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Human-aided dispersal has altered but not erased the phylogeography of the tench

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Abstract

Human-aided dispersal can result in phylogeographic patterns that do not reflect natural historical processes, particularly in species prone to intentional translocations by humans. Here, we use a multiple-gene sequencing approach to assess the effects of human-aided dispersal on phylogeography of the tench *Tinca tinca*, a widespread Eurasian freshwater fish with a long history in aquaculture. Spatial genetic analysis applied to sequence data from four unlinked loci and 67 geographic localities (38–382 gene copies per locus) defined two groups of populations that were little structured geographically but were significantly differentiated from each other, and it identified locations of major genetic breaks, which were concordant across genes and were driven by distributions of two phylogroups. This pattern most reasonably reflects isolation in two major glacial refugia and subsequent range expansions, with the Eastern and Western phylogroups remaining largely allopatric throughout the tench range. However, this phylogeographic variation was also present in all 17 cultured breeds studied, and some populations at the western edge of the native range contained the Eastern phylogroup. Thus, natural processes have played an important role in structuring tench populations, but human-aided dispersal has also contributed significantly, with the admixed genetic composition of cultured breeds most likely contributing to the introgression.

Introduction

Determining the effects of human-aided dispersal and how it overlays with natural distributional changes is essential for the effective protection of species throughout their native ranges. Translocations that occur within the limits of the natural distribution of a species do not extend its range but instead superimpose new genetic signatures on the natural diversity patterns if they involve genetically divergent populations or domestic breeds (Taylor 2004; Ferguson et al. 2007; Stone et al. 2007; Mabuchi et al. 2008; Randi 2008; Muhlfeld et al. 2009). The impacts of such translocations are therefore more difficult to detect. Molecular phylogeography offers here a powerful tool, which can also be used to resolve the 'cryptogenic' nature of species whose status in a given area may be either native or introduced but where clear evidence for either origin is absent (Carlton 1996).

The international trade and human-aided transport provides an effective dispersal mechanism in many aquatic organisms and freshwater fishes in particular. Up until now, phylogeographic studies of European freshwater fishes were largely focused on species that were not targets of aquaculture (e.g. Durand et al. 1999; Kotlík and Berrebi 2001; Šlechtová et al. 2004; Bohlen et al. 2007; Šedivá et al. 2008). Few economically important species have been studied phylogeographically across their ranges, but even in those cases, the focus has been primarily on putative native populations, assuming (or hoping for) negligible phylogeographic contribution of human-aided

dispersal (see Nesbø et al. 1999; Triantafyllidis et al. 2002; Van Houdt et al. 2005). As a result, phylogeographic information is still lacking for many common fishes, despite their role in freshwater communities and economic importance.

One such domesticated fish (Bilio 2007) with poorly known genetic structure (Lo Presti et al. 2010; Kohlmann et al. 2010) despite the ancient history in the European aquaculture and cuisine (Giovio 1524; Lebedev 1960; Steffens 1995; García-Berthou et al. 2007) is the tench *Tinca tinca* (Linnaeus, 1758). The tench is widely distributed between the British Isles and Iberian Peninsula in the west to central Siberia in the east (Fig. 1), but because it has been in cultivation in Europe for a long time (Šusta 1884; Steffens 1995), its exact native range is difficult to discern: in some areas (e.g. Spain: García-Berthou et al. 2007; Italy: Gherardi et al. 2008; Turchini and De Silva 2008), it may be either native or introduced but clear evidence for either origin is absent (i.e. it is cryptogenic there). There are records of tench introduction outside its native range from as early as the 18th century (e.g. to Ireland: Kennedy and Fitzmaurice 1970), and since then, introduced populations have been established on all continents except Antarctica (Welcomme 1988; Brylińska et al. 1999). In some countries, it is even considered as an invasive, potentially harmful species due to concerns over competition with native fish (e.g. Rowe 2004; Stokes et al. 2004; Hesthagen and Sandlund 2007; Rowe et al. 2008; DeVaney et al. 2009).

Distribution of genetic diversity of freshwater fishes is largely controlled by the island-like nature of their habitats (Bernatchez and Wilson 1998), and the present-day phylogeographic patterns of temperate species have been shaped primarily by isolation in multiple glacial refugia during the last glacial maximum (18 000–23 000 years ago), followed by range expansion and drainage isolation. Many widely distributed temperate freshwater fish species therefore show deep phylogeographic subdivisions (e.g. Durand et al. 1999; Bernatchez 2001; Kotlík and Berrebi 2001; Van Houdt et al. 2005; Kotlík et al. 2008; Hänfling et al. 2009). However, some species display only a limited or shallow phylogeographic structure, which is usually interpreted as the result of a recent dispersion from only one glacial refugium (Triantafyllidis et al. 2002; Bohlen et al. 2007). Alternatively, it can point to strong effects of human-aided translocations (Hänfling et al. 2009).

The present study uses a multiple-gene sequencing approach (Brito and Edwards 2008) and barrier-detection statistics to test whether the range-wide genetic variation of the tench shows a significant phylogeographic structure that can be explained by natural processes during the last glacial–interglacial cycle. Tench occupy all major freshwater regions in Europe, so that it should be possible to identify the contribution of different refugia (Fig. 1) to its present-day distribution. However, if human-aided dispersal significantly altered recent evolutionary history of the tench, the haplotypes could have been redistributed among populations, wiping out any natural phylogeographic structure (Sanz et al. 2006). Captive breeding can produce admixed gene pools, increasing the homogenizing effect of human-aided dispersal. To assess this effect of hatchery practices, in addition to putative native populations, we also sampled various cultured strains and known introduced populations outside the native range.

Materials and methods

Sampling

Sampled populations were chosen to cover the majority of the natural range of the tench in Europe and Asia. Fin

Figure 1 Putative native (olive) and part of non-native (violet) distribution range of the tench. Large areas where the origin is considered ambiguous are highlighted by orange. Locations of major freshwater glacial refugia in Europe, Western/Atlantic (R1), Danubian (R2), and Ponto-Caspian (R3) are indicated. Sampling countries are labeled (codes: B, Belgium; BG, Bulgaria; BIH, Bosnia and Herzegovina; CH, Switzerland; CZ, Czech Republic; D, Germany; EST, Estonia; GB, Great Britain; H, Hungary; I, Italy; P, Portugal; RO, Romania; S, Sweden; SK, Slovakia). References to the map: Urchinov 1995; Brylińska et al. 1999; Mitrofanov and Petr 1999; Savvaitova and Petr 1999, Economidis et al. 2000; Wang et al. 2004; Innal and Erk'akan 2006; Hesthagen and Sandlund 2007; Popov 2009; Mamilov et al. 2010.

tissue samples were stored in 95% ethanol. A total of 225 individuals were collected from 76 populations and included 25 hatchery stocks and several known introductions (Fig. 2; Appendix A). A single specimen (MNHN 0000–1357) from the collection of the Museum National d'Histoire Naturelle in Paris, France, was sampled. We also analyzed 16 Czech- and foreign-cultured tench breeds maintained in the live gene bank of the Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic (Gela et al. 1998, 2006; Flajšhans et al. 1999), and an Italian regional breed, the Golden hump tench of Poirino highland (Gasco et al. 2010).

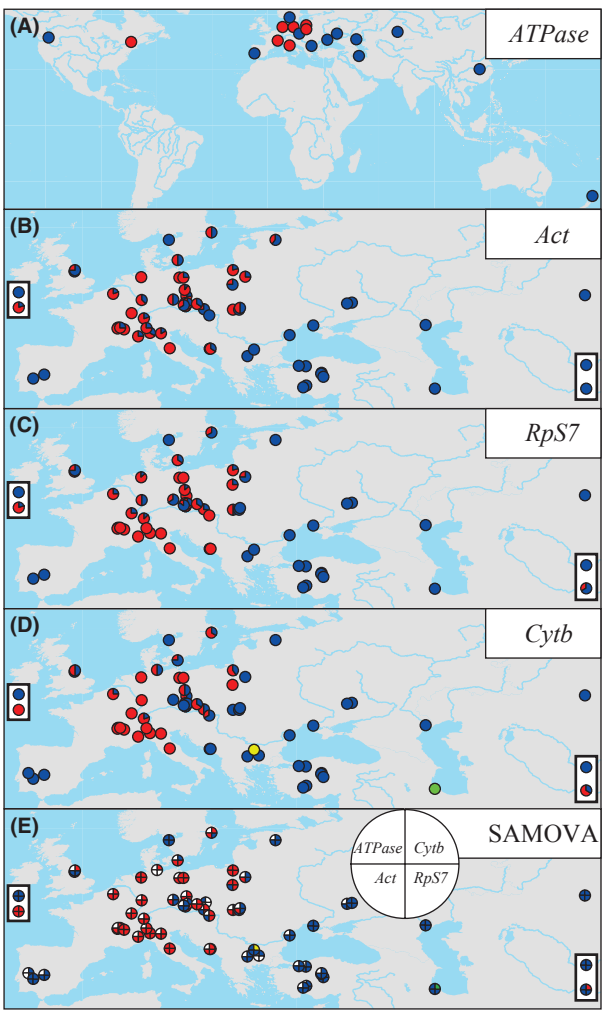

Figure 2 Geographic distribution of major clades and SAMOVA groups. Clade W is shown in red and clade E in blue for *ATPase* (A), *Act* (B), and *RpS7* (C). For *Cytb* (D), clade W is in red, clade EA in blue, clade EC in green, and clade EI in yellow. The same colors are used for the SAMOVA groups (E). Boxed data points to the right and left of the maps in (B) through (E) represent identities for two sites in North America and in China and New Zealand, respectively [see (A)]. For exact haplotype distribution and frequencies, see Appendix A.

Data collection

Introns of three nuclear genes and a complete sequence of one mitochondrial gene (Table 1) were analyzed by polymerase chain reaction (PCR) amplification from genomic DNA and direct sequencing. Total genomic DNA was extracted with QIAGEN (Valencia, CA, USA) DNeasy® Tissue kit. The PCR conditions followed standard methods (Tsigenopoulos and Berrebi 2000; Machordom and Doadrio 2001). The resulting PCR products were purified using the Millipore (Bedford, MA, USA) Montage PCR centrifugal filter devices and were directly sequenced with the ABI PRISM BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, MA, USA) and purified using DyeEx Spin kit (Qiagen). The extension products were run on ABI 3730 or 3730×l automated sequencers. Sequences were assembled using SEQMAN II (DnaStar Inc., Madison, WI) with the default options. All sequence traces were inspected visually to check the accuracy of the heterozygous base calls (Hare and Palumbi 1999). Nucleotide sequences of each unique haplotype were deposited in the GenBank database under the accession numbers HM167935–HM167965.

A part of nuclear DNA containing the second intron of the actin gene (*Act*) was amplified and sequenced using primers Act-2-R and Act-2-F described by Atarhouch et al. (2003). The intron of the gene coding for the ATP synthase beta subunit (*ATPase*) was amplified and sequenced using the primers described by Jarman et al. (2002). The first intron of the gene coding for the S7 ribosomal protein (*RpS7*) was amplified and sequenced using the primers S7RPEX1F and S7RPEX2R (Chow and Hazama 1998). Haplotypes were inferred from diploid sequence traces (Clark 1990; Won and Hey 2005) and verified by the use of fastPHASE (Scheet and Stephens 2006). The entire mitochondrial cytochrome *b* gene (*Cytb*) was amplified with the primers GluF and ThrR described by Machordom and Doadrio (2001) and sequenced with newly designed forward (5′-AAACAACCCAACAGGACT-3′) and reverse sequencing primers (5′-CAAATAGGAAATATCA TTCTG-3′).

Data analyses

Sequence analysis

For each locus, we estimated the haplotype and nucleotide diversities and their variances (Nei 1987). To explore whether intragenic recombination may have affected the patterns of variation at *Act*, *ATPase*, and *RpS7*, we used the four-gamete test (Hudson and Kaplan 1985). McDonald and Kreitman (1991) test was performed for *Cytb* to test for deviation from neutrality using an outgroup species and comparing different tench clades with each other.

Table 1. Summary of polymorphism for each gene and the results of demographic analyses.

Gene	Phylogeographical unit	*N*	Number of haplotypes	Polymorphic sites	Indels	Haplotype diversity ± SD	Nucleotide diversity ± SD (x 100)	Tajima's D	Fu's Fs	$P(SSD_{D/R})$
Cyt b	Clade E	140	12	33	0	0.228 ± 0.048	0.181 ± 0.058	$-1.940^{**/**/**}$	−1.455	0.217/0.383
(1141bp)	Clade EA	130	8	7	0	0.105 ± 0.037	0.009 ± 0.003	$-2.065^{***/***/***}$	$-13.791^{***/***/***}$	0.286/0.312
	Clade EI	5	1	0	0	0	0	–	–	–
	Clade EC	5	3	3	0	0.700 ± 0.218	0.105 ± 0.043	−1.048	−0.186	0.882/0.896
	Clade W	70	5	4	0	0.308 ± 0.070	0.029 ± 0.007	$-1.278^{*/-/-}$	$-2.988^{*/-/*}$	0.366/0.092
	Total	210	17	44	0	0.581 ± 0.029	0.687 ± 0.038	0.092	4.994	0.000/0.230
RpS7	Clade E	210	3	0	2	0.019 ± 0.013	0.002 ± 0.002	$-1.279^{-/*/*}$	$-5.178^{-/*/***}$	0.109/0.082
(868bp)	Clade W	172	5	4	1	0.666 ± 0.018	0.116 ± 0.007	0.266	0.891	0.053/0.005
	Total	382	8	15	5	0.637 ± 0.020	0.883 ± 0.013	$3.669^{+++/+/+++}$	$18.222^{++/+++/+}$	0.113/0.274
Act	Clade E	237	2	1	0	0.008 ± 0.008	0.003 ± 0.003	−0.934	$-2.952^{-/-/**}$	0.033/0.996
(289bp)	Clade W	193	2	1	0	0.010 ± 0.010	0.004 ± 0.004	−0.956	$-2.776^{-/-/*}$	0.050/0.991
	Total	430	4	6	0	0.501 ± 0.006	0.860 ± 0.009	$3.240^{++/++/++}$	$8.886^{+/++/+}$	0.000/0.008
ATPase	Clade E	26	1	0	0	0	0	–	–	–
(100bp)	Clade W	12	1	0	0	0	0	–	–	–
	Total	38	2	1	0	0.444 ± 0.058	0.444 ± 0.058	1.253	1.538	0.095/0.015

The size of DNA fragments is given below the gene names in base pairs. The superscripts indicate probability levels that values in the neutral population can be equal or lower than observed: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; equal or higher than observed: $^{+}P < 0.05$; $^{++}P < 0.01$ and '–' means nonsignificant result given by coalescent simulations based on number of segregating sites/the average number of nucleotide differences estimated by DNASP, version 4.50.3 (Rozas et al. 2003)/result given by ARLEQUIN version 3.11 (Excoffier et al. 2005), respectively. The value P(SSD) shows the probability of observing a less good fit between the model and observed distribution by chance under the demographic/spatial expansion scenario.

Tinca tinca is the only species in the family Tincidae, so that a sharpbelly species, *Hemiculter leucisculus*, from a related family Cultridae (Chen and Mayden 2009) has been used as the outgroup (GenBank Accession no. AF095608). All the calculations were performed using DNASP, version 4.50.3 (Rozas et al. 2003).

Phylogenetic and network analyses

Rooted phylogenies were reconstructed by the maximum-likelihood criterion (ML) using PhyML version 3.0.1 (Guindon and Gascuel 2003). We used Akaike information criterion and jModelTest version 0.1 (Posada 2008) to identify the HKY+G model as the most suitable model of DNA substitution for the *Cytb* data and the TrN model for the *RpS7* data. Sharpbelly *RpS7* sequence was not available, so that a sequence (AY325789) of the rosy bitterling, *Rhodeus ocellatus*, from another related family Acheilognathidae was used to root the *RpS7* tree. The robustness of the trees was assessed by the approximate likelihood ratio test (Anisimova and Gascuel 2006) and by bootstrap resampling (1000 replicates; Felsenstein 1985) using PhyML. A haplotype network was constructed for each gene by the statistical parsimony (Templeton et al. 1992) as implemented in TCS version 1.21 (Clement et al. 2000).

Inference of demographic history

To examine past population dynamics, we calculated two commonly used summary statistics *D* (Tajima 1989) and *Fs* (Fu 1997) with DnaSP and ARLEQUIN version 3.11 (Excoffier et al. 2005). Their significance was tested by generating random samples under constant population size using a coalescent simulation conditioned on the number of polymorphic sites (Ramírez-Soriano et al. 2008). For neutral markers, significant negative values can be expected in cases of population expansion (Tajima 1989; Fu 1997).

As another way of assessing signatures of refugial expansion, we considered the distribution of the number of pairwise nucleotide differences (mismatch distribution) by contrasting observed distributions with those expected from models of population size change. We tested whether the data fitted the sudden demographic expansion model (Rogers and Harpending 1992) or the instantaneous range expansion model (Excoffier 2004), using ARLEQUIN. The models were fitted to the data by a generalized nonlinear least-square approach, which allowed the estimation of the parameter $\tau = t/2\ \mu$, the expansion time scaled by the mutation rate (Schneider and Excoffier 1999). A parametric bootstrapping approach (Schneider and Excoffier 1999) was used to obtain the probability that the observed data conform to the model using the sum of square deviations (SSD) between the observed and expected mismatch distribution as a test statistic. We considered a wide range of estimated *Cytb* mutation rates for fishes of about 0.005–0.125 substitutions per site per Myr, published by

Dowling et al. (2002) and Burridge et al. (2008), respectively.

Spatial genetic analysis

Two complementary barrier-detection methods were applied to identify any discontinuities in the geographic distribution of genetic variation (Guillot et al. 2009). The geographic component of the phylogeographic pattern was first assessed by the spatial analysis of molecular variance using SAMOVA version 1.0 (Dupanloup et al. 2002). The advantage of SAMOVA is that it removes bias in population designation because it does not make *a priori* group distinction for genetic analyses. It employs a simulated annealing procedure using geographic locations of the sampling sites to cluster the sites into a user-defined number of groups (K), so that the proportion of total genetic variance between groups (F_{CT}) is maximized and the proportion of variation among sites within groups (F_{SC}) is minimized.

Major barriers to the distribution of genetic variation were then estimated by the Monmonier's (1973) maximum difference algorithm implemented in BARRIER version 2.2 (Manni et al. 2004), based on a matrix of the pairwise net genetic distances among sampling sites generated from DNA sequences using ARLEQUIN. The algorithm was applied to a network connecting the geographic coordinates of the sampling locations computed using Delaunay triangulation (Manni et al. 2004). Analyses were performed separately for each locus but on the same geographic network, and the results were then combined to identify barriers supported by multiple loci; the locus *ATPase* was excluded because of its limited geographic coverage.

Coalescent simulation

We conducted a series of simulation experiments to evaluate whether a natural population that was founded by unrelated clades at the end of the Younger Dryas, and has been isolated from other populations since then, may still carry haplotypes from different clades. This situation would correspond, for example, to tench populations inhabiting lakes in deglaciated areas of northern Europe (see Lajbner et al. 2010). In each experiment, we simulated 10 000 coalescent trees using Mesquite version 2.5 (Maddison 2008; Maddison and Maddison 2008) to estimate the distribution of the time to the most recent common ancestor (TMRCA) in such a population, and we counted the trees deeper than 3000 generations, approximately corresponding to the end of the Younger Dryas *c.* 11 500 years ago (Muscheler et al. 2008) and the generation time of 4 years (Monich 1953; Pekař 1965). We parameterized the simulations by female effective population size (N_{ef}) values corresponding to known population densities of tench (*c.* 100–500 individuals per hectare; Lusk et al. 1998) and a lake area between 10 and 400 hectares, and assuming an equal sex ratio (Monich 1953) and the ratio of the effective population size to the adult census size, N_e/N, of 0.3 (Turner et al. 2006). We focused on the female component of population, which is represented in our data by mtDNA variation, because of its relatively shallower coalescence time depth and therefore shorter expected TMRCA compared with autosomal loci. For values of N_{ef} yielding the number of deep trees that was <5% of all the trees simulated assuming that N_{ef}, we considered it unlikely that a population with that effective number of females would still contain haplotypes from different clades unless the haplotypes were recently redistributed among populations through human-mediated movement. On the other hand, a high number of deep trees (i.e. more than 95%) would indicate that there is no need to invoke recent gene flow as the likely explanation for the coexistence of divergent clades in such population, which could be the result of natural postglacial contact. Although these simulation experiments make simplifying assumptions that may not be realistic, they generate ideal benchmarks for interpreting the observed data.

Results

Sequence variation

The levels of polymorphism among sequences obtained for each of the four genes (38–430 gene copies per gene) are summarized in Table 1. There were five short (<5 bp) insertion/deletion (indel) polymorphisms segregating at the *RpS7* locus (Table 1) that were not associated with simple sequence repeats and could be unambiguously aligned. Of these, a two-base deletion was inferred to have occurred along the branch leading to clade W and a single-base deletion along the branch leading to clade E. Data sets from neither *Act*, *ATPase,* nor *RpS7* showed evidence of homoplasy and they all passed the four-gamete test, indicating that recombination has not affected the patterns of variation at the nuclear genes in our study. The McDonald–Kreitman test provided no evidence of selection on the coding sequence of the *Cytb* gene ($P > 0.05$).

Genealogical and geographic relationships

The phylogenetic and network analyses split the range-wide data set for the mitochondrial *Cytb* into two distinct phylogroups (clades W and E) separated with 1.6% of genetic distance (Fig. 3E,F), translating to a divergence time of about 64×10^3 to 1600×10^3 years ago. The Western phylogroup was found in Europe between the British Isles and Poland, whereas the Eastern phylogroup was present from Europe throughout Asia to China, with a

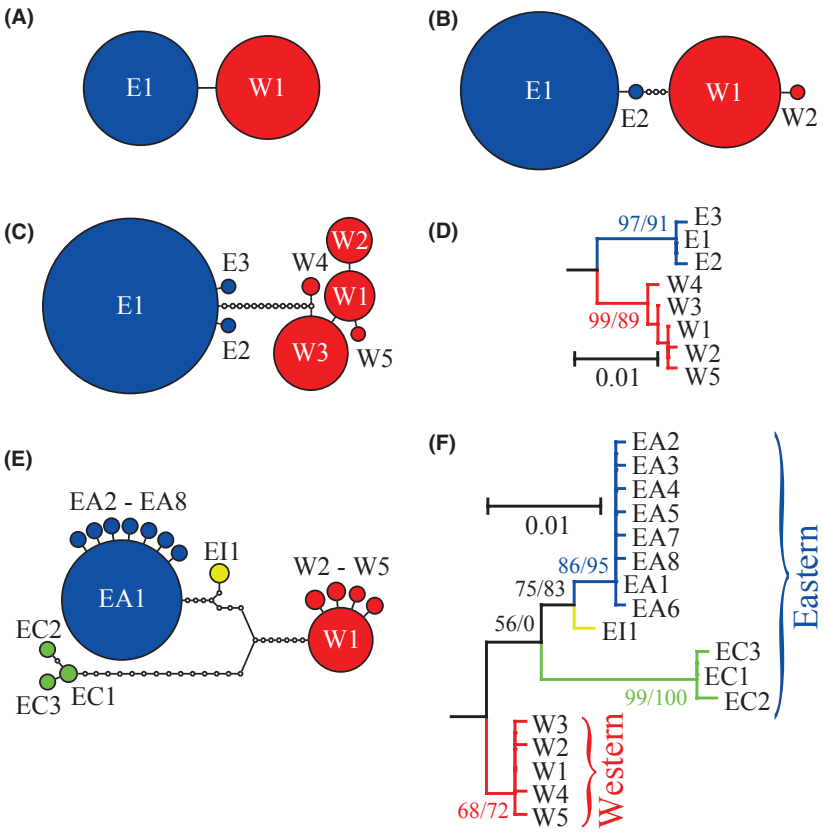

Figure 3 Haplotype relationships. Clade E is shown in blue and clade W in red for *ATPase* (A), *Act* (B), and *RpS7* (C, D). For *Cytb* (E, F), clade W is in red, clade EA in blue, clade EC in green, and clade EI in yellow. The networks were constructed under the 95% maximum parsimony criterion, and the size of the circles is proportional to the haplotype frequency; small empty circles represent unobserved haplotypes. The maximum-likelihood phylograms are shown with bootstrap (from 1000 replicates)/aLRT support for major partitions in the *RpS7* (D) and *Cytb* (F) phylogenies, with branch lengths proportional to the scale bar with the unit being a mean number of nucleotide changes per site.

broad zone of overlap with the Western phylogroup in Europe (Fig. 2D). While clade W showed very little internal structure, clade E was partitioned into three subclades (Fig. 3E,F). The majority of haplotypes were in the clade EA, while the other two clades had very restricted distributions: the EC haplotypes in the Anzalee lagoon of the Caspian Sea in Iran and the EI haplotype in the Iskar River of the Danube River drainage in Bulgaria (Fig. 2D).

We constructed a phylogenetic network for each nuclear DNA locus and a phylogenetic tree of the *RpS7* haplotypes (Fig. 3A–D). The most salient feature of the inferred genealogies is the complete lineage sorting of nuclear genes between the two phylogroups in that all genes are distinguished into two clades W and E, and the divergence between the phylogroups based on sequences of the nuclear *Act*, *ATPase,* and *RpS7* genes is geographically concordant with mitochondrial *Cytb* sequences (Fig. 2A–D). Nuclear DNA loci and mtDNA thus display striking similarities, showing a strong genealogical concordance across the distribution range of the tench. Changes in mtDNA and the three nuclear loci are concordant also across the contact zone between the two phylogroups, with only finer-scale differences being evident in phylogroup frequencies among sites (Fig. 2A–D).

The introduced populations in Turkey and China carried at all loci only clade E haplotypes, as did the overseas introduction to the state of Washington. However, the non-native populations in Bosnia and Herzegovina, in New Zealand, and in Quebec carried at one or more loci haplotypes from both clade W and clade E (Fig. 2A–D).

The phylogeographic variation observed among the tench populations was present also in the cultured breeds, with the exception of *Cytb* clades EC and EI that had very restricted geographic distributions. Each one of the 16 cultured breeds in the Vodňany live gene bank as well as the Italian regional breed carried haplotypes from both clades W and E at one or more loci, including the seven regional Czech breeds, three European breeds (German, Romanian, and Hungarian), three experimental breeds, and three ornamental breeds (Appendix A).

Population demographic history

The *D* and *Fs* statistics were negative for the major *Cytb* clades W and E as well as for clades EA and EC, reflecting the excess of rare mutations compared to the expectation under constant population size, and for clades W, E, and EA, this difference was significant (Table 1). A similar pattern was observed at the *Act* and *RpS7* genes, with a number of *D* and *Fs* values being large and negative, and with significant results for both *Act* clades and the *RpS7* clade E (Table 1).

For all four genes and clades W and E as well as for *Cytb* clades EA and EC, there was also a good fit [*P* (simulated SSD ≥ observed SSD) > 0.1] between the observed and the expected mismatch distribution from at least one expansion model (Table 1). The τ values obtained for *Cytb* clades W (0.373) and EA (3.000) translate into an expansion time of about 1308–31 134 years ago and 10 517–262 927 years ago, respectively.

Spatial genetic structure

The SAMOVA analyses identified a significant two-group spatial structure for each locus (Fig. 2E), with approximately 65% to 100% of the genetic variation proportioned between the two groups (*Cytb*: F_{CT}, 0.687, $P < 0.05$; F_{SC}, 0.606, $P < 0.001$; nuclear DNA loci: F_{CT}, 0.667–1.000, $P < 0.001$; F_{SC}, 0.000–0.080, $P < 0.001$). Assuming a four-group scenario for *Cytb* placed the Anzalee population (clade EC) and the Iskar population (clade EI) in their own separate groups (Fig. 2E), yielding higher F_{CT} (0.791, $P < 0.001$) and lower F_{SC} values (−0.095, $P < 0.001$) than those observed for this gene in the two-group scenario. Interestingly, one SAMOVA group was defined in the way that its distribution was clearly partitioned into distinct sets of sites, which belonged to that same group but which were not geographically adjacent (i.e. the British, one Swedish, and the Spanish and Portuguese sites were placed in the same group with sites from eastern Europe and Asia; Fig. 2E).

The BARRIER analysis overlaying five major barriers for each locus identified several discontinuities with a support from multiple loci (Fig. 4). The longest break divided the tench distribution into a western part and an eastern part and was fully supported by two loci and partially by all three loci (Fig. 4), depending on the local patterning of clades in the contact zone between the Western and Eastern phylogroups (Fig. 2B–D). Another barrier separated the Spanish and Portuguese sites from the rest of the sites with a complete support of all loci. The third barrier separated the British sites from the other sites with a support of two loci, and the fourth barrier separated the Swedish site Lake Öre sjö from the other sites in Sweden and around the Baltic Sea, with a complete support from two loci and a partial support of all loci (Fig. 4). Additional three short breaks supported by two loci were identified in central Europe (Fig. 4), following the transitions between phylogroups in that region (see Fig. 2E).

TMRCA distribution

The simulations of the TMRCA assuming N_{ef} of 730 produced fewer than 5% of coalescent trees that were deeper than 3000 generations. We therefore consider it unlikely that an isolated population with this effective number of females or smaller that was founded by unrelated mtDNA clades at the end of the Younger Dryas (assuming the generation time of 4 years) would still contain haplotypes from different clades, unless the haplotypes were recently redistributed among populations by human-mediated movement. However, for any N_{ef} larger than that, there was >5% chance that the TMRCA predated the origin of the population, and for N_{ef} larger than 4000, more than 95% of all coalescent trees were deeper than 3000 generations. The effective number of females of 4000 would translate to an adult census size of *c.* 25 000 individuals assuming an equal sex ratio and the ratio N_e/N of 0.3, which would correspond to a lake area of *c.* 250 hectares, assuming the population density of 100 individuals per hectare.

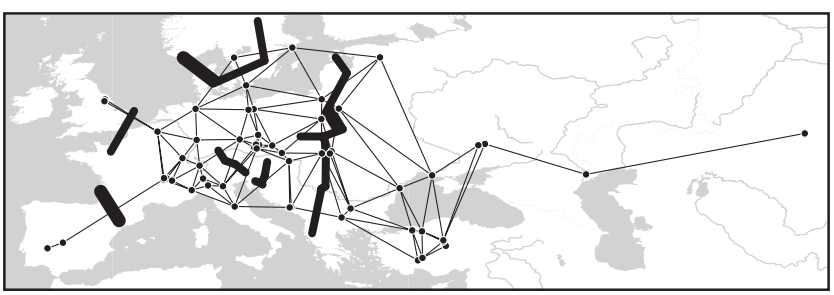

Figure 4 European phylogeographic breaks identified in tench data by BARRIER using the Monmonier's algorithm. Thin lines, Delaunay triangulations; thick lines, barriers supported by at least two loci. The thickness of the different barriers and their segments is proportional to the number of loci that supported them (two or three).

Discussion

Pleistocene phylogeographic subdivision

The statistical method in SAMOVA detected a significant phylogeographic pattern driven by the spatial orientation of the Western and Eastern phylogroups, with high congruence between mtDNA and nuclear DNA loci (Fig. 2E). The barrier-detection method in BARRIER revealed a well-supported genetic break crossing central Europe in a north–south direction (Fig. 4), paralleling the transition between the phylogroups (Fig. 2A–D). These results together provide evidence of a strong geographic component to the present phylogeographic pattern in the tench that is highly concordant among unlinked loci.

The distribution of highly divergent, reciprocally monophyletic phylogroups is strongly reminiscent of phylogeographic discontinuities modulated by refugial isolation (Taberlet et al. 1998; Hewitt 2000). It seems thus likely that, after the last glacial maximum, the Western phylogroup originated from the western European refugium, whereas the Eastern phylogroup originated from an eastern European or western Asian refugium. This conclusion is in accordance with previous phylogeographic studies indicating putative freshwater refugia in drainages of the Atlantic tributaries and of Rhone River (Durand et al. 1999; Nesbø et al. 1999; Kotlík and Berrebi 2001) and in the Black and Caspian Sea basins (Bănărescu 1991; Kotlík and Berrebi 2001; Kotlík et al. 2004, Kotlík et al. 2008). The importance of the Ponto-Caspian refugium is supported by the findings of tench fossils from glacial deposits in the Black Sea basin (Lebedev 1960). It is interesting that a distinct *Cytb* clade EC occurred in the southern Caspian Sea and only there, although the widespread clade EA occurred in the northern Caspian Sea, and all tench from both sites carried the same nuclear DNA haplotypes (Fig. 2A–D). Furthermore, clade EI occurred only at one site in the Iskar River basin in the lower Danube River drainage, where again only widespread nuclear DNA haplotypes were present (Fig. 2A–D). This shows hitherto undescribed complexities in the distribution of refugia within the Ponto-Caspian region and the Danube River, and lineage sorting and/or gene flow between them.

The signatures of population expansion in both phylogroups are consistent with a history of postglacial dispersion from formerly isolated refugia. The estimates of time from population expansion are approximately consistent with an expansion following the last glacial maximum. If, on the other hand, the significant tests reflected recent introductions, the time estimates should indicate much more recent expansion. The higher age of the expansion of the Eastern phylogroup than of the Western phylogroup is congruent with phylogeographic evidence from other fishes that the geographic range occupied by the Eastern phylogroup was much less directly affected by recent glacial advances than the western European drainages (Bernatchez 2001).

Fourteen sites in central and northern Europe were assigned to one SAMOVA group by some loci and to the other SAMOVA group by the other loci (Fig. 2E), and admixed sites carrying haplotypes of both phylogroups were observed over a large area between, roughly, Belgium and Estonia (Fig. 2A–D). Changes in mtDNA and the three nuclear loci are concordant across the contact zone, supporting that this is not a matter of primary contact and selection on some of the markers but rather of a secondary contact of populations from different refugia. But can this introgression be caused entirely by human-aided dispersal? Our TMRCA simulations indicated that there is no need to invoke recent gene flow as a likely explanation for the presence of both phylogroups even in relatively small populations. Furthermore, the location of the tench contact zone matches phylogeographic subdivisions in other species where expanding populations from different refugia meet in the same area (e.g. Taberlet et al. 1998; Hewitt 2000). We therefore consider it unlikely that the overlap between the phylogroups at the sites in central Europe has been entirely caused by human transport and release. Rather, it most likely represents a region of natural postglacial contact between lineages from the eastern and western refugia.

Evidence for human-aided dispersal

On the other hand, the contact zone is very broad and spans across several watershed divides, and there is fairly high amount of introgression in western Europe (Fig. 2B–D). The SAMOVA analysis even placed sites from three western European regions that contained particularly high proportions of the Eastern phylogroup into the same group with the sites from eastern Europe and Asia (Fig. 2E). These sites were located in Iberian Peninsula, in Britain and in Sweden, and they were separated from the other western sites with a BARRIER support of several loci (Fig. 4). All tench from the three sites in Spain and Portugal contained exclusively the Eastern phylogroup, which strongly speaks in favor of the hypothesis that tench are not a native species on the Iberian Peninsula (García-Berthou et al. 2007; Ribeiro et al. 2009), and points to the eastern Europe or Asia as their likely source. This demonstrates the ability of detailed phylogeographic studies such as ours to resolve the status of cryptogenic species where other evidence for either native or introduced origin is absent (Carlton 1996). The lack of phylogeographic resolution means, however, that we cannot confirm or reject the native status of the populations in Italy (Gherardi

et al. 2008; Turchini and De Silva 2008). The absence of strong genetic separation from more northern sites (Figs 2E and 4) suggests that tench colonization of Italy is most likely of postglacial origin.

Another site in western Europe that only contained Eastern alleles is Lake Öre sjö in southern Sweden. It may suggest that this population escaped admixture, but it may also be that the sample of only one fish (four loci) was not enough to detect the Western phylogroup if it was present in low frequency.

The British sites were separated from the other western sites by BARRIER, but they carried a mixture of the Eastern and Western phylogroups, which was reflected by their SAMOVA assignment to both groups, depending on the locus (Fig. 2E). This is probably a result of human introduction of the Eastern phylogroup to the British Isles as this phylogroup occurs in much lower frequency in western Europe. It could also be a natural colonization by both phylogroups but it would require almost complete replacement of the Eastern phylogroup in western Europe (see Searle et al. 2009).

Cultured breeds and introgression

The above evidence strongly suggests that human-aided dispersal has altered the phylogeographic structure of the tench. This implies either that tench from geographically remote populations were used for stocking, or that local source breeds carried the opposite phylogroup. Interestingly, we found that although the cultured breeds originating from different parts of Europe differed in the frequencies of the Western and Eastern phylogroups (Appendix A), all of them carried haplotypes of both phylogroups. Therefore, supplemental stocking with these or genetically related breeds would increase the probability of introgression between the phylogroups. Our recent study looked for evidence of a reproductive isolation in a postglacial lake inhabited by both phylogroups but we found no results that would point toward barriers to their interbreeding (Lajbner et al. 2010). Furthermore, at many sites within the contact zone, we observed individuals of apparently hybrid ancestry (see Fig. 2B–D). The putative hybrids were heterozygous for alternate phylogroups or were homozygous but for different phylogroups at different loci and/or carried mtDNA of the opposite phylogroup (data not shown). Finally, that both phylogroups characterized all of the examined breeds support that populations of mixed origin can persist without strong negative fitness consequences at least under cultured conditions. Therefore, the admixed genetic composition of the cultured breeds most likely contributed to the introgression between the phylogroups in natural habitats.

Phylogeography of known introductions

There is no record as to the geographic origin of tench in the Neretva River in Bosnia and Herzegovina, which is in the eastern Adriatic Sea basin where tench do not naturally occur (Glamuzina 2006). The presence of both phylogroups in the Neretva population shows that it is may have descended either from introductions from the adjacent Danube River drainage where both phylogroups occur (Fig. 2), or from genetically admixed hatchery stocks.

In Turkey, tench are probably native to some river drainages within the Black Sea basin (Brylińska et al. 1999) but it have been introduced to water systems of central and western Turkey (Korkmaz and Zencir 2005; Innal and Erk'akan 2006). The six putative non-native populations in Turkey (Appendix A) contained exclusively haplotypes of the Eastern phylogroup (Fig. 2B–D), which made them indistinguishable from the other sites in the eastern part of the range (Figs 2E and 4). This points to a local source of this introduction or to a distant source but within the range of the Eastern phylogroup.

The introduced population in China also carried only the Eastern phylogroup (Fig. 2A–D). Tench were introduced in large parts of China during the 20th century (Walker and Yang 1999; Huang et al. 2001), most probably from the Itrysh River drainage in northern China where tench naturally occur (Fig. 1). Interestingly, European cultured breeds originating from the live gene bank in Vodňany were recently imported to China to serve as a source for stocking into open waters throughout China (Wang et al. 2004). If those breeds carry both phylogroups, as did all breeds in that gene bank that we examined, this practice is likely to induce introgression of the European genes into the native populations of the tench in Asia.

The first introduction of tench from Europe to the United States occurred in 1877 (Baird 1879). By 1896, their descendants had been distributed to at least 36 states, and subsequent introductions to North America followed, including to Canada in 1986 (Quebec: Dumont et al. 2002). Both these introductions used tench from Germany (Baughman 1947; Fuller et al. 1999; Nico and Fuller 2010). Consistent with this, the population from Quebec contained both phylogroups and was placed in the same SAMOVA group with German and other western European sites (Fig. 2E). However, the Silver Lake population in the state of Washington contained only the Eastern phylogroup and it was grouped with the eastern sites by SAMOVA (Fig. 2E). This suggests that this population originated from yet another introduction to the United States that occurred in the state of Washington in

1909 (Wydoski and Whitney 2003) and which would have involved an unknown but most likely an eastern European or Asian source.

New Zealand tench were introduced several times in 19th century from Tasmania (Allport 1866; Abbott 1868; Arthur 1881; Thomson 1922; Hicks 2003), to where they had been successfully introduced from England in 1858 (Allport 1866, 1868). The North Island population contained both phylogroups (Fig. 2A–D) and it was placed in one SAMOVA group by one locus and to the other SAMOVA group by other loci (Fig. 2E). We were unable to acquire samples from Tasmania but these results suggest that England already had the Eastern phylogroup in 19th century, placing an upper limit on the time of its introduction to the British Isles.

Conclusions

The difficulty of disentangling the confounding effects of secondary dispersal from the impact of natural historical processes presents a persistent challenge for studies on the historical biogeography, particularly of species prone to intentional translocation by humans. Our study highlights that for such species, it may be useful to consider the effects of anthropogenic factors as juxtaposed with the natural phylogeographic structure rather than viewing these as mutually exclusive causes of the observed genetic and distribution patterns. We showed that natural historical processes have played an important role in genetically structuring the tench populations and that their signatures can still be detected across multiple genes. On the other hand, we demonstrated that human-aided dispersal significantly contributed to the recent evolutionary history of the tench and that the admixed genetic composition of cultured breeds most likely enhances introgression between genetically differentiated populations. It appears likely that if the current practices in open-water fisheries management continue, the human-aided migration will eventually erase the natural phylogeographic pattern for large parts of the tench range. It is also possible that, by increasing their adaptive variation, the hybridization would enhance the invasive potential of the admixed populations outside the native range, including into novel niches not occupied in the native range (Lucek et al. 2010). Within the native range, phylogroups descended from different refugia would likely show physiological adaptations to different selective environments. Stocking with individuals of the opposite phylogroup or the mixed ancestry may disrupt such adaptations, which can lead to reduction in fitness of wild populations (see Araki et al. 2008; Hutchings and Fraser 2008; Fraser et al. 2010; Marie et al. 2010; for numerous examples from salmonids). Such impacts might substantially reduce the evolutionary potential of wild populations and affect their chance of persistence (Stockwell et al. 2003; Frankham 2005).

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Appendix A

Origin of tench specimens with haplotype codes and frequencies.

Locality*	Basin	Water body	Country†	Coordinates		Haplotype codes (counts)				N	Year
				Latitude	Longitude	*CytB*	*Act*	*RpS7*	*ATPase*		
Open waters (nonfarm sites)											
Linkebeek	Scheldt/North Sea	Artificial pond	B	50.77	4.33	EA1(1), W1(3)	E1(2), W1(8)	E1(2), W2(1), W3(5)	–	5	2005
Osikovica	Danube/Black Sea	Iskar tributary	BG	42.94	24.00	EI1(5)	E1(10)	E1(10)	E1(2)	5	2005
Blagoevgrad	Struma/Aegean Sea	Struma	BG	42.02	23.09	EA1(1)	E1(2)	E1(1), E2(1)	–	1	2005
Karaotok Park Prirode	Neretva/Adriatic Sea	Canal Sunca ‡	BIH	43.05	17.80	W1(1)	E1(1), W1(1)	W3(1), W4(1)	–	1	2005
Stolac	Neretva/Adriatic Sea	Bregava ‡	BIH	43.08	17.96	EA1(3)	E1(2), W1(4)	W2(3), W3(1), W4(2)	–	3	2005
Noyan	Saint Lawrence River/ Atlantic Ocean	Richelieu River ‡	CDN	45.12	−73.26	W1(3)	E1(1), W1(5)	E1(1), W2(4), W3(1)	–	3	2005
Zurich	Rhine/North Sea	Zurich	CH	47.30	8.62	EA1(1), W1(4)	E1(2), W1(8)	E1(1), W2(6), W3(1)	–	5	2005
Lugano	Po/Adriatic Sea	Lugano	CH	45.98	8.97	W1(2)	E1(2), W1(6)	W2(2), W3(1), W5(1)	–	4	2006
Olomouc	Danube/Black Sea	Morava	CZ	49.61	17.25	–	–	–	E1(2)	1	1863
Kokořín	Elbe/North Sea	Pšovka	CZ	50.44	14.58	EA1(1), W1(1)	E1(1), W1(3)	W1(1), W2(1)	–	2	2003
Felchow	Oder/Baltic Sea	Grosser Felchowsee	D	53.06	14.13	W1(1)	E1(2), W1(6)	W1(3), W2(1)	W1(2)	5	1997
Haaven	Wesser/North Sea	Hunte	D	53.09	8.21	W1(3), W5(1)	W1(8)	E1(1), W1(3), W2(4)	W1(2)	4	2004
Hessen	Rhine/North Sea	Rhine	D	49.92	8.32	W3(4)	E1(3), W1(5)	E1(4), W2(2), W3(2)	–	4	2005
Plön	Schwentine/Baltic Sea	Vierer see	D	54.13	10.47	EA1(1), W1(1)	–	–	–	2	2007
Döllner Heide	Oder/Baltic Sea	Kleiner Döllnsee	D	52.98	13.57	EA1(2), W1(2)	E1(1), W1(7)	W3(6)	–	5	1996
Guadalupe	Guadiana/Atlantic Ocean	Guadalupejo	E	39.44	−5.31	EA1(1)	E1(2)	E1(2)	–	1	2006
Võnnu	Narva/Baltic Sea	Emajõgi	EST	58.83	27.00	EA1(5)	E1(6), W1(4)	E1(10)	–	5	2005
Priay	Rhône/Mediterranean Sea	Ain	F	46.00	5.27	W1(2)	E1(1), W1(3)	W1(1), W3(1)	–	2	2005
Belley	Rhône/Mediterranean Sea	Rhône	F	45.78	5.81	W1(2)	W1(4)	W1(4)	W1(2)	2	2005
Gérardmer	Rhine/North Sea	Gérardmer	F	48.07	6.87	W1(2)	W1(4)	E1(1), W3(3)	–	2	2005
Warbutts	Ouse/North Sea	Artificial pond	GB	54.05	−1.01	EA3(1), W1(1)	E1(3), W1(1)	E1(3), W1(1)	–	2	2005
Stillingfleet	Ouse/North Sea	Artificial pond	GB	53.86	−1.09	EA1(2)	E1(3), W1(1)	E1(2), W2(1), W4(1)	–	2	2005
Cascina Belgiardino	Po/Adriatic Sea	Adda (Cavo Roggione)	I	45.28	9.48	W1(3)	E1(2), W1(4)	W1(2), W2(1), W3(2), W4(1)	–	3	2005
Ghazian	Caspian Sea	Anzalee lagoon	IR	37.47	49.33	EC1(3), EC2(1), EC3(1)	E1(10)	E1(3), E3(1)	E1(2)	5	2005
Sadyrbay	Tengiz – Korgalzhyn	Korgalzhyn	KZ	50.59	70.29	EA1(3)	E1(6)	E1(6)	E1(2)	3	2005
Hamilton	Waikato/Tasman Sea	Hamilton Lake ‡	NZ	−37.80	175.28	EA1(1), W1(2)	E1(8)	E1(4), W3(2)	E1(2)	4	2003–2005
Lentiscais	Tejo/Atlantic Ocean	Tejo	P	39.73	−7.49	EA1(1)	–	–	–	1	2007
Sątopy-Samulewo	Pregel/Baltic Sea	Sajna	PL	54.08	21.06	EA6(1), EA7(1),W1(3)	E1(1), E2(1), W1(8)	E1(2), W1(2), W2(1), W3(5)	W1(2)	5	2006
Kurowo	Vistula/Baltic Sea	Narew	PL	53.12	22.80	EA1(2)	E1(1), W1(3)	E1(3), W1(1)	–	2	2005
Tulcea	Danube/Black Sea	Danube delta	RO	45.00	29.00	EA1(3), EA4(1)	E1(8)	E1(8)	–	4	2004
Astrakhan	Volga/Caspian Sea	Volga	RUS	46.41	48.00	EA1(4), EA8(1)	E1(10)	E1(10)	E1(2)	5	2006
Vabacken	Bäveå/North Sea	Öre sjö	S	58.31	12.13	EA1(1)	E1(2)	E1(2)	E1(2)	1	2007

Appendix Table (Continued)

Locality*	Basin	Water body	Country †	Coordinates		Haplotype codes (counts)				N	Year
				Latitude	Longitude	*CytB*	*Act*	*RpS7*	*ATPase*		
Stockholm	Mälaren/Baltic Sea	Mälaren	S	59.33	18.07	EA1(1), W1(2)	E1(3), W1(3)	E1(4), W1(1), W3(1)	–	3	2007
Börringe	Segeå/Baltic Sea	Havgårdssjön	S	55.49	13.36	EA1(3), W2(1)	E1(4), W1(4)	E1(2), W1(2), W2(1), W3(1)	–	4	2007
Moravský Svätý Ján	Danube/Black Sea	Dlhé lúky	SK	48.59	17.00	EA1(1), W1(1)	E1(3), W1(1)	E1(1), W2(1), W3(2)	–	2	2006
Buzica	Danube/Black Sea	Ida	SK	48.55	21.08	EA1(2)	W1(4)	E1(2), W3(2)	–	2	2006
Michalovce	Danube/Black Sea	Zemplínská Šírava	SK	48.76	22.07	EA1(1)	E1(1), W1(1)	E1(2)	–	1	2006
Gabčíkovo	Danube/Black Sea	Starý les	SK	47.77	17.73	EA1(2), W3(1)	E1(4)	W1(1), W3(3)	–	3	2004–2005
Oborín	Danube/Black Sea	Laborec	SK	48.54	21.90	EA1(2)	E1(4)	E1(4)	–	2	2006
Sapanca	Sakarya/Black Sea	Sapanca gölü ‡	TR	40.71	30.28	EA1(4), EA5(1)	E1(10)	E1(10)	–	5	2006
Örencik	Yenice Irmağı/Black Sea	Abant gölü ‡	TR	40.60	31.28	EA1(2)	E1(4)	E1(4)	–	2	2006
Gedikli	Göksu/Mediterraean Sea	Beyşehir gölü ‡	TR	37.91	31.33	EA1(3)	E1(6)	E1(6)	–	3	2006
Köprüköy	Kızıl Irmak/Black Sea	Köprüköy baraji ‡	TR	39.57	33.43	EA1(2)	E1(4)	E1(4)	–	2	2006
Kirikkale	Kızıl Irmak/Black Sea	Kapulukaya baraji ‡	TR	39.69	33.46	EA1(2)	E1(4)	E1(4)	–	2	2004
Toklumen	Kızıl Irmak/Black Sea	Hirfanlı baraji ‡	TR	39.13	33.71	EA1(2)	E1(4)	E1(4)	–	2	2005
Kırıntı	Aksu Çayi/Mediterraean Sea	Kovada gölü ‡	TR	37.65	30.87	EA1(3)	E1(6)	E1(6)	–	3	2006
Savincy	Donets/Azov Sea	Siverskyj Donets	UA	49.38	37.02	EA1(4)	E1(8)	E1(8)	–	4	2006
Gola Pristan	Dnipro/Black Sea	Dnipro delta	UA	46.31	32.31	EA1(4)	E1(8)	E1(8)	E1(4)	4	2006
Senkove	Donets/Azov Sea	Krasnyj Oskol	UA	49.51	37.69	EA1(2)	E1(4)	E1(4)	E1(2)	2	2006
Medical Lake	Columbia River/Pacific Ocean	Silver lake ‡	USA	47.54	−117.65	EA1(5)	E1(10)	E1(10)	E1(2)	5	2005
Fish farms											
Plovdiv	Maritsa/Aegean Sea	Fish pond	BG	42.15	24.72	EA1(2)	–	–	–	2	2007
Vegas del Guadiana	Guadiana/Atlantic Ocean	Fish pond	E	38.89	−6.88	EA1(5)	E1(10)	E1(10)	E1(2)	5	2006
Mionnay	Rhône/Mediterranean Sea	Fish pond	F	45.90	4.92	W1(2)	W1(4)	W1(1), W2(1), W3(2)	–	2	2005
Bouligneux	Rhône/Mediterranean Sea	Fish pond	F	46.02	4.99	W1(1), W2(1)	E1(2), W1(2)	W3(2)	–	2	2005
Perugia	Tiber/Tyrrhenian Sea	Trasimeno Lake §	I	43.15 §	12.10 §	W1(2)	W1(4)	W3(4)	W1(2)	2	2005
Mincio, Bonferraro di Sorga	Po/Adriatic Sea	Garda Lake §	I	45.55 §	10.70 §	W1(3)	E1(1), W1(4), W2(1)	W1(2), W2(2), W3(2)	–	3	2005
Żabieniec	Vistula/Baltic Sea	Fish pond	PL	52.05	21.03	W1(1), W5(1)	E1(3), W1(1)	E1(1), W3(3)	W1(2)	2	2005
Wuhan	Yangtze River/East China Sea	Fish pond	PRC	30.56	114.37	EA1(3), EA2(1)	E1(8)	E1(2)	E1(2)	4	2004
Italian regional breed											
Ceresole d'Alba	Po/Adriatic Sea	Fish pond	I	44.80	7.82	W1(2)	E1(1), W1(3)	W1(3), W3(1)	–	2	2005

Appendix Table (Continued)

Locality*	Basin	Water body	Country †	Coordinates		Haplotype codes (counts)				N	Year
				Latitude	Longitude	*CytB*	*Act*	*RpS7*	*ATPase*		
Live gene bank in Vodňany											
Regional breeds											
Hluboká, new stock	Elbe/North Sea	Fish pond	CZ	49.05	14.43	EA1(2), W1(1)	E1(1), W1(5)	E1(1), W1(2), W3(3)	–	3	2004
Hluboká, old stock	Elbe/North Sea	Fish pond	CZ	49.05	14.43	EA1(3)	E1(2), W1(4)	W2(2), W3(4)	–	3	2004
Mariánské Lázně	Elbe/North Sea	Fish pond	CZ	49.97	12.70	EA1(3)	E1(3), W1(3)	E1(4), W3(2)	–	3	2005
Tábor (Milevsko), new stock	Elbe/North Sea	Fish pond	CZ	49.45	14.36	EA1(2), W1(2)	E1(2), W1(4)	E1(2), W3(2)	–	4	2004
Tábor, old stock	Elbe/North Sea	Fish pond	CZ	49.40	14.69	EA1(3)	E1(2), W1(4)	E1(2), W1(1), W3(3)	–	3	2004
Velké Meziříčí	Elbe/North Sea	Fish pond	CZ	49.35	16.02	EA1(1), W1(1), W4(1)	E1(2), W1(4)	E1(2), W1(4)	–	3	2004
Vodňany	Elbe/North Sea	Fish pond	CZ	49.15	14.18	EA1(3)	E1(4), W1(2)	E1(5), W2(1)	–	3	2004
European breeds											
Königswartha (Germany)	Elbe/North Sea	Fish pond	D	51.31	14.33	EA1(1), W1(1)	E1(1), W1(9)	E1(1), W1(5), W2(2)	–	5	2004
Romania	Danube/Black Sea	Fish pond	RO			EA1(1), B1(3)	E1(5), W1(3)	E1(2), W1(1), W3(3)	–	4	2004
Hungaria	Danube/Black Sea	Fish pond	H			EA1(5)	E1(2), W1(8)	E1(1), W3(3)	–	5	2004
Experimental breeds											
Leather '92		Fish pond	CZ			W2(3)	E1(1), W1(5)	E1(6)	–	3	2004
Synthetic		Fish pond	CZ			EA1(3)	E1(3), W1(3)	E1(2), W2(2), W3(2)	–	3	2004
Gynogenetic		Fish pond	CZ			EA1(3)	W1(6)	E1(4), W3(1), W4(1)	–	3	2004
Ornamental breeds											
Golden		Fish pond	CZ			EA1(2)	E1(2), W1(2)	E1(1), W1(3)	–	2	2004
Blue		Fish pond	CZ			EA1(2)	E1(1), W1(3)	E1(1), W1(1), W3(2)	–	2	2004
Alampic		Fish pond	CZ			EA1(2)	E1(1), W1(3)	E1(1), W1(2), W3(1)	–	2	2004

*For the geographic breeds in the live gene bank, locality identifies the original source of the breed, while for the experimental and ornamental breeds, only the breed name is given.

†The countries are coded as follows: Belgium (B), Bulgaria (BG), Bosnia (BIH), Canada (CDN), Switzerland (CH), Czech Republic (CZ), Germany (D), Spain (E), Estonia (EST), France (F), Great Britain (GB), Hungary (H), Italy (I), Iran (IR), Kazakhstan (KZ), New Zealand (NZ), Portugal (P), Poland (PL), China (PRC), Romania (RO), Russia (RUS), Sweden (S), Slovakia (SK), Turkey (TR), Ukraine (UA), United States of America (USA).

‡Known introduced population.

§Information about the source population.

