

# Genetic diversity and evolutionary relationships of the troglodytic 'living fossil' *Congeria kusceri* (Bivalvia: Dreissenidae)

C. A. STEPIEN,\* B. MORTON,† K. A. DABROWSKA,\* R. A. GUARNERA,‡ T. RADJA,§ and B. RADJA¶  
\*Great Lakes Environmental Genetics Laboratory, Center for Environmental Science, Technology & Policy, Cleveland State University, 1899 East 22nd Street, MC-219, Cleveland, OH 44114–4434, USA, †The Swire Institute of Marine Science and Department of Ecology and Biodiversity, The University of Hong Kong, Hong Kong, China, ‡Cleveland Genomics, Ltd, 10515 Carnegie Avenue, Cleveland, Ohio 44106, USA, §Njegoseva 2, 21000 Split, Croatia, ¶Department of Biology, Faculty of Science, University of Split, Teslina 12/III, 21000 Split, Croatia

## Abstract

Population genetic theory predicts that long-term isolation of 'living fossils' in relic habitats might reduce genetic variability due to small population sizes and inbreeding. The recent description of a troglodytic 'living fossil' *Congeria kusceri* – the only known subterranean bivalve mollusc – from a genus thought to be extinct since the Miocene, offers a unique opportunity to examine this hypothesis. Here, we use DNA sequences from two mitochondrial genes to compare levels of genetic variability and to test phylogenetic relationships of *C. kusceri* with surface-dwelling dreissenid relatives. Phylogenetic analyses of sequences from the cytochrome oxidase 1 (COI) and 16S rDNA genes reveal that *Mytilopsis* is the sister genus to *Congeria* and this clade forms the sister taxon to *Dreissena*. Relatively high levels of DNA diversity characterized the population of *C. kusceri* (haplotypic diversity = 0.50 for 16S rDNA and 0.66 in the COI gene), in contrast to no intraspecific variability in populations of *Dreissena polymorpha*, *D. bugensis*, *Mytilopsis leucophaeta*, and *Corbicula fluminea*. Maintenance of genetic variability in *C. kusceri* may result from long-term population size stability, which merits further investigation. This underground species apparently was buffered from the climatic changes and resultant population bottlenecks that affected its surface-dwelling relatives during the Pliocene and Pleistocene Ice Ages.

**Keywords:** 16S mtDNA, cave-dwelling, *Congeria*, COI mtDNA, Dreissenidae, genetic diversity

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## Introduction

Subterranean caverns and rivers house unique aquatic faunas. They comprise long-term stable temperate freshwater environments, serving as refugia from the stochastic climatic fluctuations that have shaped surface-level communities. Subterranean habitats have no light and temperatures are relatively constant, but may experience some seasonal variations in water flow. Population genetic theory predicts that long-term isolation of 'living fossils' might reduce genetic variability due to relatively small population sizes and possible inbreeding (Avice 1994, 2000).

The bivalve mollusc *Congeria kusceri*, was recently discovered living exclusively in subterranean waters of the Dinaric karst of Slovenia, Croatia, and Bosnia-Herzegovina (Fig. 1) (Bole 1962; Morton *et al.* 1998). The Dinaric karst region of the northern Adriatic contains nearly 400 specialized stigobiont taxa in an area of only 153 400 km<sup>2</sup> (Sket 1999), suggesting long-term evolutionary stability.

Until this discovery, the dreissenoid genus *Congeria* was believed to have become extinct about 5 million years ago (Ma; Nuttall 1990), coinciding with the break-up of the Paratethys Sea and the Messinian salinity crisis (Morton 1992). *Congeria* comprised a large number of species of common coastal zone burrowing mussels during the late Miocene (Morton 1992). For example, approximately 30 species have been described from Miocene deposits in the region of Croatia and Bosnia-Herzegovina (Kochansky-Devide &

Correspondence: Carol A. Stepien. Fax: 216 687 5393; E-mail: c.stepien@csuohio.edu



Fig. 1 Map with stippling showing the range of *Congeria kusceri* in Croatia and Bosnia-Herzegovina and the location of the sampling site in Croatia.

Sliskovic 1980). Since the relationship between *Congeria* and the other dreissenoid genera has been controversial (Nuttall 1990; Morton 1992), the finding of living *C. kusceri* constitutes an important systematic discovery (Morton *et al.* 1998).

#### Life history and ecology of *C. kusceri*

Specimens of *C. kusceri* are light tan, range up to 24 mm in length, and occur in gregarious clusters (Morton *et al.* 1998). The species appears to be long-lived, reaching possible ages of approximately 30–40 years, and the shells are sometimes heavily coated with travertine (precipitated calcium carbonate) (Morton *et al.* 1998). Adaptations to a subterranean existence include loss of pigmentation, an absence of statocysts and eyespots, great reduction of the foot, loss of the posterior pedal retractor muscles, and the presence of external cleansing currents on the mantle (to remove settling loam). The ctenidia are large, the labial palps minute, the intestine short, and there are few (if any) sorting areas in the stomach – all reflecting the limited supply of potential food and the need to accept any available detritus (Morton *et al.* 1998).

*C. kusceri* is dioecious and the eggs are lecithotrophic, internally fertilized, and then brooded in the ctenidia – nourished by hypobranchial glandular secretions tissue (Morton 1977; Morton *et al.* 1998). The offspring are released as crawl-away pediveligers, which is characterized as a *K*-selected life history trait in terms of evolutionary ecology (Pianka 1999). In contrast, the more *r*-selected extant dreissenid genera *Dreissena* and *Mytilopsis* have external fertilization

and free-swimming larvae (Mills *et al.* 1996). There is thus a fundamental difference in life history traits between the opportunistic *r*-selected *Dreissena* and *Mytilopsis* (Mills *et al.* 1996), which are its closest living relatives, and *C. kusceri*.

*C. kusceri* has a tiny foot, rendering it incapable of significant locomotion (unlike juvenile *D. polymorpha*) and the byssal threads are short and thick – also indicating that *C. kusceri* moves little after attachment (Bole 1962; Morton *et al.* 1998).

#### Hypothesized evolutionary relationships of *Congeria*

*C. kusceri* is a relic of Pliocene lakes, which formed above fractured limestone and dolomite deposits of the western Paratethys. Today, *C. kusceri* lives in the caves and subterranean rivers of this karst, where it apparently followed the waters down as they eroded the porous rock (Morton *et al.* 1998). Another Paratethys species, the similarly troglodytic polychaete *Marifugia cavatica* (Serpulidae) occurs sympatrically with *C. kusceri* and encrusts the shells with its tubes (Morton *et al.* 1998).

*Congeria* belongs to the Dreissenoida, which contains a single family, the Dreissenidae and two other extant genera; the freshwater *Dreissena* and the marine *Mytilopsis* (Nuttall 1990). The Dreissenoida is believed to have arisen from an ancestor shared with the Corbiculoidea during the late Mesozoic era (Morton 1993). The dreissenoids appear united by having an apical shell septum to which the anterior adductor and anterior byssal retractor muscles are attached (Nuttall 1990; Morton 1993). They also are characterized by the neotenous retention of the byssus (a larval character) and the foot has consequently ceased to be an organ of locomotion (except in juveniles), with corresponding enlargement of the larval byssal retractor muscles and diminution of the adult pedal ones. The three dreissenid genera are believed to have diverged during the mid to late Miocene Epoch (Morton 1993).

Whether *Congeria* and *Mytilopsis* belong to two separate genera and how these groups are related has been debated (Nuttall 1990; Morton *et al.* 1998). A hypothesized sister relationship between the genera *Congeria* and *Mytilopsis* is supported by the shared character of attachment of the anterior byssal retractor muscle to a special lobe of the anterior shell septum called the apophysis (Morton 1993). In contrast, the anterior byssal retractor muscle is attached directly to the septum in *Dreissena*. *Congeria* is distinguished from *Mytilopsis* by its distinct shell form and microstructure, and different reproductive strategy (Morton 1993; Morton *et al.* 1998). It also has been disputed whether *Mytilopsis* (Nuttall 1990) or *Congeria* (Morton 1993) is more closely related to an ancestor shared with the sister superfamily Corbiculoidea.

The number of species comprising the euryhaline *Mytilopsis* are disputed; they are native to the New World,

but Old World fossils exist and some species have been introduced back into Europe (*M. leucophaeta*) (van der Velde *et al.* 1992) and Asia (*M. sallei*) (Morton 1980). The genus *Dreissena* includes the zebra mussel *D. polymorpha* and the quagga mussel *D. bugensis*, which were spread via ballast water introductions from Europe to the North American Great Lakes during the last 15 years and where they now form large established populations (Mills *et al.* 1996; Berkman *et al.* 2000).

### Objectives and hypotheses

The first objective of the present study was to test the phylogenetic relationships of the Dreissenidae, comprising *Congeria* and the genera *Dreissena* and *Mytilopsis*, using sequence data sets from two mitochondrial DNA (mtDNA) genes. The relationship of the Dreissenidae with the Corbiculoidea (represented by the Asian clam *Corbicula fluminea*), which is their hypothesized sister superfamily, was also examined and the results compared with morphological hypotheses. The second objective was to compare the relative level of DNA genetic variability in a population of the troglodytic living fossil *C. kusceri* with its surface-dwelling close relatives (*D. polymorpha*, *D. bugensis*, and *M. leucophaeta*), in order to examine the possible influences of long-term isolation in a climatically stable environment (*C. kusceri*) vs. larger extant populations that were subjected to historic climatic changes (*Dreissena* spp. and *M. leucophaeta*).

### Materials and methods

Ten individuals of *Congeria kusceri* were collected from the pit 'Jama u Predolcu', near Metkovic, Croatia in August 1998 (Fig. 1) and placed in 95% ethanol. The pit is about 10 m deep, with a subterranean lake at its bottom (Jalzić 1998). Genomic DNA was extracted from nine specimens using standard methods (Stepien *et al.* 1999). The remaining sample was kept as a whole voucher. Shells from the nine specimens sequenced were archived. The mitochondrial cytochrome c oxidase subunit I (COI) gene was polymerase chain reaction (PCR) amplified following Baldwin *et al.* (1996) and the purified products sequenced by Cleveland Genomics, Ltd. using an ABI 377XL automated sequencer. Sequences of the COI gene comprising 577 bp were aligned manually according to Baldwin *et al.* (1996) with their data from the same region for the related dreissenids *Dreissena polymorpha*, *D. bugensis*, and *Mytilopsis leucophaeta*, the corbiculid *Corbicula fluminea*, and the mytilid *Mytilus edulis* (GenBank Accession nos U47647–53). The mitochondrial 16S rRNA gene was PCR amplified and 477 bp were sequenced using methods previously described by Stepien *et al.* (1999). Sequences for the 16S rDNA region in the present study were aligned manually with those from the same region sequenced by Stepien *et al.* (1999) for

*D. polymorpha*, *D. bugensis*, *M. leucophaeta*, and *C. fluminea* (GenBank Accession nos AF0389989–92). The 16S rRNA sequences were rooted to those published for *M. edulis* (Hoffman *et al.* 1992), which was used as the outgroup for both gene regions. The alignments for the 16S rDNA gene data are given in Stepien *et al.* (1999).

Both gene regions were analysed separately and the resulting phylogenetic trees compared for congruency of relationships among taxa. Genetic distances, including Kimura (1980) 2-parameter, Tamura & Nei (1993), and uncorrected pairwise distances (following Nei 1987), between each pair of taxa were calculated and clustered using the neighbour-joining (NJ) algorithm in PAUP\* 4.0A4b (Swofford 2000). Maximum parsimony (MP) trees were determined using exhaustive searches in PAUP\* (Swofford *et al.* 1996; Swofford 2000), with *M. edulis* designated as the outgroup. Taxa contained in the MP trees were compared using strict and 50% majority-rule consensus analyses (Margush & McMorris 1981). Support of the data sets for the nodes of the NJ and MP trees were determined from 1000 bootstrap replications (Kumar *et al.* 1993; Swofford *et al.* 1996). Measures of nucleotide variability were calculated according to Nei (1987) and included the number of polymorphic nucleotide sites per nucleotide position  $pn$  (eqn 10.1)  $\pm$  its variance over the stochastic process (eqn 10.3), haplotypic diversity (eqn 8.5)  $\pm$  its variance (eqn 8.8), and the average number of nucleotide differences among haplotypes (nucleotide diversity) (eqn 10.5)  $\pm$  its variance due to stochastic errors (eqn 10.9).

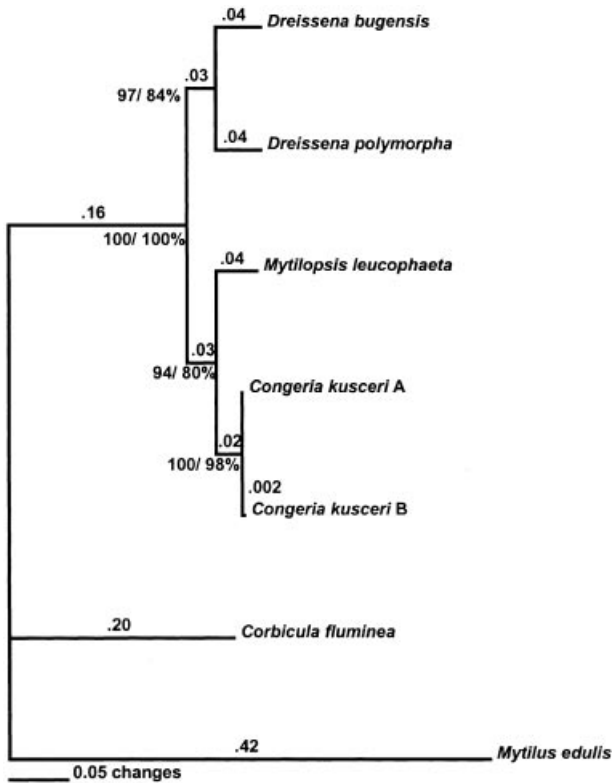
### Results

#### Interspecific divergences

Our sequencing results from the mtDNA COI (reported in GenBank as Accession no. AF325444) and the 16S ribosomal gene (GenBank Accession no. AF320601) regions showed that *Congeria*, *Mytilopsis*, and *Dreissena* comprise a monophyletic lineage (see Figs 3 and 4), confirming their morphological classification together as the Dreissenidae (Nuttall 1990; Morton 1993). The NJ trees from both genes and the single MP tree from 16S rDNA indicated that *Congeria* and *Mytilopsis* are sister genera, and this clade then forms the sister group to *Dreissena*. Genetic distance estimates from Kimura (1980) 2-parameter, Tamura & Nei (1993), and uncorrected pairwise distances (following Nei 1987), were very similar (within 0.003), and the former was used in Table 1 and to construct the NJ tree (Fig. 2). The *Dreissena* and the *Mytilopsis*/*Congeria* clade appear to have diverged between 10 and 17 Ma, using a molecular clock calibrated to fossil record dates for this group given in Stepien *et al.* (1999). The genera *Mytilopsis* and *Congeria* then diverged from each other approximately 5–15 Ma and *D. bugensis* and *D. polymorpha* speciated about 10–13

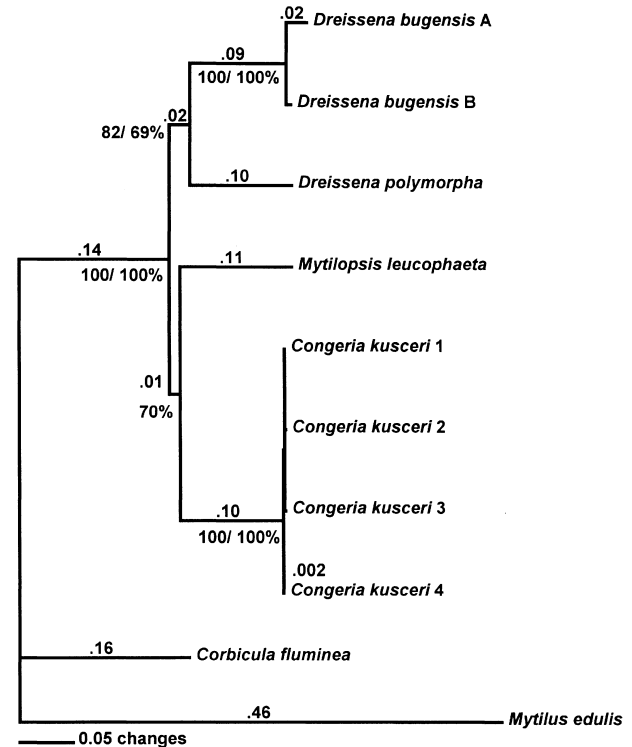
**Table 1** Pairwise Kimura (1980) 2-parameter genetic distances  $\pm$  standard error among taxa, from the mtDNA 16S RNA gene above the diagonal and the COI gene below the diagonal

Taxon	<i>Congerina kusceri</i>	<i>Mytilopsis leucophaeta</i>	<i>Dreissena polymorpha</i>	<i>Dreissena bugensis</i>	<i>Corbicula fluminea</i>	<i>Mytilus edulis</i>
<i>C. kusceri</i>	—	0.059 $\pm$ 0.012	0.111 $\pm$ 0.017	0.113 $\pm$ 0.017	0.405 $\pm$ 0.038	0.617 $\pm$ 0.038
<i>M. leucophaeta</i>	0.205 $\pm$ 0.021	—	0.125 $\pm$ 0.018	0.130 $\pm$ 0.018	0.399 $\pm$ 0.037	0.638 $\pm$ 0.056
<i>D. polymorpha</i>	0.226 $\pm$ 0.021	0.204 $\pm$ 0.021	—	0.079 $\pm$ 0.014	0.423 $\pm$ 0.039	0.638 $\pm$ 0.056
<i>D. bugensis</i>	0.245 $\pm$ 0.023	0.243 $\pm$ 0.024	0.236 $\pm$ 0.023	—	0.402 $\pm$ 0.038	0.639 $\pm$ 0.056
<i>C. fluminea</i>	0.404 $\pm$ 0.033	0.444 $\pm$ 0.036	0.414 $\pm$ 0.055	0.445 $\pm$ 0.036	—	0.613 $\pm$ 0.054
<i>M. edulis</i>	0.723 $\pm$ 0.033	0.704 $\pm$ 0.053	0.732 $\pm$ 0.056	0.727 $\pm$ 0.055	0.625 $\pm$ 0.047	—



**Fig. 2** Neighbour-joining (NJ) tree for relationships among the dreissenoid and corbiculoid taxa from mtDNA COI sequence data, using Kimura (1980) 2-parameter genetic distances. This tree was congruent with the maximum-parsimonious (MP) tree. The two different haplotypes of *Congerina kusceri* are designated A and B. Percentage values at the nodes denote the bootstrap support values, with those for the NJ analysis preceding those for the MP analysis. The *g*-1 skewness value from the exhaustive MP search was  $-1.46$ , indicating significant skew, and thus high probability that the data set had sufficient signal to resolve the MP trees (see Swofford *et al.* 1996). The MP tree had 359 steps, a consistency index of 0.91, and a consistency index excluding uninformative characters of 0.79.

Ma (Stepien *et al.* 1999). The MP strict consensus tree from the three most parsimonious trees for the COI gene alone failed to distinguish the relationships among the three genera (Fig. 3).



**Fig. 3** Neighbour-joining (NJ) tree for relationships among the dreissenoid and corbiculoid taxa from mtDNA COI sequence data, using Kimura (1980) 2-parameter genetic distances. This tree was congruent with the strict consensus tree from the most parsimonious trees ( $n = 3$ ), except for an unresolved trichotomy denoting the relationships among the genera *Congerina*, *Mytilopsis*, and *Dreissena*. The four different haplotypes of *Congerina kusceri* are numbered, and the two haplotypes for *Dreissena bugensis* are lettered. Percentage values at the nodes denote the support values from 1000 bootstrap replications, with those for the NJ analysis preceding those for the MP analysis. The *g*-1 skewness value for the tree distribution produced during the MP search was  $-0.80$ , indicating significant skew (Swofford *et al.* 1996). The two MP trees had 605 steps, consistency indices of 0.84, and consistency indices excluding uninformative characters of 0.75.

#### Intraspecific variation

Our investigation revealed two haplotypes in the mtDNA 16S RNA gene region for *C. kusceri*. The haplotypes differ

by a transitional substitution from cytosine (the apparent ancestral condition found in six individual *C. kusceri* and in all specimens of *Dreissena* and *Mytilopsis*) to thymine (in three individuals). Measures of DNA polymorphism (Nei 1987) for the 16S RNA gene in *C. kusceri* included the number of polymorphic nucleotide sites per nucleotide position  $pn = 0.0024 \pm 0.0001$ , haplotypic diversity  $h = 0.503 \pm 0.005$ , and the average number of nucleotide differences among haplotypes (nucleotide diversity)  $\pi = 0.00050 \pm 0.00017$ . The Kimura 2-parameter genetic distance between the haplotypes was  $0.0022 \pm 0.0018$ . This difference indicates a possible divergence of approximately  $189\,000 \pm 150\,000$  years, suggesting their differentiation during the Pleistocene Ice Ages.

The COI gene sequences revealed four different haplotypes of *C. kusceri*. The haplotypes each differ by single substitutions, including two transitions and one transversion. None of these substitutions produces an amino acid change (Baldwin *et al.* 1996). Haplotype 1 characterized five individuals, haplotype 2 represented two individuals, and haplotypes 3 and 4 each characterized single individuals. Measures of DNA polymorphism (Nei 1987) for the COI gene included  $pn = 0.0052 \pm 0.0010$ ,  $h = 0.659 \pm 0.014$ , and  $\pi = 0.0006 \pm 0.0001$ . The Kimura 2-parameter genetic distance between the pairs of haplotypes was  $0.0017 \pm 0.0010$ . This difference indicates a possible genetic divergence of  $130\,000 \pm 77\,000$  years, which is congruent with the above estimate from the 16S RNA sequences.

## Discussion

### *Systematic relationships of Congeria*

Phylogenetic trees from mtDNA 16S RNA and COI sequences support monophyly of the Dreissenidae, consistent with morphological hypotheses (Nuttall 1990; Morton 1993). *Dreissena* is the sister group to a *Congeria*/*Mytilopsis* clade. The genera *Congeria* and *Mytilopsis* are sister taxa, which was hypothesized by Morton (1993) on the basis of anterior pedal retractor muscle attachment. The molecular clock calibration indicates that the genera diverged approximately 5–15 Ma, consistent with the mid to late Miocene separation hypothesized by Morton (1993).

### *Comparative genetic diversity of Congeria and its relatives*

Our study discerned two haplotypes for 16S rDNA and four for the COI gene, revealing considerably higher genetic diversity in *C. kusceri* than for the same sequence regions in populations of its surface-dwelling dreissenoid relatives (Baldwin *et al.* 1996; Stepien *et al.* 1999). The latter taxa have considerably greater distributional ranges and larger

population sizes today (Morton 1993; Stepien *et al.* 1999). A study of the same 16S rDNA region found no intra-specific variability in samples representing the ranges of *D. polymorpha* ( $n = 24$  individuals) and *D. bugensis* ( $n = 20$ ), yielding values of  $h = 0.00$  (Stepien *et al.* 1999) vs. 0.50 for *C. kusceri* (Results). Similarly, no intraspecific variation was found in sequences for the same region of the 16S rDNA gene in three samples of *M. leucophaeta* and seven *Corbicula fluminea* (both from single sites; Stepien *et al.* 1999).

Likewise, a previous study of the same region of the COI gene by Baldwin *et al.* (1996) found no intraspecific sequence variability in *D. polymorpha*, *M. leucophaeta*, or *C. fluminea*. The sole intraspecific variation discerned by Baldwin *et al.* (1996) was two different haplotypes of *D. bugensis* alone, differentiating the deeper-water white-coloured 'profundal' ecomorph in Lake Erie from the 'shallow' and more widespread morph in the lower Great Lakes ( $pn = 0.0065$ ). However, the profundal *D. bugensis* was not genetically divergent from the shallow form using nuclear random amplified polymorphic DNA (RAPD) markers, which evolve more quickly than the mtDNA COI and 16S RNA genes examined in the present study (Stepien *et al.* submitted). The profundal ecomorph of *D. bugensis* in Lake Erie is a close genetic match for specimens from the Ukraine. The lack of divergence between the two Great Lakes ecomorphs of *D. bugensis* suggests they are not biogeographically separated in their native range (Stepien *et al.* submitted), indicating that they are a naturally occurring single population (like the sample of *C. kusceri* in the present investigation). The profundal and shallow water ecomorphs of *D. bugensis* in Lake Erie probably were introduced from similar founding source populations, and the divergence between them is thus not greater than that within a single population (Stepien *et al.* submitted).

A study of North American unionid bivalves by King *et al.* (1999) using 710 bp of the same region of the COI gene discerned three haplotypes (differing by a single transition and a single transversion) for *Lasmigona subviridis* from nine locations and a total of 37 individuals ( $pn = 0.0028$ ). The species ranges south of the area covered by the Pleistocene glaciations in North America and was presumed less affected by climatic changes than were more northerly species (King *et al.* 1999). The level of variation for *C. kusceri* in our study thus appears relatively high, in comparison with the same genes in other bivalves.

Why is a troglodytic species, which is restricted to the Dinaric karsts of the former Yugoslavia (Morton *et al.* 1998), more variable in these slowly evolving genes? Greater genetic diversity may be explained by the buffering of the population from climatic fluctuations in the unique subterranean habitat of *C. kusceri*. Insulation from the climatic changes during the Pleistocene Ice Ages apparently

preserved its accumulated genetic variability and its population sizes and levels of gene flow have been large enough to maintain it. Our genetic distances (calibrated according to Stepien *et al.* 1999) suggest that the haplotypes of *C. kusceri* diverged from each other between approximately 100 000–200 000 years ago, corresponding to this period.

In contrast, populations of dreissenoids inhabiting the cold temperate surface waters were subjected to extreme climatic variations. During the Pliocene and Pleistocene Ice Ages, surface-water species contracted into small isolated populations in restricted southerly refugia (Cooper *et al.* 1995; Hewitt 1996; Taberlet *et al.* 1998). Such populations underwent bottlenecks and much of their genetic variability was lost (Nei *et al.* 1975), apparently due to stochastic lineage extinctions (Avice 1994, 2000). Subsequent rapid range expansion during the interglacials may have further decreased genetic variability, according to the 'leading edge hypothesis' (Hewitt 1996, 2000). This hypothesis suggests that the first colonizing individuals to follow the retreating glaciers would have founded the new populations, leading to the rapid exponential growth of their genotypes (Hewitt 1996; Stepien *et al.* 1998). Subsequently arriving individuals would have contributed less to the gene pools of the new populations, which had already reached their carrying capacities (Hewitt 1996). This population genetic pattern apparently characterizes many *r*-selected colonizing species, which tend to be excellent invaders – such as *Dreissena*, *Mytilopsis*, and *Corbicula* (Morton 1996).

In contrast, the subterranean populations of *C. kusceri* have existed for 5 million years, apparently uninterrupted by environmental disturbances (Morton *et al.* 1998). These populations maintained higher genetic diversity, derived from the steady accumulation of random mutations in selectively neutral areas, as identified in our study. Since mtDNA is very sensitive to population bottlenecks (Avice 1994, 2000), the constant subterranean environment and lack of fluctuations in population sizes would have preserved selectively neutral genetic changes. High genetic variability despite the smaller population sizes of *C. kusceri* compared to its surface-dwelling relatives (including species of *Dreissena*, *Mytilopsis*, and *Corbicula*), suggests that it may be the long-term maintenance of population size, rather than the current one, that is crucial to retaining genetic diversity. This hypothesis merits considerable further testing.

### Summary and conclusions

In our present-day human quest to rescue endangered species, we must keep in mind that once they have undergone marked reductions in effective population sizes, much of their genetic diversity is likely lost. This also

is true of present-day large populations, which appear to have rebounded from past bottlenecks, such as *Dreissena polymorpha*. Such decreased genetic diversity may not negatively affect the survival of the species, as evidenced by the colonizing success of *D. polymorpha*.

Retention of variability in slowly evolving genes may be indicative of constant historic environments and sustained population sizes, as found in the cold underground caves and rivers of the Dinaric karst of the former Yugoslavia. In contrast, climatic changes and resultant range alterations in surface-level habitats during the Pleistocene Ice Ages likely led to genetic drift and founder effects that significantly reduced variability in other dreissenoid molluscs. Changes in the genetic diversity and composition of populations, coupled with vicariant isolations – such as those produced by glaciation patterns – may lead to speciation (Avice 1994, 2000). In contrast, long-term maintenance of population size, a relatively constant reproductive rate, a presumably moderate gene flow, and *K*-selected species character traits coupled with isolation in an extreme environment can apparently lead to long-term retention of genetic diversity. Therefore, it is not predictive that a relatively small, specialized population will necessarily have low genetic diversity. Investigations of the genetic diversity of *Congerius kusceri* throughout its range will be valuable for further addressing these questions, along with analysis of nuclear DNA variation.

The cave faunas of the Dinaric karst of Slovenia, Croatia, and Bosnia-Herzegovina are highly endangered by ground-water pollution (Morton *et al.* 1998; Sket 1999). For example, the pit location from which our samples were collected has been used as a rubbish dump (Jalžic 1998). The *K*-selected population of *C. kusceri* houses significant genetic diversity that merits considerable further study, along with comparisons that are needed with other inhabitants of this unique environment (Sket 1999). Their 5 million years of troglodytic survival away from the extreme climatic fluctuations characteristic of surface-level aquatic habitats promises to reveal many secrets about the long-term effects of environmental constancy. Hopefully, we will not lose *C. kusceri* to anthropogenic disturbances before this can be accomplished.

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Carol Stepien's 'Great Lakes Environmental Genetics Laboratory' focuses on the conservation genetics of aquatic molluscs and fishes. Kora Dabrowska is an undergraduate research student in the laboratory. Brian Morton specializes in the Bivalvia. This study is part of an ongoing collaboration between Stepien and Morton. Stepien and Biljana Radja further plan to test population genetic diversity in *Congeria kusceri* from other locations, with the latter conducting that part of her Ph.D. dissertation in the Stepien laboratory.

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