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INTRODUCTION

Historically, rainbow trout have been an important component of the fishery in Lake McConaughy and its feeder streams in the North Platte River basin. Although numerous strains of rainbow trout have been introduced into this system, the migratory McConaughy strain has been credited for much of the past success of the fishery. The decline of this strain over the last three decades is a major concern. Genetic material representing these two rainbow trout strains were collected from fish taken from the U.S. Fish and Wildlife Service National Fish Hatchery at Innis, Montana. In this study, PCR-RFLP's (polymerase chain reaction-restriction fragment length polymorphisms) were used to generate specific markers for McConaughy and Kamloop strains of rainbow trout. These markers were used to document the present status of the two different strains in two different drainages, one in the North Platte River basin (9 Mile Creek) and the other feeding into Lake McConaughy (Otter Creek). Otter Creek was found to consist of a mixture of McConaughy and Kamploop strains, whereas 9 Mile Creek was found to contain no McConaughy strain of rainbow trout.

MATERIAL AND METHODS

We isolated DNA from the following rainbow trout sources; twenty samples of the McConaughy strain from the U.S. Fish and Wildlife Service, National Fish Hatchery in Innis, Montana and twenty-two samples of the Kamloop strain from the hatchery in U.S. Fish and Wildlife Service, National Fish Hatchery in Innis, Montana. Two mixed populations in Nebraska were examined; one from the 9 Mile Creek and the other from Otter Creek (n=19 and n=21, respectively).

For this analysis, we examined the non-coding region found in mitochondrial DNA (mtDNA) known as the D-loop. This maternally inherited region is known to mutate 5-10 times faster than nuclear DNA and is suitable for phylogenetic analysis of closely related or recently diverged species (Gyllensten and Wilson, 1987). Our results demonstrate the use of restriction fragment length polymorphisms that differentiates two strains of rainbow trout (McConaughy and Kamloop) in a mixed population, based on five-four base restriction endonucleases (HinP1I, HpaI, NlaIII, RsaI, Sau3A1).

DNA Extractions

Total DNA (nuclear and mitochondrial) was extracted from fin clips using a modification of the proteinase K-phenol/ chloroform method outlined in Ausubel, et al., 1994, except for Brook trout in which eggs were used for a source of total DNA. After the DNA was extracted, the resultant DNA pellet was resuspended in TE (10 mM Tris, 1 mM EDTA, pH 7.4) and stored at -30°C until needed for PCR.

Amplification, Digestion, and Visualization of the PCR-RFLP's

The mitochondrial D-loop was amplified using primers designed by Kocher et al. (1989) and listed in Martin et al., 1992 which are as follows: CB3-L15560 (5'-CATATTAAACCCGA ATGATATTT-3') and 12SA-H1067 (5'-ATAATAGGGTATCTAATCCTAGTTT-3') . Amplication reactions were set up using 5 ul 10 x reaction buffer, 4 ul dNTP's (10 mM) , 0.5 ul of each primer (0.2 nM/ul), 1.0 ul of BSA (25 mM), 8 ul of $MgCl_2$ (10 mM), 28.5 ul of ddH_20, 0.5 ul Taq polymerase (2.5 U). Amplifications were performed in a Perkin Elmer 480 thermocycler using 30 cycles of 94°C for 40 seconds, 55°C for 25 seconds and 72°C for 120 seconds. Samples were denatured for 4 minutes at 95°C prior to amplification. After completion of PCR, all reactions were examined for quantity and size of amplicon by removing 1/6th of the reaction mixture and running it on a 0.8% agarose gel at 125 volts for 45 minutes with subsequent EtBr staining to visualize the DNA fragments. Negative controls (no DNA) were performed with each amplification to test for possible contamination. At no time did a negative control yield any detectable amplicon. All samples visualized by agarose gel electrophoresis demonstrated the sample size (~2000 base pair) amplicons. After testing for correct amplification, the rest of the reaction mixtures were subjected to restriction digestion using the aforementioned enzymes. The restriction digests were performed per manufactureres protocol (New England BioLabs) and incubated between 4 to 16 hours. After completion of the restriction digestion, the fragmented DNA was separated by polyacrylamide gel electrophoresis using a 6% polyacrylamide gel in a Hoefer gel electrophoresis apparatus. The gel was ran at 200 volts, for 2.5 hours. Following gel electrophoresis, the DNA fragments were visualized by silver staining using a modification of the protocol by Milligan, 1992. Briefly, the gel was soaked in ddH_2O for 20 minutes, then incubated in a silver nitrate solution (0.2 g silver nitrate in 125 ml of ddH_2O to which 500 ul of 1 M NaOH and 500 ul of NH_4OH was added). After the silver nitrate incubation, the solution was removed, the gel briefly rinsed in ddH_2O for 10 seconds and a sodium carbonate solution (4 g of sodium carbonate in 200 ml ddH_2O which 125 ul of formaldehyde was added). The gel was incubated in the sodium carbonate solution until the DNA bands were visualized. The reaction was stopped by removing the solution and soaking in ddH_2O for at least 10 minutes. Gels were placed onto 3MM Whatman paper and dried using a Labconco Model 433-0100 gel dryer. DNA fragments were sized by use of a DNAStar digitizer and software.

RESULTS

The haplotypes generated were assigned a letter based on the RFLP patterns generated, the most common RFLP pattern was given the letter "A", the next most common pattern "B", etc. The data for the five populations are as follows:

Otter Creek		*9 Mile Creek*		*McConaughy*		*Kamloop*	
AAAAA	6/21	AAAAA	0/19	AAAAA	20/20	AAAAA	0/6
AABAA	12/21	AABAA	10/19	AABAA	0/20	AABAA	6/6
BAAAA	3/21	BAAAA	7/19	BAAAA	0/20	BAAAA	0/6
CAAAA	0/21	CAAAA	2/19	CAAAA	0/20	CAAAA	0/6

The resultant haplotype freguencies are as follows:

	Otter Creek	*9 Mile Creek*	*McConaughy*	*Kamloop*
AAAAA	0.2857	0.0000	1.0000	0.0000
AABAA	0.5714	0.5263	0.0000	1.0000
BAAAA	0.1429	0.3684	0.0000	0.0000
CAAAA	0.0000	0.1053	0.0000	0.0000

A haplotypic diversity was derived using the following formula: $h = n(1-\sum_i^2)/n-1$ (Nei and Tajima, 1981). This diversity index was used to evaluate intrapopulational mtDNA lineages based on the haplotype frequencies found in the populations.

Diversity Index

Otter Creek	*9 Mile Creek*	*McConaughy*	*Kamloop*
0.60	0.61	0.00	0.00

DISCUSSION

In our study, hatchery stocks of two strains (McConaughy and Kamploop) of rainbow trout (Oncorhynchus) from the U.S. Fish and Wildlife National Fish Hatchery at Innis, Montana were demonstrated to possess only one haplotype. Each of these strains possess a unique haplotype which is the result of a single restriction site variation (Nla II). The McConaughy strain has an AAAAA haplotype (HinP1 I, Hpa I, Nla III, Rsa I and Sau3A1) whereas the Kamploop strain haplotype is AABAA (HinP1 I, Hpa I, Nla III, Rsa I and Sau3A1). We then used these haplotypic designations as markers to analyze the trout populations of two feeder streams (Otter Creek and 9 Mile Creek).

Analysis of the Otter Creek population demonstrated that this population was comprised of approximately 57% Kamloop and 28.6% McConaughy strains. There was also an approximately 14% haplotype (BAAAA) whose source has yet to be identified. The 9 Mile Creek was to be comprised of 53% Kamloop, 37% of the unidentified BAAAA haplotype and approximately 10% of a CAAAA haplotype whose source has also yet to be identified.

Future studies will need to identify the source of the two unique haplotypes. Since fairly accurate records of which strains have been introduced to these feeder streams, those strains which still exist can be analyzed and compared with the aforementioned data.

Although this has been a preliminary study, the results are promising. With further work, these haplotype designations will be useful in identifying the relative contributions of rainbowtrout strains in specific feeder streams. This data could be used in the management of hatchery transfers and introductions in new streams.

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