Measuring associations between polymorphisms in the GREB1-L gene and the development of different migratory phenotypes in *Oncorhynchus mykiss*

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Abstract

Rainbow trout, *Oncorhynchus mykiss,* exhibits two life history strategies: resident rainbow trout and migratory steelhead trout. Previous research has shown that the decision to migrate is highly heritable. Recently, interest has focused on the GREB1-L gene as studies in several populations of rainbow trout have found alleles that are associated with migration. This project aimed to measure allelic associations between GREB1-L and migratory life-history in rainbow trout from Sashin Creek, Alaska. Sequence data suggests that all individuals, regardless of their migratory trajectory, had the alleles associated with migration. These results confirm that there are population specific genetic effects that determine the migratory life history of rainbow trout.

Introduction

Rainbow Trout

- *Oncorhynchus mykiss* is comprised of two different migratory strategies[1]:
  - Resident rainbow trout (Figure 1)
  - Migratory steelhead trout (Figure 2)

Migration

- Is the movement of individuals to new areas that return to the natal area to breed.
- Proven to be heritable in *Oncorhynchus mykiss*[2].
  - Regions of genome associated with migration[3].
  - Differential gene expression[4,5].
  - Identification of specific genes involved in migration has proven difficult.
- **GREB1-L**
  - Associated with variation in migratory strategies in rainbow trout[6,7].
  - Key regulator in the migratory decision.

Figure 1: Photograph showing a resident rainbow trout

Figure 2: Photograph showing a migratory steelhead trout

Objective

To identify polymorphic positions (SNPs) within GREB1-L that are associated with migration in a model population of rainbow trout from Sashin Creek, Alaska.

Methods

Sampling

- 1579 samples were taken from Sashin Creek, Alaska.
  - Samples were F1 individuals from two separate crosses:
    - Between mature steelhead trout
    - Between resident rainbow trout
  - Categorized into one of four phenotypes (Figure 3)

Sexing

- *OmyY1* gene amplified to identify male samples (Figure 4).
- PCR and gel electrophoresis were run on a subset of samples.
- Gel was interpreted to determine sex of individuals.

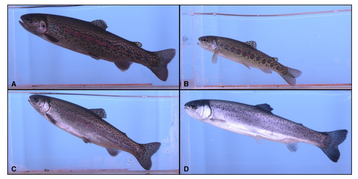

Figure 3: Examples of four phenotypes. A: Mature resident males. B: Immature resident. C: Indeterminate. D: Smolt[8].

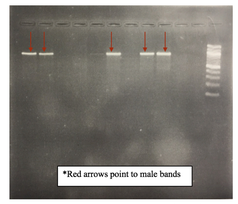

Figure 4: Agarose gel showing OmyY1 amplification

Sequencing

- Various samples were sequenced using designed primers, PCR, ExoSap clean up, and Big Dye reactions.
- Primer pairs designed from a scaffold constructed in a previous study proved most successful.
  - Three primer pairs were used to sequence 13 polymorphic positions according to a previous study[6].
- Sequence data was then analyzed using Sequencher.

Results

- All individuals, regardless of their migratory trajectory, showed the resident allele at each of the 13 positions that matched a polymorphic position from the previous study (Table 2).
  - Residents, mature males, and smolts all showed the resident allele (Table 3).
  - All sequence data originated from good quality PCR products (Figure 5) produced from the three primer pairs (Table 1).
- Sexing results confirm that there were more female migrants than male migrants (Figure 6).

Gene	Left Primer	Right Primer	Size	Location in Scaffold
GREB1_A2	GTGGCCACTGCTTCAACTGT	TGATACAGTGAAATAATCTG	594	649062;649656
GREB1_A3	AGCGTGAACACTCCAAAGGA	CTGAACTTGCTTCCCGACTC	650	594922;595551
GREB1_A1.1	GGAGCTGACCCTGTTCTCTG	GACTGGGTCCCTCACACCTA	665	592277;592940

Table 1: The three primer pairs used to sequence the polymorphic positions found in a rainbow trout scaffold. The forward and reverse primer sequences, the size of the product , and the target of each primer pair within the region are listed.

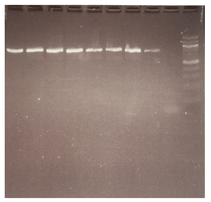

Figure 5: An agarose gel showing the sequence quality bands amplified using the A2 primer pair at 64°C annealing temperature.

GREB1-L Position	Migrant Allele	Resident Allele
568978	T	C
592595	G	A
592596	G	T
592627	T	C
595076	T	C
595084	A	G
595186	G	T
595253	A	G
649195	A	T
649286	T	C
649350	T	A
649428	A	G
649467	G	A
649544	A	C
780205	G	T
780229	T	C

Table 2: The 16 polymorphic positions found within the constructed scaffold containing GREB1-L[6]. The alleles associated with migrants and the alleles associated with residents are each shown. The positions highlighted in light blue are the positions targeted by the three designed primer pairs.

GREB1-L Position	Sashin Creek Migrant Allele	Sashin Creek Resident Allele	Resident Allele
592595	A	A	A
592596	T	T	T
592627	C	C	C
595076	C	C	C
595084	G	G	G
595186	T	T	T
595253	G	G	G
649195	T	T	T
649286	C	C	C
649350	A	A	A
649428	G	G	G
649467	A	A	A
649544	C	C	C

Table 3: The resulting alleles in all of the 13 positions in each of the migratory phenotypes after sequencing and analysis. All alleles found showed the resident allele, regardless of the migratory trajectory.

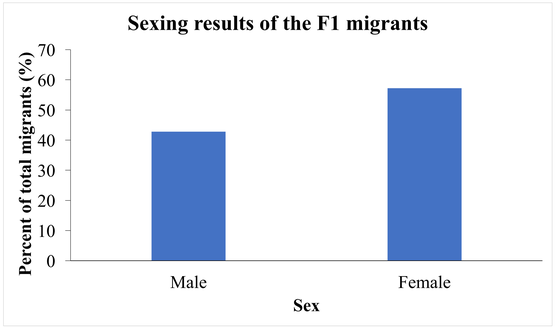

Figure 6: The percentage of males and female migrants in the Sashin Creek population. Mature resident samples are not included in this figure.

Discussion

- Results confirm that population specific genetic effects determine the migratory life-history of rainbow trout.
- Locally adapted genes like GREB1-L may play a role in the decision to migrate.
- Difference in polymorphic data between different populations show that this gene is not conserved.
- Ultimately, the environment may have different effects on the development of migration.

Future Directions

- Finding and genotyping polymorphisms in the GREB1-L gene of the Sashin Creek samples.
- Genotyping different populations of rainbow trout for GREB1-L.
- Possible that GREB1-L is not variable at higher latitudes.

Implications

- To know more about the inheritance of migratory strategies of rainbow trout.
  - Help the species from losing its migratory population.
  - May help in conservation efforts if necessary.
- Results found in this project may carry over into other species of migratory trout and salmon.
  - Can narrow the gap between genetics and migration in migratory fish species.

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Acknowledgements

Michael Miller at UC Davis for supplying the scaffold containing GREB1-L and TCU SERC for funding this whole research project.