Genetic Identity, Phylogeography, and Systematics of Ruffe Gymnocephalus in the North American Great Lakes and Eurasia

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ABSTRACT. The objectives of this study were to determine possible source regions for the newly invasive ruffe Gymnocephalus cernuus (Percidae: Teleostei), and to compare its level of genetic variability and divergence with Eurasian populations and congeners. Sequencing the entire mitochondrial DNA control region (1,024 base pairs) showed that G. baloni and G. schraeber were distinguished by six and seven fixed sequence substitutions, respectively, and were estimated to have diverged from G. cernuus by about 1.2 million years. Five Eurasian haplotypes of G. cernuus were identified, each distinguished by fixed sequence substitutions, and only one intrapopulational polymorphism was discerned. The five haplotypes of G. cernuus were divided into two primary groups—a western (Danube River and the introduced Bassenthwaite Lake, England populations) and an eastern (Baltic Sea area and Ob' River, Siberia populations) group—diverged by seven fixed sequence differences and an estimated 450,000 years. The two groups were probably isolated in different glacial refugia during the mid-Pleistocene Epoch. The population from the Danube River appeared genetically identical to the North American exotic population. The exotic population from Bassenthwaite Lake was distinguished from the Danube River/North American type by a fixed base substitution. Within the eastern group, the Baltic Sea area samples were separable from the Siberia population by a fixed substitution, repeated 17 times. Lack of genetic variability in most Eurasian populations of ruffe may be due to rapid expansion and founder events during post-Pleistocene recolonization.

INDEX WORDS: Ruffe, Gymnocephalus, Percidae, genetics, founder effect, Eurasia, Great Lakes, exotic species, source regions.

INTRODUCTION

The Eurasian ruffe Gymnocephalus cernuus (Teleostei: Percidae; Fig. 1A) was introduced to the St. Louis Harbor region of Lake Superior in the North American Great Lakes (Fig. 2) during the mid-1980s (Simon and Vondruska 1991, Pratt et al. 1992). The likely vector was ballast water discharged from a vessel arriving from a Eurasian port (Simon and Vondruska 1991). The ruffe is found in a wide variety of Eurasian fresh and brackish water habitats, variable depths, temperatures, and water flow rates (Ogle 1995). Its primary Eurasian range encompasses northeastern France, England, the rivers entering the Baltic and White seas, most of Siberia, and the Black Sea region (Ogle 1995). Its most recent Eurasian introductions include Loch Lomond, Scotland (Maitland and East 1989) and Bassenthwaite Lake, England (Winfield et al. 1996). The Lake Superior population is now well established and has been expanding eastward along Lake Superior’s southern shore (Ogle 1995). The ruffe was apparently transported by intra-lake shipping to Thunder Bay Harbour, Canada, 300 km northeast of the St. Louis River (Pratt et al. 1992). It also was apparently introduced to Lake Huron at Thunder Bay River, Michigan, by the summer of 1995 (Busiahn 1997; see Fig. 2). The potential North American range of ruffe is predicted to extend from the Great Plains to the eastern seaboard and north into Canada (Busiahn 1997). Its habitat types, temperature tolerance, food, and trophic rela-

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relationships appear similar to those of the North American yellow perch *Perca flavescens*, to which it poses a potential competitive threat (Ogle et al. 1995).

The ruffe is morphologically variable throughout its native range and questions have arisen as to whether morphological variants constitute separable species (Holčík and Hensel 1974). Genetic data offer an independent test and a means to quantify degree of variation within and among populations and species (Avise 1994). Identifying the Eurasian source of the North American introduction is important for making ecological comparisons between native and introduced populations. If the ruffe appears at sites downstream of Lake Superior, as in the case of Lake Huron (Busiahn 1997), it will be important to know whether populations arise from dispersal within the Great Lakes or from new introductions from Eurasia. Genetic characterization of exotic populations in Europe (i.e., Loch Lomond, Scotland and Bassenthwaite Lake, England) and North America may help to determine whether a specific variant type is more likely to be ecologically successful and may aid in developing strategies for control.

The genus *Gymnocephalus* contains three additional species: *G. baloni* (Fig. 1B), endemic to fast-flowing areas of the Danube and Dnieper Rivers; *G. schraetser* (Fig. 1C), endemic to deeper waters of the Danube basin and estuaries leading into the Black Sea; and *G. acerina*, found in drainage basins of rivers emptying into the northern Black Sea (Holčík and Hensel 1974, Shevtsova et al. 1986). Relationships among the species were hypothesized by Holčík and Hensel (1974) based on meristic and morphometric characters, but remain unresolved, and were further tested in this study. It was not possible to obtain *G. acerina* for our genetic analyses. The present investigation examined DNA sequences and morphological characters of *G. cernuus*, *G. baloni*, and *G. schraetser* in order to gauge species-level separations in comparison with intraspecific variation in *G. cernuus*.

This study examined sequence substitutions in the highly variable mitochondrial (mt) DNA control region (or D-loop), which has been shown to have appropriate variability to address population genetic questions in the walleye *Stizostedion vitreum*—another percid (Stepien 1995, Faber and Stepien 1997)—and in other fishes (e.g., Meyer et al. 1990, Arnason and Rand 1992, Sturmbauer and Meyer 1992, Brown et al. 1996, Nielsen et al. 1997). Variation in the control region among fish populations thus has been relatively well studied (summarized in Stepien and Kocher 1997) and a DNA sequence data set is readily augmentable, allowing direct comparisons with studies by other investigators (Avise 1994, Stepien 1995, Hillis et al. 1996). The vertebrate control region ranges from 888 to 1,235 base pairs (bp) in percids (Faber and Stepien 1997) and is involved in regulating mtDNA replication and RNA transcription (Palumbi 1996). The control region also is called the displacement loop (D-loop) because during replication, one of the two strands of the helix is displaced by the synthesis of a third strand (Palumbi 1996). A central conserved section of the control region separates two variable flanking segments; the left and right domains (Palumbi
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FIG. 2. Present distribution of the ruffe and sample sites in the Great Lakes. 1 = St. Louis River, Minnesota/ Lake Superior Harbor, Wisconsin area (point of North American origin), 2 = Amnicon River, Wisconsin, 3 = Lake Huron at Alpena, Michigan.

that were divergent at the population level (and above) in percids (Stepien 1995, Faber and Stepien 1997). The proline tRNA end of the left domain contained tandemly repeated sequences at the R1 site (near the stop site for the d-loop, adjacent to the proline tRNA gene; Fumagalli et al. 1996) in percids (Stepien 1995, Faber and Stepien 1997). The number of copies of tandem repeats varied among individual walleye S. vitreum, and did not vary intraspecifically in other percids examined (Stepien 1995, Faber and Stepien 1997). Tandemly repeated sequences at the R1 site of the mtDNA control region have been identified in some other groups of fishes, including the American shad (Bentzen et al. 1988), the Atlantic cod (Arnason and Rand 1992), and species of sturgeon (Brown et al. 1996). The central conserved section and the two conserved sequence blocks located downstream (towards phenylalanine tRNA) of the right domain facilitated sequence alignment of the control region among fishes (Stepien 1995, Lee et al. 1995, Faber and Stepien 1997).

The purpose of this investigation was to compare mtDNA control region sequences of ruffe from the Great Lakes with Eurasian populations in order to assess relative levels of intraspecific variability and to test for distinguishing markers among areas of its present distribution. Specific objectives of the study were to: (1) characterize the genetic structure of the founding population of ruffe in the Great Lakes and establish groundwork for continued monitoring, (2) provide baseline data for genetic identification of potential future colonization sites, whether due to spread or to additional, independent introductions, and (3) determine whether samples from Eurasian and North American sources are genetically similar or different, in order to evaluate potential founding sources.

METHODS

Ruffe (Gymnocephalus cernuus) collections for mtDNA sequencing and morphological measurements included three sites off Lake Superior, representing the initial North American introduction.
(Fig. 2): the St. Louis River, Minnesota 46° 57' N, 92° 58' W (N = 4), Superior Harbor, Wisconsin 46°, 44' N, 92° 06' W (N = 15), and the Amicon River, Wisconsin 46° 35' N, 91° 56' W (N = 2). Individuals also were examined from a new population area in Thunder Bay River, a tributary of Lake Huron at Alpena, Michigan 45° 05' N, 83° 30' W (N = 6; Fig. 2); Danube River, Slovakia 47° 48' N, 17° 35' E (N = 12); Bassenthewaite Lake, United Kingdom 54° 25' N, 2° 59' W (N = 11); St. Petersburg, Russia 59° 57' N, 30° 20' E (N = 13), including the Neva River Embankment (N = 3) and Komsomolskoe Lake (N = 10); and the Ob' River at Novosibirsk, Siberia, Russia 54.5° N, 82.5° E (N = 12; Fig. 3). Samples included one specimen of *G. baloni* and two specimens of *G. schraetser* from the Danube River site at Gabicikovo, Slovakia (Fig. 3). All specimens were either stored frozen at -80°C or at room temperature in 95% ethanol.

Meristic and morphometric data were taken with a metric caliper and a stereo microscope in order to confirm species identification and to test for variation in morphological characters among genetic types. Measurements followed Holčík and Hensel (1974) and included: numbers of dorsal and anal fin rays and spines; numbers of opercular and preopercular spines; lengths of dorsal and anal fins; and standard (SL), total (TL), head (HL), and caudal peduncle (CP) lengths (see Fig. 1). All individuals were examined for the anal fin cleft, supraorbital bulge, and shape of the dorsal margin of the dorsal fin that distinguish *G. baloni* from *G. cernus* (Fig. 1; Holčík and Hensel 1974). Whole specimens were sexed by microscopic examination of gonadal tissues. Student’s t-tests and one-way ANOVA tests (Sokal and Rohlff 1981) in Microsoft Excel (vers. 4.0) tested for differences in morphological characters among North American and Eurasian populations, and between North American males and females.

Samples of approximately 500 mg muscle tissue were ground in liquid nitrogen using a cylindrical stainless steel mortar and pestle (Stepien 1995). Samples were immediately placed in guanidine thiocyanate buffer to circumvent degradation and incubated overnight at 55°C in a shaking water bath. Genomic DNA was precipitated from the buffer solution using 95% ethanol. The DNA pre-
The 1,024 bp mtDNA control region (D-loop; Fig. 4) was amplified in two overlapping sections from extracted DNA using the polymerase chain reaction (PCR) with conserved primers (Mullis and Faloona 1987) adapted from other fish studies (Kocher et al. 1989, Meyer et al. 1990, Stepień 1995, Faber and Stepień 1997). The left domain of the control region, extending from the tRNA-proline gene to the central conserved section (Fig. 4), was amplified using the oligonucleotide primers L15926 (5'-TCA-AAG-CTT-ACA-CCA-GTC-TTG-TAA-ACC-3') for the light chain (Kocher et al. 1989) and H16498 (5'-CCT-GAA-GTA-GGA-ACC-AGA-TG-3') for the heavy chain (Meyer et al. 1990; Fig. 4). The right domain, which continues from the central conserved section to the bordering tRNA-phenylalanyl-gene, was amplified with the light chain complement of H16498, L16498 (5'-CAT-CTG-GTT-CCT-CTT-AG-3'; Faber and Stepień 1997) and the heavy chain primer 125a-H1067 (5'-AAA-CTC-GGA-CCA-GAT-ACC-CCA-CTA-T-3'; Palumbi 1996). The entire control region also was amplified using primers L15926 and 125a-H1067. The central conserved section then was sequenced with an additional set of primers designed from reference sequences of percids (Faber and Stepień 1997); light chain CCRL (5'-AAT-GTA-GTA-AGA-GCC-TA-3') and heavy chain CCRH (5'-GGG-TAA-CGA-GGA-GTA-TG-3').

The amplification program for the left domain of the control region was 34 cycles of 92°C for 40 s, 52°C for 20 s, and 72°C for 60 s. The program for the right domain was 38 cycles of 94°C for 45 s, 52°C for 45 s, and 72°C for 60 s. The program for amplifying the entire control region was 38 cycles for 94°C for 45 s, 50°C for 50 s, and 72°C for 2 min. All programs ended with a final extension step of 72°C for 5 min to ensure that chains were fully polymerized.

Primers for the heavy chain were end-labeled with biotin and the double-stranded PCR products were separated using Dynal streptavidin magnetic beads (Dynal, No. 112.05, Oslo, Norway). Single stranded DNA templates were sequenced using the Sanger dideoxy-chain termination method with Amersham Sequenase II PCR Product Sequencing Kits (#70170, Cleveland, OH.) and 35S labeled dATP. Both strands were sequenced in most cases for verification. Sequencing reactions were run on 6% polycrylamide gels with a 1X glycerol tolerant gel buffer (Amersham Sequenase PCR Product Sequencing Kit Protocols, 1st ed.) at 50°C and 60 W on Owl Model S2S sequencing rigs (Owl Scientific Plastics, Inc., Cambridge, MA). Gels were run for 2, 5, and 8 h time periods in order to resolve sequences at various distances from the primer. Gels then were fixed in a 5% acetic acid/15% methanol solution and transferred to chromatography paper (Whatman International Ltd., Maidstone, England). Gels were vacuum dried at 80°C for 2 h and autoradiographed using Kodak X-Omat AR film for 72 h or longer.

Sequences were entered using a IBI/Kodak digitizer into a Macintosh computer and aligned to other percids following Faber and Stepień (1997). The central conserved section and other structural elements such as the termination associated sequence (TAS) and conserved sequence blocks (CSB) were located by comparing the ruffe data.

FIG. 4. A schematic diagram of the mtDNA control region of Gymnocephalus. Regions are marked that show homology with percid and other fish sequences, adapted from Faber and Stepień (1997). Lines indicate polymorphic characters among haplotypes of the three species, G. cernuus, G. baloni, and G. schraetser. Asterisks denote intraspecific polymorphisms within G. cernuus surveyed (see Table 1 for corresponding substitutions).
with mtDNA control region sequences from other percids (Faber and Stepien 1997) and teleosts (Stepien 1995, Lee et al. 1995).

Genotypes were compared using pairwise \((p)\) distances, which are the number of nucleotide differences between each pair of sequences divided by the total number of nucleotides, using the computer package program MEGA (Molecular Evolutionary Genetics Analysis Vers. 1.01; Kumar et al. 1993). Standard errors of \(p\)-distance values were calculated as the square-root of \(V(p) = p(1 - p) / n\) (Nei et al. 1985, Kumar et al. 1993). Genetic distances also were used to estimate possible divergence times separating haplotypes (haploid genotypes), using a mean rate of sequence divergence of two percent per million years (reviewed by Avise 1994). This rate of sequence divergence has been applied to mtDNA restriction enzyme data for other percids (Billington et al. 1990) and to control region sequences of \(S. \text{vitreum}\) (Faber and Stepien 1997), at best providing a rough estimate.

The neighbor joining (NJ) clustering method (Saitou and Nei 1987) was used to construct an evolutionary distance tree based on the \(p\)-distances in the program MEGA (Kumar et al. 1993). Neighbor joining began with a star-like tree of equal relationships and each pair of most closely related taxa were progressively collapsed together as neighbors (Saitou and Nei 1987). “Neighbors” were pairs of taxa that were combined to yield the smallest sum of branch lengths (Kumar et al. 1993). Each pair of taxa then was considered as a single group, and the next pair that produced the smallest sum of the branch lengths was grouped together (Saitou and Nei 1987).

The genetic similarity analysis presented here included mtDNA control region sequences from two other percids, walleye (\(S. \text{vitreum}\); Stepien 1995, Faber and Stepien 1997) and yellow perch (\(P. \text{flavescens}\); Faber and Stepien 1997), that were used for comparison and as outgroup taxa to root the phylogenetic trees. Bootstrapping was used to test how well the data set supported the interior branches (nodes) of the NJ tree (Kumar et al. 1993). In this analysis, the data set was randomly sampled with replacement from the original sequence data, and the reshuffled data were used to produce a new NJ tree, that was compared to the topology of the original NJ tree. Interior branches of the original NJ tree that gave the same division of sequences as that of the bootstrap tree were given an identity value of 1 and those that did not match received a 0 non-identity value. This entire process was repeated 500 times, and the percentage of times that there was a match between the bootstrap trees and the NJ tree constituted the bootstrap confidence level (Kumar et al. 1993).

Parsimony analysis was conducted with PAUP* 4.0 (Phylogenetic Analysis Using Parsimony, Swofford 1997) using an exhaustive search, in order to compare results from the character-based cladistic analysis with the NJ tree. Unlike NJ, parsimony trees are based on analyzing the minimum number of character state changes among the taxa necessary to explain their relationships (see summary by Swofford et al. 1996). Parsimony allows comparisons to be made among the possible solutions, i.e., among the shortest trees, whereas NJ produces only a single tree. Support of the data set for nodes of the parsimony tree was evaluated with 1,000 bootstrap permutations using the branch-and-bound algorithm (see discussion by Swofford et al. 1996).

RESULTS

Morphological Variation

All specimens of \(G. \text{cernuus}\), \(G. \text{baloni}\), and \(G. \text{schaetser}\) in this study fit the morphological characters for those species described by Holčík and Hensel (1974; Fig. 1). All specimens of \(G. \text{cernuus}\) had a single opercular spine and an oblique positioning of the dorsal margin of the dorsal fin (see Fig. 1A). The single specimen of \(G. \text{baloni}\) whose mtDNA was sequenced had the characteristic anal fin cleft, a supraorbital bulge, a perpendicular positioning of the dorsal margin of the dorsal fin relative to the caudal peduncle, and two opercular spines (Fig. 1B; Holčík and Hensel 1974).

Morphological characters of \(G. \text{cernuus}\) that differed significantly among regions are summarized in Table 1 (see Fig. 1 for locations of these characters). Four significant differences were found among the five population groups examined—North America, Danube River, Bassenthwaite Lake, St. Petersburg, and Ob’ River—including the number of soft spines in the dorsal fin, number of preopercular spines, the relative length of the anal fin, and the relative length of the caudal peduncle (Table 1). All other counts and measurements were not significantly different. Counts and measurements between males and females also did not differ significantly.

Variation in the mtDNA Control Region

The mtDNA control region (Fig. 4) was 1,024 bp in length in all examined individuals of three
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TABLE 1. One-way ANOVA test for differences in morphological characters among populations of Gymnocephalus cernuus. North America (Lake Superior/St. Louis Harbor region -site 1 on Fig. 2) N = 20, Danube River (site 4 on Fig. 3) N = 10, Bassenthwaite Lake, United Kingdom (site 5 on Fig. 3) N = 16, St Petersburg, Russia (site 6 on Fig. 3) N = 7 (Some samples used for DNA were from tissues), Ob’ River, Siberia (site 7 on Fig. 3) N = 16. P = Probability.* = Significant at P < 0.05.

<table>
<thead>
<tr>
<th>Character</th>
<th>North America Mean ± s.e. (range)</th>
<th>Danube River Mean ± s.e. (range)</th>
<th>Bassenthwaite Lake Mean ± s.e. (range)</th>
<th>St. Petersburg Mean ± s.e. (range)</th>
<th>Ob’ River, Siberia Mean ± s.e. (range)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Soft rays in dorsal fin</td>
<td>12.5 ± 0.2 (12–14)</td>
<td>13.3 ± 0.2 (13–14)</td>
<td>12.6 ± 0.1 (12–13)</td>
<td>12.6 ± 0.4 (11–14)</td>
<td>12.9 ± 0.2 (12–14)</td>
<td>3.03</td>
<td>0.024*</td>
</tr>
<tr>
<td>N Preopercular spines</td>
<td>9.9 ± 0.2 (8–12)</td>
<td>8.9 ± 0.2 (8–10)</td>
<td>9.1 ± 0.2 (8–10)</td>
<td>9.7 ± 0.5 (9–12)</td>
<td>10.5 ± 0.4 (10–12)</td>
<td>8.02</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Length of anal fin in % SL</td>
<td>13.9 ± 0.2 (13.0–15.4)</td>
<td>12.5 ± 0.4 (10.7–14.4)</td>
<td>13.3 ± 0.3 (12.2–16.4)</td>
<td>12.2 ± 0.3 (11.1–12.9)</td>
<td>12.1 ± 0.3 (8.7–13.7)</td>
<td>8.35</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Length of caudal peduncle in % SL</td>
<td>19.1 ± 0.3 (16.3–21.5)</td>
<td>19.2 ± 0.7 (16.2–23.1)</td>
<td>19.5 ± 0.6 (16.2–23.1)</td>
<td>22.9 ± 0.9 (19.7–25.6)</td>
<td>19.0 ± 0.5 (13.4–21.4)</td>
<td>6.45</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

species: G. cernuus, G. baloni, and G. schraeater. Sequence differences among species and locations were summarized in Table 2, and the entire sequences were deposited in GenBank (Accession Numbers AF025355 through AF025362). Locations of conserved and variable sequence areas in the mtDNA control region and inter- and intra-specific variations were mapped on Figure 4. Within the Gymnocephalus data set, 31 nucleotide sites contained single substitutions and two sites had two substitutions each (Table 2). Eleven of the 33 substitutions in Gymnocephalus were intraspecific changes, and 22 occurred among species.

The three species were distinguished by a number of unique base substitutions, including seven for G. schraeater (at nucleotide positions 305, 349, 379, 726, 764, 810, and 841), six for G. baloni (314, 654, 726, 727, 755, and 810), and 11 for all G. cernuus (262, 277, 433, 726, 734, 759, 810, 811, 812, 813, and 837; see Table 2). Nine nucleotides were uniquely shared by G. schraeater and G. baloni (262, 277, 433, 734, 759, 811, 812, 813, and 837). Five bases were uniquely shared by G. baloni and G. cernuus (305, 349, 579, 764, and 841), and four by G. schraeater and G. cernuus (314, 654, 727, and 755) alone (Table 2).

The mtDNA sequence of the left domain of the three species of Gymnocephalus contained the putative termination associated sequence (TAS; Doda et al. 1981) 5’-AAA CTA AAC TAT T-3’, that was followed by 17 exact copies of a 10 bp “perfect” repeat. The sequence of the perfect repeat was 5’-GCA AGT ATT T-3’ for G. schraeater, G. baloni, and haplotypes A (North America and the Danube River, Slovakia), B (Bassenthwaite Lake, United Kingdom), and D and E (Ob’ River, Siberia) of G. cernuus. The sequence of the perfect repeat was 5’-GCA AGT AYT T-3’ for all samples of G. cernuus from the St. Petersburg, Russia area (haplotype C; site 3; Fig. 3). A transversion from thymine (found in G. schraeater, G. baloni, and haplotypes A, B, D, and E of G. cernuus) to guanine (haplotype C of G. cernuus) was identical in all 17 copies. Number of copies of tandem repeats did not vary in Gymnocephalus. The perfect repeat sequence array ended with the nine base sequence, 5’-GCAAATAC-3’, termed the “imperfect” repeat (following Faber and Stepień 1997).

MtDNA sequence data revealed five different haplotypes of Eurasian G. cernuus; A, B, C, D, and E, that had fixed differences among the four sample sites. Only one intrapopulational polymorphism was found (between haplotypes D and E), in the Ob’ River site, Siberia (site 7, Fig. 3). Haplotype A characterized all specimens examined from North America (see map of Fig. 2) and all individuals from the Danube River (site 4, Fig. 3). Type B differed from A by a fixed transition (from T to C) at base 221 (Table 2), and was found in all individuals tested from Bassenthwaite Lake (site 5, Fig. 3).
TABLE 2. Haplotypes of mtDNA control region sequence data for Gymnocephalus cernuus, G. baloni, and G. schraetser. Numbers in the top row correspond to the nucleotide positions of polymorphisms in the data set. * Indicates fixed difference in the first of 17 copies of a 10 bp repeated sequence region. This difference is repeated in all 17 copies, at nucleotide positions 38, 48, 58, 68, 78, 88, 98, 108, 118, 128, 138, 148, 158, 168, 178, and 188. Haplotypes are lettered A through G. Sites correspond to those on maps in Figs. 2 and 3.

<table>
<thead>
<tr>
<th>Species, Location, and Haplotype</th>
<th>Nucleotide Position</th>
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<tbody>
<tr>
<td>G. cernuus</td>
<td></td>
</tr>
<tr>
<td>North America sites 1-3</td>
<td>T T A C A A A G T C T C C A A A T A A A T T G A T G A G T T C</td>
</tr>
<tr>
<td>haplotype A (N = 24)</td>
<td></td>
</tr>
<tr>
<td>Eurasia</td>
<td></td>
</tr>
<tr>
<td>haplotype A (N = 12)</td>
<td></td>
</tr>
<tr>
<td>haplotype B (N = 11)</td>
<td></td>
</tr>
<tr>
<td>haplotype C (N = 13)</td>
<td></td>
</tr>
<tr>
<td>haplotype D (N = 8)</td>
<td></td>
</tr>
<tr>
<td>G. baloni</td>
<td>T T A A C A A G T T T T T A A A T A A A T T G G T G A G T T T</td>
</tr>
<tr>
<td>haplotype E (N = 4)</td>
<td></td>
</tr>
<tr>
<td>G. schraetser</td>
<td>T T T C A C A A T C C C T A G G G T A G C C G G C T T A A T C</td>
</tr>
<tr>
<td>Danube River</td>
<td></td>
</tr>
<tr>
<td>haplotype F (N = 1)</td>
<td></td>
</tr>
<tr>
<td>Danube River</td>
<td>T T T C A C T G A T C C T G A A A A A G T C A G A T T A A C C</td>
</tr>
<tr>
<td>haplotype G (N = 1)</td>
<td></td>
</tr>
<tr>
<td>haplotype H (N = 1)</td>
<td>T T T C A C T G A T C C T G A G A A A G T C A G A T T A A C C</td>
</tr>
<tr>
<td>haplotype H (N = 1)</td>
<td></td>
</tr>
</tbody>
</table>
Type C characterized all specimens from the St. Petersburg, Russia region (site 6, Fig. 3). The Ob' River, Siberia location (site 7, Fig. 3) was the sole polymorphic population of *G. cernuus* discerned, with eight specimens having type D and four having E (Table 2). Types C, D, and E diverged from A and B by seven fixed sequence differences, including two transversions and five transitions (Table 2). Type C differed from D and E by a fixed transversional substitution at the eighth base of the 10 bp tandemly repeated sequence, that occurred in all 17 copies (see Fig. 4 and Table 2). Types D and E diverged by a single transitional substitution at base position 730 (Table 2). The two specimens of *G. schraetser* varied from each other by a transversional substitution, with one haplotype having a guanine and one a cytosine at base position 706 (Table 2).

**Genetic Divergences**

The genetic distances between haplotypes A (North America/ Danube River) and B (Bassenthwaite Lake, United Kingdom), between C (St. Petersburg, Russia) versus D/E (Ob' River, Siberia), and between D and E were 0.001 ± 0.0005, corresponding to an estimated 50 ± 25 thousand yrs of divergence (Table 3; see Methods for calibration). The distance between the two groups of haplotypes—A and B versus C, D, and E was about ten times greater, between 0.009 ± 0.003 to 0.011 ± 0.004, about 450 to 500 ± 200 thousand yrs of separation. Divergences of *G. cernuus* from *G. baloni* and *G. schraetser* were significantly larger, corresponding to an estimated 1.2 ± .25 million yrs and 1.25 ± .3 million yrs of separation, respectively. *G. baloni* and *G. schraetser* were more closely related, separated by a genetic distance of 0.015 ± 0.004, and about 750 ± 200 thousand yrs (Table 3).

Neighbor joining (NJ) of *p*-distances (MEGA, Kumar *et al.* 1993) and parsimony analyses (PAUP* 4.0, Swofford 1997) of the five *G. cernuus* mtDNA haplotypes, *G. baloni*, *G. schraetser*, and other percdids (in which only one copy of the perfect repeat was used) produced the tree shown in Figure 5. The NJ tree had identical topology to the single most parsimonious tree from an exhaustive search using PAUP* 4.0 (Swofford 1997), and the latter thus is not shown. The most parsimonious tree had 145 steps and a consistency index excluding uninformative characters of 0.79. The g-l skewness statistic of the frequency of distribution of trees was -1.03, indicating highly significant skew and showing that the data were significantly structured (Hillis and Huelsenbeck 1992). There were no trees with 146 steps. The three next-most parsimonious trees had 147 steps.

*Gymnocephalus* was a monophyletic group, with 100% bootstrap support, in the NJ and most parsimonious trees (Fig. 5). Within the genus, *G. baloni* and *G. schraetser* were sister taxa (84% bootstrap support in NJ and 81% in the PAUP analysis), comprising the sister group to the *G. cernuus* clade (99% bootstrap support in NJ and PAUP). Within *G. cernuus*, haplotypes A (North America and Danube River) and B (Bassenthwaite Lake) were sister groups (supported by 99% of bootstrap replications in NJ and 87% in PAUP). Haplotypes C (St. Petersburg), D (Ob' River), and E (Ob' River) formed a clade (supported by 99% of NJ and 100% of the PAUP bootstrap replicates), that was the sister group of haplotypes A and B (Fig. 5).

**DISCUSSION**

**Morphological Differences**

Significant differences were found in four meristic and morphometric characters among the different North American and Eurasian sites (Table 1). The mean number of soft rays in the dorsal fin was significantly greater in the Danube River specimens than in the other sites, although the ranges overlapped. There also were fewer preopercular spines in the Danube River and Bassenthwaite Lake populations than in the other samples. The length of the anal fin was longer on average in North America and Bassenthwaite Lake populations. The St. Petersburg samples had a longer caudal peduncle (Table 1). Most of the ranges of these characters overlapped among populations, and they may well represent different temperature-related growth patterns, as is often true of meristic and morphometric characters (Hubbs 1922, Holčík and Jedlička 1994). Morphological variation needs to be surveyed throughout Eurasia using additional sites and larger sample sizes. The morphological differences found among populations (Table 1) did not mirror the degree of genetic divergences discussed below.

**Structure and Substitutions in the Control Region**

The mtDNA control region of *Gymnocephalus* had similar relative proportions of guanine (16%), adenine (32%), thymine (33%), and cytosine (19%) bases to those found in the control region of other
TABLE 3. Pairwise (p-) genetic distances among haplotypes and comparisons with congeners and other percids. p-distances and standard errors are given below the diagonal, approximate years since separation are above the diagonal. Haplotypes are lettered and their sequences are given in Table 2. Type A = all North America (sites 1 through 3 on Fig. 2; N = 24) and Danube River (site 4 on Fig. 3; N = 12) samples. Type B = all Bassenthwaite Lake, United Kingdom samples (site 5 on Fig. 3; N = 11), C = all samples from St. Petersburg/Baltic Sea (site 6 on Fig. 3; N = 13) area. D = eight and E = four samples from the Ob' River, Siberia (site 7 on Fig. 3; N = 12).

<table>
<thead>
<tr>
<th>Taxon and Haplotype</th>
<th>G. cernuus A</th>
<th>G. cernuus B</th>
<th>G. cernuus C</th>
<th>G. cernuus D</th>
<th>G. cernuus E</th>
<th>G. baloni F</th>
<th>G. schraeitser G</th>
<th>Stizostedion vitreum</th>
<th>Perca flavescens</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. cernuus A</td>
<td>—</td>
<td>50,000 ± 25,000</td>
<td>500,000 ± 150,000</td>
<td>450,000 ± 150,000</td>
<td>500,000 ± 200,000</td>
<td>1,100,000 ± 250,000</td>
<td>1,250,000 ± 300,000</td>
<td>4,100,000 ± 500,000</td>
<td>4,400,000 ± 500,000</td>
</tr>
<tr>
<td>G. cernuus B</td>
<td>0.001 ± .0005</td>
<td>—</td>
<td>560,000 ± 200,000</td>
<td>500,000 ± 200,000</td>
<td>560,000 ± 200,000</td>
<td>1,200,000 ± 300,000</td>
<td>1,300,000 ± 300,000</td>
<td>4,100,000 ± 500,000</td>
<td>4,400,000 ± 500,000</td>
</tr>
<tr>
<td>G. cernuus C</td>
<td>0.010 ± .003</td>
<td>0.011 ± .004</td>
<td>—</td>
<td>50,000 ± 25,000</td>
<td>125,000 ± 200,000</td>
<td>1,400,000 ± 300,000</td>
<td>1,350,000 ± 300,000</td>
<td>4,200,000 ± 500,000</td>
<td>4,300,000 ± 500,000</td>
</tr>
<tr>
<td>G. cernuus D</td>
<td>0.009 ± .003</td>
<td>0.010 ± .004</td>
<td>0.001 ± .005</td>
<td>—</td>
<td>50,000 ± 25,000</td>
<td>1,300,000 ± 300,000</td>
<td>1,300,000 ± 300,000</td>
<td>4,450,000 ± 500,000</td>
<td>4,550,000 ± 500,000</td>
</tr>
<tr>
<td>G. cernuus E</td>
<td>0.010 ± .003</td>
<td>0.011 ± .004</td>
<td>0.003 ± .002</td>
<td>0.001 ± .005</td>
<td>—</td>
<td>1,350,000 ± 300,000</td>
<td>1,350,000 ± 300,000</td>
<td>4,300,000 ± 500,000</td>
<td>4,300,000 ± 500,000</td>
</tr>
<tr>
<td>G. baloni F</td>
<td>0.022 ± .005</td>
<td>0.024 ± .006</td>
<td>0.027 ± .006</td>
<td>0.026 ± .006</td>
<td>0.027 ± .006</td>
<td>—</td>
<td>750,000 ± 200,000</td>
<td>4,000,000 ± 500,000</td>
<td>4,250,000 ± 500,000</td>
</tr>
<tr>
<td>G. schraeitser G</td>
<td>0.025 ± .006</td>
<td>0.026 ± .006</td>
<td>0.027 ± .006</td>
<td>0.026 ± .006</td>
<td>0.027 ± .006</td>
<td>0.015 ± .004</td>
<td>—</td>
<td>3,950,000 ± 500,000</td>
<td>4,300,000 ± 500,000</td>
</tr>
<tr>
<td>S. vitreum</td>
<td>0.082 ± .010</td>
<td>0.082 ± .010</td>
<td>0.084 ± .010</td>
<td>0.089 ± .010</td>
<td>0.086 ± .010</td>
<td>0.080 ± .010</td>
<td>0.079 ± .010</td>
<td>—</td>
<td>4,900,000 ± 505,000</td>
</tr>
<tr>
<td>P. flavescens</td>
<td>0.088 ± .010</td>
<td>0.088 ± .010</td>
<td>0.086 ± .010</td>
<td>0.091 ± .010</td>
<td>0.086 ± .010</td>
<td>0.085 ± .010</td>
<td>0.086 ± .010</td>
<td>0.086 ± .010</td>
<td>—</td>
</tr>
</tbody>
</table>
fishes (Sturmbauer and Meyer 1992, Lee et al. 1995, Stepieen 1995), including percids (15% G, 31% A, 33% T, and 21% C for walleye *S. vitreum*; Stepieen 1995, Faber and Stepieen 1997). The ratio of transitional to transversional substitutions was 1.46, and was somewhat lower than that found among haplotypes of *S. vitreum* (1.77; Stepieen 1995). Most transversional substitutions occurred among the three species (*G. cernuus*, *G. baloni*, and *G. schraetser*), suggesting longer periods of separation (summarized by Avise 1994, Stepieen and Kocher 1997). Transition: transversion ratio within *G. cernuus* was 2.33 (Table 2). Most variation was concentrated in the left and right variable domains of the control region, similar to *Stizostedion* (Faber and Stepieen 1997) and other teleost fishes (Lee et al. 1995). Two polymorphisms occurred in the central conserved section of *Gymnocephalus* (one inter- and one intra-specific character; Fig. 4), at approximately the same location as an intraspecific polymorphism in *S. vitreum* (Faber and Stepieen 1997).

The TAS site of *Gymnocephalus*- 5'-AAA CTA AAC TAT T-3' (Table 2, Fig. 4) was identical to that of the percids sauger (*S. canadense*), zander (*S. lucioperca*), and yellow perch (*P. flavescens*), and differed in *S. vitreum* by one transitional substitution (Faber and Stepieen 1997). A 10 bp repeated sequence in *Gymnocephalus*- 5'-GCA AGT ATG T-3' was located at the R1 site (Fig. 4; Arnason and Rand 1992), where tandemly repeated sequences were identified in all percids sequenced (Stepieen 1995, Faber and Stepieen 1997). Turner (1997) independently corroborated the presence of repeats at this site in darters and some other percids, but his sequences were incorrect for sauger and yellow perch (Faber and Stepieen 1997). Tandem repeats at the R1 site have been found in some other fishes (Bentzen et al. 1988, Arnason and Rand 1992, Brown et al. 1996). Tandem repeats at the R1 site are believed to result from imperfect terminations of strand replication (Arnason and Rand 1992, Fumagalli et al. 1996).

All *Gymnocephalus* examined had 17 copies of the perfect repeat, in contrast to *S. vitreum*, which alone among the percids examined had varying numbers—ranging from 6 to 14 copies per individual (Stepieen 1995, Faber and Stepieen 1997). A transversional substitution distinguished all 17 copies of the 10 bp perfect repeat of haplotype C of *G. cernuus* from St. Petersburg, Russia—5'-GCA AGT ATG T-3' (Table 2). This transversion was regarded as a single substitution in calculating genetic distances and phylogenetic analyses in our study. It is probable that this mutation occurred as a single evolutionary event in all copies due to concerted evolution (Wilkinson and Chapman 1991, Broughton and Dowling 1994), which is the non-independent evolution of repetitive DNA sequences resulting in their sequence similarity. It has been hypothesized that the mechanisms responsible for creating repeat arrays also may mediate concerted evolution (Dover 1982). A single base substitution also was found within the perfect repeats of *S. vitreum*, in the case of the highly diverged populations from the Ohio River and the Great Lakes (0.029, about 1.4 million yrs; Stepieen and Faber, unpublished). Substitutions in the perfect repeat may occur only in widely diverged populations, as indicated here and in walleye (Faber and Stepieen 1998).

A single variant copy, termed the imperfect repeat, 5'-GCAATACA-3', followed the perfect repeats in all *Gymnocephalus*. The imperfect repeat appeared to be evolutionarily derived from the perfect repeat sequence, as hypothesized for other percids (Faber and Stepieen 1997, 1998). Like *Gymnocephalus*; the sauger (*S. canadense*), zander (*S. lucioperca*), banded darter (*E. zonale*), bluebreasted darter (*E. camurum*), and blackside darter (*P. maculata*), possessed a single imperfect repeat (Faber and Stepieen 1997). *Stizostedion vitreum* had two to five copies of imperfect repeats, that varied in base
composition and number of nucleotides among individuals (Faber and Stepień 1997, 1998). Unlike S. vitreum, the number and sequence of the imperfect repeats did not vary among Gymnocephalus.

Phylogenetic Relationships and Interspecific Divergences of Gymnocephalus

MtDNA control region sequences confirmed species-level separations of G. cernuus, G. baloni, and G. schraetser (Table 3, Fig. 5) and suggested that G. cernuus comprised two species or subspecies (see Intraspecific Divergences below). The genus Gymnocephalus appeared to have diverged from other percids about 4 to 4.5 million yrs ago (+/- 500 thousand yrs; Table 3), during the Pliocene Epoch. A study of lactate dehydrogenase (Ldh) allozyme-isozyme migration patterns from G. baloni, G. cernuus, and G. schraetser by Šlechtová and Slechtá (1995) described unique bands in G. baloni for Ldh-A and B. The migration pattern in G. baloni was different than in the other two species, supporting genetic separation (Šlechtová and Slechtá 1995), as found in the present study.

 Parsimony and NJ analyses showed that G. baloni and G. schraetser were more closely related to each other than either was to G. cernuus (Table 3, Fig. 5). Relationships among these species differed from the meristic character hypothesis of Holčík and Hensel (1974), that placed G. baloni and G. cernuus as sister taxa. The hypothesis of Holčík and Hensel (1974) that G. schraetser and G. acerina were more primitive than G. cernuus and G. baloni was refuted by our mtDNA control region sequence data (Fig. 5). Holčík and Hensel (1974) also described several characters that appeared to support a sister relationship between G. baloni and G. schraetser, including number of branched rays in the dorsal fin, number of preopercular spines, number of branchiostegal rays, and number of gill rakers. Those morphological characters (Holčík and Hensel 1974) are congruent with the results of the present study. Ráb et al. (1987) found that G. baloni and G. schraetser shared a derived chromosomal karyotype with more metacentric elements that differed significantly from the apparently pleiomorphic condition of G. cernuus. The results of the Ráb et al. (1987) study thus were congruent with our findings. According to our data and calibration, divergence of the G. schraetser-G. baloni clade from G. cernuus apparently occurred during the early Pleistocene Epoch, about 1.1 to 1.4 million yrs ago (+300 thousand yrs; Table 3). Divergences suggest that speciation of G. schraetser and G. baloni occurred during the mid-Pleistocene Epoch, about 750 ± 200 thousand yrs ago (Table 3).

Genetic distances among Gymnocephalus species were significantly less (about 1/3) than that separating the congeners walleye (S. vitreum) and sauger (S. canadense; 0.078 ± 0.009, about 3.9 million yrs; Faber and Stepień 1997), suggesting that speciation in Gymnocephalus occurred more recently. The three species (G. cernuus, G. baloni, and G. schraetser) appeared to have shared a common origin in the Paleodanube River region (Holčík and Hensel 1974). The Paleodanube was a large and unique freshwater fish refuge during the Quaternary glaciations and comprised the main source of freshwater recolonization of most European rivers (Lindberg 1972). The Danube River today has the richest and the most diversified fish fauna in Europe (Lindberg 1972). It would be desirable to include the fourth species, G. acerina, in further analyses. Juraj Holčík (personal communication, June 1997) hypothesized that G. acerina is a sister species of G. schraetser; as the Dniester River, together with the Dniester and Southern Bug rivers, shared a Paleodanube drainage during the Quaternary Period (Lindberg 1972).

Intraspecific Divergences and Phylogeography of G. cernuus

This study identified low intrapopulational genetic variation in the mtDNA control region of G. cernuus in all but one population surveyed (Table 2). Populations of G. cernuus in the Ob’ River, Siberia, and G. schraetser in the Danube River were polymorphic (Table 2). Apparent low intrapopulational genetic variability of G. cernuus in four of the five populations contrasts with high population genetic diversity of S. vitreum in the mtDNA control region (Stepień 1995, Faber and Stepień 1997), for which 79 haplotypes have been identified from 199 individuals in the Great Lakes region (Faber and Stepień 1998). Marine fishes, such as the scorpænid Sebastolobus alascanus, the pleuronectid Microstomus pacificus, and the serranid Paralabrax maculatofasiatus have significantly higher levels of intrapopulation sequence polymorphism in the mtDNA control region than does Gymnocephalus (Stepień 1995). Among freshwater fishes, a study of sequence variation in 188 bp of the mtDNA control region from 354 Onocorhynchus mykiss revealed eleven haplotypes from sample sites in California, Baja California, and the
Rio Yaqui (Nielsen et al. 1997). Some populations, such as the Rio Yaqui basin of northwestern Mexico and the San Pedro Martir basin in Baja California, were monomorphic for given haplotypes (Nielsen et al. 1997). Results of Nielsen et al. (1997) were similar to those of the present study in that there were a limited number of haplotypes and mtDNA control region sequences revealed significant biogeographic structuring.

Šlechtová and Slechtá (1993) described two rare allozymic polymorphisms at the Ldh-A locus for G. cernuus from the Elbe (N = 247 sampled) and the Danube rivers (N = 61 sampled); one variant was found in only two individuals from the Elbe River and the frequency of the other variant (found in both the Elbe and the Danube rivers) was not reported. No intraspecific variability was discerned at the Ldh-B locus for G. cernuus, and neither locus was variable in G. schraetser (N = 73 sampled) or in G. baloni (N = 6 surveyed). The Slechtová and Slechtá (1993) study was not very informative since only two loci were sampled (most comprehensive allozyme/isozyme studies routinely survey 40 to 50 or more; Avise 1994) and the frequencies of one of the Ldh-A variants were not given. It would be useful to compare levels and patterns of genetic variability in the present mtDNA study with nuclear DNA variation and/or a more extensive allozyme/isozyme survey.

The recently introduced North American and Bassenthwaite Lake (England) populations of ruffe may have undergone a founding event which could explain the lack of mtDNA intrapopulation variability found here. However, no variability was detected within two of the three other Eurasian populations examined. This suggests either that the ruffe is characterized by low genetic diversity, in comparison with G. schraetser and other percids (Faber and Stepien 1997); or alternatively, that populations of G. cernuus lost genetic diversity. Loss of genetic variation may have occurred from bottlenecks during isolation in glacial refugia and/or during subsequent rapid range expansion following the Ice Ages (Hewitt 1996). Present populations of ruffe are large; for example, a five-year investigation by Holčik and Bastl (1976) of populations in the Danube River indicated that ruffe comprised 17.2% of the total number of fishes and 8.3% of the fish biomass. Loss of genetic diversity during the glaciations and subsequent range expansions may be supported by the greater polymorphism of the southeasterly Ob’ River population (Fig. 3), which is in an area that was less affected by glacialation (Hewitt 1996). Higher levels of polymorphism in southeastern European populations of G. cernuus are congruent with trends in polymorphism found by Hewitt (1996) in the grasshopper Chorthippus parallelus.

Separations of G. cernuus into small, isolated populations during the Pleistocene Ice Ages may account for marked divergences among sites and reduced intrapopulation variability. Ice, steppe, and tundra covered much of northern and middle Europe at the height of the last Ice Age, which lasted until 18 thousand yrs ago (summarized in Hewitt 1996). Taxa that are presently common and widespread over Europe are believed to have survived only in sheltered southern refugia (Cooper et al. 1995, Hewitt 1996). The refugia populations then evolved in allopatry, accumulating independent genetic differences (Cooper et al. 1995), as indicated here. Following climate amelioration, the refugia populations expanded northwards when suitable habitats opened up again (Cooper et al. 1995). Leading edge colonization during this rapid expansion led to homozygosity and spatial assortment of genomes, as hypothesized by Hewitt (1996) and supported by our results.

The leading edge hypothesis (Hewitt 1996) states that expansion from southerly refugia populations was rapid and early long distance dispersants established founding populations well ahead of the main distribution. The founding populations increased rapidly and dominated the genome of the leading populations (Hewitt 1996). Later migrants contributed little to the gene pool, since they entered established populations at carrying capacity and their reproductive rate was logistically low compared to the exponential growth of the original colonizers (Hewitt 1996). Spreading from the leading edge involved a series of bottlenecks for the colonizing genome, leading to a loss of alleles and a tendency to homozygosity (Nei et al. 1975). Lack of genetic diversity would be especially apparent in the mitochondrial genome, due to its smaller effective population size (1/4 the nuclear genome), lack of recombination, and more rapid extinction of lineages (Avise 1994).

Patterns of mtDNA variation in the brown trout Salmo trutta across Europe (Bernatchez 1995, Bernatchez and Osinov 1995) matched those discerned here for G. cernuus, suggesting their common vicariant isolation and divergence during the Ice Ages. The Danube River area and northwestern Atlantic areas of S. trutta appeared to be ancestral and were more closely related to each other.
(Bernatchez 1995), as were haplotypes A and B of _G. cernuus_ in our study. Patterns of mtDNA and allozyme variation in the grasshopper _Chorthippus parallelus_ also revealed phylogeographic relationships (Hewitt 1996) similar to populations of _G. cernuus_, suggesting comparable history of refuge and expansion.

The fixed intraspecific difference between the two primary haplotype groups of _G. cernuus_- A and B versus C, D, and E located in the central conserved section is unusual (Fig. 4), since studies of teleost fishes have identified it as a highly conserved site (Lee et al. 1995). For example, Faber and Stepien (1997) found only one polymorphic nucleotide in the central control section of _S. vitreum_ among 79 haplotypes. Variation in this conserved section and the large number of fixed differences (N = 7) between the haplotype groups of _G. cernuus_, suggests a species or subspecies level separation. The mean genetic distance separating the groups of haplotypes, A/B versus C/D/E (with only one repeat considered) was 0.010 ± 0.0035 (Table 3, Fig. 5), which was significantly larger (by about 5 times) than the average intraspecific genetic distance calculated for haplotypes of _S. vitreum_ 0.002 ± 0.002 from the control region (Faber and Stepien 1997). The time of separation of the two groups of _G. cernuus_ haplotypes was estimated as 500 ± 180 thousand yrs. This corresponds to a particularly prolonged period of cold temperatures, that is believed to have produced pronounced divergences in other European taxa that were isolated in southern glacial refugia (Hewitt 1996). The genetic difference separating the two groups of _G. cernuus_ haplotypes was somewhat less than that separating _G. baloni_ and _G. schraetser_ (0.016 ± 0.005), but not significantly so. These distances suggest that the two groups of _G. cernuus_ were isolated in different glacial refugia and either have not been in subsequent contact and/or have evolved reproductive barriers. Bruce Collette (National Marine Fisheries Service Systematics Laboratory, Washington, D.C.; pers. commun, January 1997) offered the opinion that the Danube River specimens of _G. cernuus_ appeared morphologically different from _G. cernuus_ from other European regions. Holčík and Hensel (1974) also stated that “_G. cernuus_ from the Danube basin seems to (morphologically) stand apart from populations in other watersheds.” The Danube River _G. cernuus_ was designated as a separate morphological form (_G. cernuus_ natio _danubica_) by Vladykov (1931), a category of classification that is no longer recognized (Kottelat 1997). If further genetic and/or morphological evidence indicate significant divergence, it may be that our haplotypes A and B, including all specimens from North America, Bassenthwaite Lake, and the Danube River area, may comprise a separate species or subspecies.

The invasive population of Bassenthwaite Lake (haplotype B) was genetically diverged from the North America/Danube River specimens (haplotype A) by one fixed substitution and an estimated 50 ± 25 thousand yrs, corresponding to a later Pleistocene separation. It is possible that the Bassenthwaite Lake population was introduced from southern England, where a native population of _G. cernuus_ occurs that presumably diverged from the Danube River type (A). Six individuals were sequenced from the invasive population in Loch Lomond, Scotland, that were genetically identical to the Bassenthwaite Lake samples. It appears likely that both the Loch Lomond and Bassenthwaite Lake populations were introduced from southern England, where the ruffe is a popular bait fish and is frequently transported by fishermen (Maitland and East 1989, Winfield et al. 1996).

The Ob' River, Siberia population (haplotype D) was genetically diverged from the St. Petersburg, Russia samples (haplotype C) by a fixed difference, a transversion in the repeated sequences of haplotype C (Table 2, Fig. 4). The haplotypes were diverged by an estimated 50 ± 25 thousand yrs, also corresponding to a later Pleistocene separation. At one time, the populations of the Ob' and Yenisey rivers were described as the species _G. essipovi_ (Holčík and Hensel 1974), suggesting a similar pattern of morphological divergence that should be further examined.

Lack of intrapopulational variability and the presence of fixed differences among the five Eurasian haplotypes of _G. cernuus_ suggests that these populations have been isolated from each other for some time, due to two different vicariant events during the Quaternary Period. Haplotypes A and B were vicariantly separated from C, D, and E during the early to mid-Pleistocene Epoch; probably in different glacial refugia. They appear to have remained isolated in different drainage systems. Haplotype A diverged from B and haplotype C from D and E during the later Pleistocene Epoch, again vicariantly in separate refugia and then in different drainages. Vicariant separations often lead to fixed differences (as seen here) and/or significantly different frequencies of haplotypes among populations (as seen in _S. vitreum_, Faber and Stepien...
1997). Low intrapopulation polymorphism and the fixed differences among populations suggest a history of repeated founder effects and/or population bottlenecks. Whether intermediate populations with more than one mtDNA haplotype occur in other Eurasian areas, remains to be discerned.

The presence of a single genotype of *G. cernuus* (Haplotype A) in North America suggests that colonization occurred from only one of the four Eurasian areas examined in our study. The 1995 appearance of ruffe in Lake Huron either resulted from intralake shipping from Lake Superior, since only haplotype A was present, or from a separate colonization from the same Eurasian region. It is probable that haplotype A also may occur in the Black Sea watershed, in addition to the Danube River, since the Danube River drains into the Black Sea. The North American Great Lakes may have been founded from a source population in the rivers that empty into the Black Sea watershed (Thomas Busiann, pers. commun., March 1997). Juraj Holčík (pers. commun., June 1997) postulated that possible Danubian sources for the Great Lakes population are the following shipping ports where ballast water is taken; Galati (Romania), Tulcea (Romania), Izmayil (Ukraine), and the entire lower and middle reaches of the Danube River north to the city of Komarno (Slovakia; more than 1,700 km). Holčík further suggested the ports of Kherson and Kakhovs’ke (Ukraine) on the Dnieper River and Bilhorod-Dnistrovs’kyi (Ukraine) on the Dniester River as possible sources. Genetic analyses of samples from these places may enable the founding origin of the North American exotic population to be determined.

Genetic Variability of Invasive Species

Nevo (1983) hypothesized that cosmopolitan and widespread animals should be genetically more variable than narrow-ranging and endemic species, and that habitat generalists should be more polymorphic than habitat specialists. Invading species are expected to be in the first category of both comparisons (Gray 1986, Williamson 1996). The ruffe appears to fit these criteria, since it is the most widespread of the genus *Gymnocephalus*, has a generalized diet, and occupies a variety of habitats (Ogle 1995, Ogle et al. 1995). However, the present study suggested lower intra-population genetic variability in the ruffe, as compared to the walleye *S. vitreum* (Stepien 1995, Faber and Stepien 1997). Lack of intrapopulation genetic variability in *G. cernuus* may have resulted from their rapid range expansion into new habitats during re-colonization of northerly areas after the Ice Ages, as is postulated to have occurred in other European species (Hewitt 1996). The considerable number of fixed sequence differences among areas of the range of *G. cernuus* appear to have originated during isolation in different glacial refugia and suggests that the populations have not substantially mixed.

Apparent low genetic variability does not appear to negatively affect the invasive capability and population growth of ruffe in either the North American Great Lakes or Bassenthwaite Lake, England. In fact, low variability within populations may be indicative of the capacity for rapid range expansion that characterized successful invaders following the Ice Ages (Hewitt 1996). Some of the other invaders in the Great Lakes, including the introduced pink salmon (Gharrett and Thomason 1987), alewife (Ihssen et al. 1992), round goby (Wallis and Beardmore 1984), and the spiny water flea (Wieden 1991) also exhibit low genetic variability. In contrast, the sea lamprey (Wright et al. 1985) and zebra mussel (Marsden et al. 1996) possess relatively high levels of genetic polymorphism. The role of genetic variability in predicting successful colonization of new populations in these and other species thus remains to be further investigated.

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