ANALYSIS OF GENOMIC DIVERSITY BETWEEN BLUEGILL POPULATIONS IN PLATTE COUNTY STREAMS BY RANDOM AMPLIFICATION OF POLYMORPHIC DNA

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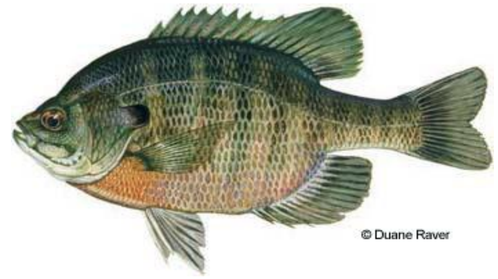


ABSTRACT

We hypothesized that the genomic diversity between 4 different creek populations of bluegill (*Lepomis machrochirus*) would vary directly with the relative distance between each creek mouth along the course of the Missouri River. We employed random amplification of polymorphic DNA (RAPD) analysis using the 10mer GGGCAATGAT to examine ten fish collected from each site (Brush, Rush, White Aloe, and Burlington Creeks at least 250 yards upstream of the mouth). DNA was extracted and amplified with the Redi-PCR tissue kit (Sigma-Aldrich) using 40 cycles of 1 min X 94 ^{O}C, 1 min X 36 ^{O}C, 1 min X 56 ^{O}C, 1.5 min at 72 ^{O}C. PCR product analysis was performed with 2% (w/v) 1X TAE agarose gels and Syber-Green staining. The position of each distinct and reproducible UV-visualized DNA band was determined within +/- 0.5 mm. The relative mobility (Rm) of each band versus standard DNA markers was tabulated for each fish and standard cluster analysis was performed to compare the relatedness of the different fish profiles. Our results proved to be more complex than anticipated. Fish from Brush and White Aloe Creeks were quite similar, Burlington Creek fish were somewhat similar to these, but Rush creek fish were different than all others. Furthermore, these similarities and differences were not correlated with the relative distances between creek mouths. Instead, observed relatedness among the 4 fish populations may best be explained in terms of how far upstream each sample site was from the confluence of the creek with the Missouri River..

Small fish were seined or hooked from the indicated locations. Each fish was frozen separately in a water-filled bag at -70^{o} C. Samples from each creek were assigned numbers 1-10, bu = burlington, br = brush, wa = white aloe, & r = rush. Approximately 10 mg of muscle from individual fish were DNA extracted with the Sigma-Aldrich Redi-PCR Tissue Extract Kit. A small sample (2 μl) of this extract was amplified by PCR (as indicated above) to generate a banding profile for each fish.

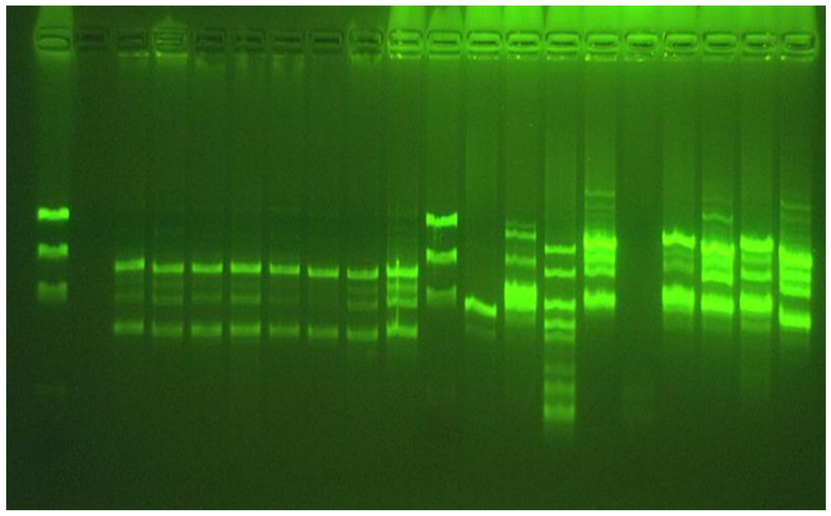
Four 10mers were evaluated for PCR:

Primer A GCGGCTGGAG	**Primer B** GTGACGCCGC
Primer C GGGCAATGAT	**Primer D** CTCGGGTGGG

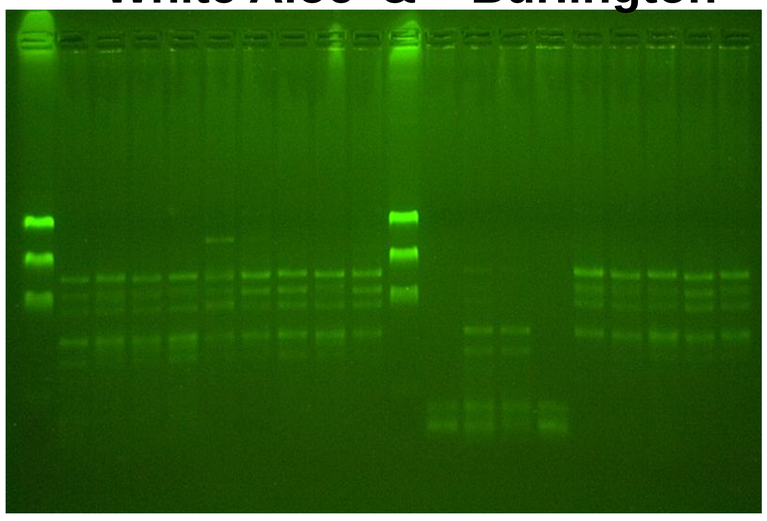
Primer C
GGGCAATGAT

was chosen

Brush & Rush



White Aloe & Burlington



SUMMARY of BANDING PROFILES

Bands	1	2	3	4	5	6	7	8	9	10	11
Br2			1	1	1	1					
Br3			1	1	1	1					
Br4			1	1	1	1					
Br5			1	1	1	1					
Br6			1	1	1	1					
Br7			1			1					
Br8			1	1	1	1					
Br9			1	1	1	1					
Ru1					1	1					
Ru2	1	1		1	1						
Ru3		1	1		1	1	1	1	1	1	
Ru4	1	1	1		1						
Ru5									1	1	
Ru6	1	1	1		1	1					
Ru7		1	1	1	1	1					
Ru8	1	1	1		1	1					
Ru9		1	1	1		1					
WA1			1	1	1	1	1				
WA2			1	1	1	1					
WA3			1	1	1	1	1				
WA4			1	1	1	1	1				
WA5	1		1	1	1	1					
WA6			1	1	1	1					
WA7			1	1	1	1	1				
WA8			1	1	1	1	1				
WA9			1	1	1	1					
Bu1										1	1
Bu2			1		1	1	1			1	1
Bu3						1	1			1	1
Bu4										1	1
Bu5			1	1	1	1					
Bu6			1	1	1	1					
Bu7			1	1	1	1	1				
Bu8			1	1	1	1	1				
Bu9			1	1	1	1					

CONCLUSIONS

Cluster analysis represents our initial attempt to determine likeness among the DNA profiles. Unfortunately, this result does not indicate much more of a pattern than can be discerned by careful visual inspection of the simple banding profiles.

Brush and White Aloe Creeks have somewhat homogenous populations of fish that resemble each other. Burlington and Rush Creeks both show more variation between individual fish and show both similarities to and important differences from the other creeks.

Overall analysis suggests that the main differences between the DNA profile of the 4 fish populations correlates more with the distance upstream than it does from the distances separating the creek mouths along the Missouri.