

Molecular Genetic Investigations of Harbor Seals in Alaska

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Management and Conservation of Alaskan Harbor Seals

Maintain species and population stocks as significant functioning elements of their ecosystem, and not allow them to diminish below optimum sustainable population levels

MMPA, 1994

Conserving and sustaining the harbor seal for our cultural well-being

ANHSC, 1995

To achieve these goals, by designing an effective management strategy, we need to :

Improve our knowledge of harbor seal biology

distribution

population abundance & trends

life history & breeding behaviour

diet & foraging ecology

population structure

movements and dispersal patterns

health, & levels of genetic diversity

... and gain this knowledge using the following methods

Traditional Ecological Knowledge (TEK)

aerial and land-based surveys

tagging, telemetry

necropsy and biosampling

fatty acid analysis, blood chemistry, etc.

genetic analysis

Genetic methods provide data on [in green] :

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How can genetic analysis inform us about population structure, dispersal patterns and breeding behaviour?

Genetic markers (DNA) are inherited from parent to offspring and remain, usually unchanged, with each individual their entire lives.

These markers may act as 'tags' that track the dispersal and breeding behaviour of individuals

Extensive interbreeding or dispersal by animals between 2 areas is reflected in a high level of mixing of these tags = **high gene flow**

By measuring **the level of genetic differentiation** between 2 areas, we can estimate **the level of dispersal or interbreeding**.

Method

1. collect adequate samples from representative locations
2. examine patterns of variation at several genetic markers
3. measure genetic differences between locations
4. estimate dispersal/interbreeding between locations

What are the properties we are looking for in a Genetic Marker ?

To be informative in studies of population structure, dispersal and breeding patterns, a marker needs to be **variable**, **heritable** and **selectively neutral**.

Traits	Variable	Heritable	Selectively neutral
eye colour	X	X	
plumage patterns in birds	X	X	
coat colour in mammals	X	X	
language, dialects	X		
last names	X	X	
mitochondrial DNA	X	X	X
nuclear DNA	X	X	X

Mitochondrial DNA

An ideal genetic marker in studies of population structure

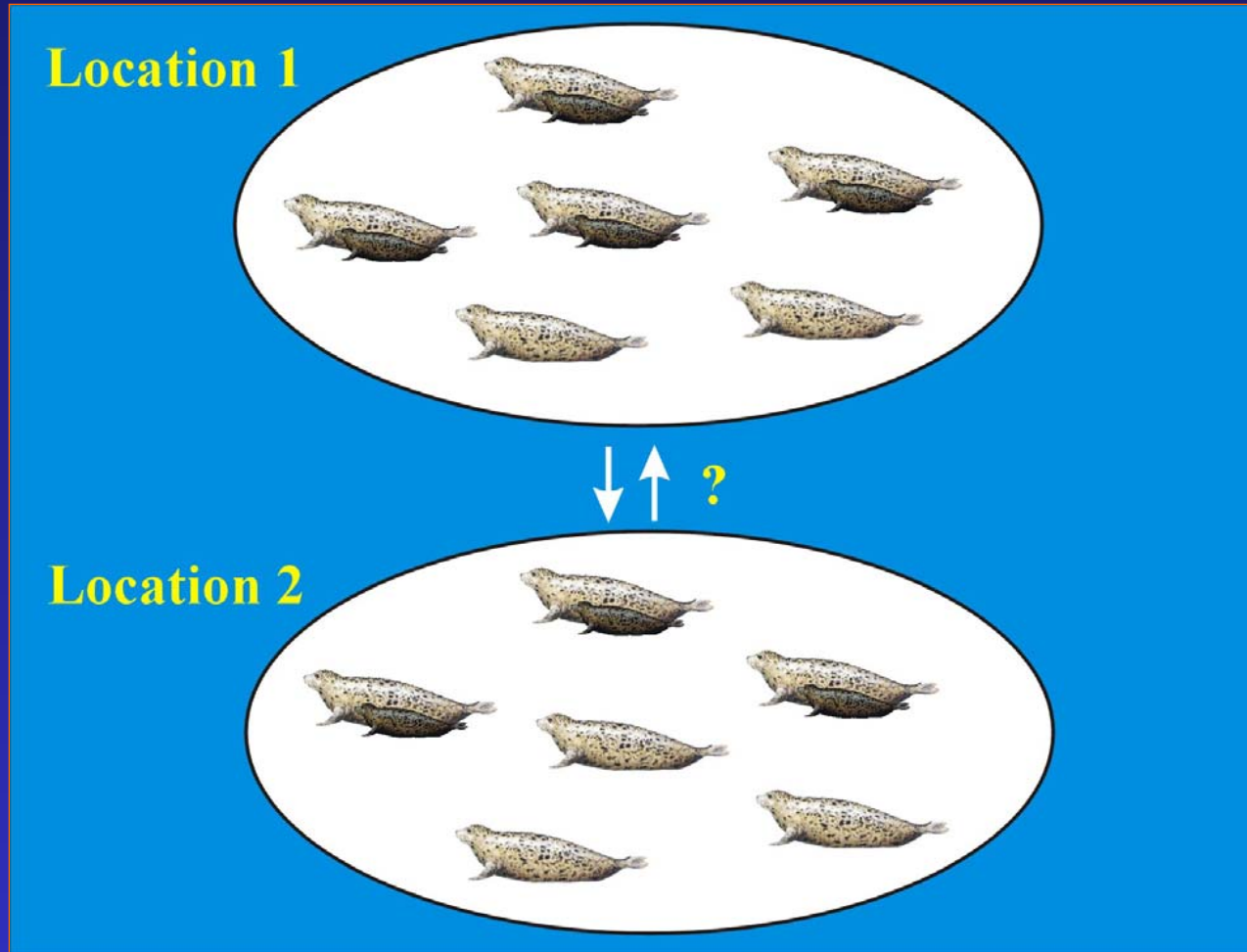
Highly variable - several different variants, termed **haplotypes**, may occur within a single population.

Maternally inherited - each individual inherits its **haplotype** from its mother. Similar to the inheritance of last names from the father.

Used to:

Estimate level of movement between populations by examining the distribution of **haplotypes**

A critical component in understanding population structure and identifying management stocks in Alaskan harbor seals is estimating the level of dispersal between groups of seals



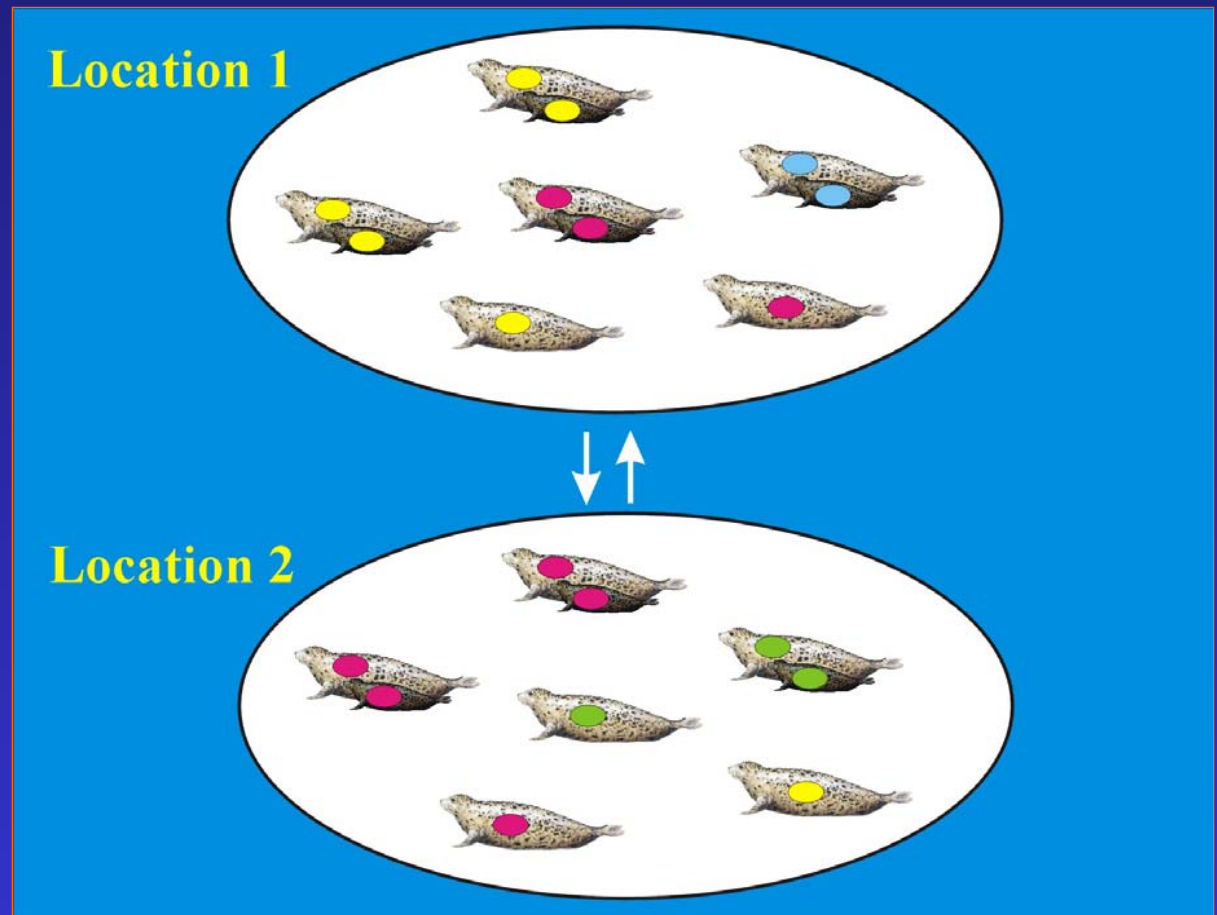
This can be achieved by estimating the level of differences in mitochondrial DNA among locations

Mitochondrial DNA

- * The colored dot on each seal indicates which haplotype that individual carries.
- * Because mtDNA is maternally inherited, each pup has the same haplotype as its mother
- * The frequencies of the 4 haplotypes differ substantially between the two locations.

For instance, in location 1, 5 out of the 10 seals have the yellow haplotype, while in location 2, the yellow haplotype was only detected in 1 out of 9 seals.

This indicates that dispersal between these two locations is limited



Application of Molecular Genetics to the Management and Conservation of Alaskan Harbor Seals

- * **Identify demographically discrete sub-populations**

- aid in providing a framework for the identification of stocks

- determine whether a sub-population or stock is hunted at more than one location or time

This knowledge of population structure and dispersal patterns will help interpret:

- * **the spread and modeling of disease**

- * **population size and range estimates**

- * **population trends**

- * **level of take and impacts of habitat loss, development, pollution, etc.**

Range of North Pacific Harbor Seals, *Phoca vitulina*





The primary Harbor Seal sampling locations across Alaska
 (The dashed lines represent the current stock boundaries)

Sample Collection

Tissue samples were collected from throughout Alaska using the following methods and resources:

Biosampling

Tagging

Strandings

Museum archives



ANHSC



National Geographic



ANHSC

Laboratory procedures: **DNA extraction**

Back at the lab, we first extracted DNA from each tissue sample.....



High Latitude Molecular Ecology Group - *Genetics Program, SWFSC, La Jolla*

G.O'Corry-Crowe - NMFS

Laboratory procedures: **DNA amplification**

.... and then amplified the region of the DNA we were interested in.



PCR setup - Genetics Program, SWFSC, La Jolla

Laboratory procedures: **DNA sequencing**

Finally, the amplified DNA was sequenced, producing the final genetic data for analysis

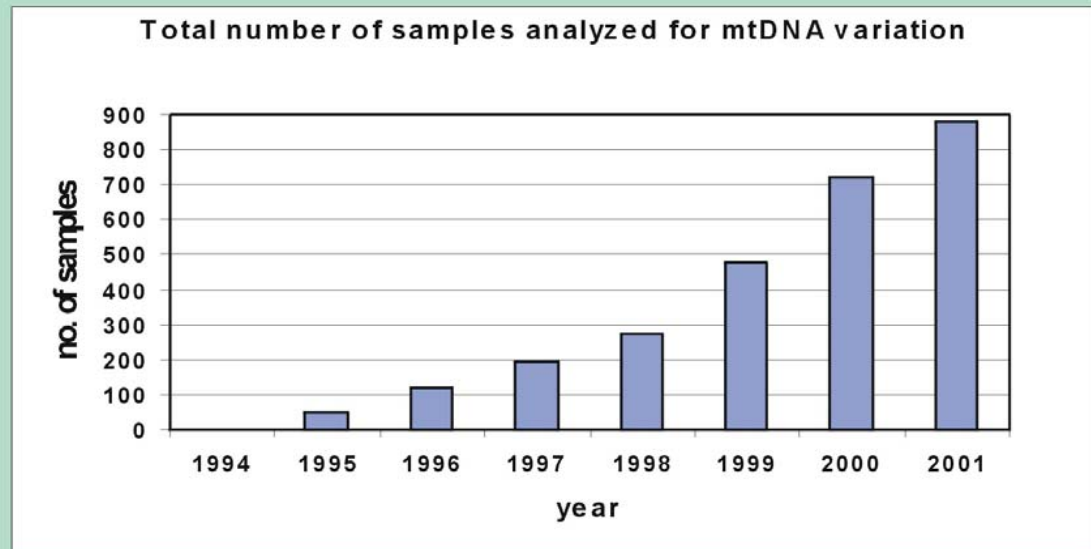


ABI 3100 and 377 Automated Sequencers - Genetics Program, SWFSC, La Jolla

Study Milestones

- 1994** initial proposal, initiation of sample collection and development of lab methods
- 1995** early detection of high haplotypic diversity (H)
- 1996 to 1998** detection of broad-scale population structure and an isolation by distance pattern to genetic differentiation
- 1999** discovery of micro-geographic structure
- 2000** development of new analytical techniques.

The most recent research (**2001 & 2002**) has further clarified the genetic and demographic relationships among well-sampled areas and highlighted the need for more samples from the poorly sampled areas.



Proposals

Sampling

Method dev.

Data collection

High H

large - scale structure

isolation by distance

micro - geo Structure

evidence of structure at least at 3 PBR areas

more evidence of micro-geo structure

new analytical techniques

further clarify structure

well sampled v. poor sampled areas

Analysis of Population Structure

Analysis of Molecular Data

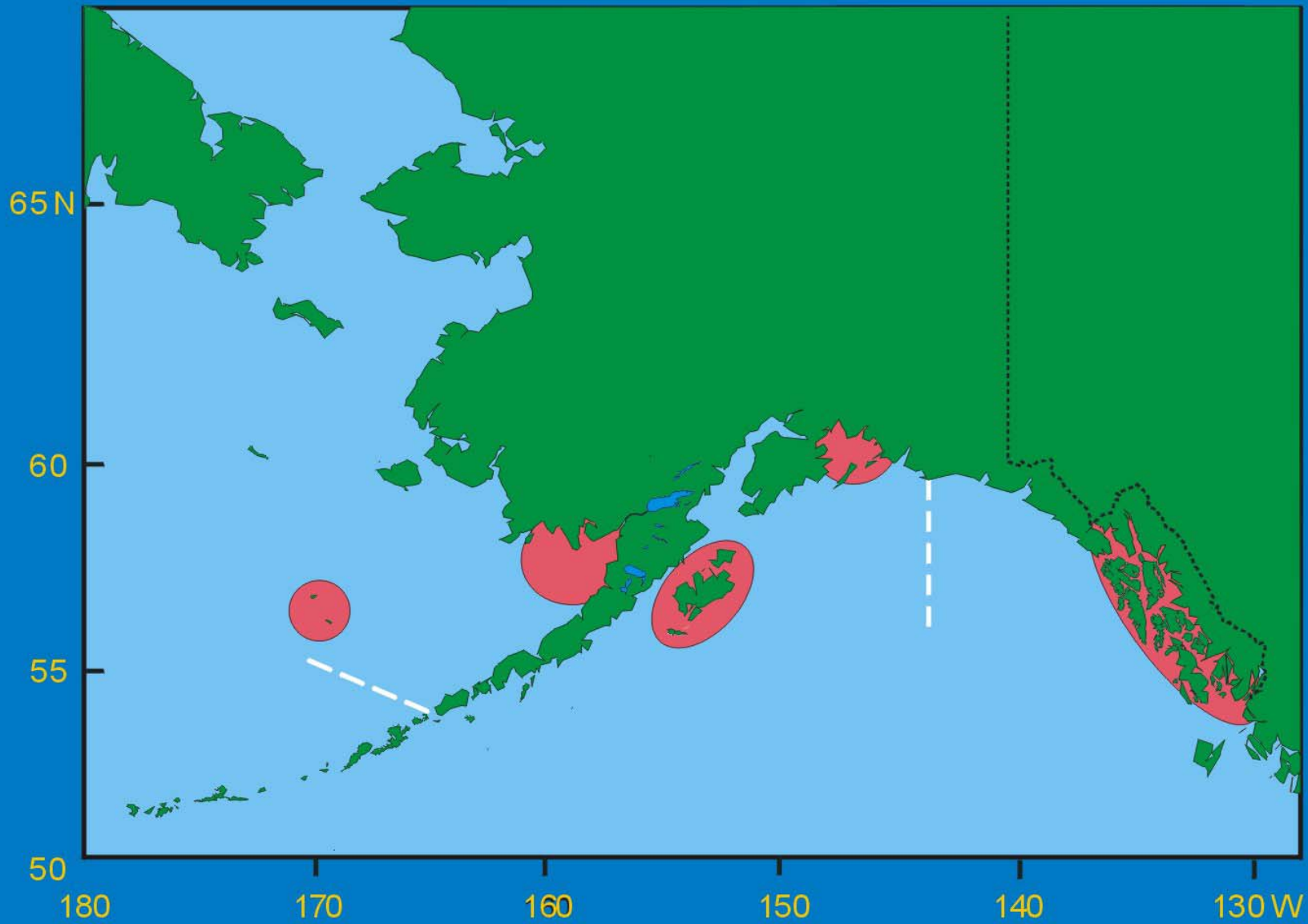
To date, we used:

- * **the traditional approach of hypothesis testing**
 - which requires the scientist to define the populations to be tested
- * **a new approach called Boundary Rank**
 - which uses the genetic data to suggest population structure hypothesis

Both approaches suggest that there are more than the currently defined 3 stocks

Total Evidence

**genetic data
movements and foraging behaviour
distribution
habitat - bathymetry, oceanography
TEK**



Comparison among 5 major centers of abundance of harbor seals in Alaska
(note: dashed lines represent current stock boundaries)

Estimates of genetic differentiation (below diagonal), and significance values (P) from 10,000 permutations (above diagonal) among major centers of abundance of harbor seals in Alaska

		I	II	III	IV	V
Φ_{st}						
I	Southeast Alaska	-	0.009	0.000	0.008	0.009
II	Prince William Sound	0.015	-	0.140	0.056	0.026
III	Kodiak Archipelago	0.035	0.004	-	0.110	0.056
IV	Bristol Bay	0.048	0.020	0.012	-	0.297
V	Pribilof Islands	0.085	0.049	0.028	0.006	-
F_{st}						
I	Southeast Alaska	-	0.011	0.002	0.000	0.000
II	Prince William Sound	0.004	-	0.006	0.000	0.000
III	Kodiak Archipelago	0.007	0.006	-	0.000	0.001
IV	Bristol Bay	0.031	0.031	0.027	-	0.000
V	Pribilof Islands	0.065	0.065	0.044	0.085	-

Macrogeographic Population Structure in Alaskan Harbor Seals

Extensive population structure, indicating little or no dispersal, was found between centers of distribution

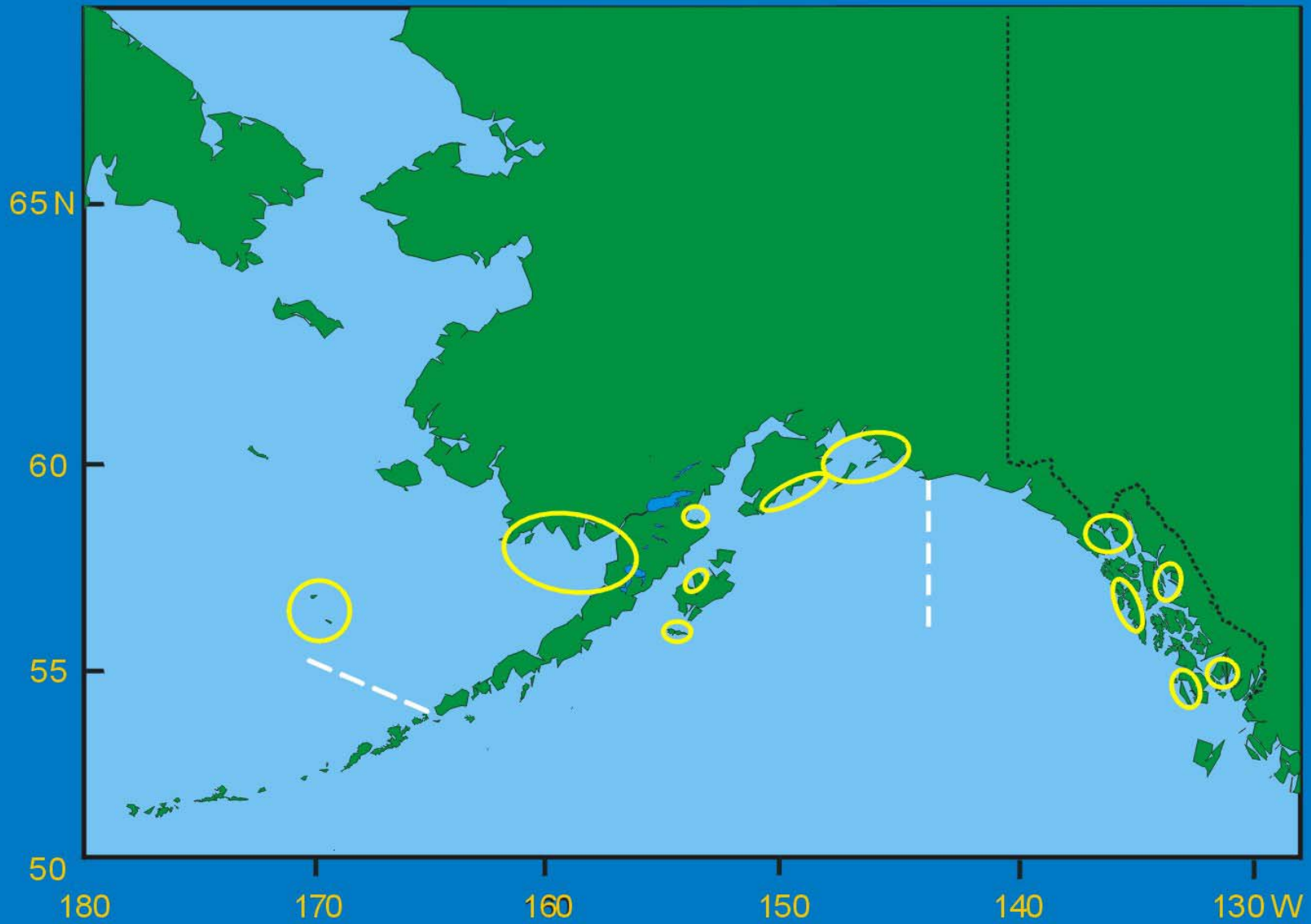
supported by data on movements and trends

The level of differentiation was correlated with geographic distance

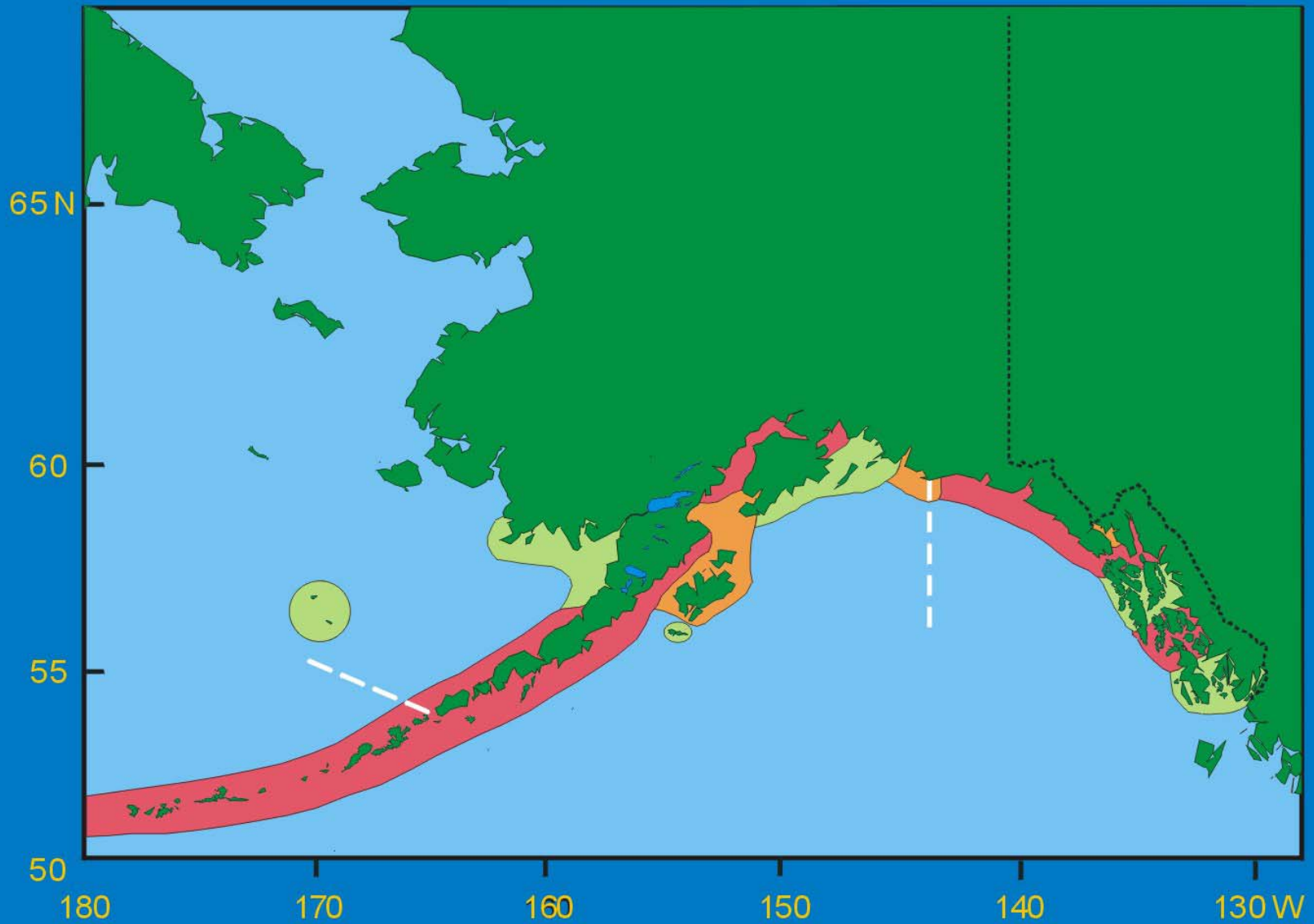
suggests that dispersal, when it occurs, is among neighboring sub-populations

Differentiation between the Kodiak archipelago and PWS and between Bristol Bay and the Pribilof Islands

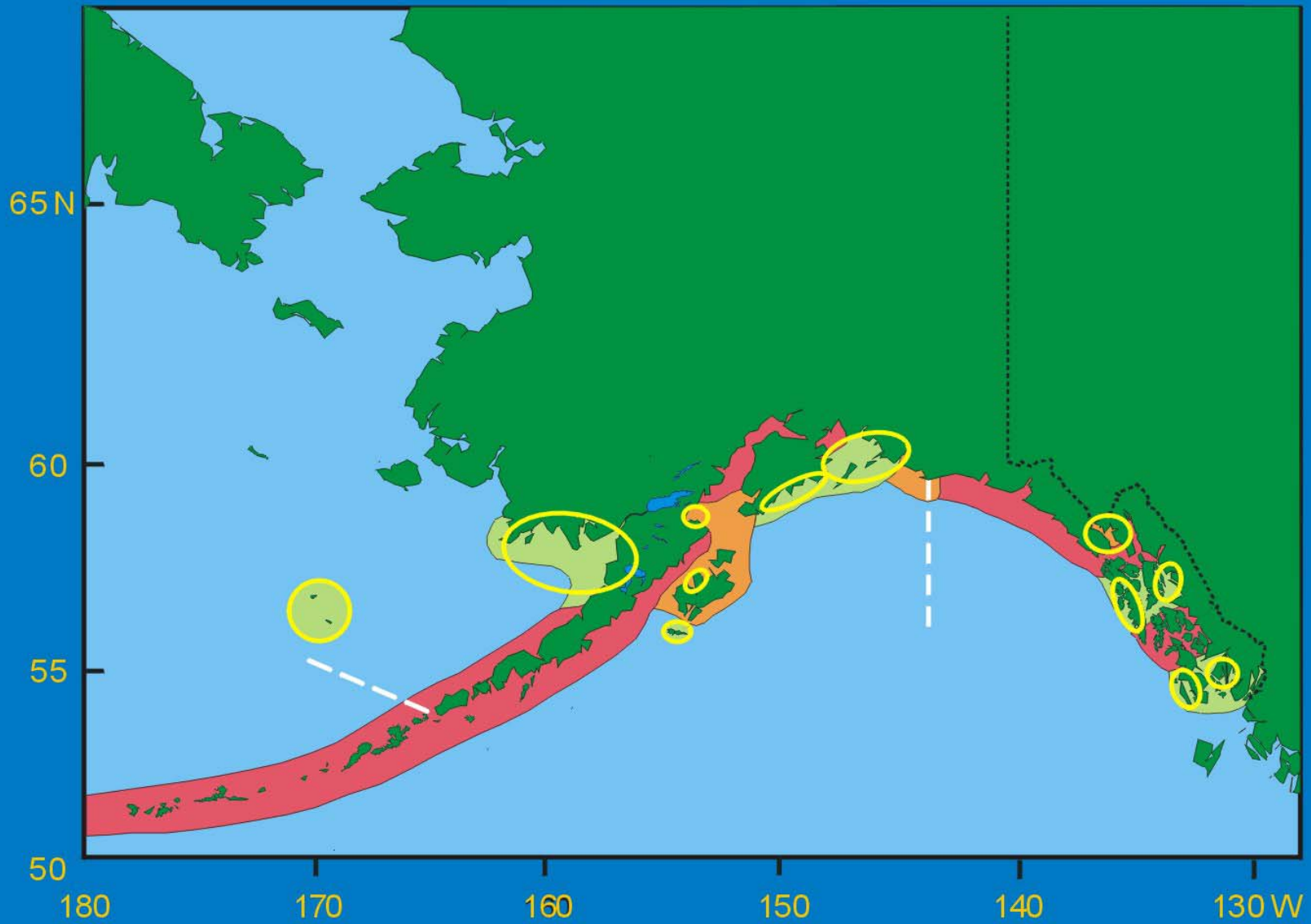
indicate that current management stocks are incorrect



Results of Boundary Rank cluster analysis. All differentiated at $P < 0.1$

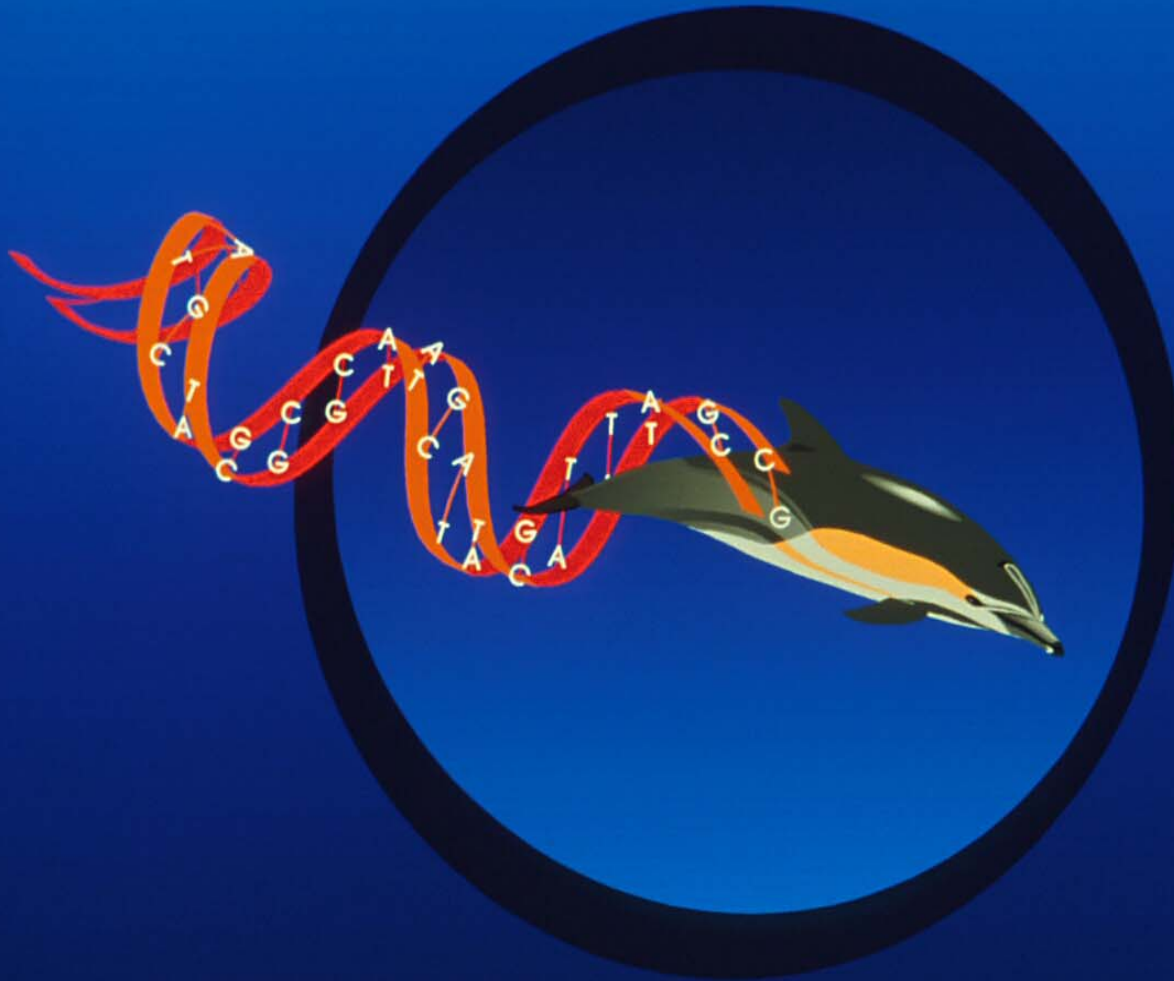


Harbor seal sample coverage in Alaska, April, 2002
Green = good, amber = fair, red = poor



The results of the cluster analyses relative to sample coverage

MARINE MAMMAL DIVISION



Southwest Fisheries Science Center, La Jolla, California

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