

# Genetic effects of habitat fragmentation on blue sucker populations in the upper Missouri River (*Cycleptus elongatus* Lesueur, 1918)

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**Abstract** The blue sucker, *Cycleptus elongatus*, is a large catostomid fish that occurs in main stem rivers throughout the Mississippi basin of North America. Although not federally listed as threatened or endangered, populations are not considered stable in any of 21 states where they occur. Included in the range is the Missouri River, which flows more than 3,200 km from Montana to St. Louis, Missouri. Historically, *C. elongatus* was distributed continuously throughout the main stem Missouri and its major tributaries, but from 1952 to 1963, six major impoundments were constructed on the upper Missouri by the US Army Corps of Engineers. The resulting reservoirs have inundated and fragmented large riverine habitat from Yankton, South Dakota to the headwaters. *C. elongatus* still occurs in remnant stretches between reservoirs; however, little is known of the impacts of the dams on these populations. In order to test for such effects, 231 individuals from nine sites were genotyped at 14 variable microsatellite loci. An additional 142 individuals from six sites in the Mississippi River were also genotyped for comparative purposes. In the Missouri, allelic richness was reduced in inter-reservoir sites relative to those in the free flowing lower river. In addition, significant isolation by distance occurs in the Missouri, a pattern not present in the unimpounded Mississippi. These results are consistent with reduced intradrainage gene flow in the Missouri River and are the first to indicate effects of impoundments on genetic

structure in the system. This information will assist governing agencies in making informed decisions regarding conservation of *C. elongatus* in the Missouri River drainage and throughout the range.

**Keywords** *Cycleptus* · Conservation · Impoundment · Microsatellite · Isolation by distance

## Introduction

The past 100 years have witnessed a great deal of anthropogenic change to natural waterways in North America. Many rivers have been impounded to store water and regulate flow while others have been channelized to facilitate navigation. In addition, locks and dams have been constructed in a number of systems to further control water levels for navigation. Consequently, migration and reproduction in many riverine fishes have been negatively impacted because routes to historical spawning grounds have been compromised (Ickes et al. 2001; Jungwirth et al. 1998; Laroche and Durand 2004).

The blue sucker, *Cycleptus elongatus*, is a large catostomid fish native to main stem rivers throughout the Mississippi basin and is one of the most widespread lotic fishes in North America. Its elongate, hydrodynamic body form, with paired anteriorly rounded fins, enables it to maintain its position in swift current while expending little energy (Wolter and Arlinghaus 2003). Historically, the species occurred in 21 conterminous states, but is now endangered or extirpated in four (New Mexico, West Virginia, Ohio, and Pennsylvania) (NatureServe 2005) and is a candidate for federal listing (Elstad and Werdon 1993).

The range of *C. elongatus* has diminished greatly over the past 100 years and it is thought that impoundment of

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main stem rivers has played a major role (Boschung and Mayden 2004; Robison and Buchanan 1998). In the Missouri River drainage, *C. elongatus* formerly inhabited all reaches of the main stem from its confluence with the Mississippi River at St. Louis to the far northern headwaters of the Missouri and Yellowstone rivers in Montana (Brown 1971; Underhill 1959), a distance of over 3,200 km. From 1952 to 1963, six major impoundments were constructed on the upper Missouri River by the Army Corps of Engineers to control flooding and provide hydroelectric power (Hesse et al. 1982). The resulting reservoirs have inundated and fragmented large riverine habitat from Yankton, South Dakota to the headwaters in Montana, reducing main stem riverine habitat in the intervening stretches by an estimated 70% (based on length of reservoirs at full pool—see USACE 2001). Following construction of the dams, blue suckers and other migratory species have amassed in the tailwaters at times coinciding with seasonal spawning movements (Eitzmann et al. 2005; Walburg et al. 1971). Blue suckers are, indeed, riffle spawners that swim up tributaries to reach suitable habitat (i.e., flooded gravel bars) and are known to travel great distances to spawn (Moss et al. 1983; Peterson et al. 2000).

Although *C. elongatus* still occurs in stretches between reservoirs, it is unknown whether these represent viable populations and whether any gene flow occurs between them. A “best case” hypothesis is that there is enough reproduction and recruitment to maintain genetic diversity and allow for at least some level of downstream gene flow while an alternative is that there is no reproduction and recruitment with concordant genetic isolation between inter-reservoir stretches. It is important to note that there are no modifications to allow upstream migration (i.e., no “fish ladders”) over or around any of these dams. A third alternative, based on the biology of these fishes, is that little, if any downstream gene flow occurs because adults are not suited to the lentic characteristics of reservoirs and probably avoid them. Also, larval drift from tributaries above reservoirs may settle to the bottom or be consumed by predators before it can pass through floodgates or turbines, thus limiting gene flow and recruitment.

From this point hereafter, the term “population” refers to the evolutionary paradigm set forth by Waples and Gaggiotti (2006). In this context, the following specific hypotheses regarding impoundment effects were tested in Missouri River populations: (1) inter-reservoir populations in the upper Missouri River will show an overall reduction in genetic diversity compared to those in open sections of the lower river; (2) population subdivision will be non-negligible based on one or more analyses of molecular variance and Bayesian cluster analysis (due to reduced gene flow and increased effects of genetic drift); (3) Missouri River populations will show a significant pattern of

isolation by distance (IBD) due to a lack of homogenizing gene flow; and (4) inter-reservoir populations will show a signal of recent decline in number as evidenced with an excess in observed heterozygosity.

## Materials and methods

### Study location and individual collection

Two-hundred and thirty-one blue suckers were collected from six main stem localities and three tributaries of the Missouri River, spanning a distance of 3,021 river km. For comparative purposes, an additional 142 individuals were collected from six sites in the Mississippi River and its tributaries, spanning 2,593 river km. The Mississippi main stem is the single most appropriate choice for comparison as it covers a similar length and latitudinal gradient but is not subject to impassable dams and impoundments. Although locks and dams have been put in place at various points, fish passage still readily occurs through these corridors (Ickes et al. 2001). To ensure adequate representation throughout the two systems, all inter-reservoir populations were targeted in the upper Missouri while stratified sampling was employed in the lower reaches of the Missouri (i.e., below Lewis and Clark Reservoir, the southern-most dam and impoundment) as well as the Mississippi (Table 1; Fig. 1). Note that Missouri site A is the same as Mississippi site 3. When direct statistical comparisons between the two systems were conducted, analyses were performed with and without this site. At no time did the inclusion or exclusion of the site alter conclusions of significance. In global analyses (i.e., summaries in Appendix 1; Bayesian STRUCTURE analyses), this site was included only once.

All sampling occurred between November 2004 and November 2005. Individuals were captured using hoop nets, gill nets, and electroshocking devices. A small (1 cm<sup>2</sup> or less) fin clip was removed from each specimen and preserved in 95% EtOH for subsequent genetic work. All tissues are vouchered in the personal collection of MLB and are freely available upon written request.

### DNA preparation and amplification

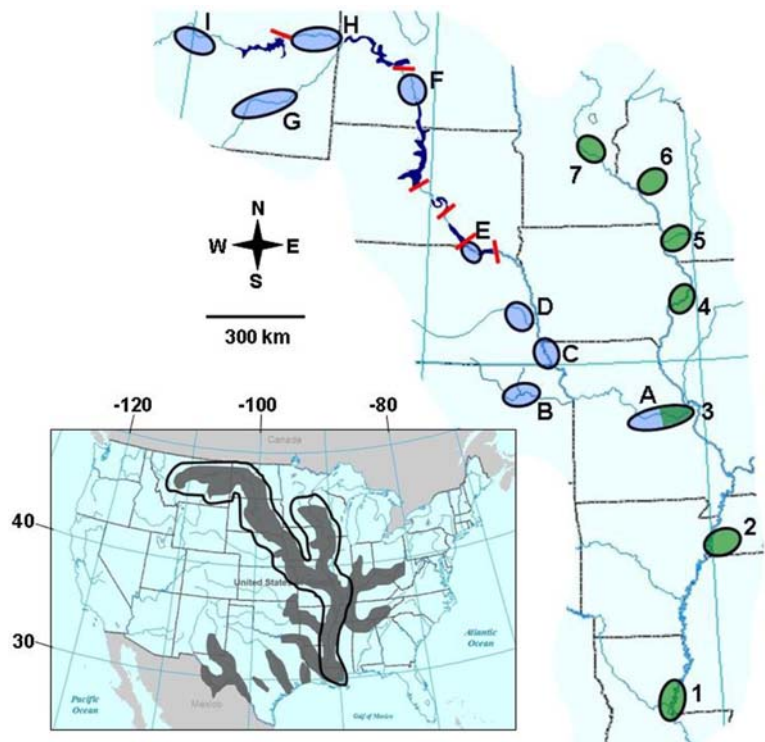
DNA was extracted from tissue samples using either a standard phenol–chloroform protocol (Sambrook et al. 1989) or a DNeasy<sup>®</sup> Tissue purification kit (Qiagen, Valencia, CA). DNAs were eluted in either water or buffer (EB) supplied by Qiagen. A small number of samples were randomly selected and (1–2 µl) electrophoresed through a 1% agarose gel to check for quality (high molecular

**Table 1** Site-specific summary statistics

	River/state	CL	n	na	Gene diversity ( $H_O$ )	$H_E$	A
A	Missouri/Missouri	38°68'48"N 90°66'96"W	30	140	0.700	0.688	7.340
B	Kansas/Kansas	39°11'20"N 96°31'03"W	6	62	0.576	0.729	–
C	Missouri/Nebraska	40°81'49"N 95°84'43"	38	138	0.704	0.745	7.290
D	Platte/Nebraska	41°05'13"N 96°10'83"W	23	128	0.698	0.661	7.311
E	Missouri/South Dakota	42°76'47"N 97°98'81"W	29	142	0.717	0.692	7.271
F	Missouri/North Dakota	47°25'42"N 101°24'34"W	14	96	0.690	0.698	6.343
G	Yellowstone/Montana	46°43'36"N 96°31'03"W	30	134	0.691	0.732	6.629
H	Missouri/Montana	48°03'42"N 106°91'72"W	31	132	0.699	0.727	6.902
I	Missouri/Montana	47°73'71"N 109°61'83"W	29	125	0.686	0.675	6.591
Missouri summary			$n = 231$	Total $na = 194$	Mean = 0.698	Mean = 0.702	Mean = 6.960
1	Mississippi/Louisiana	31°03'51"N 91°35'37"W	32	127	0.679	0.700	7.510
2	Hatchie/Tennessee	35°31'77"N 89°15'96"W	19	101	0.751	0.759	7.088
3	Missouri/Missouri	38°68'48"N 90°66'96"W	30	140	0.700	0.688	7.340
4	Mississippi/Iowa	41°27'12"N 90°47'36"W	11	92	0.727	0.767	7.667
5	Wisconsin/Wisconsin	43°12'31"N 90°75'11"W	32	123	0.713	0.746	7.226
6	Chippewa-Red Cedar/Wisconsin	44°48'60"N 92°05'71"W	30	121	0.730	0.765	7.302
7	Minnesota/Minnesota	44°53'71"N 93°90'09"W	18	101	0.751	0.754	7.149
Mississippi summary			$n = 172$	Total $na = 189$	Mean = 0.722	Mean = 0.740	Mean = 7.326

CL central locality (lat/long) of sampling area,  $n$  number of individuals sampled,  $n_a$  number of alleles, Gene diversity ( $H_O$ ) observed heterozygosity,  $H_E$  expected heterozygosity, A allelic richness

**Fig. 1** Sampling area for nine localities (A–I) in the Missouri River and Mississippi River (1–7) main stems. Letters correspond to localities listed in Tables 3 and 5. Solid lines indicate Missouri River dam locations. Note that site A in the Missouri and site 3 in the Mississippi are the same site. As indicated in the text, care was taken regarding the inclusion or exclusion of this site in relevant comparative analyses



weight). All samples were quantified with a GeneQuant II spectrophotometer (Pharmacia Biotech, Piscataway, NJ) and a portion of each was diluted to a working stock concentration of 100 ng/ $\mu$ l. The remainder of each elution was placed in  $-70^{\circ}\text{C}$  for long-term storage. As with tissue samples, DNA extractions are maintained in the personal collection of the author (MLB) and are freely available upon written request.

Eleven microsatellite loci previously isolated from *C. elongatus* (Bessert et al. 2007) were chosen for this study. In addition, 16 primer pairs designed for other catostomid taxa were screened and optimized in *C. elongatus*. This resulted in the addition of four more loci: Mox294, Mox306, and Mox329 (Lippe et al. 2004), and Dlu4235 (Tranah et al. 2001).

In order to increase the efficiency of data collection, primer pairs were screened for potential cross-reactivity in multiplex reactions using the software AutoDimer (Vallone and Butler 2004) and two multiplex reactions were precisely optimized to provide for relatively equal signal strength according to guidelines provided by Henegariu et al. (1997). If two loci (in the same reaction) were labeled with the same fluorophore, special care was taken that the allelic size ranges were non-overlapping. In this way, 15 loci were successfully amplified in only two reactions, as follows (also see Table 2). Reaction "A" (Table 2) contained the following: 1.60  $\mu$ l dNTPs (1 mM), 1.20  $\mu$ l PCR

reaction buffer (10 $\times$ ), 0.80  $\mu$ l MgCl (50 mM), forward and reverse primers for eight loci (see Table 2 for specific loci and concentrations), 0.12  $\mu$ l Taq DNA polymerase (Gibco BRL), 1.0  $\mu$ l DNA (=100 ng), and ddH<sub>2</sub>O to a total volume of 10  $\mu$ l. Thermal cycling conditions were as follows: 94 $^{\circ}\text{C}$  (1 min), 30 cycles of 94 $^{\circ}\text{C}$  denature for 30 s, 55.8 $^{\circ}\text{C}$  anneal for 40 s, and 65 $^{\circ}\text{C}$  extension for 2 min, followed by a final extension of 65 $^{\circ}\text{C}$  for 5 min and a holding temperature of 4 $^{\circ}\text{C}$ . Reaction "B" (Table 2) contained the following: 1.60  $\mu$ l dNTPs (1 mM), 1.20  $\mu$ l PCR reaction buffer (10 $\times$ ), 0.88  $\mu$ l MgCl (50 mM), forward and reverse primers for seven loci (Table 2), 0.14  $\mu$ l Taq DNA polymerase (Gibco BRL), 1.0  $\mu$ l DNA (=100 ng), and ddH<sub>2</sub>O to a total volume of 10  $\mu$ l. Thermal cycling conditions were the same as those for reaction "A" except the annealing temperature was 54.8 $^{\circ}\text{C}$ .

Note that a seven nucleotide "PIG-tail" (GTTTCTT) was added to the 5' end of the reverse (unlabeled) primers for three of the four dinucleotide loci (Ce35, Ce63, and Ce104) in order to improve adenylation, reduce stutter, and ease scoring (see Brownstein et al. 1996). The fourth dinucleotide locus, Ce126, elicited discrete bands in preliminary screenings (Bessert et al. 2007) while the remainder 11 loci possessed either tri- or tetranucleotide repeat motifs (Table 2) which tend to stutter far less frequently and are more easily scored (O'Connell and Wright 1997), as was the case here.

**Table 2** Locus name, repeat type, allelic size range in screening population, fluorophore label, and picomoles of primer used in each reaction for 15 microsatellite markers used in this study

Locus	Repeat type	Allelic size range (bp)	Fluorophore	Units in final volume (pmol)
Reaction "A"				
Ce13S	tetra	120–192	6-FAM	3.0
Ce35	di	124–140	PET <sup>a</sup>	3.2
Ce52	tetra	233–245	6-FAM	3.0
Ce126	di	166–178	NED <sup>a</sup>	3.2
Ce215	tetra	215–303	NED <sup>a</sup>	5.0
Mox306	tetra	175–227	VIC <sup>a</sup>	4.8
Mox329	tetra	158–218	PET <sup>a</sup>	3.2
Ce104	di	144–152	VIC <sup>a</sup>	2.0
Reaction "B"				
Ce13L	tetra	142–162	6-FAM	2.6
Dlu4235	tetra	131–187	PET <sup>a</sup>	4.4
Ce49	tri	106–109	6-FAM	3.0
Ce63	di	152–268	VIC <sup>a</sup>	4.8
Ce146	tetra	144–156	NED <sup>a</sup>	2.8
Ce195	tetra	240–244	NED <sup>a</sup>	3.2
Mox294	tetra	227–271	PET <sup>a</sup>	5.4

<sup>a</sup> Proprietary dyes from Applied Biosystems Inc.

### Sample preparation and data collection

Prior to genotyping, PCR products were purified with a Mini-Elute (Qiagen) or Microarray PCR purification kit (Telechem International Inc., Sunnyvale, CA) to remove residual unincorporated fluorescently labeled primers that can obscure allele signatures in an electropherogram (Butler 2002). Products were prepared for electrophoresis by mixing (0.5  $\mu$ l PCR product) with 0.5  $\mu$ l LIZ 500 size standard (Applied Biosystems, Foster City, CA) and 9.0  $\mu$ l deionized formamide. Samples were denatured at 95 $^{\circ}\text{C}$  for 3 min and quenched on ice for 2 min prior to capillary electrophoresis on an ABI 310 fragment analyzer. Allele sizes were scored with GeneMapper 3.7 (Applied Biosystems) and the data transformed to GenePop format (Raymond and Rousset 1995) with GMCONVERT 0.32 (Faircloth 2006).

Genotypic data were checked for typographical errors, evidence of null alleles [Hardy–Weinberg equilibrium (HWE)], and adherence to the Stepwise Mutation Model (SMM) (Ohta and Kimura 1973) with MicroChecker 2.2.3 (VanOosterhout et al. 2004) and MSAnalyzer 4.0 (Dieringer and Schlötterer 2002). Additional transformations of the data set to other formats were performed with

CONVERT 1.3.1 (Glaubitz 2004) and Genepop 3.3 (Raymond and Rousset 1995).

#### Data analysis: summary statistics and fixation indices

In addition to observed and expected heterozygosities for each locality, deviations from HWE and pairwise  $F_{ST}$  estimates (Weir and Cockerham 1984) were calculated for each locality and globally for each locus using GenePop3.3 (Raymond and Rousset 1995). The program FSTAT 2.9.3.2 (Goudet 2002) was also used to calculate the average allelic richness ( $A$ ) at each locality and for each locus individually. Allelic richness is a useful parameter for comparing samples of different sizes because it scales results to the smallest sample included (Goudet 2002). Pairwise  $t$ -tests were then performed to test for differences in mean allelic richness between free-ranging (sites A–D) and impounded populations (E–I) in the Missouri and between all free-ranging (sites A/3, B–D, 1–2, and 4–7) and impounded (E–I) populations. In addition, FSTAT was used to calculate pair-wise  $F_{ST}$  values for sites within each river system. Since 12 loci did not deviate from expectations of the SMM (Ohta and Kimura 1973; Valdes et al. 1993; Wehrhahn 1975), they were used to calculate  $\rho$ , an estimator of  $R_{ST}$  values (Valdes et al. 1993), with RST-CALC (Goodman 1997), for comparative purposes.

#### Population structure

In order to investigate the distribution of genetic variation within and among collection sites as well as that between impounded (sites E–I) and unimpounded (sites A–D) sites in the Missouri, an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed with Arlequin 3.1 (Excoffier et al. 1992; Schneider et al. 2000). This test was executed under both the standard model (“different alleles are considered mutationally equidistant from each other”) and the SMM. As before, two loci were excluded from the latter due to inconsistencies with the SMM (i.e., gaps in allelic distribution).

Another useful method that does not require a priori assumptions regarding population structure is the Bayesian clustering technique implemented in STRUCTURE 2.0 (Pritchard et al. 2000). Here, genetic data can be used to define the number of subpopulations ( $k$ ) in the absence of any locality data. The method assumes HWE within subpopulations and seeks combinations of individuals that maximize HWE at a pre-determined value of  $k$ . Four simulations were executed using the full Missouri + Mississippi data set ( $n = 373$ ) at  $k = 1$  to 9 under the admixture model with independent allele frequencies and  $\lambda$  fixed at 1;

that is, the model assumes that allele frequencies in each subpopulation are independent from one another (i.e., independent draws from a distribution defined by  $\lambda$ ). Preliminary simulations were performed to determine appropriate burn-in and run lengths and real time plots of  $\alpha$  (parameter for the degree of admixture) and other parameter estimates were monitored to determine when convergence occurred. For all subsequent simulations, a conservative burn-in period of 250,000 generations was followed by 1,000,000 generations of data collection. Posterior probabilities for  $k$  were computed based on the mean log likelihood of the data from 4 to 5 simulations at each value of  $k$  (see Pritchard and Wen 2004).

Several analyses were performed to determine whether the data from each of the two river systems were concordant with a model of genetic IBD (Wright 1943). This model predicts that gene flow will be negatively correlated with geographic distance between populations. That is, when the homogenizing effects of gene flow are reduced, the effects of drift become more pronounced and distant populations tend to diverge at neutral genetic loci. In order to test for IBD, central localities (CL) for each sampling area were estimated from locality plots of each individual collected, and a matrix of linear distances between the CL for all pairs of populations was calculated. The web-based software package IBDWS (Jensen et al. 2005) was used to calculate a matrix of pairwise genetic differentiation between populations ( $F_{ST/1} - F_{ST}$ ) (Rousset 1997) and to perform a Mantel test with 10,000 random permutations between matrices of pairwise linear and genetic distances between populations. Additional Mantel tests were also performed between each of the following matrix combinations for each of the two river systems: genetic differentiation versus ln (linear distance); ln (genetic differentiation) versus linear distance; and ln (genetic differentiation) versus ln (linear distance).

#### Demography

BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) was used to investigate the possibility of recently reduced effective population sizes in the Missouri River sampling areas. The method compares gene diversity (expected heterozygosity,  $H_e$ ) with the expected equilibrium gene diversity ( $H_{eq}$ ) that is computed from the observed number of alleles ( $n_a$ ) under mutation/drift equilibrium. If a significant number of loci show an excess in gene diversity then the population has likely undergone a recent bottleneck. A Wilcoxon sign-rank test was used to make this determination. The two-phase mutation model (TPM) was used because it more realistically describes microsatellite evolution than either the strict SMM or infinite alleles (IAM) model. In the TPM, a 95%

frequency of stepwise mutations was assumed with a 12% variance of multiple-step mutations.

## Results

### Marker screening

One locus, Ce63, showed a heterozygote deficit across all sample sites. This pattern is consistent with the presence of null alleles; thus, Ce63 was excluded from further analyses. In addition, two loci, Mox329 and Ce215 exhibited significant gaps in their allele frequency distributions. While neither deviated from HW expectations, the mutational patterns clearly deviated from SMM expectations. Consequently, these two markers were withheld from any analyses that assumed the stepwise model.

### Summary statistics and fixation indices

Summary statistics for each locality are presented in Table 1 while global estimates for each individual locus

(based on the full data set;  $n = 373$ ) are shown in Appendix 1. With the exception of site B, which had a very small sample size ( $n = 6$ ), gene diversity (i.e., observed heterozygosity) was only slightly reduced in impounded populations, and not significantly so ( $\text{mean}_{A-D} = 0.701$ ,  $\text{mean}_{E-I} = 0.697$ ;  $t = 0.544$ ,  $df = 6$ ,  $p = 0.61$ ). However, in a similar site comparison of mean allelic richness ( $A$ ), the impounded upper Missouri harbored significant reductions relative to the lower Missouri ( $\text{mean}_{A-D} = 7.31$ ,  $\text{mean}_{E-I} = 6.75$ ;  $t = 2.68$ ,  $df = 6$ ,  $p = 0.036$ ); thus, supporting the hypothesis of reduced genetic diversity in populations isolated by impoundments. When this analysis was extended to include additional unimpounded sites from the Mississippi main stem and lower tributaries (see Table 1), the pattern was even more highly significant ( $\text{mean}_{\text{others}} = 7.32$ ,  $\text{mean}_{E-I} = 6.75$ ;  $t = 3.68$ ,  $df = 15$ ,  $p = 0.002$ ). Pairwise  $F_{ST}$  and  $R_{ST}$  comparisons for both river systems are shown in Table 3. Sequential Bonferroni adjustments for multiple comparisons (Rice 1989) resulted in adjusted values of 0.001786, 0.000358, and 0.000036 in the Missouri and 0.002381, 0.000476, and 0.000048 in the Mississippi for  $\alpha = 0.05$ , 0.01, and 0.001, respectively.

**Table 3** Pairwise  $R_{ST}$  and  $F_{ST}$  comparisons for Missouri and Mississippi River sample sites

	$R_{ST}$							
	A	C	D	E	F	G	H	I
$F_{ST}$								
Missouri River								
A	–	0.0007	–0.0039	–0.0001	0.0068	0.0085	0.0118	0.0720***
C	–0.0007	–	0.0011	–0.0019	0.0173	0.0111	0.0141	0.0580***
D	–0.0039	–0.0030	–	0.0043	0.0159	0.0063	0.0093	0.0732***
E	0.0005	–0.0009	–0.0029	–	–0.0065	–0.0040	–0.0019	0.0281
F	0.0113	0.0108*	0.0068	–0.0035	–	–0.0108	0.0050	0.0215
G	0.0150***	0.0093***	0.0095	0.0013	0.0056	–	–0.0019	0.0273
H	0.0109***	0.0105***	0.0074*	0.0006	0.0001	0.0011	–	0.0524**
I	0.0371***	0.0291***	0.0363***	0.0108	0.0094	0.0102	0.0214***	–
Mississippi River								
	1	2	3	4	5	6	7	
1	–	–0.0048	0.0128	–0.0036	0.0110	–0.0001	0.0228	
2	0.0027	–	0.0334	0.0187	0.0213	0.0150	0.0219	
3	0.0020	0.0037	–	–0.0155	0.0081	0.0084	0.0115	
4	–0.0014	–0.0027	–0.0055	–	–0.0013	–0.0099	0.0034	
5	0.0048	0.0014	0.0002	–0.0023	–	0.0062	0.0308	
6	0.0061	0.0030	0.0019	–0.0025	–0.0012	–	0.0208	
7	0.0130	0.0057	0.0117	0.0000	0.0085	0.0036	–	

Above diagonal:  $R_{ST}$ ; below diagonal:  $F_{ST}$ . Sequential Bonferroni adjustments (Rice 1989) for  $\alpha = 0.05$ , 0.01, and 0.001 were 0.001786, 0.000358, and 0.000036 for the Missouri and 0.002381, 0.000476, and 0.000048, for the Mississippi, respectively. Note that Missouri site B was excluded due to small sample size

\* Significant at the 5% level

\*\* Significant at the 1% level

\*\*\* Significant at the 0.1% level

Following these corrections, ten significant values remained, all within the Missouri River. Eight occurred between pairs that were among the most distant from one another and seven of these were significant at  $\alpha = 0.001$ , suggesting the possibility of genetic IBD.

Population structure and demography

Both analyses of molecular variance revealed that more than 98% of the total variation occurred within populations, that is, within Missouri River sample sites (Table 4). Roughly 1% occurred among populations above and below the lowest impoundment (sites A–D versus E–I), while a very modest amount (est. 0.38 and 0.31%) occurred between populations.

In Bayesian analyses of population structure (Pritchard et al. 2000) in the full data set,  $k = 3$  yielded the highest mean posterior probability over 4–5 separate simulations. The results of the single run with the highest posterior probability are shown in Fig. 2, which indicates that the Mississippi data set (including site 3/A) forms a single cluster while the Missouri is split into two clusters based in the upper and lower portions of the river with highly

admixed populations between. Pritchard and Wen (2004) suggest caution in interpreting results when a value of  $k = 3$  or less is indicated. In order to validate the results from the Missouri River, Nei’s standard distance (1978) was calculated for all population pairs (Table 5) and the results were used to construct a neighbor joining dendrogram (Fig. 3).

Importantly, Mantel tests for IBD in the Missouri River were highly significant ( $p \leq 0.001$ ) with and without site 3/A, i.e., genetic distance ( $F_{ST}$ ) and  $\ln$  (genetic distance) versus geographic distance and  $\ln$  (geographic distance). The correlation between untransformed  $F_{ST}$  and geographic distance is presented in Fig. 4a ( $Z = 306.2966$ ,  $r = 0.6917$ ; null hypothesis of  $r \leq 0$ : one sided  $p = 0.0010$  from 10,000 randomizations). Slopes and negative correlations were similar for the other three Mantel tests, indicating a clear pattern of IBD in the Missouri River.

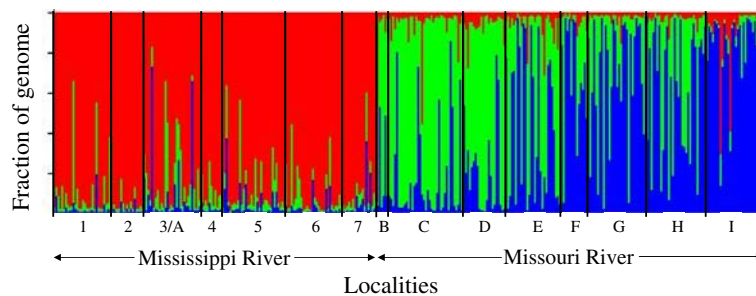
Conversely, the Mississippi River data set, which spans 2,493 river km (versus 3,021 from the Missouri) and covers a similar latitudinal gradient, elicited no signal of IBD (again, with and without site 3/A). The results of a Mantel test for IBD in this data set are presented in Fig. 4b, where untransformed  $F_{ST}$  and geographic distances are plotted against one another. The correlation coefficient was much

**Table 4** Missouri River AMOVA summaries under the standard model followed by the microsatellite (SMM) model, as implemented in Arlequin 3.1 (Schneider et al. 2000)

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices
Among groups	1	17.177 (13.270)	0.05165 Va (0.03944 Va)	1.05 (0.98)	$F_{CT}$ : 0.01045* (0.01045*)
Among populations within groups	7	40.303 (31.978)	0.01871 Vb (0.01237 Vb)	0.38 (0.31)	$F_{SC}$ : 0.00383* (0.00383)
Within populations	437	2,128.168 (1,739.923)	4.86995 Vc (3.98152 Vc)	98.58 (98.72)	$F_{ST}$ : 0.01424* (0.01424*)
Total	445	2,185.648 (1,785.170)	4.94031 (4.03333)		

The group level refers to (1) impounded and (2) unimpounded populations. Fixation  $p$ -values are derived from Mantel tests (1,023 permutations) calculated with Arlequin 3.1

\* Significant at  $\alpha = 0.05$



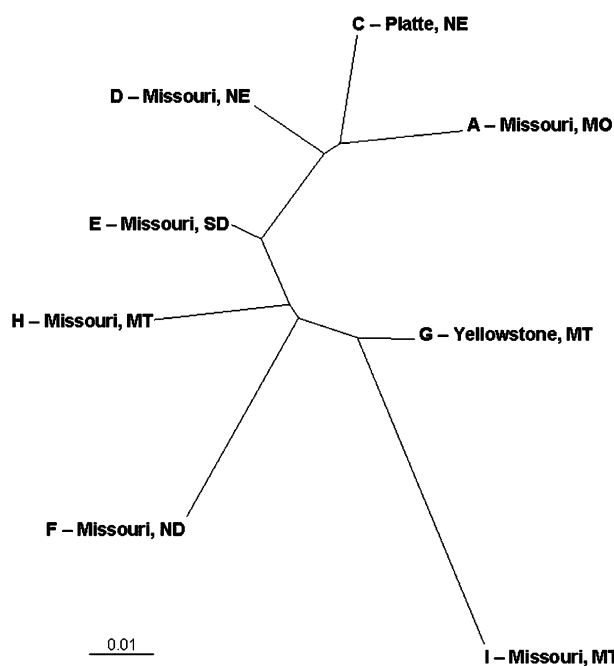
**Fig. 2** Bayesian inference of population structure in the Missouri and Mississippi Rivers as determined with STRUCTURE 2.0 (Falush et al. 2003; Pritchard et al. 2000);  $k = 3$  elicited the highest posterior probability for the number of genetic subgroups as indicated here.

Vertical colored bars indicate the fraction of an individual’s genome that has ancestry in a given subgroup (one of two subgroups in this case). Localities correspond to Table 1

**Table 5** Pairwise genetic distance (Nei’s standard distance) between Missouri River populations (Nei 1978) as calculated with the Phylip software package (Felsenstein 1989)

Sites	C	D	E	F	G	H	I
A	0.0333						
C	0.0359	0.0341					
D	0.0422	0.0352	0.0403				
E	0.0854	0.0808	0.0818	0.0535			
F	0.0727	0.0555	0.0655	0.0421	0.0702		
G	0.0644	0.0599	0.0620	0.0420	0.0592	0.0395	
H	0.1291	0.1052	0.1324	0.0657	0.0791	0.0599	0.0883

Site B was excluded due to small sample size



**Fig. 3** Neighbor joining dendrogram for Missouri River sample sites based on Nei’s (1978) standard distance

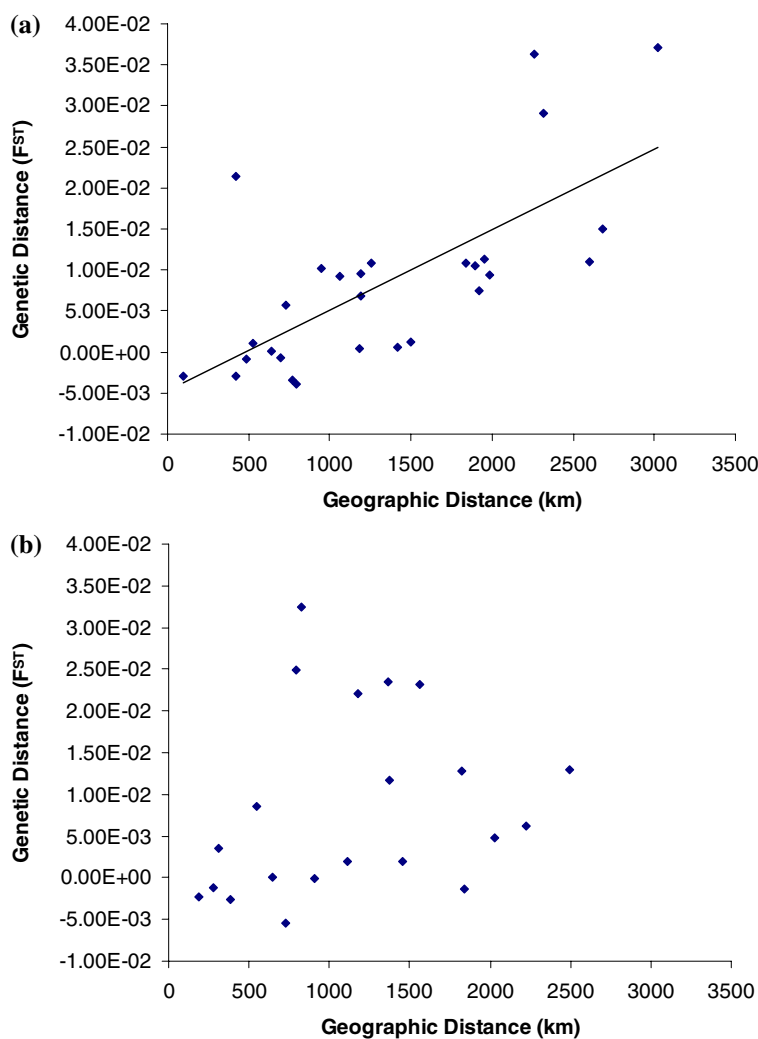
reduced ( $Z = 148.1843$ ,  $r = 0.2359$ ) in comparison to the Missouri River and the relationship was clearly non-significant (null hypothesis of  $r \leq 0$ : one sided  $p = 0.1790$  from 10,000 randomizations). The other three possible matrix combinations—pairwise  $F_{ST}$  versus  $\ln$  (linear distance);  $\ln(F_{ST})$  versus linear distance; and  $\ln(F_{ST})$  versus  $\ln$  (linear distance)—were tested and also proved non-significant. When the analysis was restricted to include only sites above the confluence, the IBD signature in the two rivers was only slightly less disparate ( $p = 0.1749$  in Mississippi versus  $p = 0.001$  in Missouri).

Finally, multilocus frequencies of observed heterozygosity were slightly higher than expected at seven of the nine sampling locations; however, no recent bottlenecks were detected at any of the sample sites via the methods used in BOTTLENECK 1.2.02 (Cornuet and Luikart 1996).

### Discussion

Our results suggest that impoundments have significantly impacted genetic structure in *C. elongatus* populations of the upper Missouri River. Gene diversity is somewhat uniform throughout and there is no evidence for recent bottlenecks; however, allelic richness differs significantly between populations above and below the lowest impoundment (Table 1). A reduction in allelic diversity is frequently associated with invasive (Genton et al. 2005) or founding populations (Ramstad et al. 2004), but may also indicate population decline (Faugeron et al. 2004). Although it is possible that this type of structure was present prior to the construction of the dams, this would be inconsistent with allelic richness estimates from other northerly drainages within the Mississippi Basin (Table 1). Indeed, one of the highest estimates of allelic richness occurred in the upper Wabash River of central and northern Indiana ( $40^{\circ}76'65''N/85^{\circ}95'42''$ ;  $A = 7.638$ ), an area not included in the present study. This area, like the upper Missouri, was covered with ice during Pleistocene glaciations (Hewitt 2000). In the Missouri River drainage, it is not difficult to imagine a loss of genetic diversity through the physical loss of individuals (i.e., one-way gene flow through the dams) or through the enhanced effects of genetic drift in populations that are either recently reduced or isolated from others of their kind (i.e., limited gene flow, in this case due to impassable dams). One peculiarity in these estimates is the elevated level of allelic richness at site E (Table 1) relative to other impounded populations. Indeed, when site E is excluded from comparisons of allelic richness between impounded and unimpounded sites, the difference between the two is even greater ( $\text{mean}_{A-D} = 7.31$ ,  $\text{mean}_{E-I} = 6.61$ ;  $t = 6.0$ ,  $df = 3$ ,  $p = 0.004$ ). It is difficult to explain the elevated allelic richness at site E, but one possibility is that this population is simply not as profoundly affected by the dams because the area contains a relatively long, unmodified inter-reservoir stretch with access to high quality spawning areas in

**Fig. 4** Mantel test for matrix correlation between genetic distance ( $F_{ST}$ ) and geographic distance. Pairwise  $F_{ST}$  is plotted against geographic distance; **(a)** Missouri River samples:  $Z = 306.2966$ ,  $r = 0.6917$ ; null hypothesis of  $r \leq 0$ : one sided  $p = 0.0010$  from 10,000 randomizations; **(b)** Mississippi River samples:  $Z = 129.1548$ ,  $r = 0.3067$ ; null hypothesis of  $r \leq 0$ : one sided  $p = 0.1679$  from 10,000 randomizations



at least one prominent tributary, the Niobrara River (see site E, Fig. 2). In other words, it may still harbor a large population relative to other inter-reservoir areas. Upstream passage through the lowest dam is extremely doubtful; however, it is also possible that allelic richness is bolstered here because the area may act as a “sink” for larval drift that survives passage from upstream populations.

The time period for accumulation of genetic signatures originating from construction of dams seems relatively short (60 years); however, the array of molecular tools employed is powerful enough to detect such effects, if present (e.g., Table 2; mean number of alleles ( $n_a$ ) = 122 per site). Still, it is also important to consider the generation time for *C. elongatus*, which is ~10 years (Becker 1983). Given the accompanying lag time, detection of a significant bottleneck (Cornuet and Luikart 1996) would indicate an extreme situation for the affected populations, and this was not the case. In other vertebrates that have been subject to recent anthropogenic habitat fragmentation, fixation measures and coalescent techniques have revealed

highly significant and dynamic population subdivision after as little as 100–200 years (Hitchings and Beebee 1997) or even shorter time frames (Poissant et al. 2005; Stamford and Taylor 2005). Here, AMOVA based on both Rho and  $F$ -statistics indicated that the vast majority of variance (>98%) occurred within populations, not between. In addition, average  $F_{ST}$  among impounded populations (0.006) was only slightly elevated over that between unimpounded populations in the lower Missouri (effectively, zero) and the difference was not significant ( $p = 0.54$ ).

Conversely, a highly significant pattern of IBD was detected among Missouri River populations (Fig. 4a). Pairwise comparisons of both types of fixation statistics ( $F_{ST}$ ,  $R_{ST}$ ) were also consistent with this pattern as significant divergence occurred between (generally) the most geographically distant populations in the Missouri. While this may seem intuitive or trivial given that sample sites span a distance of nearly 3,050 river km, it is important to note that cycleptid fishes are known to migrate hundreds of kilometers to spawning grounds (Mettee and Shepard

1997). In an unobstructed waterway of similar length, it is conceivable that these behaviors could mitigate distance effects and elicit a signature of—or tending toward—panmixia. In this light, it is worthy to note that the majority of main stem tributaries (e.g., Red, Missouri, Ohio, and Tennessee) within the Mississippi Basin have been impounded at some point (Benke and Cushing 2005) while the main stem Mississippi itself has not. A long series of locks and dams was constructed over a distance of several hundred kilometers in the upper Mississippi, but fish passage is still possible through these corridors (Ickes et al. 2001); therefore, the lack of IBD and the concurrent lack of significant pairwise  $F_{ST}$  and  $R_{ST}$  estimates (Table 3) in this system does not come as a surprise.

Bayesian analysis of structure in the combined Missouri/Mississippi data set yielded an estimate of three genetic clusters. Clearly, the Mississippi data set (including site 3/A) forms a single distinct cluster with only moderate admixture. This is consistent with high gene flow due to unconstrained bidirectional movement in the system. The other two genetic clusters occur in the Missouri River. The signal here is somewhat ambiguous in that a fair number of individuals were admixed (Fig. 2); however, the pattern of admixture is not randomly distributed throughout the drainage. Instead, some individuals on either geographic “end” of the drainage (i.e., sites B + C and I) are strongly assigned to one population or the other; that is, a high proportion of their genome is consistent with one of the two genetic clusters while a higher frequency of admixed individuals occurs in intermediate areas. Although more difficult to interpret, the pattern is not inconsistent with the results of pairwise fixation and IBD analyses. Furthermore, the neighbor joining dendrogram (Fig. 3) does not contradict these conclusions as the three unimpounded lower sites (A, C, and D; recall that B was excluded from most analyses due to low sample size) consistently cluster together to form a monophyletic clade separate from impounded populations. That is, there are no major deviations from the expected linear order along the river. The only minor exception is that site H from eastern Montana consistently clusters more closely with site D (South Dakota) than does site F, which is geographically closer.

In this study, the accessory measures (e.g., AMOVA, etc.) are interesting in and of themselves while IBD and STRUCTURE comparisons with the unimpounded Mississippi River more strongly warrant concern. A recent study in European gallinaceous birds with a similar generation time (10 years) revealed declines in genetic diversity and concomitant shifts toward a metapopulation structure with IBD after only 50 years of anthropogenic fragmentation (Segelbacher et al. 2003). In the Missouri River, these highly significant correlations, Bayesian clustering patterns, and coincidental reductions in allelic

richness in the upper river, may be the first detectable signals of genetic change due to the impoundments. Although genetic diversity remains relatively high, careful monitoring of populations in the upper Missouri with recurring physical and genetic surveys, including the use of coalescent-based methods to disentangle lingering historical signals from recent demography, is strongly encouraged. This study provides a strong foundation for the latter.

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