Geographic Variation in a *Clock* Gene Polymorphism in Pink and Chum Salmon & Coho and ^Chinook

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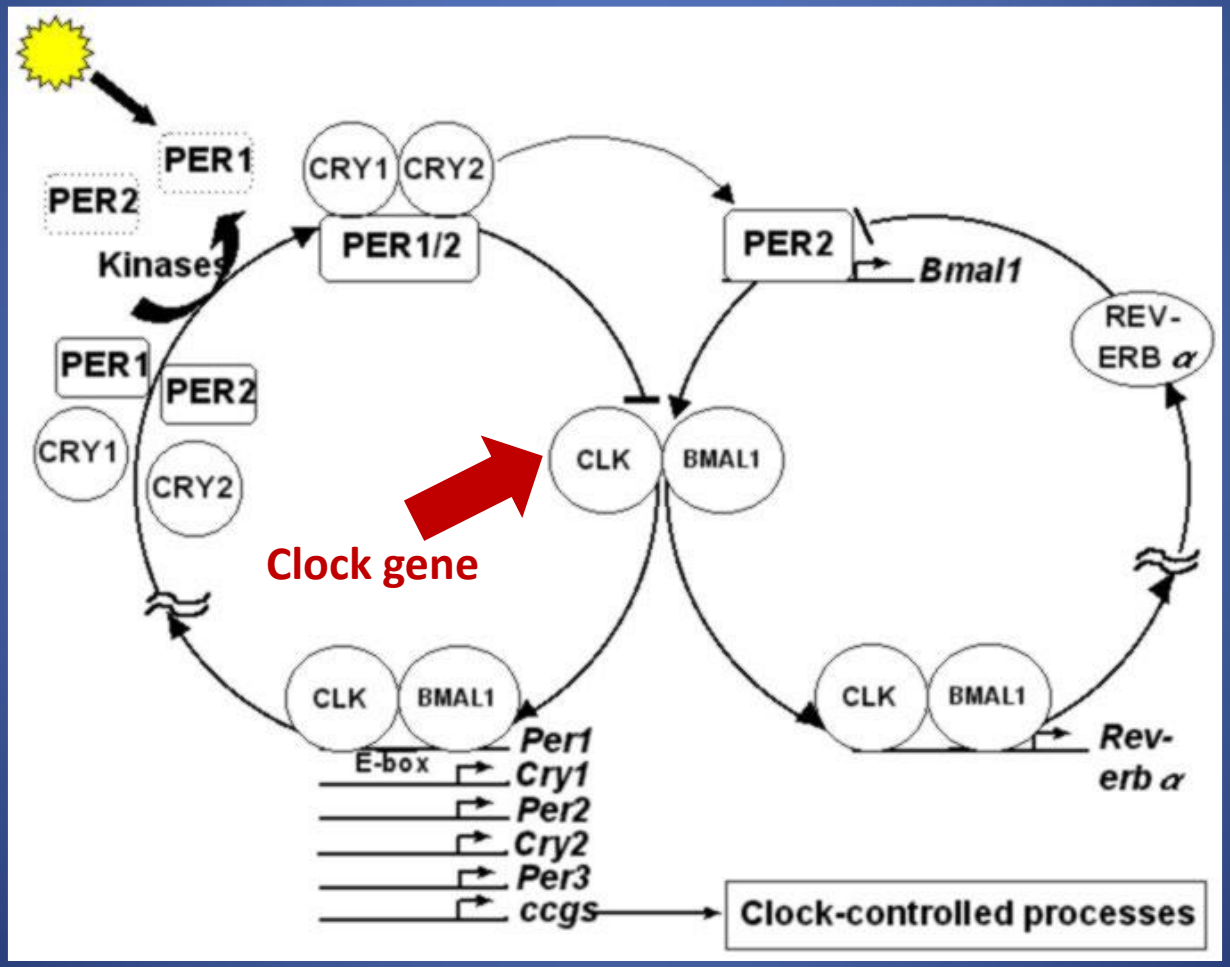
NE Pink & Chum Salmon Workshop
Juneau, AK
13-15 February 2012

Day length is used as an environmental cue to regulate seasonal behaviors

The circadian clock, which is modulated by day length, may serve as a molecular mechanism for timing of seasonal events[1]

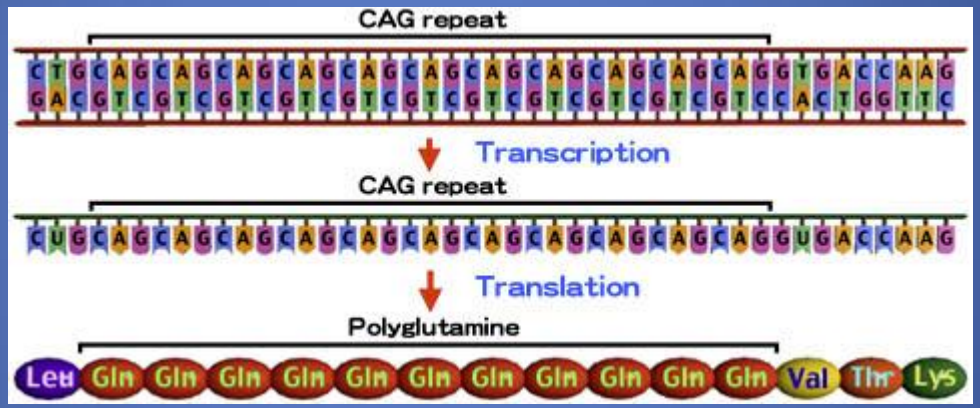
[1]Lincoln *et al.* (2003)

Molecular Mechanisms Underlying The Circadian Clock



Clock Polyglutamine Domain (PolyQ)

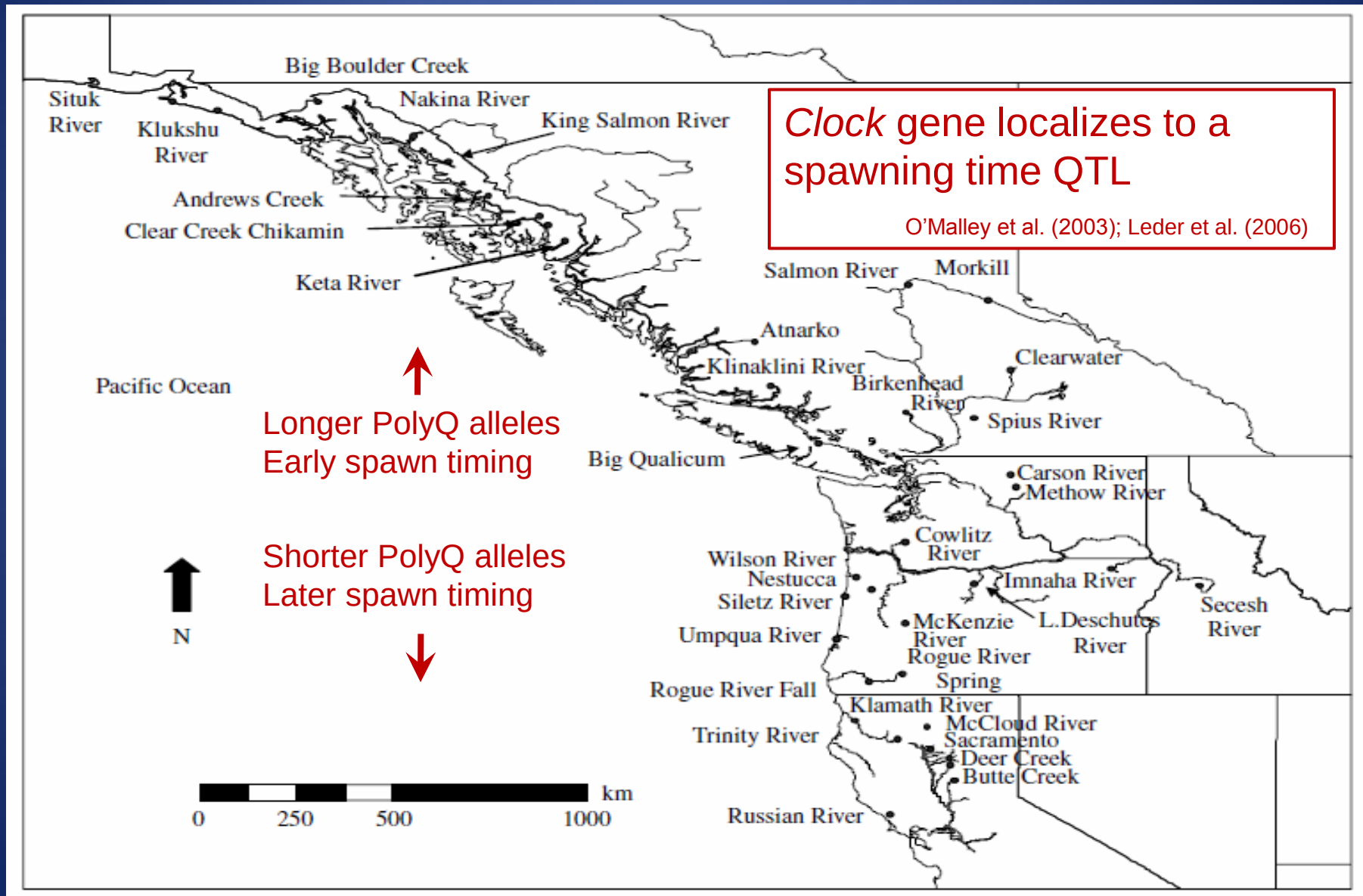
Series of $(CAG)_N$ repeats that code for the amino acid glutamine:



Variation in the number of repeats affects the transcription-activating potential of *Clock*[1]

[1]Darlington *et al*. (1998)

Latitudinal Cline in *Clock* PolyQ



Clock gene localizes to a spawning time QTL

O'Malley et al. (2003); Leder et al. (2006)

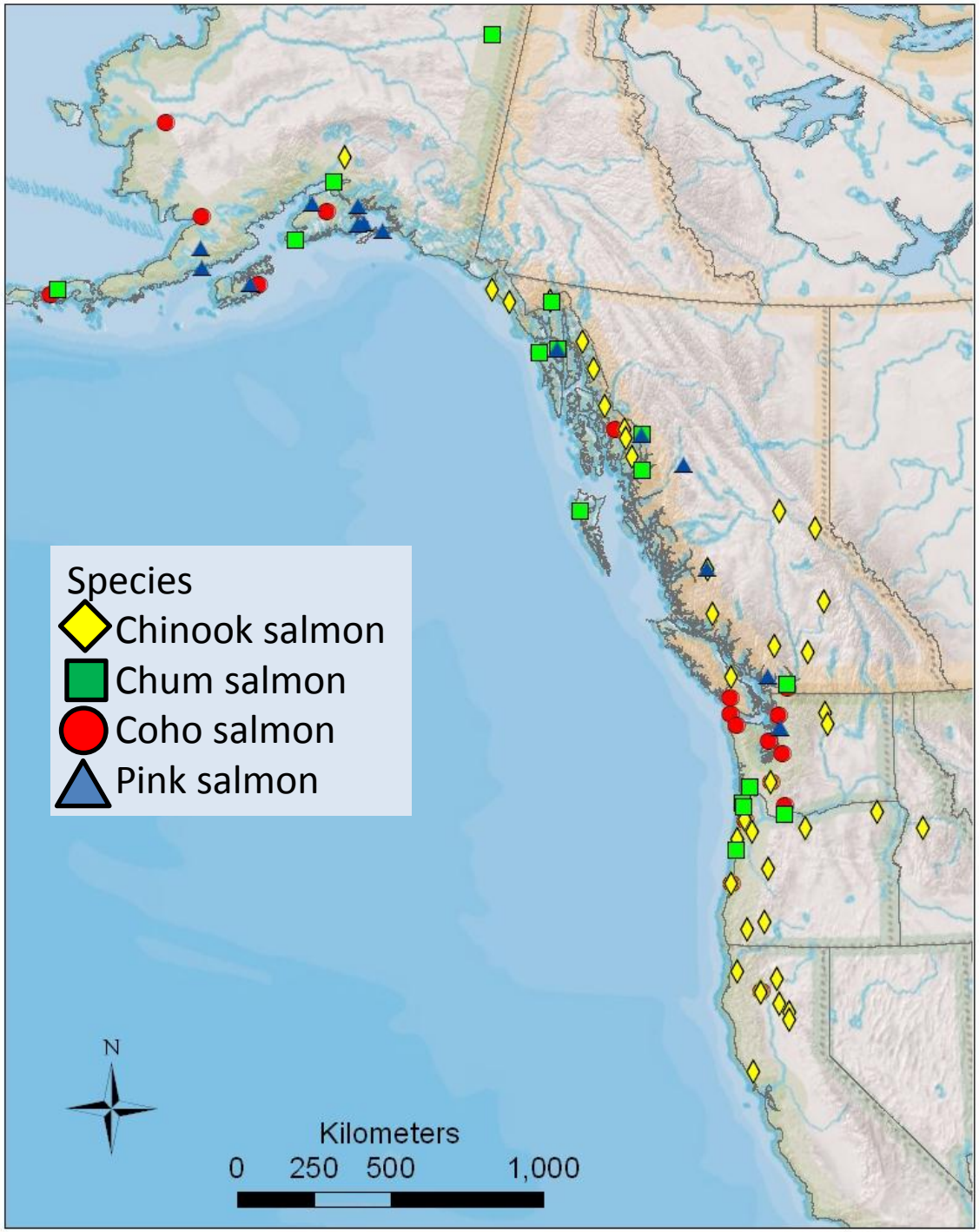
If the latitudinal cline in *Clock* PolyQ reflects an adaptation to day length, influencing reproductive timing, then the strength of the cline should correspond to clinal variation in reproductive timing in other Pacific salmon species

Objectives

1) Test for a latitudinal cline in *Clock* PolyQ among chum, coho, and pink salmon populations; determine if it corresponds to clinal variation in reproductive timing

2) Test for evidence of selection by comparing clinal variation in *Clock* PolyQ and allozyme loci

3) Identify potential ecological factors influencing patterns in *Clock* PolyQ in these species



O'Malley *et al.* (2010)

Objective 1 Predictions: Test for Latitudinal Cline in *Clock*

Species	Age at spawning (yr)	Geographic variation in spawning age	Geographic variation in spawn timing	Predicted cline in *Clock*
Chum	3 - 5	Increases with latitude	Advances with increasing latitude	Strong
Coho	2-4	Weak increase with latitude	No pattern	None
Pink	2	None	Weak advance with increasing latitude	Weak
Chinook	1 - 8	Increases with latitude	Advances with increasing latitude	Strong

Groot & Margolis (1991), Weitkamp *et al.* (1995), Hard *et al.* (1996), Johnson *et al. (*1997), Myers *et al.* (1998), Quinn (2005)

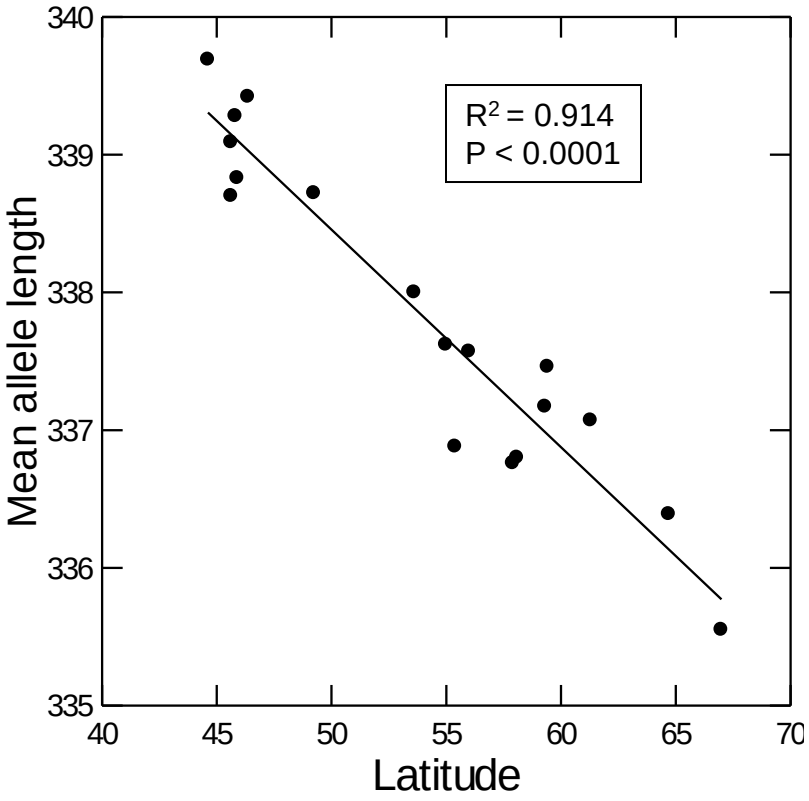
Objective 1 Results: PolyQ Variation

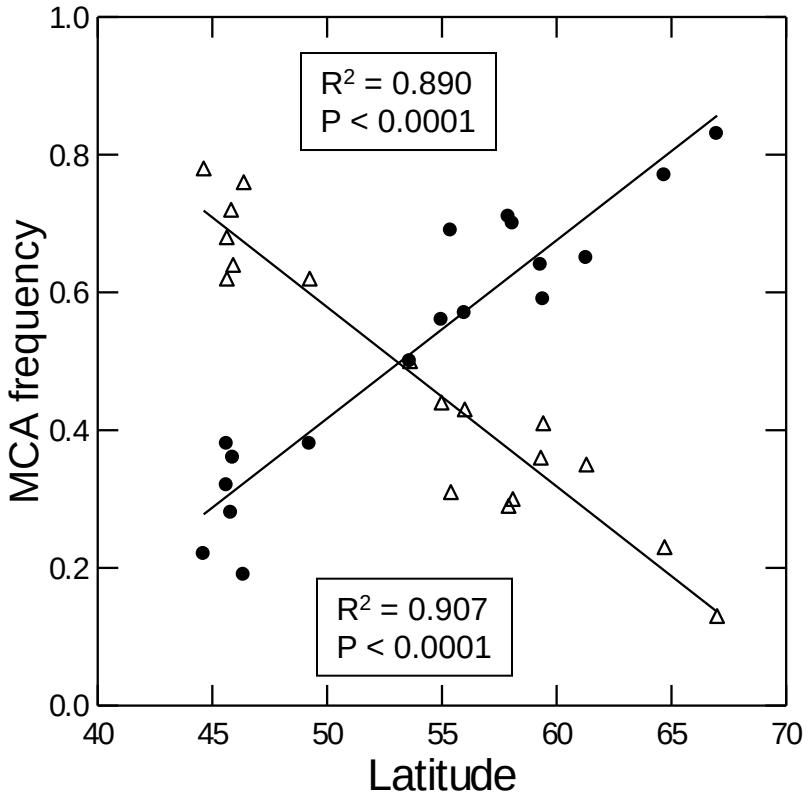
Chum	18 populations, 4 alleles 335 bp (0.519), 341 bp (0.476)
Coho	19 populations, 6 alleles 335 bp (0.761), 338 bp (0.174)
Pink	16 populations, 10 alleles 452 bp (0.923), 428 bp (0.049)
Chinook	42 populations, 8 alleles 335 bp (0.737), 359 bp (0.225)

(MAL = Mean Allele Length MCA = Most Common Allele)

Objective 1 Results: Chum salmon

Prediction: strong latitudinal cline in *Clock*

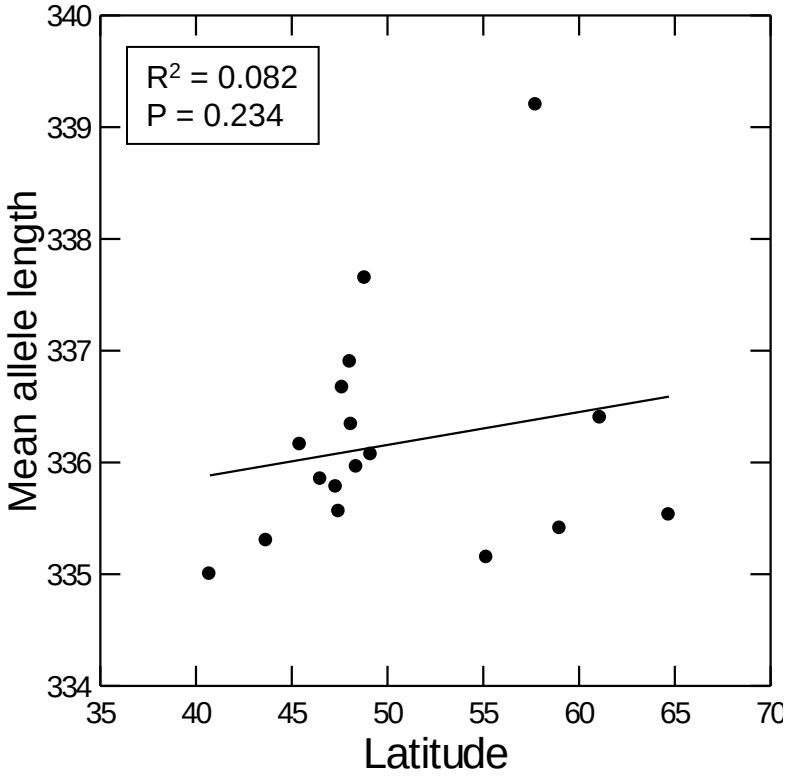


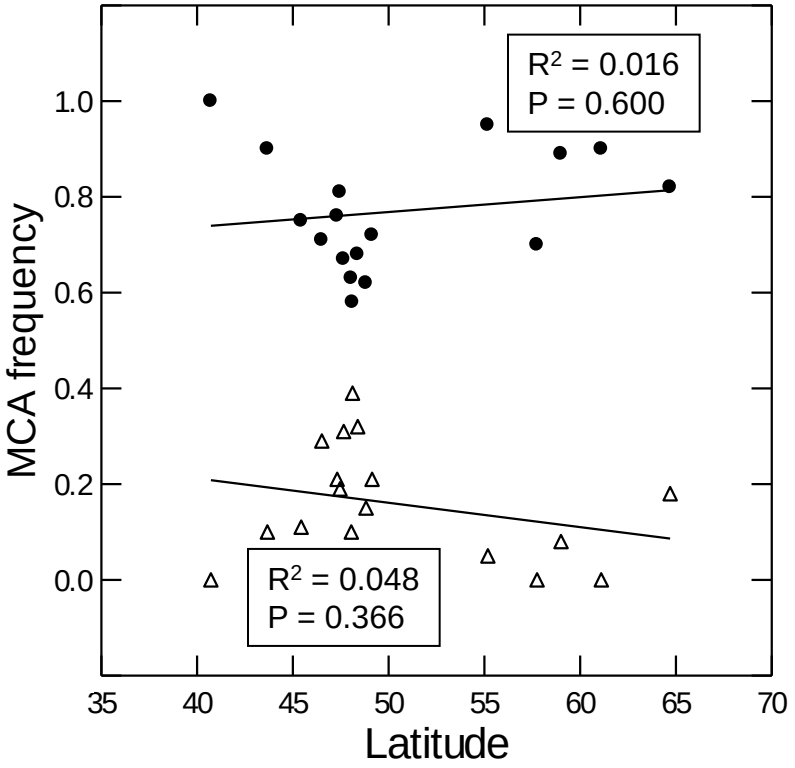


Allele
● 335bp
△ 341bp

Objective 1 Results: Coho salmon

Prediction: no latitudinal cline in *Clock*

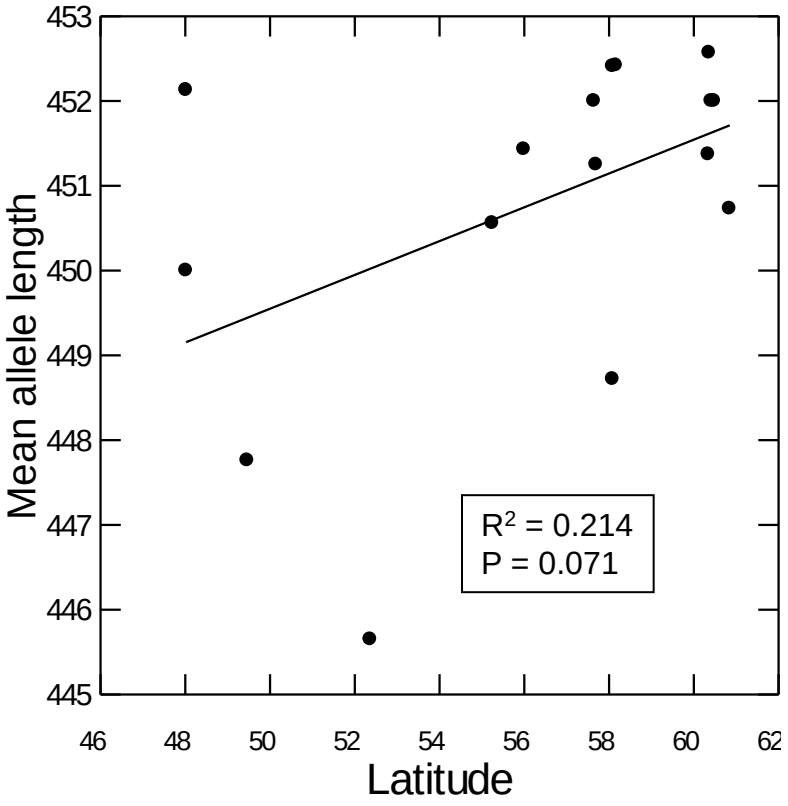


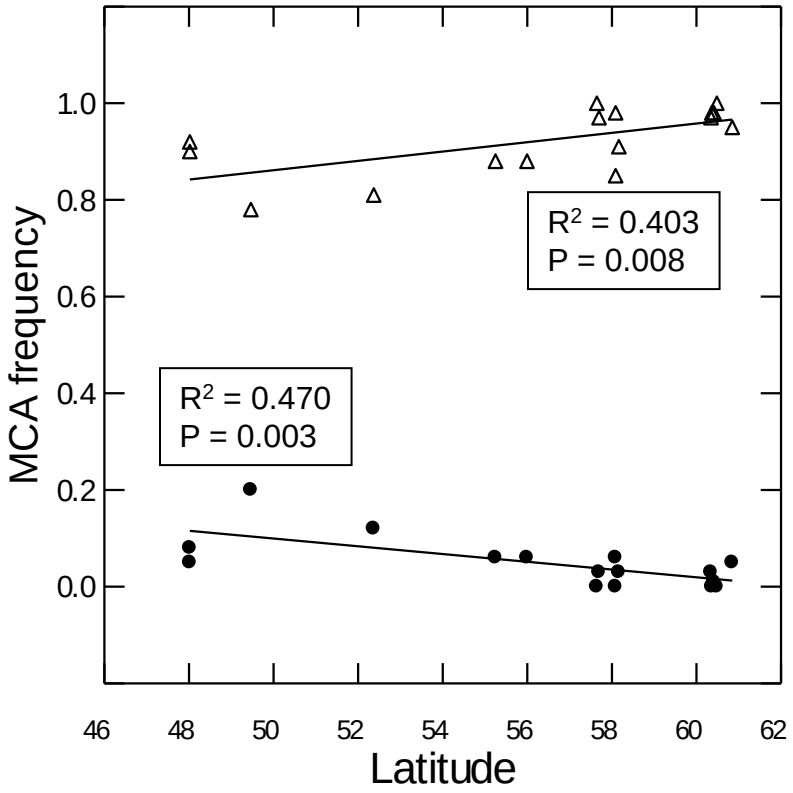


Allele
● 335bp
△ 338bp

Objective 1 Results: Pink salmon

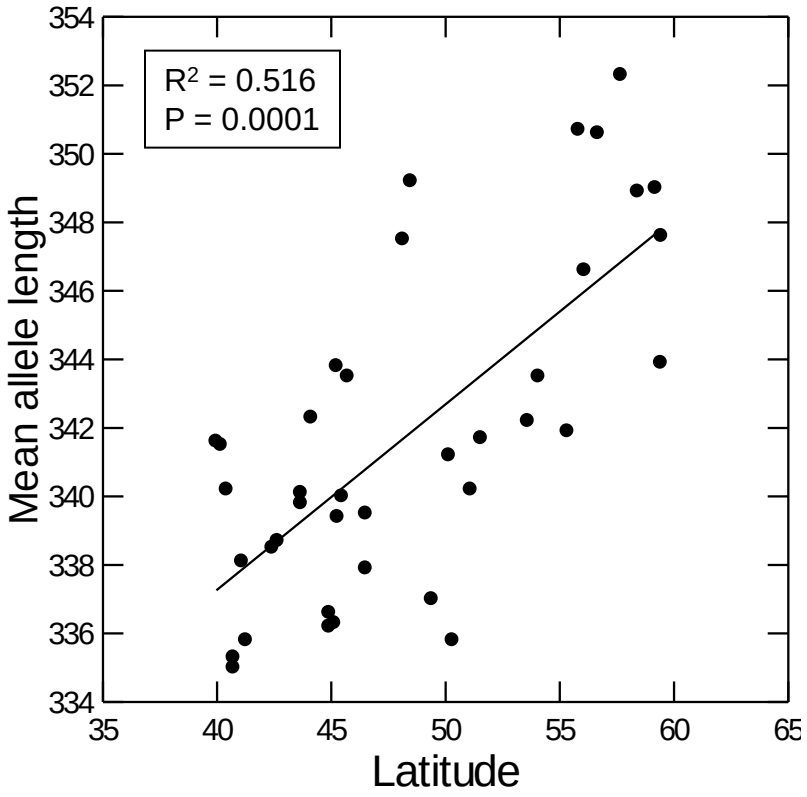
Prediction: weak latitudinal cline in *Clock*

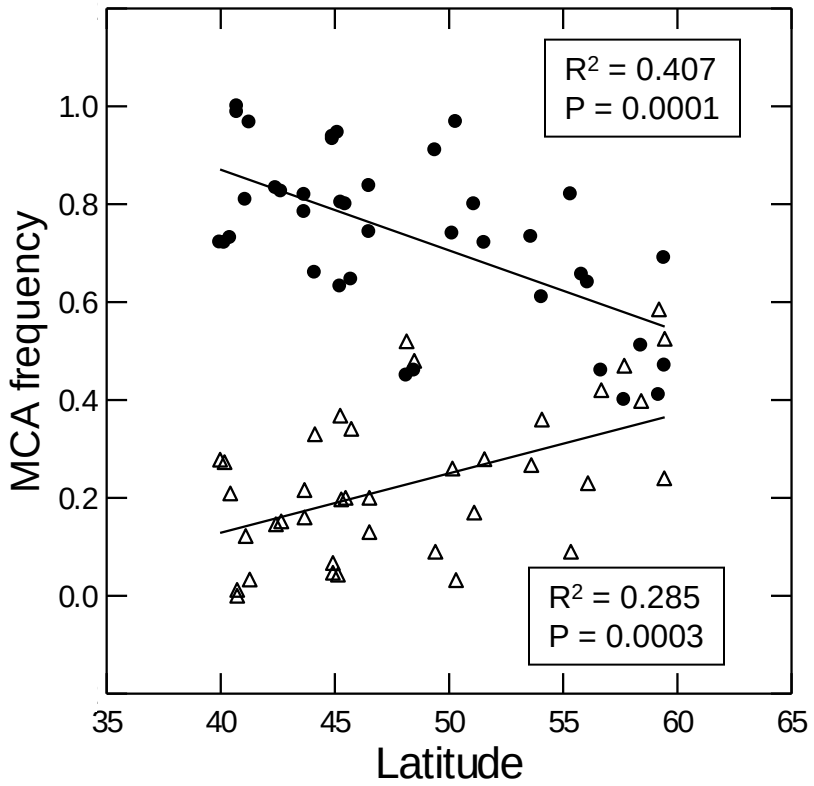




Objective 1 Results: Chinook salmon

Prediction: strong latitudinal cline in *Clock*





Allele
● 335bp
△ 359bp

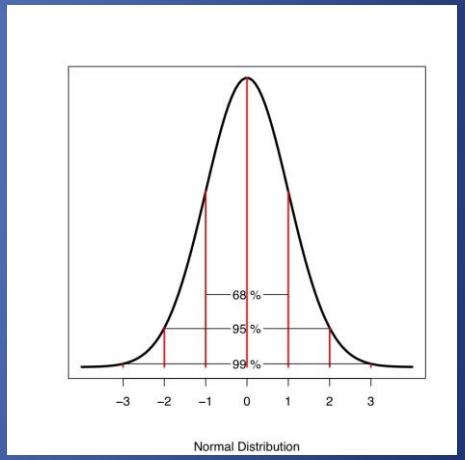
Objective 2: Test for Selection

Compare regression fits (r^2; MCA frequency on latitude) of two *Clock* alleles to the distribution of a larger set of allozyme loci

Chum salmon: 21 loci, 45 populations
(C. Kondzela, NOAA Fisheries)

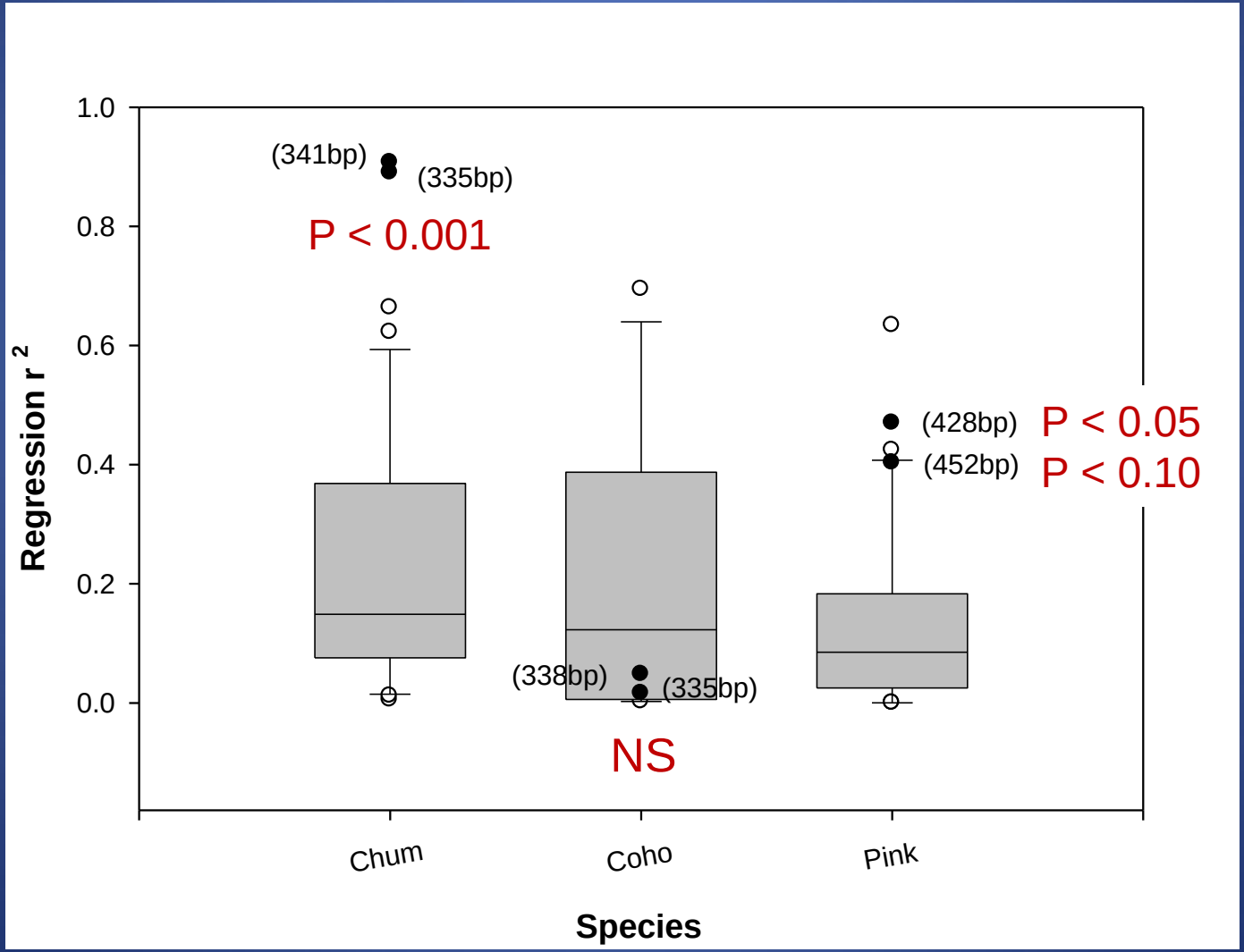
Coho salmon: 12 loci, 28 populations
(D. Teel, NOAA Fisheries)

Pink salmon: 27 loci, 76 populations
(S. Wildes, NOAA Fisheries)



Deviation from neutral expectation is evidence for selection

Objective 2 Results: Comparison of *Clock* and Allozyme r^2 Values



Summary of Objectives 1 & 2

Species	L. Cline in spawning age	L. Cline in spawn timing	L. Cline in *Clock*	Evidence for selection
Chum	Strong	Strong	Strong	Yes
Coho	Weak	No pattern	None	No
Pink	None	Moderate	Weak	Yes
Chinook	Strong	Strong	Strong	Yes

Objective 3: Potential Ecological Factors Influencing Variation in *Clock*

Univariate Regression Tree Analysis -

Partition populations into groups for which the clinal variation in *Clock* PolyQ among them best corresponds to the ecological factors:

1) Day length on the date of peak spawning (DL)
2) Index of freshwater migration (M)
   (distance to and elevation at spawning grounds)

Cross-validated predictive error (CV Error) measures the predictive value of the tree (0 = poor, 1 = perfect predictor)

Most Common Allele (MCA) frequency for *Clock* PolyQ

Objective 3 Results: Univariate Regression Trees

Chum: Day length explains 87.1% of the variance in MCA

CV Error = 0.661; moderately high

Coho: Day length and freshwater migration index explain 45.5% of the variance in MCA

CV Error = 0

Pink: Day length explains 53.2% of the variance in MCA

CV Error = 0

Chinook: Day length and freshwater migration index explain 67.8% of the variance in MCA

CV Error = 0.28; low

Conclusions

- *Clock* PolyQ length diversity does not vary uniformly with latitude among four closely related species
- Clinal variation in *Clock* PolyQ corresponds to variation in reproductive timing
- *Clock* PolyQ variation may be maintained by selection in three of the four species
- Concordance between day length and *Clock* PolyQ allele frequency in two species that show robust clines in reproductive timing

Conclusions

- Clinal variation in *Clock* PolyQ *may* reflect an adaptation to seasonally changing day length and influence geographic variation in reproductive timing in some of these highly migratory species

- Climate change could result in the decoupling of day length and optimal timing, requiring adaptive shifts in key life history traits involving photoperiodic response

- These results are strictly correlative and could alternatively be explained by parallel evolution or historical contingency

- Future research is directed towards understanding the functional significance of the polyglutamine repeat motif in the salmon *Clock* gene, as well as characterizing other candidate loci

Acknowledgments

- National Research Council

- Samples and allozyme loci data provided by:
  O. Johnson, D. Van Doornik, and D. Teel (NWFSC),
  C. Habicht, B. Templin (Alaska Dept of Fish and Game, ADFG),
  J. Olsen (U.S. Fish and Wildlife Service, USFWS),
  C. Kondzela and S. Wildes (NOAA Fisheries, Juneau),
  S. Blankenship (Washington Dept of Fish and Wildlife, WDFW),
  T. Beacham (Dept of Fisheries and Oceans DFO)

- J. Cowen (NWFSC) for performing the GIS analyses