5	506	0	1-9	506^{S}	727^{S}						
			I-T	611^{L}	310						
			1-2-3-5-6-7-8 Yes: res. gene flow/disp.			**2-3**	874^{S}	996^{S}			
DAs1r18	25^{S}	171^{S}									
DAs11r18	381^{S}	359^{S}									
I-T	−356	−188									
1-2-3-5-15 No: past frag.			**1-10**	171^{S}	150^{S}						
DAs1r24	0	0	1-11	0	141^{S}						
DAs13r23	0	0	1-12	0	237^{S}						
			I-T	171^{S}	−38						
			1-2-3-5-6-7-8 Yes: rest. gene log/disp.			2-4	279^{S}	824^{S}			
						I-T	595^{L}	172			
						1-2-3-5-15 No: past frag.			**3-2**	940^{S}	1047^{S}
DAs1r4	141^{S}	137^{S}									
DAs1r28	0	0									
I-T	141^{S}	137^{S}									
1-2-3-6-7-8 No: inad. Sampling			**1-13**	132^{S}	187^{S}						
DAs1r14	0	0	1-14	0^{S}	238^{S}						
DAs10r8	0	0	1-15	0	276^{S}						
			I-T	132^{S}	−70						

TABLE 9. Continued.

Haplotypes			One-step clades			Two-step clades			Three-step clades		
No	Dc	Dn	No	Dc	Dn	No	Dc	Dn	No	Dc	Dn
			1-2-3-5-6-7-8			**2-5**	203[S]	1165			
			Yes: rest. gene flow/disp								
DAs4r4	0	0	1-16	0	0						
DAs9r7	0	0									
DAs9r13	0	0	1-17	0	0	2-6	0[S]	1294			
DAs1r3	0	0									
DAs3r3	0	0									
DAs8r3	0	0	**1-18**	0[S]	698[S]						
DAs1r11	0	0									
DAs1r12	0	0	1-19	0[S]	1351						
			I-T	0	−653						
			1-2-3-5-15 No: past frag.			2-7	915	1067			
DAs1r15	812[S]	913[S]									
DAs1r10	0[S]	624[S]									
I-T	812[L]	289									
1-2-3-5-15 No: past frag.			**1-20**	812[S]	774[S]						
DAs1r6	0[S]	330[S]									
DAs6r6	0	330									
DAs1r26	678[S]	607[S]									
I-T	−339[S]	−139									
1-2-3-5-15 No: past frag.			1-21	927[S]	1277						
			I-T	−114	−502[S]						
			1-2-3-5-15 No: past frag.			2-8	947[S]	1266			
						I-T	−417[S]	−44			
						1-2-3-5-15 No: past frag.			3-3	1321	1891[L]
									I-T	−12	−143
									1-2-3-5-15 No: past frag.		

Literature Cited

Andersen, B. G., and H. W. Borns Jr. 1994. The Ice Age world. Scandinavian Univ. Press, Oslo, Norway.

Apostolidis, A., Y. Karakousis, and C. Triantaphyllidis. 1996a. Genetic differentiation and phylogenetic relationships among Greek *Salmo trutta* L. (brown trout) populations as revealed by RFLP analysis of PCR amplified mitochondrial DNA segments. Heredity 77:608–618.

———. 1996b. Genetic divergence and phylogenetic relationships among *Salmo trutta* L. (brown trout) populations from Greece and other Europeans countries. Heredity 76:551–560.

Apostolidis, A. P., C. Triantaphyllidis, A. Kouvatsi, and P. S. Economidis. 1997. Mitochondrial DNA sequence variation and phylogeography among *Salmo trutta* L. (Greek brown trout) populations. Mol. Ecol. 6:531–542.

Arkhipov, S. A., J. Ehlers, R. G. Johnson, and H. E. Wright Jr. 1995. Glacial drainages towards the Mediterranean during middle and late Pleistocene. Boreas 24:196–206.

Avise, J. C. 1998. The history and purview of phylogeography: concepts and applications. Mol. Ecol. 7:371–380.

Avise, J. C., D. Walker, and G. C. Johns. 1998. Speciation durations and Pleistocene effects on vertebrate phylogeography. Proc. Roy. Soc. Lond. B. 265:1707–1712.

Banarescu, P. 1990. Zoogeography of freshwater: general distribution and dispersal of freshwater animals. Aula Verlag ed. Wiesbaden 1:1–511.

Berg, L. S. 1948. Freshwater fishes of the Soviet Union and adjacent countries. Vol. 1. Akademy Nauk, Moscow.

Bernatchez, L. 1995. A role for molecular systematics in defining evolutionarily significant units in fishes. Pp. 114–132 *in* J. L. Nielsen, ed. Evolution and aquatic ecosystem: defining units in population conservation. American Fisheries Society Symposium no.17, Bethesda, Maryland.

Bernatchez, L., and R. G. Danzmann. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). Mol. Biol. Evol. 10:1002–1014.

Bernatchez, L., and J. J. Dodson. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution 45: 1016–1035.

———. 1994. Phylogenetic relationships among Palearctic and Nearctic whitefish (*Coregonus* sp.) populations as revealed by mitochondrial DNA variation. Can. J. Fish. Aquat. Sci. 51: 240–251.

Bernatchez, L., and A. Osinov. 1995. Genetic diversity of trout (genus *Salmo*) from its most eastern native range based on mitochondrial DNA and nuclear gene variation. Mol. Ecol. 4: 285–297.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic freshwater fishes. Mol. Ecol. 7:431–452.

Bernatchez, L., J. J. Dodson, and S. Boivin. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination. J. Fish Biol. 35:233–244.

Bernatchez, L., R. Guyomard, and F. Bonhomme. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. Mol. Ecol. 1:161–173.

Bernatchez, L., A. Chouinard, and G. Lu. 1999. Integrating molecular genetics and ecology in studies of adaptive radiation: whitefish, *Coregonus*, as a case study. Biol. J. Linn. Soc. 68: 173–194

Berrebi, P. 1995. Étude génétique des truites de Corse. Laboratoire Génome et Populations, Université de Montpellier pour Parc Naturel Régional de Corse.

Bianco, P. G. 1990. Potential role of the paleohistiory of the Mediterranean and Paratethys basins on early dispersal of Euro-Mediterranean freshwater fishes. Ichthyol. Explor. Fresh. 1:167–184.

Donaldson, K. A., and R. R. Wilson Jr. 1999. Amphi-Panamic geminates of snook (Percoidei: Centropomidae) provide a calibration of the divergence rate in the mitochondrial DNA control region of fishes. Mol. Phyl. Evol. 13:208–213.

Durand, J. J., H. Persat, and Y. Bouvet. 1999a. Phylogeography and postglacial dispersion of the chub (*Leuciscus cephalus*) in Europe. Mol. Ecol. 8:989–998.

Durand, J. D., A. R. Templeton, B. Guinand, A. Imsiridou, and Y. Bouvet. 1999b. Nested clade and phylogeographic analyses of chub, *Leuciscus cephalus* (Teleostei, Cyprinidae), in Greece: implications for the Balkan Peninsula biogeography. Mol. Phyl. Evol. 13:566–580.

Economidis, P. S., and P. M. Banarescu. 1991. The distribution and origins of freshwater fishes in the Balkan Peninsula, especially in Greece. Intern. Rev. Der Gesam. Hydrobiol. 76:257–283.

Englbrecht, C. C., J. Freyhof, A. Nolte, K. Rassmann, U. Schliewen, and D. Tautz. 2000. Phylogeography of the bullhead *Cottus gobio* (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations. Mol. Ecol. 9: 709–722.

Excoffier, L., and P. E. Smouse. 1994. Using allele frequency and geographic subdivision to reconstruct gene trees within a species: molecular variance parsimony. Genetics 136:343–359.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Ferguson, A. 1989. Genetic differences among brown trout, *Salmo trutta*, stocks and their importance for the conservation and management of the species. Fresh. Biol. 21:35–46.

Ferguson, A., and C. C. Fleming. 1983. Evolutionary and taxonomic significance of protein variation in the brown trout (*Salmo trutta* L.). Pp. 85–99 *in* G. S. Oxford and D. Rollinson, eds. Protein polymorphisms: adaptive and taxonomic significance. Academic Press, London.

García-Marín, J.-L., and C. Pla. 1996. Origins and relationships of native populations of *Salmo trutta* (brown trout) in Spain. Heredity 77:313–323.

García-Marín, J.-L., F. M. Utter, and C. Pla. 1999. Postglacial colonization of brown trout in Europe based on distribution of allozyme variants. Heredity 82:46–56.

Gibbard, P. L. 1988. The history of the great northwestern European rivers during the past three million years. Phil. Trans. Roy. Soc. Lond. 318:559–602.

Giuffra, E., L. Bernatchez, and R. Guyomard. 1994. Mitochondrial control region and protein coding genes sequence variation among phenotypic forms of brown trout *Salmo trutta* from northern Italy. Mol. Ecol. 3:1161–1171.

Giuffra, E., R. Guyomard, and G. Forneris. 1996. Phylogenetic relationships and introgression patterns between incipient parapatric species of Italian brown trout (*Salmo trutta* L. complex). Mol. Ecol. 5:207–220.

Grosswald, M. G. 1980. Late weichselian ice sheet of northern Eurasia. Quat. Res. 13:1–32.

Guyomard, R. 1989. Diversité génétique de la truite commune. Bull. Fr. Pêche Piscic. 314:118–135.

Hamilton, K. E., A. Ferguson, J. B. Taggart, and T. Tómasson. 1989. Post-glacial colonization of brown trout, *Salmo trutta* L.: *Ldh-5* as a phylogeographic marker locus. J. Fish Biol. 35: 651–664.

Hansen, M. M., and V. Loeschcke. 1996. Genetic differentiation among Danish brown trout populations, as detected by RFLP analysis of PCR amplified mitochondrial DNA segments. J. Fish Biol. 48:422–436.

Hansen, M. M., and K.-L. D. Mensberg. 1998. Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations. Heredity 81: 493–504.

Harpening, H. 1994. Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66:591–600.

Hewitt, H. G. M. 2000. The genetic legacy of the Quaternary ice ages. Nature. 405:907–913.

Hudson, R. R. 1990. Gene genealogies and the coalescent process.

Pp. 1–44 *in* D. Futuyama and J. Antonovics, eds. Oxford surveys in Evolutionary Biology, Vol.7. Oxford Univ. Press, Oxford.
Hynes, R. A., A. Ferguson, and M. A. McCann. 1996. Variation in mitochondrial DNA and post-glacial colonization of north western Europe by brown trout. J. Fish Biol. 48:54–67.
Ibrahim, K. M., R. A. Nichols, and G. M. Hewitt. 1996. Spatial patterns of genetic variation by different forms of dispersal during range expansion. Heredity 77:282–291.
Keith, P. 1998. Évolution des peuplements ichtyologiques de France et stratégies de conservation. Ph.D. diss., Université de Rennes I, France.
Kottelat, M. 1997. European freshwater fishes. Biol. Brat. 52(suppl. 5):1–271.
Largiadèr, C. R., and A. Scholl. 1996. Genetic introgression between native and introduced brown trout *Salmo trutta* L. populations in the Rhône River basin. Mol. Ecol. 5:417–426.
Lavery, S., C. Moritz, and D. R. Fielder. 1996. Genetic patterns suggest exponential population growth in a declining species. Mol. Biol. Evol. 13:1106–1113.
Lu, G., and L. Bernatchez. 1998. Experimental evidence for reduced hybrid viability between dwarf and normal ecotypes of lake whitefish (*Coregonus clupeaformis* Mitchill). Proc. Roy. Soc. Lond. Series B 265:1025–1030.
———. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. Evolution 53:1491–1505.
McElroy, D. P., P. Moran, E. Bermingham, and I. Kornfield. 1992. REAP: an integrated environment for the manipulation and phylogenetic analysis of restriction data. J. Hered. 83:157–158.
Merilä, J., M. Björklund, and A. J. Baker. 1997. Historical demography and present day population structure of the greenfinch, *Carduelis chloris.* Evolution 51:946–956.
Moritz, C., and E. Bermingham. 1998. Comparative phylogeography: concepts and applications. Mol. Ecol. 7:367–370.
Nei, M. 1987. Molecular evolutionary genetics. Columbia Univ. Press, New York.
Nesbo, C. L., T. Fossheim, L. A. Vollestad, and K. S. Jakobsen. 1999. Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglcial colonization. Mol. Ecol. 8:1387–1404.
Osinov, A. G. 1990. The levels of genetic variation and differentiation of the brown trout (*Salmo trutta* L.) in Tadjikistan. Biol. Sci. Bull. 45:37–41.
Osinov, A., and L. Bernatchez. 1996. Atlantic and Danubean phylogenetic groupings of brown trout (*Salmo trutta* L.) complex: genetic divergence, evolution, and conservation. J. Ichthyol. 36: 762–786.
Persat, H., and P. Berrebi. 1990. Relative ages of present populations of *Barbus barbus* and *Barbus meridionalis* (Cyprinidae) in southern France: preliminary considerations. Aquat. Living Resour. 3:253–263.
Petit, E., L. Excoffier, and F. Mayer. 1999. No evidence of bottleneck in the postglacial recolonization of Europe by the noctule bat (*Nyctalus noctul*a). Evolution 53:1247–1258.
Poteaux, C., D. Beaudou, and P. Berrebi. 1998. Temporal variations of genetic introgression in stocked brown trout population. J. Fish Biol. 53:701–713.
Presa, P. M. 1995. Déterminisme et polymorphisme génétiques des séquences microsatellites de *Salmo trutta* et d'autres salmonidés. Comparaison avec le polymorphisme des locus enzymatiques. Ph.D. diss., Université de Paris-Sud, Orsay, France.
Rogers, A. R. 1995. Genetic evidence for a Pleistocene population explosion. Evolution 49:608–615.
Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.
Ryman, N. 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture 33:1–21.
Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.
Schneider, S., D. Roessli, and L. Excoffier. 1999. Arlequin, version 2.0: a software for genetic data analysis. Genetics and Biometry Laboratory, Univ. of Geneva.
Smith, G. R. 1992. Introgression in fishes: significance for paleontology, cladistics, and evolutionary rates. Syst. Biol. 41: 41–57.
Templeton, A. R. 1998. Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. Mol. Ecol. 7:381–398.
Templeton, A. R., and C. F. Sing. 1993. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonucleases mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659–669.
Templeton, A. R., E. Boerwinkle, and C. F. Sing. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila.* Genetics 117:343–351.
Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.
Templeton, A. R., E. Routman, and C. Phillips. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotytpes in the tiger salamander, *Ambystoma tigrinum.* Genetics 140:767–782.
Turner, T. F., J. C. Trexler, J. L. Harris, and J. L. Haynes. 2000. Nested cladistic analysis indicates population fragmentation shapes genetic diversity in a freshwater mussel. Genetics 154: 777–785.
Villinger, E. 1986. Untersuchungen zur Flussgeschichte von Aare-Donau/Alpenrhein und zur Entwicklung des Malm-Karts in Südwestdeutschland. Germany.
Watterson, G. 1975. On the number of segregating sites in genetical models without recombination. Theor. Popul. Biol. 7:256–276.
Webb, T., III, and P. J. Bartlein. 1992. Global changes during the last 3 millions years: climatic controls and biotic responses. Annu. Rev. Ecol. Syst. 23:141–173.
Weiss, S., A. Antunes, C. Schlötterer, and P. Alexandrino. 2000. Mitochondrial haplotype diversity of brown trout *Salmo trutta* L. in Portuguese rivers supports a simple broad-scale model of the Pleistocene recolonization of northern Europe. Mol. Ecol. 9:691–698.
Wilson, C. C., P. D. N. Hebert, J. D. Reist, and J. B. Dempson. 1996. Phylogeography and postglacial dispersal of arctic charr *Salvelinus alpinus* in North America. Mol. Ecol. 5:187–197.
Zink, R. M. 1996. Comparative phylogeography in North American birds. Evolution 50:308–317.

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APPENDIX.

Sample size, locations, and distribution of composite haplotypes among native populations of brown trout. References: 1. Apostolidis et al. (1996a, 1997); 2. Bernatchez and Osinov (1995); 3. Bernatchez et al. (1992); 4. Giuffra et al. (1994); 5. Hynes et al. (1996); 6. Osinov and Bernatchez (1996); 7. this study.

No.	Lineage	Population	*N*	Coordinates		Basin	Sampling	Composite haplotypes (numbers)	Reference
				Latitude	Longitude				
1	AD	Zrmanja R.	4	44°08′N	16°10′E	Adriatic Sea	1996	ADs4r8	7
2	AD	Muskovci R.	3	44°12′N	15°45′E	Adriatic Sea	1996	ADs4r8	7
3	AD	Krupa R.	3	44°10′N	15°52′E	Adriatic Sea	1996	ADs4r8	7
4	AD	Tohma R.	3	38°47′N	36°55′E	Persian Gulf	1993	ADs6r1	7
5	AD	Zamanti R.	3	38°45′N	36°30′E	Mediterranean Sea	1993	ADs1r3	7
6	AD	Ceyhan R.	1	37°45′N	36°40′E	Mediterranean Sea	1993	ADs1r1	7
7	AD	Anamur R.	1	36°15′N	32°50′E	Mediterranean Sea	1993	ADs1r1	7
8	AD	Göksu R.	2	36°40′N	32°38′E	Mediterranean Sea	1993	ADs5r4	7
9	AD	Köprü R.	3	37°14′N	31°10′E	Mediterranean Sea	1993	ADs1r3	7
10	AD	Prepsa L.	26	40°50′N	21°15′E	Adriatic Sea	1993	ADs1r10	1, 7
11	AD	Taravo R.	8	41°52′N	09°07′E	Mediterranean Sea	1991	ADs3r3	3, 7
12	AD	Ohrid L.	38	41°00′N	20°40′E	Adriatic Sea	1991	ADs1r11(32)-ADs10r11(6)	1, 3, 7
13	AD	Drance R.	8	46°24′N	06°30′E	Mediterranean Sea	1990	ADs1r1(7)-ADs2r1	3, 7
14	AD	Sangro R.	1	41°45′N	13°50′E	Adriatic Sea	1996	ADs4r8	7
15	AD	Fibreno L.	6	41°42′N	13°40′E	Tyrrhenian Sea (Med.)	1996–1997	ADs1r1	7
16	AD	Sinni R.	2	40°05′N	15°55′E	Ionian Sea (Med.)	1996	ADs4r8	7
17	AD	Pardu R.	2	39°50′N	09°30′E	Sardinia	1996	ADs3r3	7
18	AD	Osu R.	1	41°35′N	09°20′E	Corsica	1996	ADs1r1	7
19	AD	Liamone R.	1	42°15′N	08°45′E	Corsica	1996	ADs1r1	7
20	AD	Fangu R.	1	42°30′N	08°38′E	Corsica	1996	ADs3r3	7
21	AD	Ascu R.	2	42°33′N	09°05′E	Corsica	1996	ADs3r3	7
22	AD	Louros R.	1	39°20′N	20°50′E	Adriatic Sea	1993	ADs1r3	7
23	AD	Drosopigi (Loudhias R.)	5	40°00′N	21°25′E	Aegean Sea	1994	ADs1r6	7
24	AD	Skopos R. (Loudhias R.)	8	40°50′N	21°40′E	Aegean Sea	1994	ADs1r3	7
25	AD	Valbona R. (Albania)	5	42°20′N	20°05′E	Adriatic Sea	1993	ADs1r1	7
26	AD	Ese	4	41°57′N	09°03′E	Mediterranean Sea	1996	ADs3r3	7
27	AD	Veraculungu	5	41°52′N	09°09′E	Mediterranean Sea	1996	ADs3r3	7
28	AD	Golo Castrila	4	42°22′N	09°08′E	Mediterranean Sea	1996	ADs3r3(3)-ADs3r7	7
29	AD	Krkic R.	4	44°05′N	16°12′E	Adriatic Sea	1997	ADs1r1	7
30	AD	Stura di Demonte	15	44°25′N	07°10′E	Adriatic Sea	1991	ADs1r1	4
31	AD	Alfios	5	37°37′N	21°27′E	Ionian Sea	—	ADs7r9	1
32	AD	Evinos	6	38°27′N	21°40′E	Ionian Sea	—	ADs1r1	1
33	AD	Thyamis	25	39°50′N	20°30′E	Ionian Sea	—	ADs1r9	1
34	AD	Tripotamos	26	40°35′N	22°15′E	Aegean Sea	—	ADs1r1(4)-Ads8r1(22)	1
35	AD	Dimcay R.	1	36°34′N	32°17′E	Mediterranean Sea	1998	ADs2r1	7
36	AD	Adour (Gaube)	5	42°50′N	00°09′W	Atlantic	1996	ADs1r1(4)-ADs1r5	7
37	AD-AT	Artesiaga R.	9	43°03′N	01°33′W	Atlantic	1992	ADs1r1(5)-ATs1r1(3)-ATs1r8	7
38	AD-MA	Krka R.	4	43°50′N	15°58′E	Adriatic Sea	1996	ADs1r1-ADs4r8-MAs1r1(2)	7
39	AD	Nestos R.	25	41°20′N	24°40′E	Aegean Sea	1993	ADs9r1(11)	1, 7
40	AD-MA-ME	Garda L.	23	45°50′N	10°40′E	Adriatic Sea	1990–1991	ADs1r1(15)-MAs2r1(5)-MAs3r1-Mes2r1(2)	3, 4, 7
41	AD-MA-ME	Chisone R.	23	44°50′N	07°10′E	Adriatic Sea	1990–1991	ADs1r1(12)-MAs2r1(8)-MEs2r1(3)	3, 4, 7
42	AD-ME	Vecchio R.	6	42°13′N	09°12′E	Mediterranean Sea	1991	ADs3r3(5)-MEs2r1	3, 7
43	AT	Aubonne R.	5	46°27′N	06°23′E	Mediterranean Sea	1990	ATs1r1	3
44	AT	Redon R.	3	46°40′N	07°00′E	Mediterranean Sea	1990	ATs1r1	3
45	AT	Tsna R.	6	57°20′N	34°00′E	Caspian Sea	1994	ATs1r1(3)-ATs1r2(2)-ATs1r7	6
46	AT	Bresle R.	8	46°50′N	01°35′E	Atlantic	1990	ATs1r1(4)-ATs1r2(4)	3, 7
47	AT	Saar R.	8	49°00′N	07°00′E	Atlantic	1990	ATs1r1(6)-ATs1r2(2)	3, 7

APPENDIX. Continued.

No.	Lineage	Population	*N*	Coordinates		Basin	Sampling	Composite haplotypes (numbers)	Reference
				Latitude	Longitude				
48	AT	Vitava R.	8	49°20′N	14°10′E	Atlantic	1991	ATs1r3	3, 7
49	AT	Slupsk R.	4	54°40′N	17°00′E	Atlantic	1990	ATs1r2(2)-ATs1r1-ATs1r13	3, 7
50	AT	Swibno R.	2	54°45′N	18°50′E	Atlantic	1990	ATs1r1	3, 7
51	AT	Triasheno L.	14	69°00′N	36°40′E	Atlantic	1993	ATs1r2	2, 6
52	AT	Nil'ma R.	7	66°30′N	33°15′E	Barents Sea	1993	ATs1r1(6)-ATs1r2	2, 6
53	AT	Medja R.	8	56°15′N	32°40′E	White Sea	1993	ATs1r2(5)-ATs1r3(3)	2
54	AT	Viasheno L.	2	69°00′N	36°70′E	Baltic Sea	1994	ATs1r2	6
55	AT	Vorob'yev R.	3	66°25′N	33°20′E	Barents Sea	1994	ATs1r1	6
56	AT	Luven'ga R.	6	67°20′N	32°10′E	White Sea	1994	ATs1r2(4)-ATs1r1(2)	6
57	AT	Jegesslerg-drammer	7	60°10′N	11°20′E	White Sea	1992	ATs1r1	3, 7
58	AT	Dalälven R.	8	60°30′N	15°10′E	Atlantic	1992	ATs1r3(6)-ATs1r2(2)	3, 7
59	AT	Leksa R.	10	63°20′N	10°55′E	Atlantic	1994	ATs1r1(2)-ATs1r2(7)-ATs1r3	7
60	AT	Tavlaa R.	10	64°27′N	11°36′E	Atlantic	1994	ATs1r1(4)-ATs1r2(6)	7
61	AT	Jylland (Denmark)	30	56°10′N	09°00′E	Atlantic	—	ATs1r1	5
62	AT	Kallsjön	10	63°40′N	13°00′E	Atlantic	—	ATs1r2	5
63	AT	Fälpfjällt-jämarna	10	59°35′N	14°50′E	Atlantic	—	ATs1r2	5
64	AT	Blanktjärn	5	59°35′N	14°25′E	Atlantic	—	ATs1r1(2)-ATs1r3(3)	5
65	AT	Leba	18	58°00′N	19°00′E	Atlantic	—	ATs1r1(5)-ATs1r2(11)-ATs1r3(2)	5
66	AT	Crumlin	56	53°25′N	10°05′E	Atlantic	—	ATs1r2(3)-ATs1r1(6)-Ats8r14(17)	5
67	AT	Oued Berrem	4	32°40′N	04°47′W	Mediterranean Sea	1995	ATs5r12	7
68	AT	Bou Hamed	2	35°13′N	05°01′W	Mediterranean Sea	1995	ATs1r1	7
69	AT	Oued el Kanar	2	35°14′N	05°05′W	Mediterranean Sea	1995	ATs1r1	7
70	AT	Oued Ziz	5	32°25′N	04°45′W	Atlantic	1992	ATs6r4(2)-ATs7r5(3)	7
71	AT	Oued Oum er Rbia	6	33°05′N	05°12′W	Atlantic	1992	ATs6r4	7
72	AT	Arizakum R.	7	43°15′N	02°40′W	Atlantic	1992	ATs1r9	7
73	AT	Trubia R.	7	43°15′N	06°00′W	Atlantic	1992	ATs1r1(5)-ATs1r9(2)	7
74	AT	Elorn R.	8	48°27′N	04°16′W	Atlantic	1990	ATs1r2	3, 7
75	AT	Riabhaich	1	50°30′N	04°40′W	Atlantic	1994	ATs1r3	7
76	AT	Iceland (13 pop)	133	64°20′N	18°30′W	Atlantic	1994	ATs1r2	5
77	AT	Melvin L	140	54°30′N	08°10′W	Atlantic	1994	ATs1r2(108)-ATs1r10(3)-ATs4r2(16)-ATs1r6(9)-ATs1r1(4)	5
78	AT	Glynn R.	102	54°45′N	05°50′W	Atlantic	1994	ATs1r2(101)-ATs1r3	5
79	AT	Basque region	5	43°17′N	01°33′W	Atlantic	1996	ATs1r1(4)-ATs1r9	7
80	AT	Glenariff R.	151	55°05′N	06°05′W	Atlantic	1994	ATs1r3(107)-ATs1r2(39)-ATs1r1(3)-ATs1r13(2)	5
81	AT	Carnlough	54	55°00′N	06°00′W	Atlantic	—	ATs1r2(50)-ATs1r3(4)	5
82	AT	Glencloy	15	54°55′N	06°00′W	Atlantic	—	ATs1r2(9)-ATs1r3(6)	5
83	AT	Sutherland	32	58°30′N	04°50′W	Atlantic	—	ATs1r3	5
84	AT	Loch Ness	15	57°30′N	04°50′W	Atlantic	—	ATs1r1(14)-ATs1r2	5
85	AT	Erne	50	54°25′N	07°50′W	Atlantic	—	ATs1r2	5
86	AT-DA	Eulenback R.	6	48°20′N	11°00′E	Black Sea	1990	ATs1r1(4)-DAs2r2(2)	3, 7
87	DA	Cetina R.	4	43°42′N	16°44′E	Adriatic Sea	1996	DAs12r22	7
88	DA	Kokra R.	3	46°23′N	14°29′E	Black Sea	1996	DAs1r1	7
89	DA	Gurk R.	5	46°50′N	14°03′E	Black Sea	1991	DAs7r9	7
90	DA	Bohing L.	1	46°17′N	13°50′E	Black Sea	1991	DAs2r2	3
91	DA	Abant L.	2	40°38′N	31°16′E	Black Sea	1993	DAs1r17	7
92	DA	Coruh R.	2	40°30′N	41°30′E	Black Sea	1992	DAs7r9	7
93	DA	Fyrat R. (Euphrates)	3	39°35′N	38°40′E	Persian Gulf	1992	DAs5r5	7

APPENDIX. Continued.

No.	Lineage	Population	*N*	Coordinates		Basin	Sampling	Composite haplotypes (numbers)	Reference
				Latitude	Longitude				
94	DA	Sava R.	1	46°26′N	13°53′E	Black Sea	1993	1DAs11r18	7
95	DA	Lavant Br.	1	47°04′N	14°36′E	Black Sea	1993	DAs7r9	7
96	DA	Vellach Stream	6	46°35′N	14°35′E	Black Sea	1994	DAs1r1-Das7r9(2)-DAs1r18(3)	7
97	DA	Sava R.	6	46°28′N	13°46′E	Black Sea	1994	DAs12r19-DAs11r18-DAs12r22(3)-DAs13r23	7
98	DA	Radovna R.	2	46°23′N	14°20′E	Black Sea	1995	DAs11r18	7
99	DA	Vilsan R.	5	45°17′N	24°46′E	Black Sea	1995	DAs2r20	7
100	DA	Doamnei R.	4	45°21′N	24°49′E	Black Sea	1995	DAs1r18	7
101	DA	Topolog R.	3	45°28′N	24°30′E	Black Sea	1995	DAs2r21(2)-DAs1r1	7
102	DA	Gören R.	1	39°45′N	27°10′E	Aegean Sea	1993	DAs5r5	7
103	DA	Menderes R.	1	39°45′N	26°50′E	Aegean Sea	1993	DAs2r25	7
104	DA	Kolpa R.	1	45°30′N	15°00′E	Black Sea	1996	DAs1r24	7
105	DA	Zavrsnica R.	1	46°24′N	14°10′E	Black Sea	1996	DAs1r18	7
106	DA	Begunje R.	2	46°23′N	14°14′E	Black Sea	1996	DAs1r18	7
107	DA	Arpa R.	8	39°40′N	45°35′E	Caspian Sea	1982	DAs1r15	2
108	DA	Terek R.	10	43°45′N	44°28′E	Caspian Sea	1982–1991	DAs1r10(8)-DAs1r11-DAs1r12	2
109	DA	Apshak R.	14	53°30′N	58°30′E	Caspian Sea	1994	DAs1r15	6
110	DA	Kuterzi R. (Ural)	7	53°40′N	58°30′E	Caspian Sea	1994	DAs1r15	6
111	DA	Sevan L.	6	40°35′N	45°00′E	Caspian Sea	1979–1982	DAs1r6(5)-DAs6r6	2
112	DA	Kodori R.	9	43°10′N	41°30′E	Black Sea	1982	DAs5r5(3)-DAs7r5(3)-DAs1r16(3)	2
113	DA	Crimea (different tributaries)	5	45°30′N	34°20′E	Black Sea	1992	DAs7r9(4)-DAs1r16	2
114	DA	Sardamiana R.	7	39°08′N	68°15′E	Aral Sea	1987	DAs3r3-DAs8r3-DAs1r3(2)-DAs1r14(3)	2
115	DA	Sofidaron R.	6	38°50′N	69°55′E	Aral Sea	1987	DAs4r4-DAs9r7-DAs1r8-DAs9r13-DAs1r4(2)	2
116	DA	Neretva R.	2	43°40′N	18°00′E	Adriatic Sea	1991	DAs1r1	3, 7
117	DA	Drava R.	1	46°37′N	15°05′E	Black Sea	1997	DAs1r18	7
118	DA	Ludrana R.	2	46°25′N	14°55′E	Black Sea	1997	DAs7r9	7
119	DA	Bistra R.	2	46°23′N	14°50′E	Black Sea	1997	DAs12r22	7
120	DA	Topla R.	4	46°30′N	14°45′E	Black Sea	1997	DAs7r9-DAs12r22(3)	7
121	DA	Drava R.	1	46°37′N	15°05′E	Black Sea	1997	DAs1r18	7
122	DA	Ludrana R.	2	46°25′N	14°55′E	Black Sea	1997	DAs7r9	7
123	DA	Bistra R.	2	46°23′N	14°50′E	Black Sea	1997	DAs12r22	7
124	DA	Balik Golu L.	2	39°50′N	43°30′E	Caspian Sea	1997	DAs2r27	7
125	DA	Golbelen R.	3	41°05′N	43°05′E	Caspian Sea	1997	DAs1r26	7
126	DA	Firtina R.	4	41°00′N	41°00′E	Black Sea	1997	DAs5r5	7
127	DA	Balkli R.	1	39°55′N	40°00′E	Persian Gulf	1997	DAs7r9	7
128	DA	Goren R.	1	39°45′N	27°10′E	Aegian Sea	1997	DAs5r5	7
129	DA	Zeytinili R.	2	39°40′N	26°59′E	Aegian Sea	1997	DAs5r5	7
130	DA	Manastir R.	2	39°40′N	26°50′E	Aegian Sea	1997	DAs1r26	7
131	DA	Topkhana R.	9	36°11′N	70°57′E	Aral		DAs1r28-DAs1r4(8)	7
132	DA	Kapuzbasi R.	1	37°46′N	35°25′E	Mediterranean Sea	1998	DAs1r1	7
133	DA	Meza R.	2	46°27′N	14°43′E	Black Sea	1998	DAs1r1	7
133b	DA	Kyzylsu	3	39°40′N	73°00′E	Aral	1999	Das1r4	7
134	MA	Socha R.	4	46°20′N	13°40′E	Adriatic Sea	1994	MAs1r1	7
135	MA	Pellice R.	27	44°50′N	07°38′E	Adriatic Sea	1990–1991	MAs2r1	3, 4, 7
136	MA	Toce R.	18	46°10′N	08°10′E	Adriatic Sea	1990	MAs1r1(9)-MAs2r1(9)	3, 4, 7
137	MA	Hudda Grappa	3	46°10′N	14°00′E	Mediterranean Sea	1996	MAs1r1	7
138	MA	Zadlascica	5	46°10′N	13°50′E	Adriatic Sea	1996	MAs1r1	7
139	MA	Stura di Lanzo	19	45°06′N	07°44′E	Adriatic Sea	1991	MAs2r1	4

APPENDIX. Continued.

No.	Lineage	Population	*N*	Coordinates		Basin	Sampling	Composite haplotypes (numbers)	Reference
				Latitude	Longitude				
140	MA	Brenta R.	8	45°50′N	11°40′E	Adriatic Sea	1991	MAs2r1	4
141	MA	Gesso R.	16	44°24′N	07°33′E	Adriatic Sea	1991	MAs2r1	4
142	MA	Sarca R.	8	45°52′N	10°52′E	Adriatic Sea	1991	MAs2r1	4
143	MA	Stura di Demonte (Vinadio)	15	44°20′N	07°20′E	Adriatic Sea	1991	MAs2r1	4
144	MA	Acheloos	48	38°20′N	21°06′E	Ionian Sea	—	ADs1r1(5)-MAs1r1(43)	1
145	MA	Mornos	15	38°25′N	21°50′E	Ionian Sea	—	MAs1r1	1
146	MA	Idrija R.	1	46°00′N	13°55′E	Adriatic Sea	1998	MAs1r1	7
147	MA	Zala R.	2	45°55′N	14°05′E	Adriatic Sea	1998	MAs1r1	7
148	ME	Voidomatis R.	27	39°55′N	20°40′E	Adriatic Sea	1993	MEs2r1(25)-MEs3r1(2)	1, 7
149	ME	Rio Llobregat	2	42°15′N	01°57′E	Mediterranean Sea	1995	MEs1r1	7
150	ME	Réverotte R.	6	47°15′N	06°40′E	Mediterranean Sea	1986	MEs2r1	3, 7
151	ME	Tes R.	6	43°50′N	03°15′E	Mediterranean Sea	1990	MEs1r1(4)-MEs2r1(2)	3, 7
152	ME	Calore R.	1	40°46′N	15°00′E	Tyrrhenian Sea (Med.)	1996	MEs1r1	7
153	ME	Carença	5	42°26′N	02°13′E	Mediterranean Sea	1996	MEs1r1	7
154	ME	Aude	4	42°45′N	02°08′E	Mediterranean Sea	1996	MEs1r1	7
155	ME	Sorgue (Vaucluse)	3	43°55′N	05°10′E	Mediterranean Sea	1996	MEs1r1	7
156	ME	Haut Golo	4	42°18′N	08°53′E	Mediterranean Sea	1996	MEs1r1	7
157	ME	Fontanaccia R.	8	44°12′N	10°45′E	Adriatic Sea	1991	MEs1r1	4
158	ME	Tagliole R.	8	44°12′N	10°37′E	Adriatic Sea	1991	MEs1r1	4
159	ME	Rio Ara	2	42°30′N	00°10′W	Mediterranean Sea	1992	MEs1r1	7
160	ME-AD	Ripa R.	6	44°57′N	06°47′E	Adriatic Sea	1991	MEs2r1(5)-ADs1r1	4
161	ME-DA	Venetikos	22	40°03′N	21°33′E	Aegean Sea	—	MEs2r1(2)-DAs14r1(20)	1