Improved Resolution of Chum Salmon Genetic Stock ID

Final Report to PCCRC

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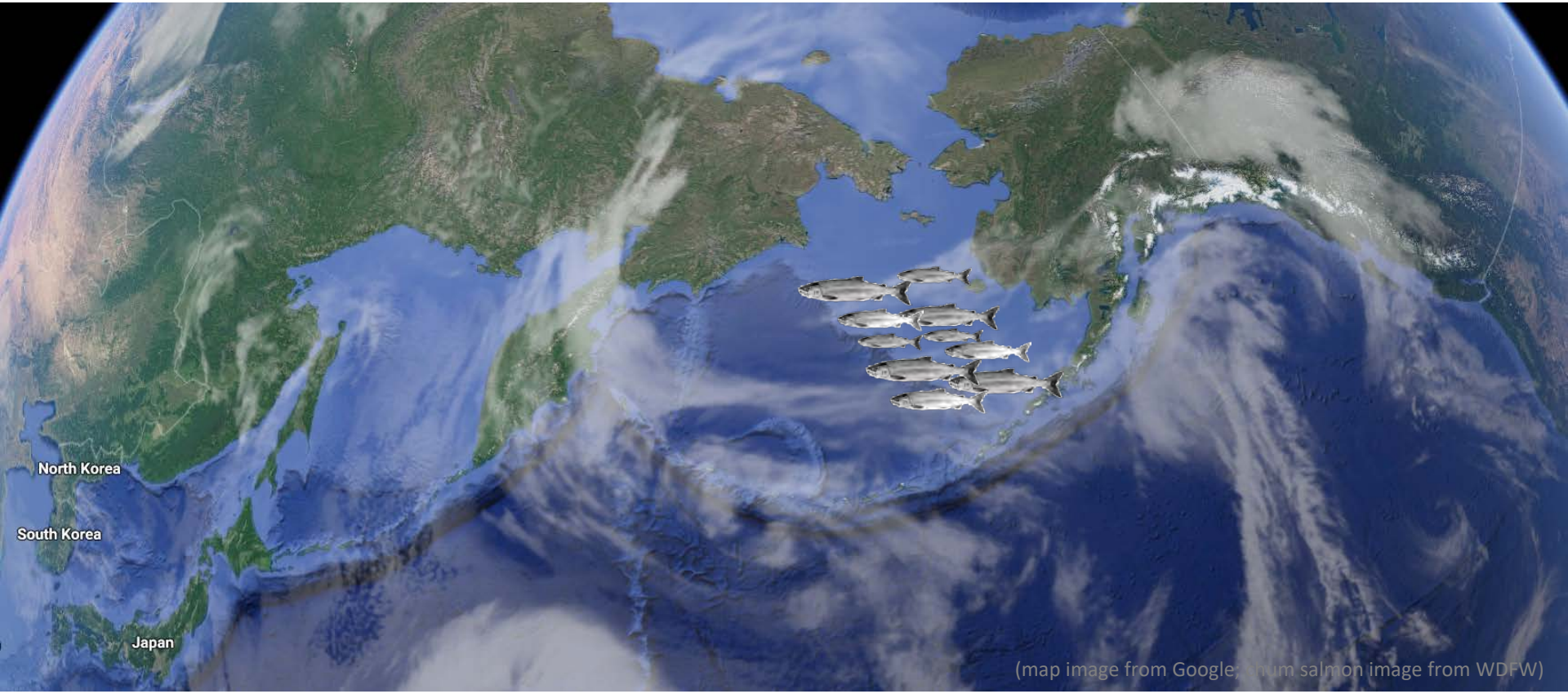
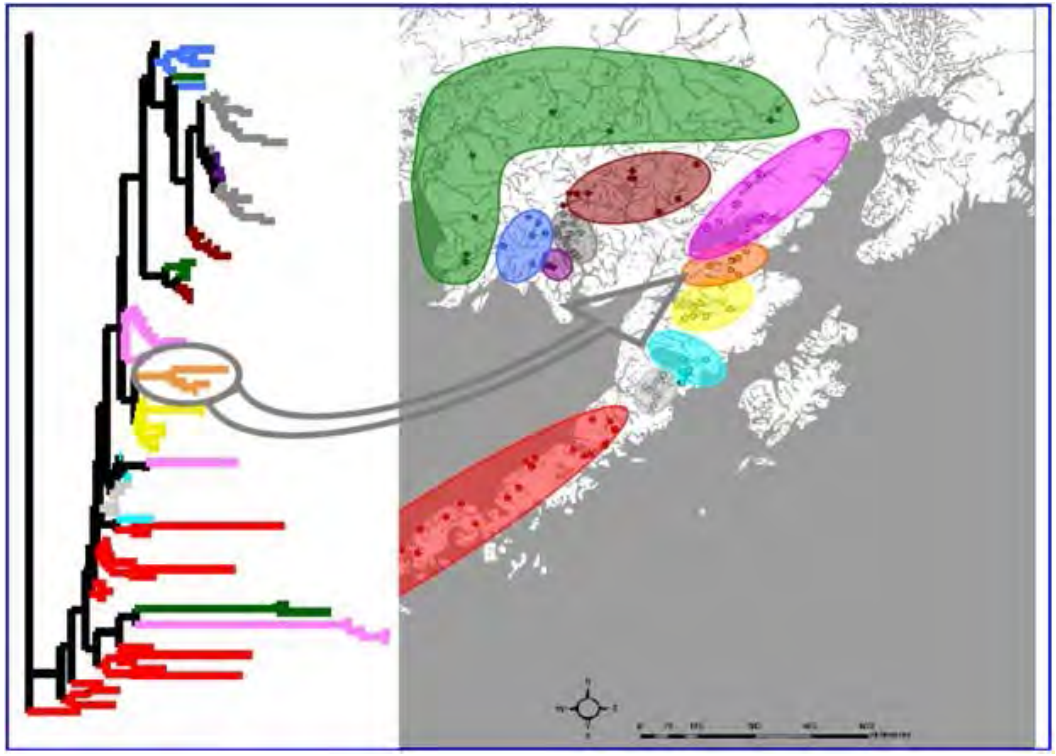
The problem?


North Korea
South Korea
Japan
(map image from Google; chum salmon image from WDFW)

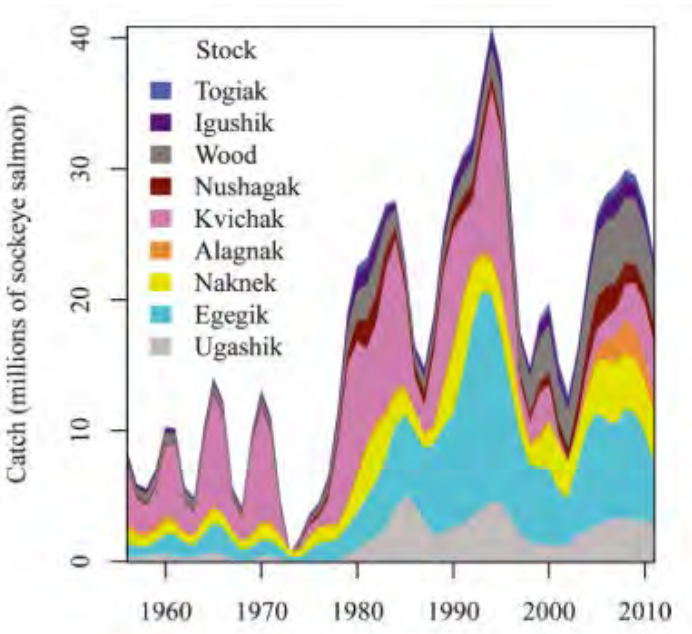
Genetic stock identification

… in a perfect world (Bristol Bay sockeye salmon)



(from ADF&G Gene Conservation Lab)

(NB: colors don't match between figures)

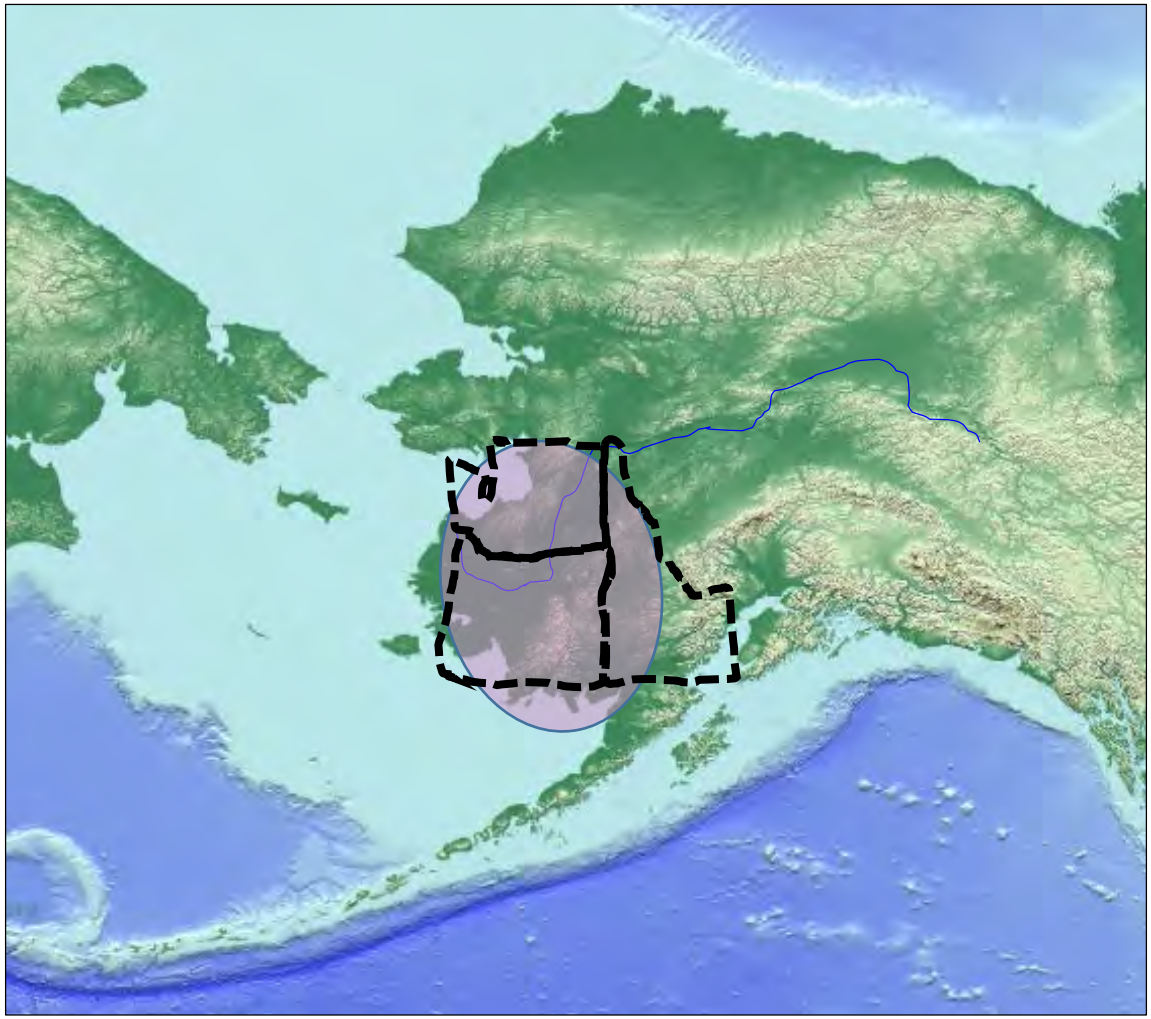


(Dann et al. 2009)

Western Alaskan chum salmon

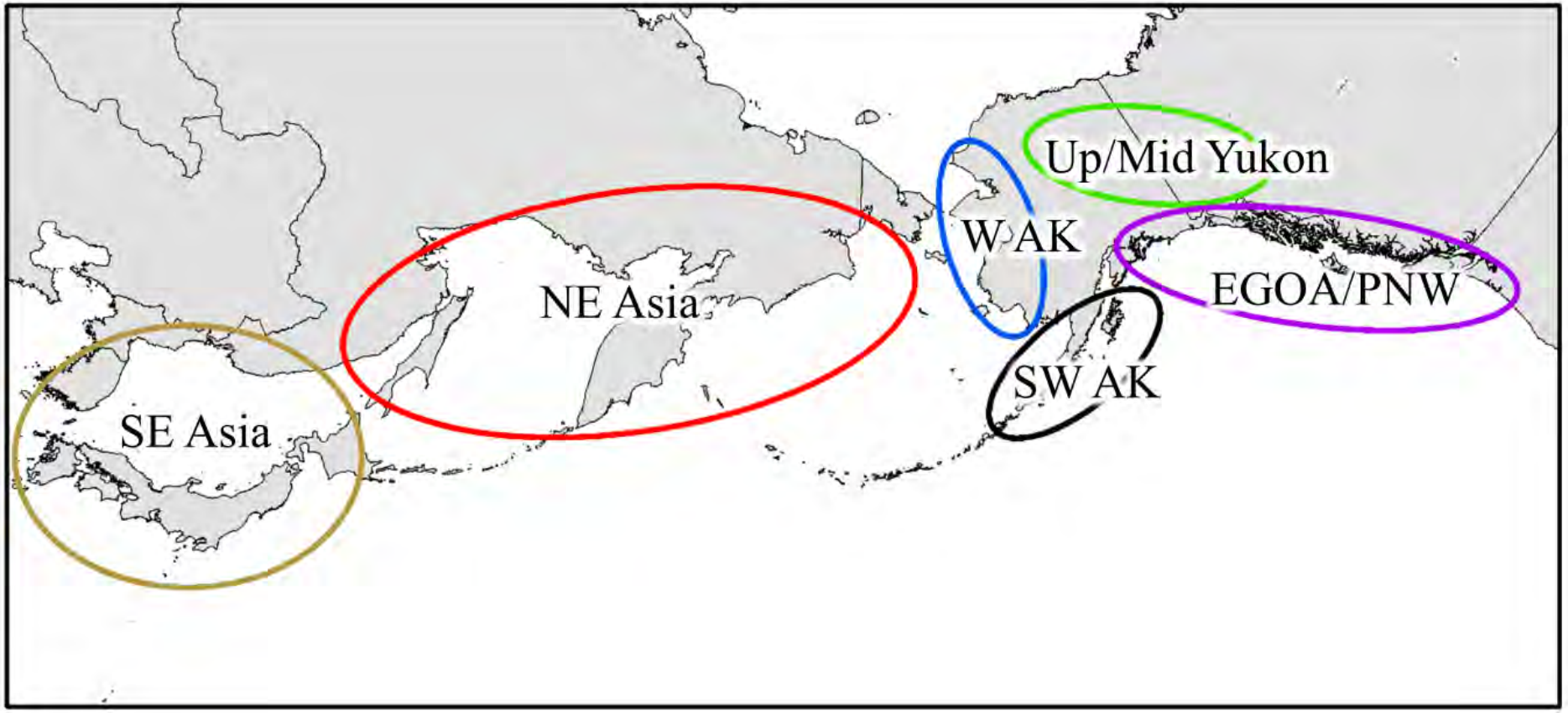
… not so perfect!

"Coastal Western Alaska" – summer-run chum are genetically indistinct across Norton Sound, Lower Yukon, Kuskokwim, and Bristol Bay



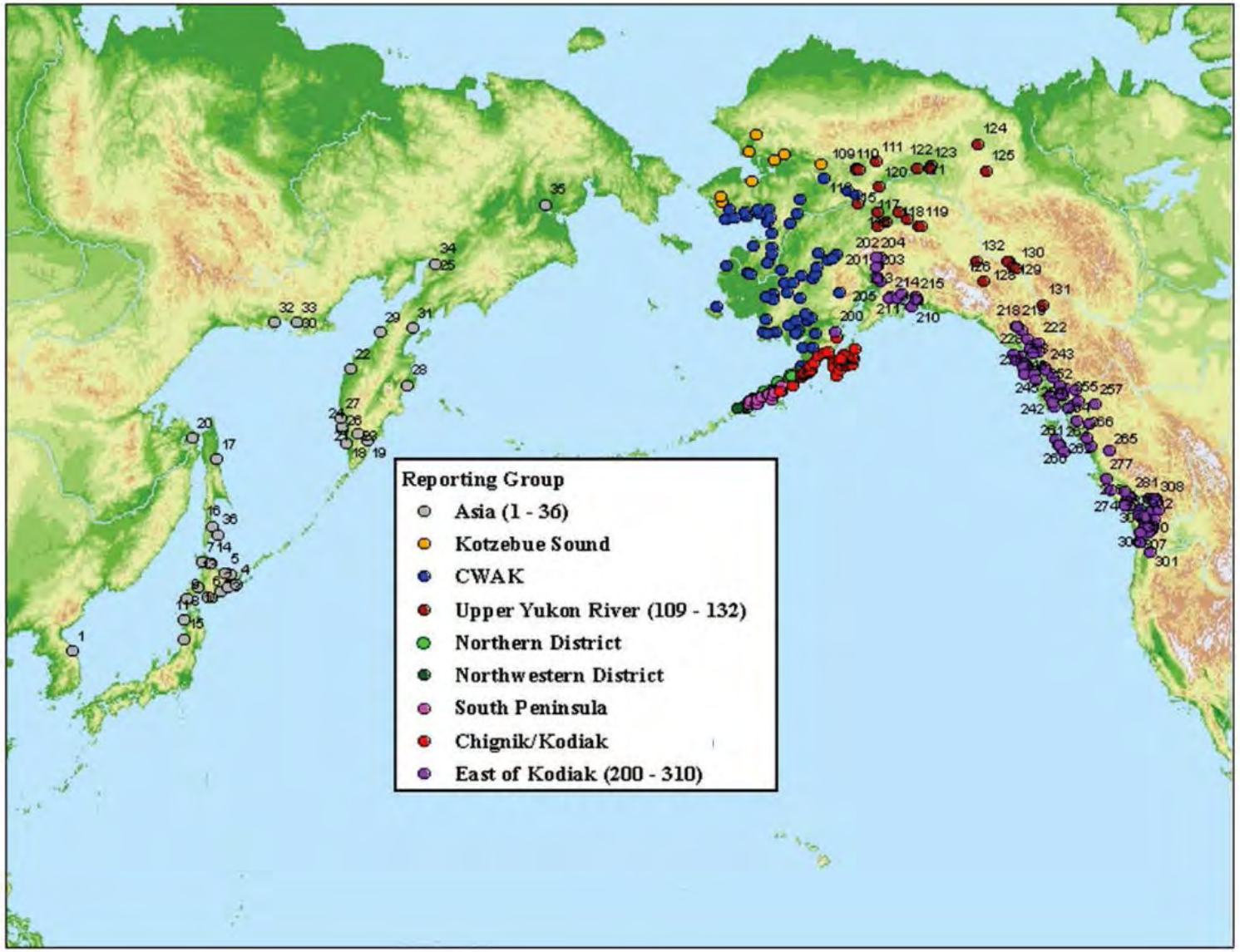
(from M. Garvin)

NMFS Baseline – 11 microsatellites



(Whittle et al. 2018)

ADF&G Baseline – 96 SNPs

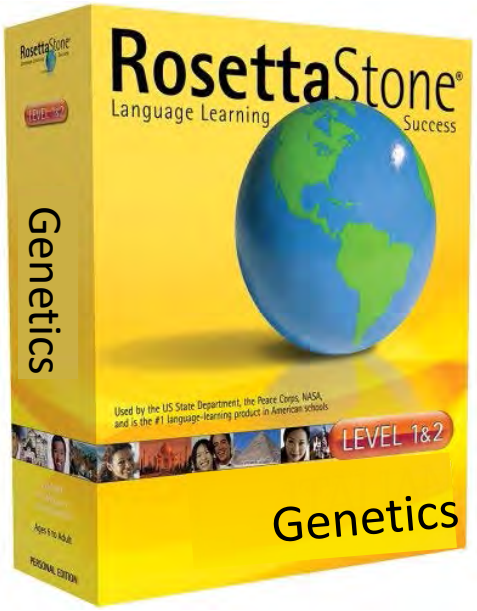


(DeCovich et al. 2012)

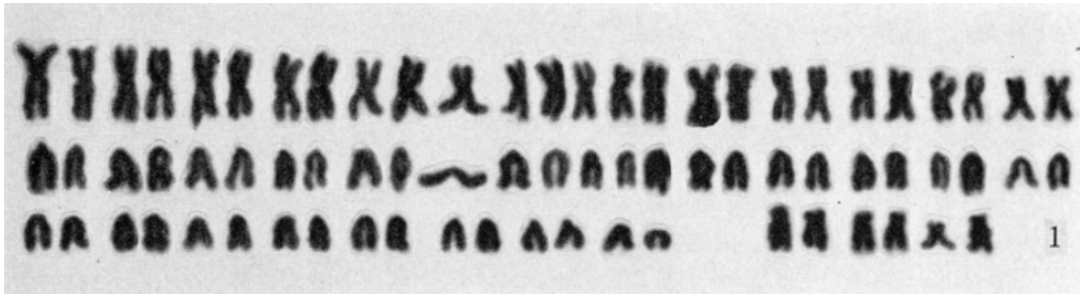
Our objectives

- To develop, test, and optimize a ‘GT-seq’ panel of 500 SNPs for Coastal Western Alaska chum salmon;

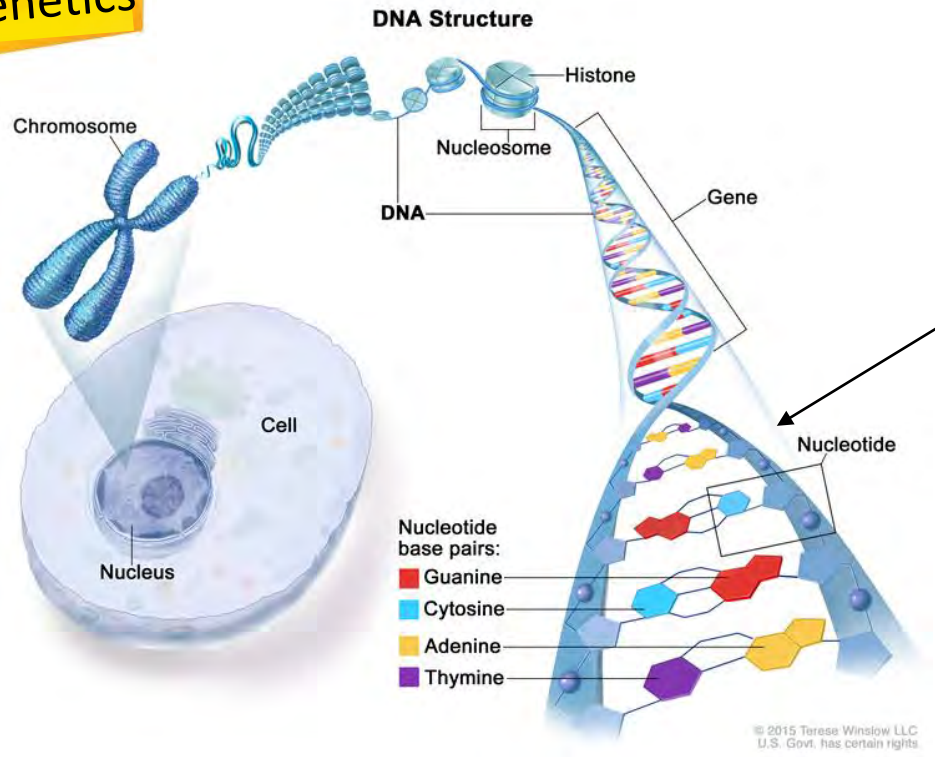
- Provide the optimized panel and protocols to resource managers throughout Alaska and adjacent areas



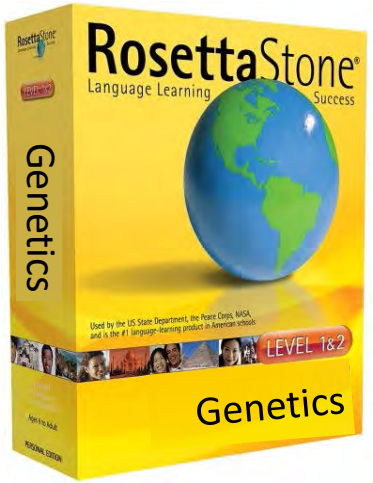
Genome: creature's DNA packaged in many chromosomes



(chum salmon 2N = 70, Sasaki et al. 1968)



DNA sequence: order of base pairs
e.g., TACGA



Marker: a place in the genome where we can assess genetic differences (sometimes also called 'locus/loci')

Microsatellite: differences are based on number of repeated motifs

5'-CGTAGGTATTTATTTATTTATTGCTAGGTT
5'-CGTAGGTATTTATTTATTGCTAGGTT

SNP ('snip'): differences are based on a single base pair in the DNA sequence (single nucleotide polymorphism)

5'-CGTAGGGCTAGGTT vs. 5'-CGTAGAGCTAGGTT

Allele: a variant at a given marker (e.g., G & A above)

Genotype: what 2 alleles does an individual possess? (1 from ma, 1 from pa)

GG 

GA 

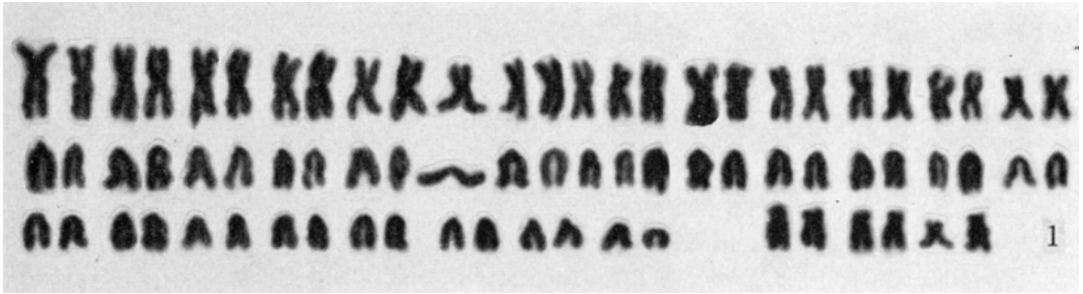
AA 

What is a GT-seq panel??

- GT-seq: “genotyping in thousands” by DNA sequencing – Nate Campbell & colleagues at Columbia River Inter-Tribal Fish Commission
- pool 1000s of individuals/100s of SNPs into single DNA sequencing run
- use DNA “bar codes” to identify individual and marker
- rapidly generate genotype data for 800-1000 individuals at 300-500 SNPs
- now running at ADF&G Gene Conservation Lab

Methods

1. identify SNPs across the chum salmon genome using “RAD-seq”



= 2.4 billion base pairs!

RAD-seq splices up the genome into manageable fragments, which are then sequenced

“Restriction-site associated DNA sequencing” – restriction enzymes cut at specific DNA sequences interspersed throughout genome; pieces are then sheared to smaller size for sequencing

Samples for SNP discovery

48 individuals/site

- Norton Sound (2)
- Yukon (2)
- Kuskokwim (1)
- Bristol Bay (1)

Methods

1. identify SNPs across the chum salmon genome using “RAD-seq”
2. Filter SNPs down to manageable number of useful markers (well-behaved and informative)

Filtering

initial: **94,002 RAD tags | 222,668 SNPs**

removed loci that did not genotype well *(including paralogs)*
removed loci that had very uneven allele frequencies *(MAF < 0.05)*

step 2: **31,919 RAD tags | 45,639 SNPs**

applied more stringent criteria for genotyping rate *(>90% of samples)*

step 3: **22,693 RAD tags | 30,006 SNPs**

Next: evaluated all 30,006 SNPs plus 500-SNP subsets using simulations

Results - all 30,006 SNPs

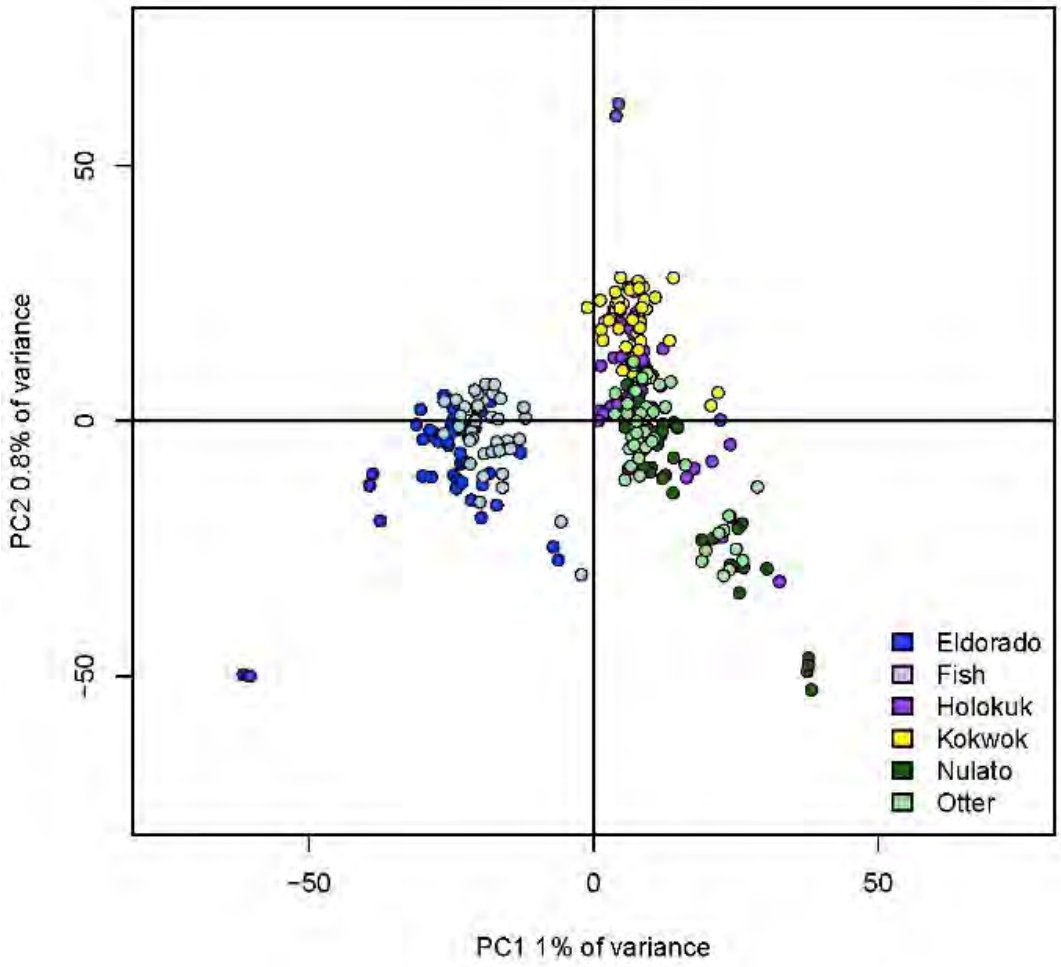
Norton Sound emerges as distinct

Norton Sound

Lower Yukon

Kuskokwim

Bristol Bay



500-SNP panel evaluations

Three different modes of marker selection:

1) individual SNP F_{ST}

2) Linked-SNP F_{ST}

3) “Random forest” marker selection *(Sylvester et al. 2016)*

Simulated individuals based on observed allele frequencies & evaluated accuracy of self-assignment in mixture analyses *(100% simulations, GSIsim)*

Results – panel evaluations

Accuracy (and 95% confidence intervals) of self-assignment by marker selection method

Selection Method	Norton Sound	Lower Yukon	Kuskokwim & Bristol Bay
Individual SNP F_{ST}	0.90 (0.87-0.94)	0.92 (0.89-0.95)	0.83 (0.78-0.88)
Linked-SNP F_{ST}	0.85 (0.82-0.89)	0.92 (0.89-0.95)	0.83 (0.78-0.88)
Random forest marker selection	0.92 (0.89-0.95)	0.82 (0.78-0.87)	0.68 (0.63-0.74)

Methods

1. identify SNPs across the chum salmon genome using “RAD-seq”
2. Filter SNPs down to manageable number of useful SNPs (well-behaved and informative)
3. Optimize and evaluate GT-seq panel

Panel selection

initial: **700 SNPs (highest individual F_{ST})**

removed loci unlikely to genotype well in GT-seq
(e.g., transposable elements, frequent primer sequences)

step 2: **533 SNPs (including 31 ADFG)**

Ran through 2 rounds of sequencing and optimization
Removed loci that did not work well when pooled

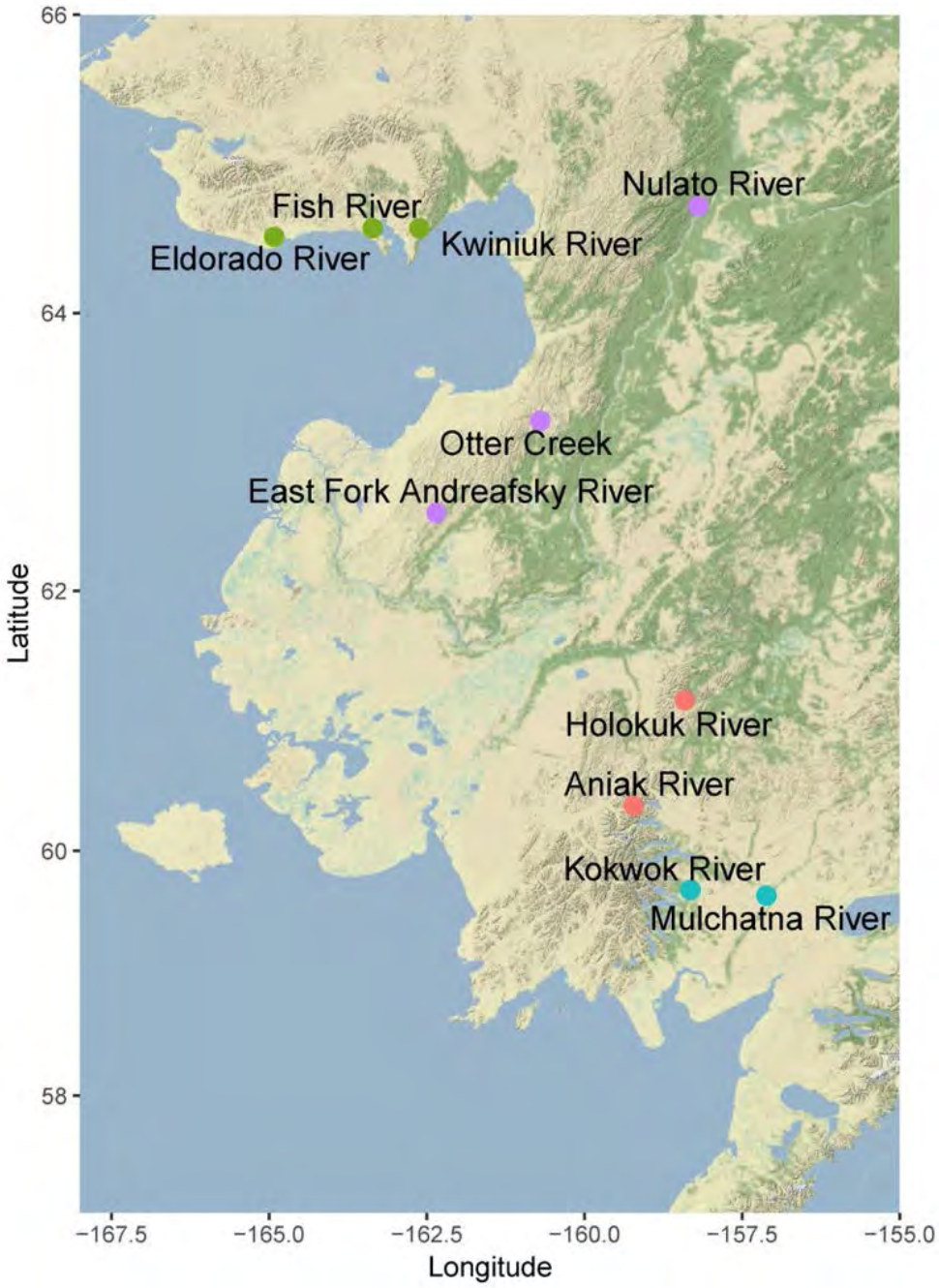
step 3: **448 SNPs (including 29 ADFG)**

Next: evaluated panel using increased number of populations and individuals

Panel evaluation

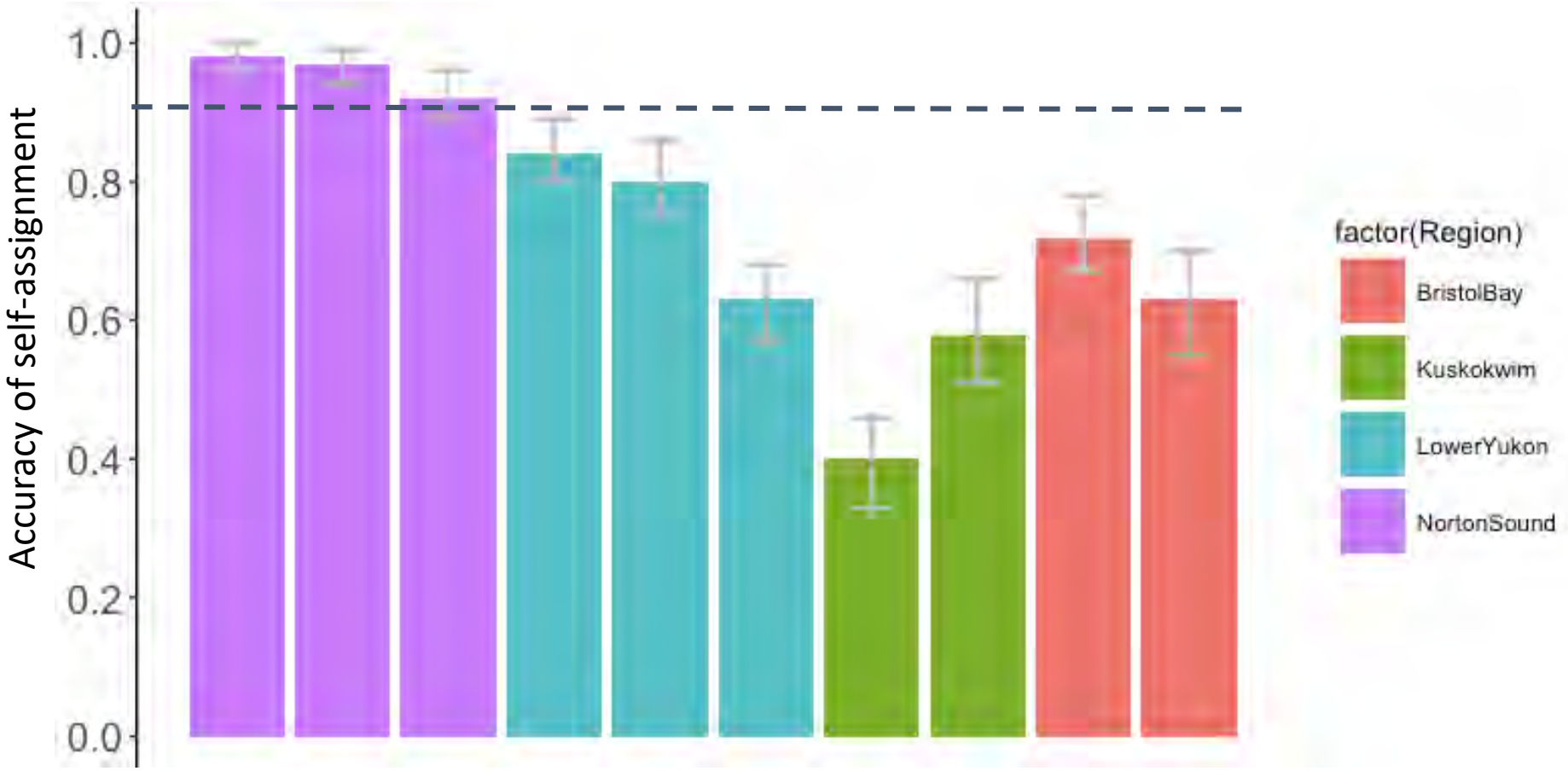
~80 individuals/site

- Norton Sound (3)
- Yukon (3)
- Kuskokwim (2)
- Bristol Bay (2)

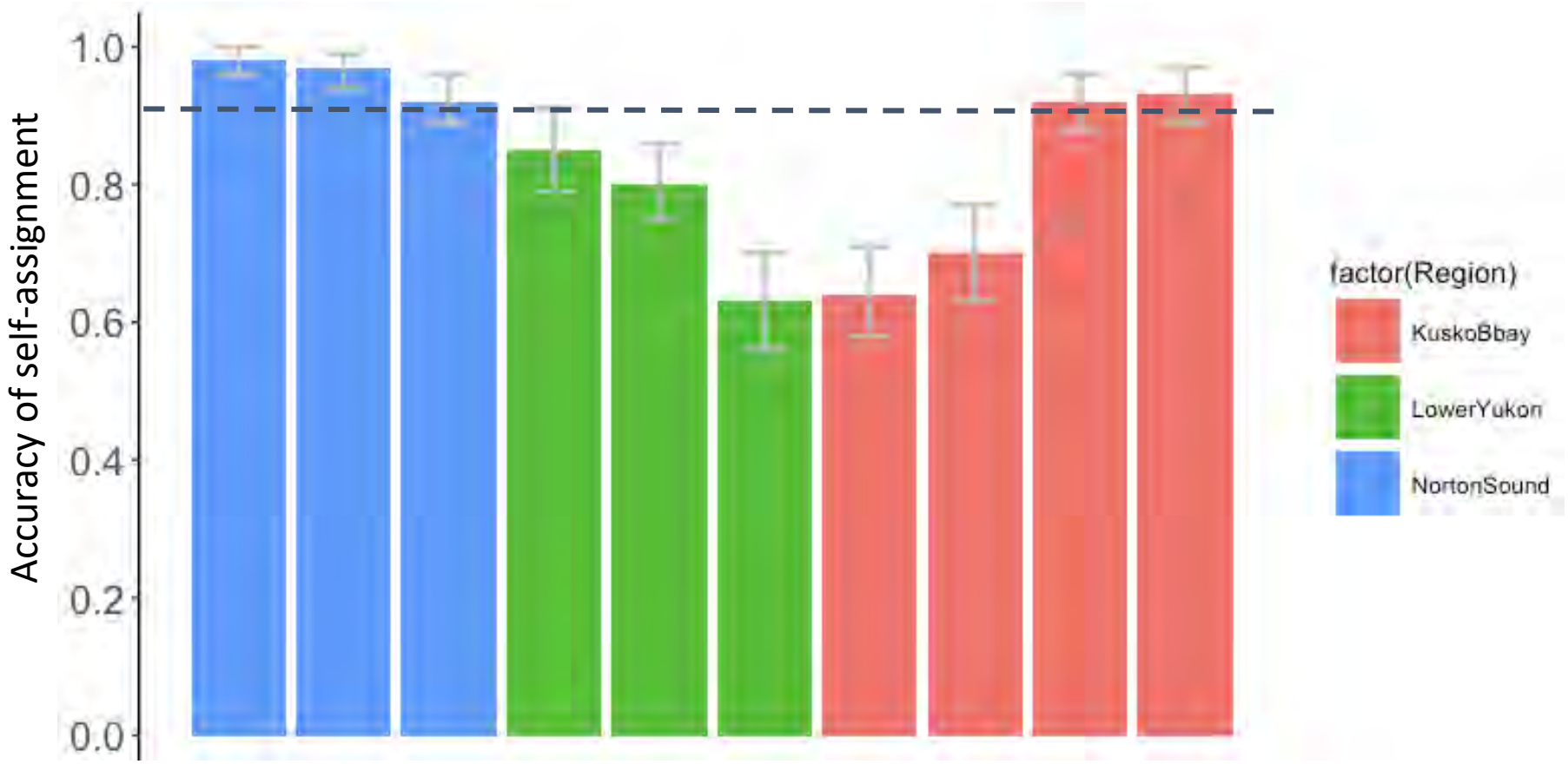


Region:
- Norton Sound
- Lower Yukon
- Kuskokwim
- Bristol Bay

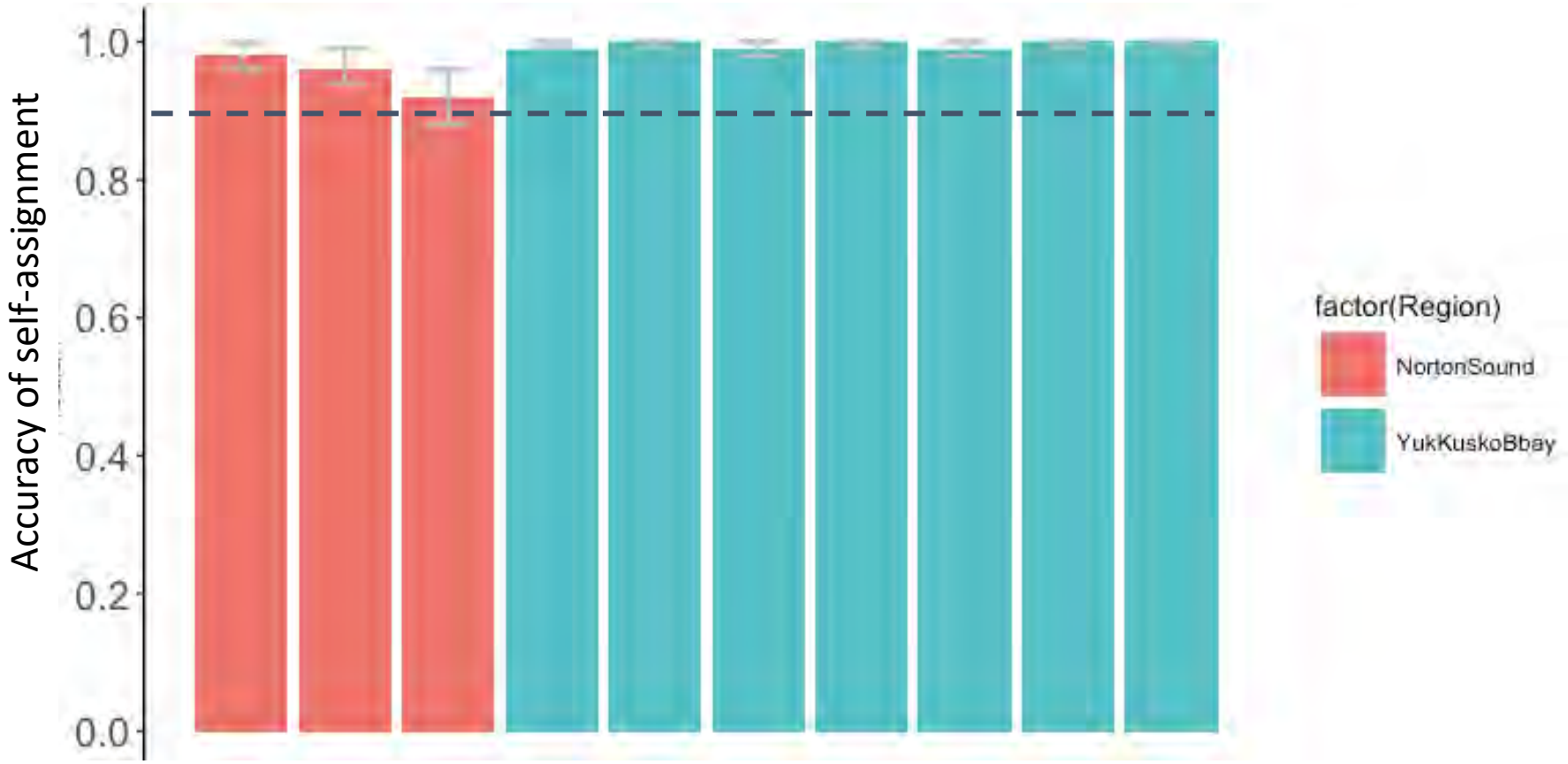
Results – 4 reporting groups



Results – 3 reporting groups



Results – 2 reporting groups



Why is coastal Western Alaska so difficult?

(Llamas et al. 2016)

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Connection between Yukon & Kuskokwim

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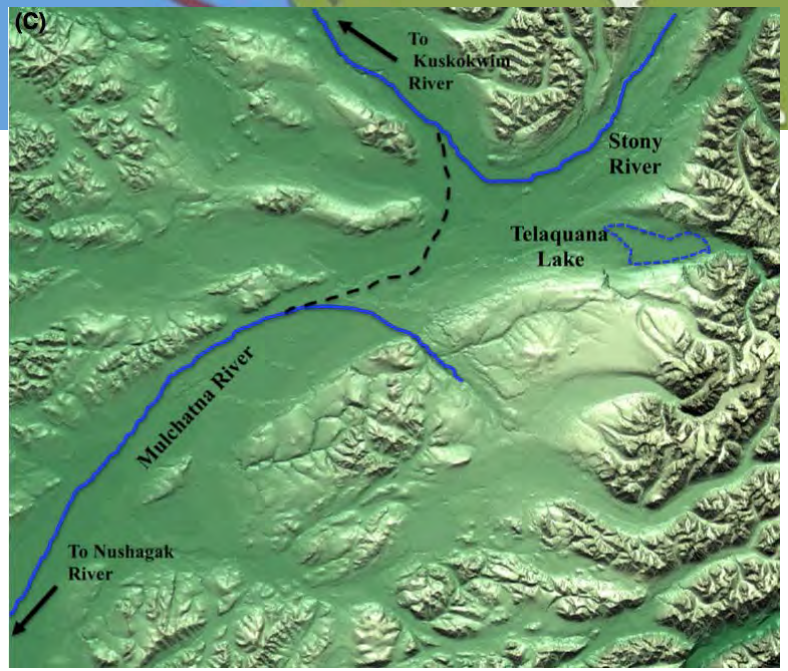

Connection between Kuskokwim & Bristol Bay (Nushagak R.)

Conclusions

- New panel allows for splitting of Coastal Western Alaska summer chum into 2 reporting groups: Norton Sound + Yukon/Kusko/Bristol Bay
- RAD-seq panel of 448 SNPs is available now for future genetic stock identification work
- Additional applications of panel – parentage-based tagging (e.g.)

Acknowledgments

- PCCRC (+ BOEM for previous funding)
- Jim & Lisa Seeb, Carita Pascal (UW)
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