

Genetic structure of Japanese pink salmon populations inferred from nucleotide sequence analysis of mitochondrial DNA



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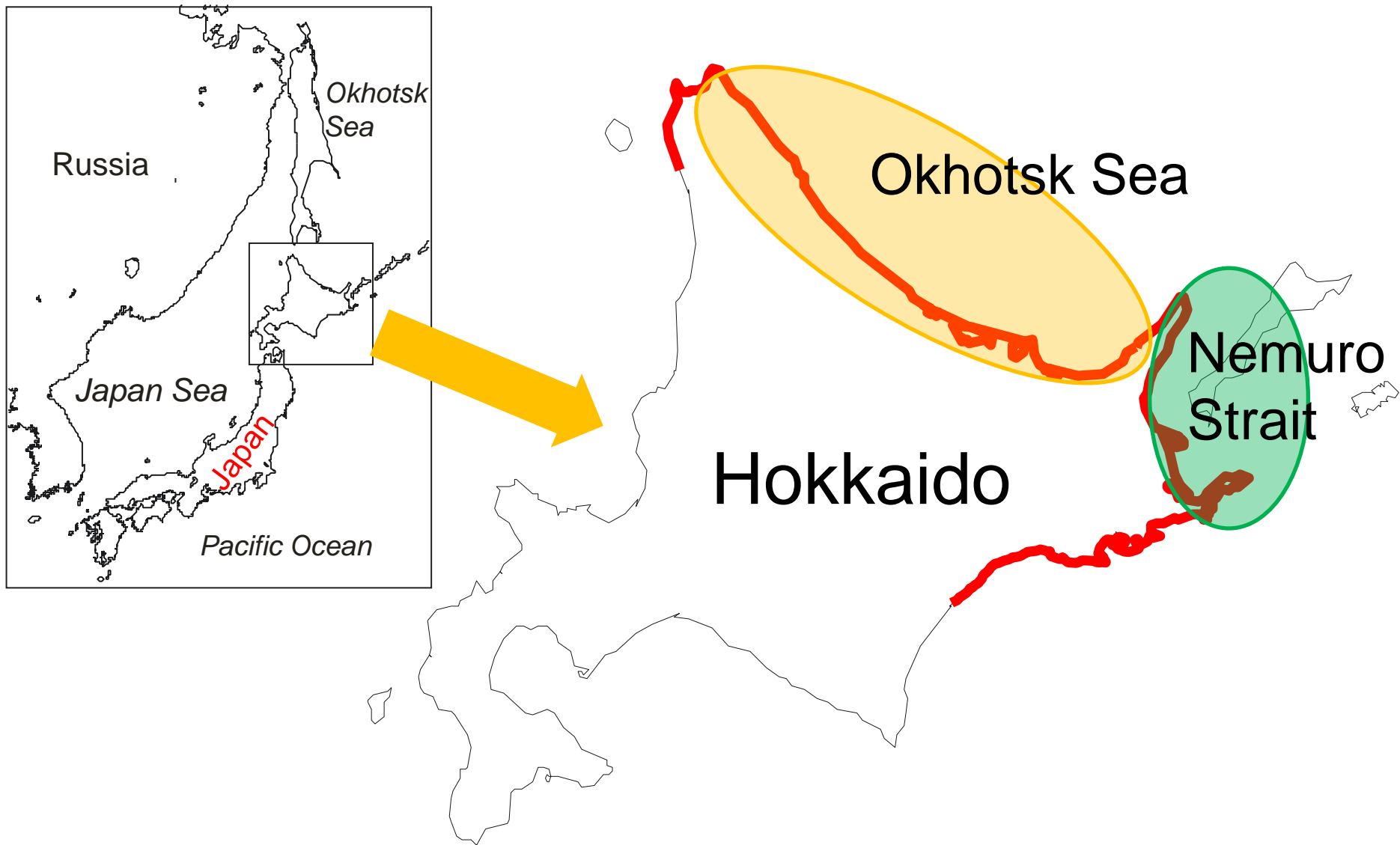
Hokkaido National Fisheries Research Institute
Fisheries Research Agency

25th Pink & Chum Salmon Workshop
Centennial Hall, Juneau AK, USA
February 13, 2012

Presentation outline

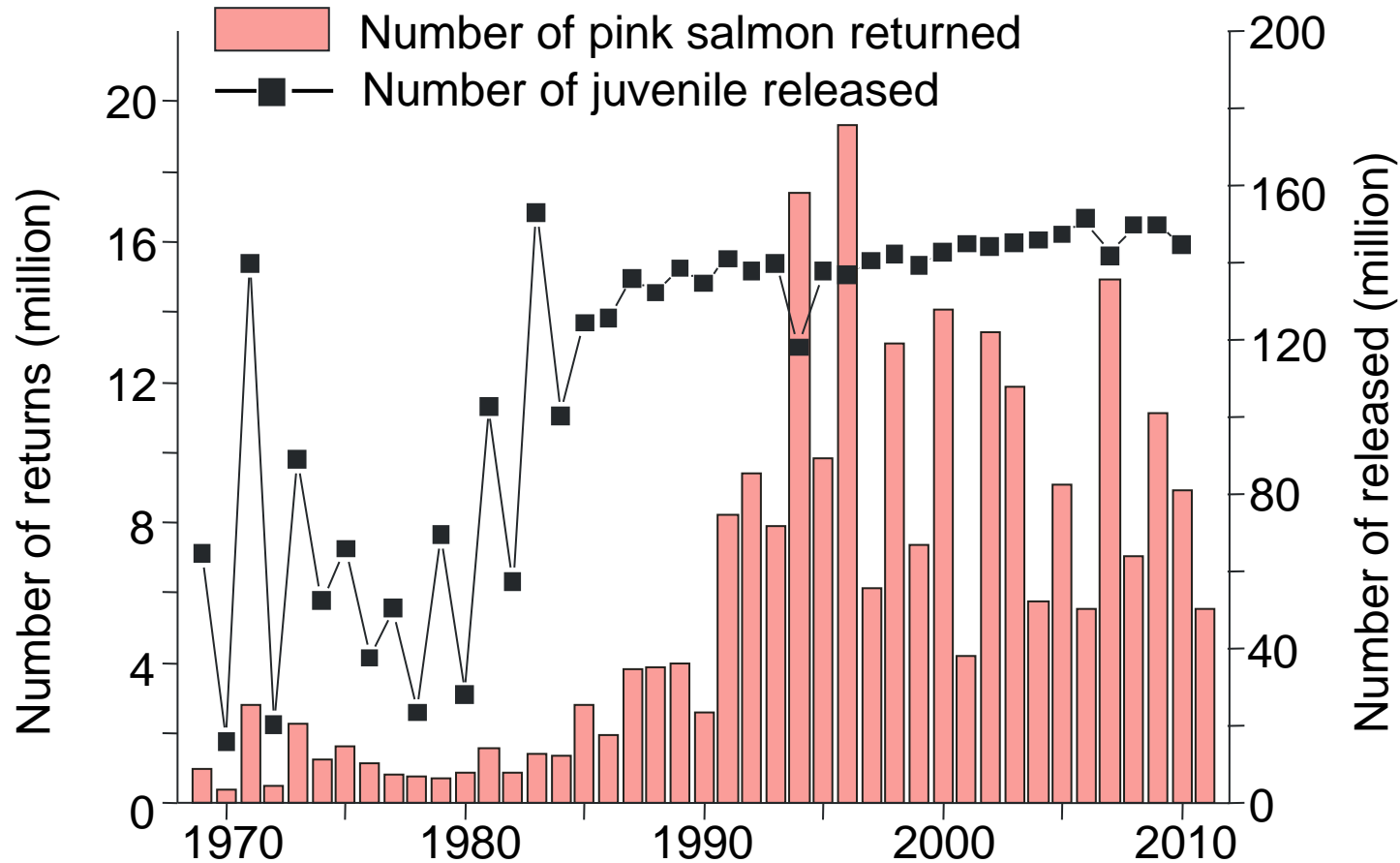
1. Background and Objectives
 - Features of Japanese pink salmon
2. Materials and Methods
 - Sample collection (location, year, number, etc)
 - Genetic analysis
3. Result
 - Haplotype data
 - Gene diversity
 - Genetic structure
4. Summery

Main spawning area of pink salmon in Japan



- Return and spawning period: August-October
- Main spawning field: Okhotsk Sea and Nemuro strait in Hokkaido

Number of hatchery released fry and returning pink salmon in Hokkaido



- Release: consistent at 140 million per year since 1985
- Return: fluctuated greatly since early-1990s

Status of Japanese pink salmon

Since 1990s,

- The constant number of pink salmon fry has been released

However,

- Show fluctuation of returning numbers between even- and odd-year broodlines



Should clear up the cause of year-to-year differentiation to

- stabilize fisheries resources of Japanese pink salmon
- improve the Japanese hatchery program management

Require more detailed and basic information of stock assessment, ecological, and **genetic data** of Japanese pink salmon

Status of Japanese pink salmon

However, genetic data of Japanese pink salmon is...



Objectives

Estimation of genetic structure of Japanese pink salmon populations using mitochondrial (mt) DNA

Sampling locations, years, & sample numbers

1. Kitamihorobetsu R.

2. Tokusibetsu R.

3. Yubetsu R.

4. Tokoro R.

5. Shari R.

6. Ichani R.

7. Shibetsu R.

8. Nishibetsu R.

9. Kushiro R.

Collection Year (N)

Even / Odd

1. 2006 (52)/07 (48)

2. 2007 (46)/08 (46)

3. 2006 (46)/07 (44)

4. 2006 (47)/07 (49)

5. 2006 (43)/07 (48)

6. 1998 (39)/99 (48)

7. 2006 (39)/07 (49)

8. 2006 (49)/07 (47)

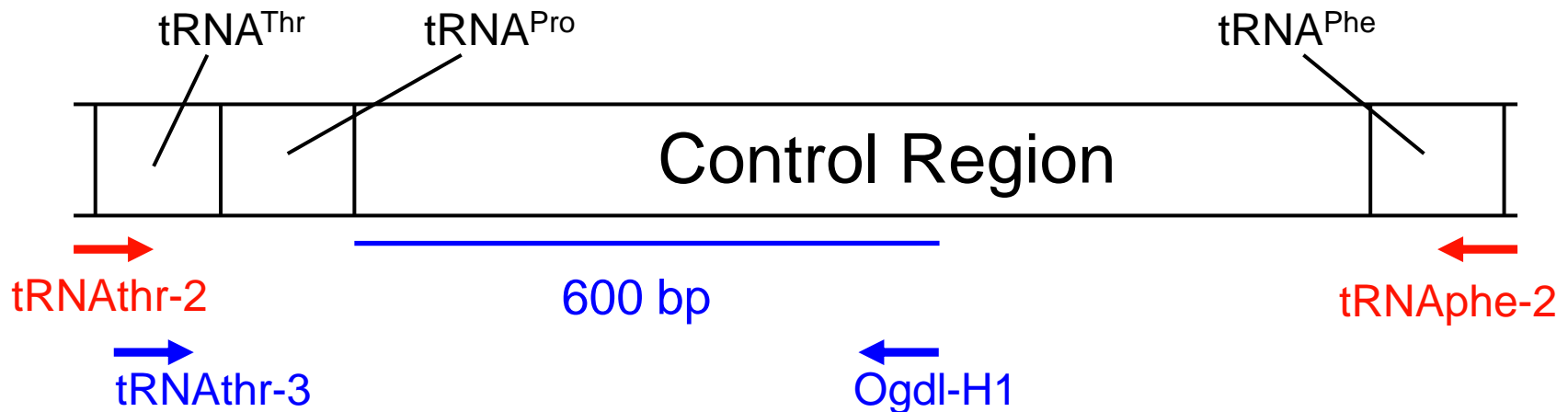
9. 2006 (45)/07 (47)

Even-year: total of 354 individuals from 9 river populations

Odd-year : total of 426 individuals from 9 river populations

Genetic analysis

1. Genetic samples: pectoral fins or liver tissues
2. DNA extraction
3. PCR: mtDNA control region
4. Nucleotide sequence: direct sequencing
5. Haplotype detection



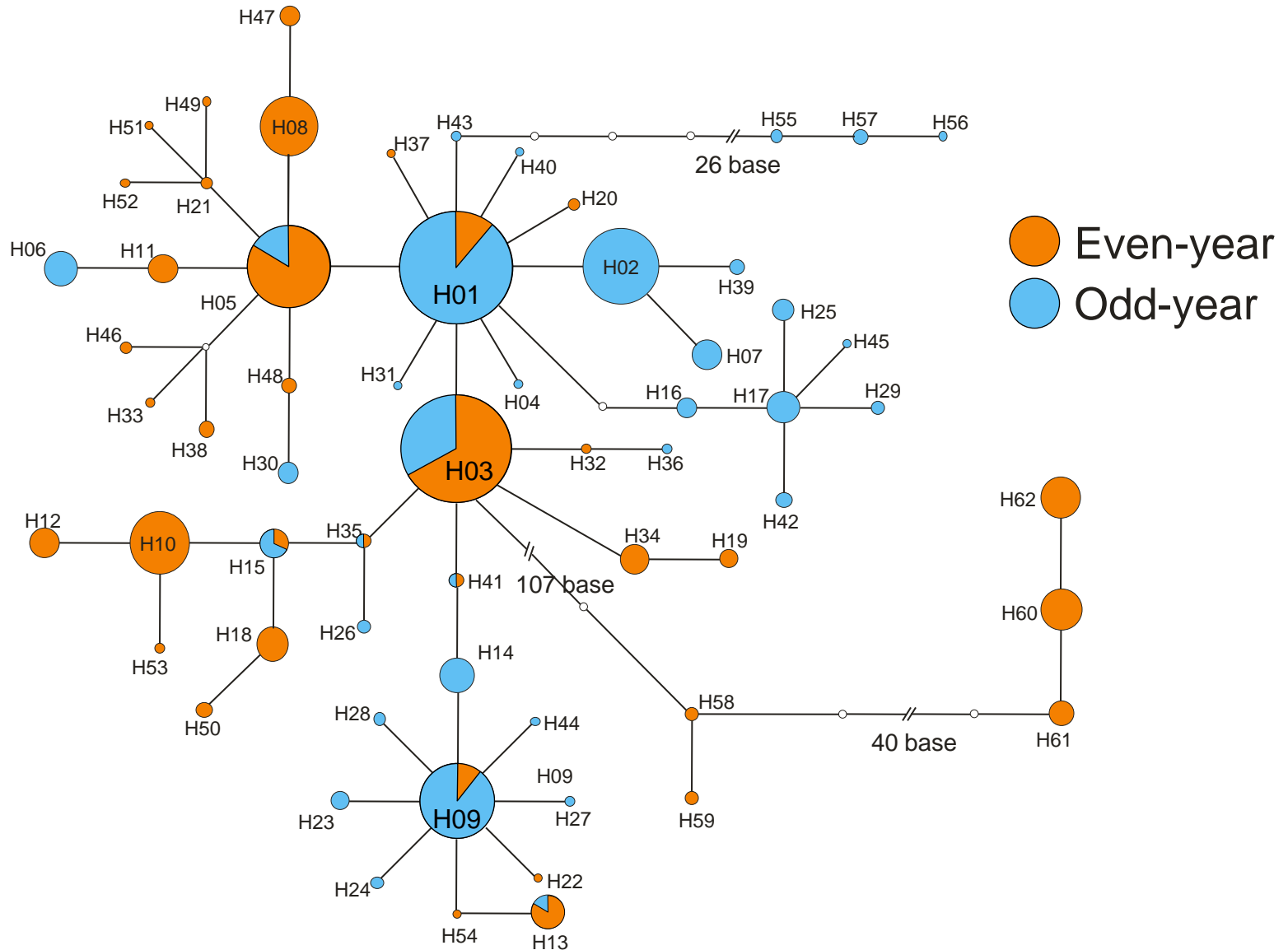
PCR condition

35 cycles

94°C	94°C - 58°C - 72°C			72°C
(5m)	(30s)	(30s)	(1m)	(7m)

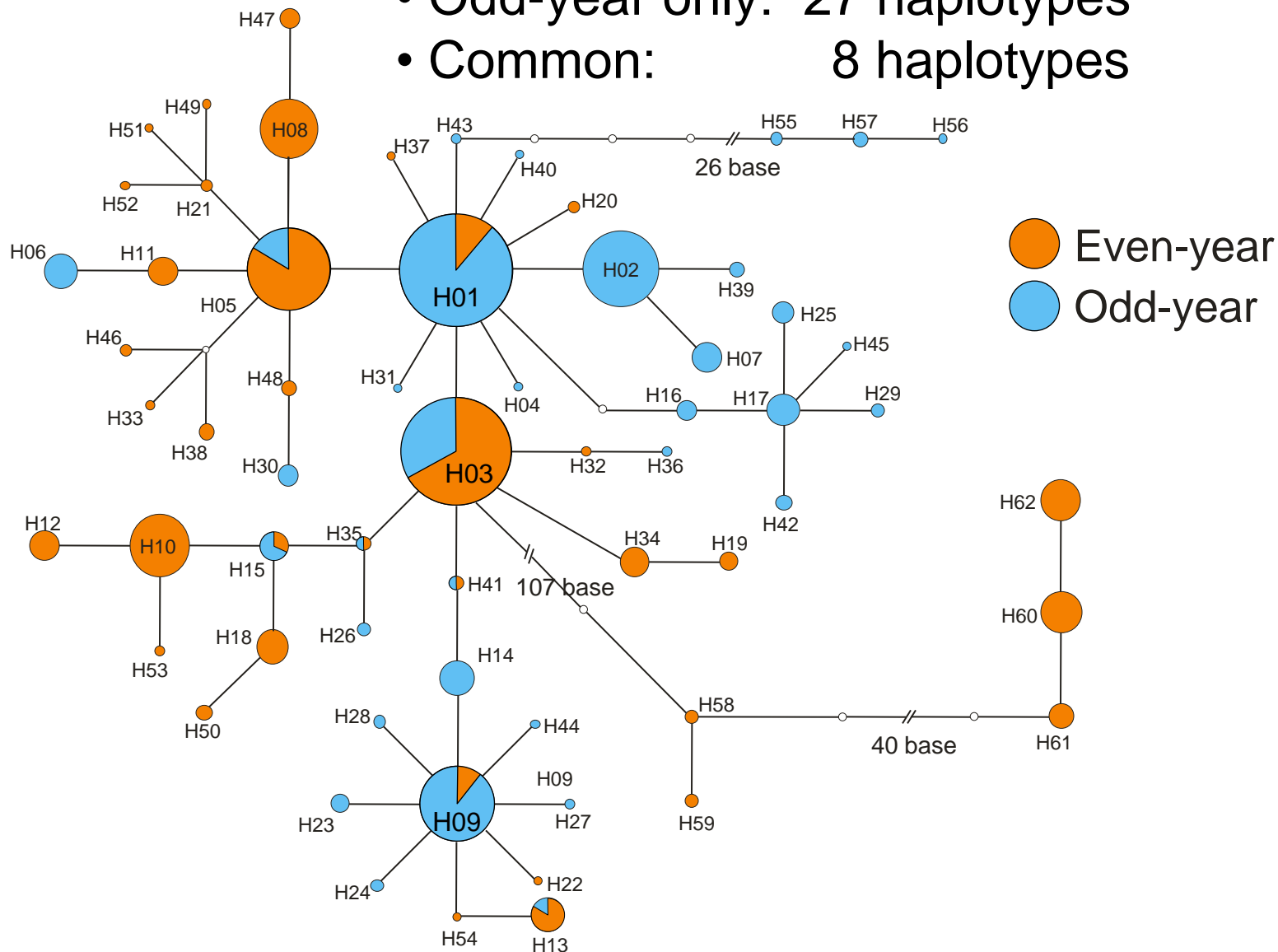
Haplotype tree (Maximum parsimony)

- Total 62 haplotypes



Haplotype tree (Maximum parsimony)

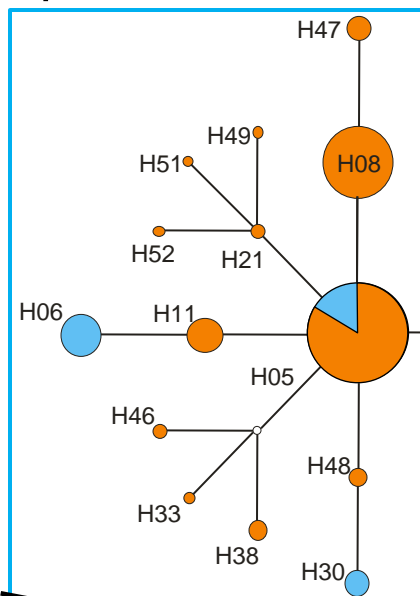
- Even-year only: 27 haplotypes
- Odd-year only: 27 haplotypes
- Common: 8 haplotypes



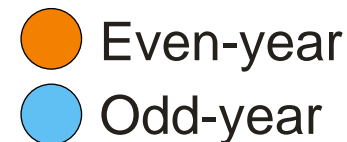
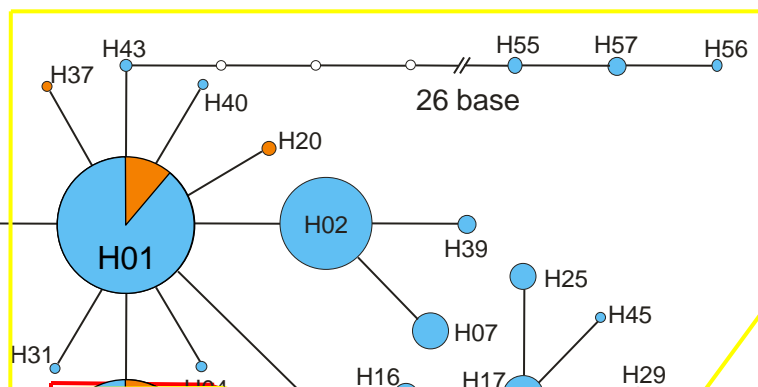
Haplotype tree (Maximum parsimony)

- 62 haplotypes were grouped in 6 clades

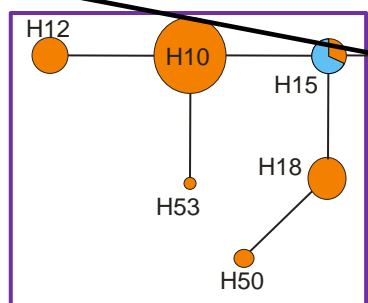
Hap05 clade



Hap01 clade

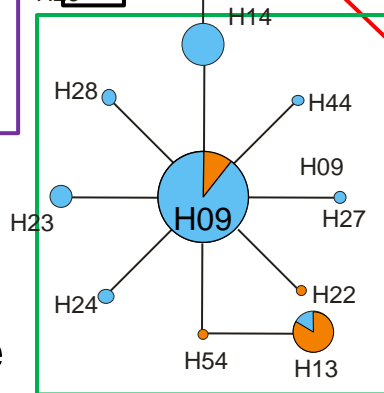


Hap35 clade

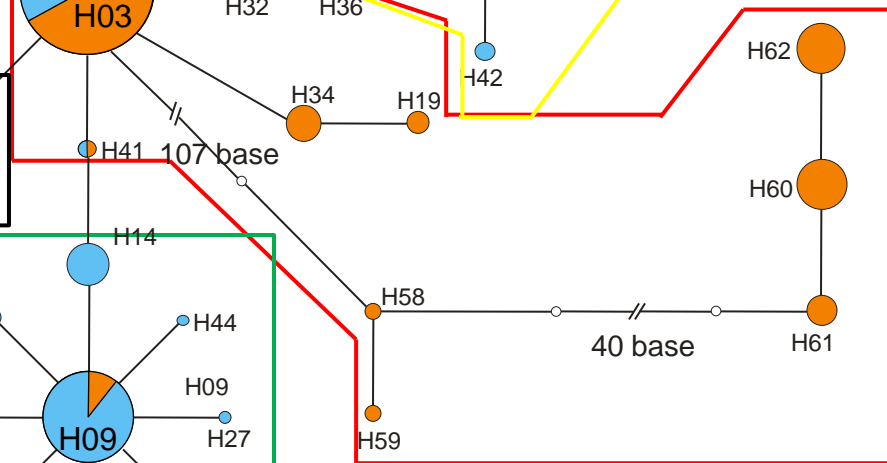


Hap15 clade

Hap09 clade

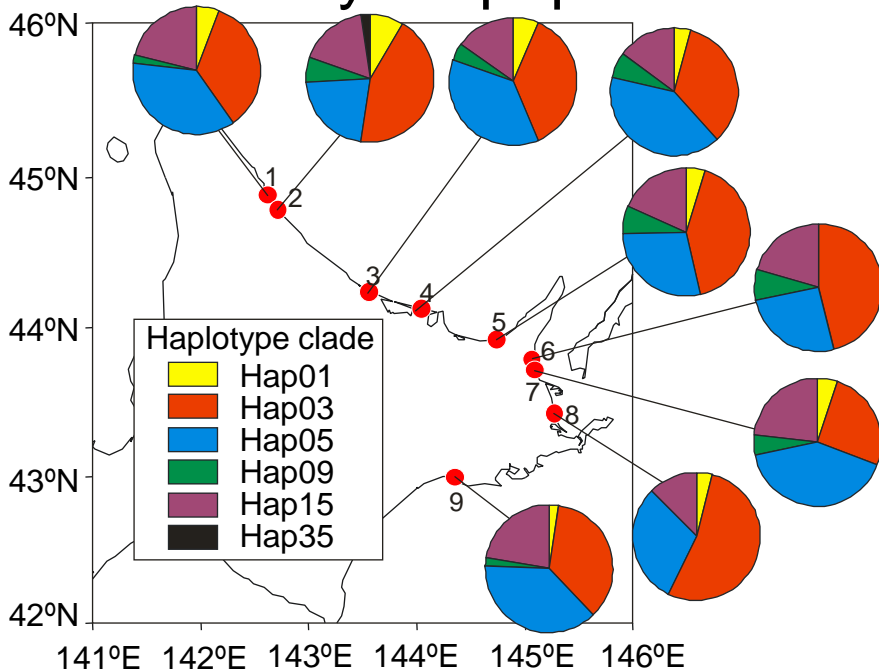


Hap03 clade

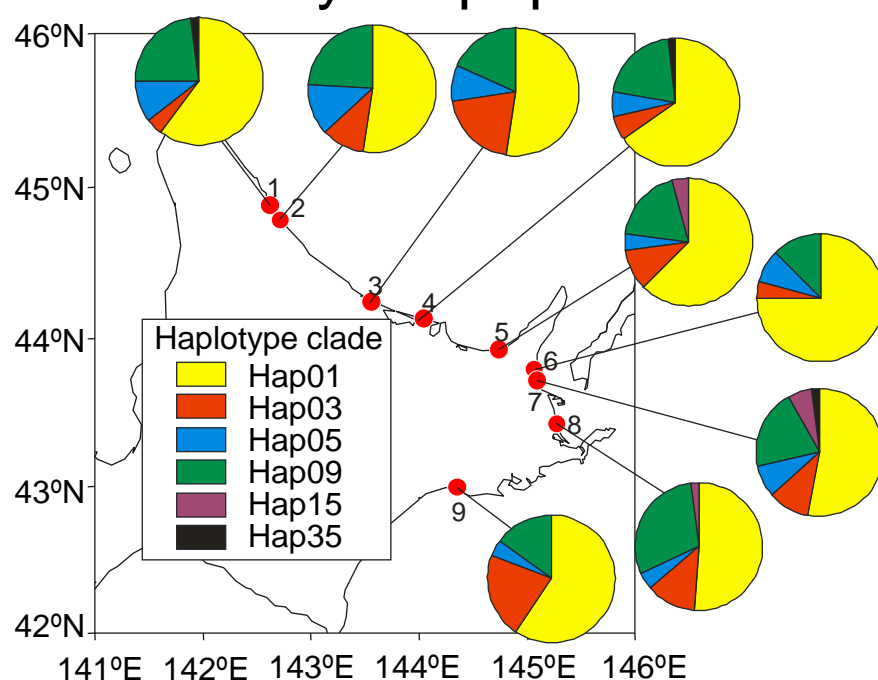


Haplotype distribution of pink salmon in Hokkaido

Even-year populations



Odd-year populations



- Main distribution of haplotype clades
Even-year: Hap03 (red), Hap05 (blue), Hap15 (purple)
Odd-year : Hap01 (yellow), Hap09 (green)
- Significant differentiation between odd- and even-year populations (contingency χ^2 test, $P < 0.001$)
- No differentiation among populations within same year

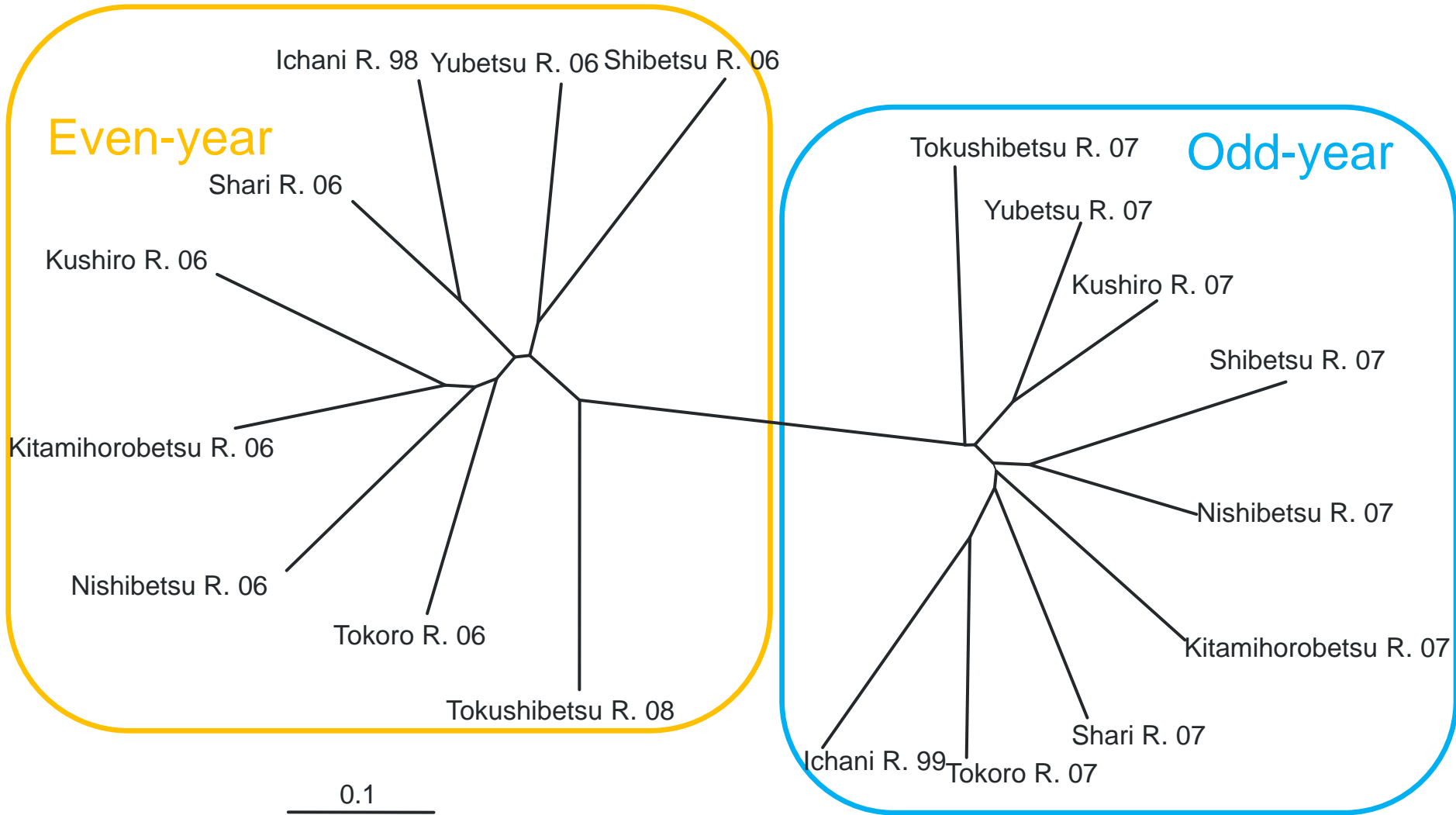
Haplotype diversity of pink salmon populations in Hokkaido

River name	Even-year	Odd-year
1. Kitamihorobetsu	0.901 ± 0.020	0.878 ± 0.029
2. Tokushibetsu	0.884 ± 0.035	0.896 ± 0.026
3. Yuubetsu	0.912 ± 0.024	0.875 ± 0.028
4. Tokoro	0.883 ± 0.028	0.853 ± 0.029
5. Shari	0.885 ± 0.032	0.799 ± 0.051
6. Ichani	0.860 ± 0.032	0.719 ± 0.054
7. Shibetsu	0.907 ± 0.025	0.881 ± 0.027
8. Nishibetsu	0.898 ± 0.022	0.833 ± 0.038
9. Kushiro	0.925 ± 0.018	0.859 ± 0.027
Total	0.893 ± 0.008	0.846 ± 0.011

Haplotype diversity: even-year > odd-year

➡ Grater genetic variation in even-year than odd-year

Neighbor-joining tree of 18 populations of pink salmon in Hokkaido



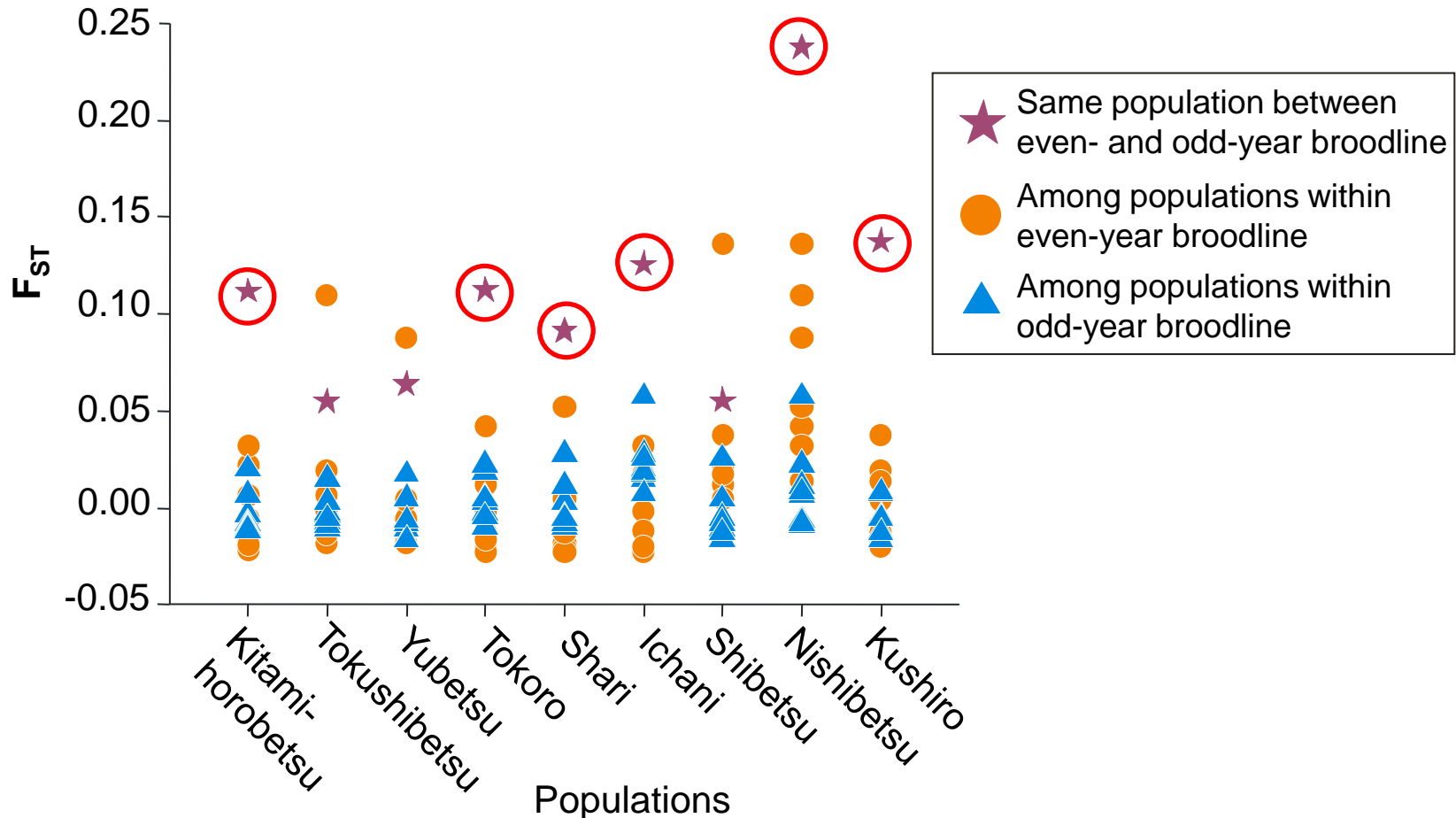
- Showed strong divergence between even- and odd-year broodlines
- No geographical structure among populations within broodline

Hierarchical analysis of molecular variance (AMOVA) for pink salmon broodlines in Hokkaido

	%	P
Between broodlines	12.23%	< 0.001
Among populations within broodline	1.31%	> 0.05
Within population	86.47%	< 0.001

- Percentages of genetic variation was higher **between broodlines** than **among populations within broodline**
- **No significant genetic structure** among populations within broodline

Comparison of pairwise F_{ST} values among populations within or between even- and odd-year broodlines



F_{ST} : Same population between even- and odd-year broodline
> among populations within the same year broodline

➔ Strong genetic differentiation between even- and odd-year broodlines

Summery

1. Haplotype data

- Total of 62 haplotypes:
 - Even-year: 27 haplotypes were unique
 - Odd-year: 27 haplotypes were unique
 - Both years: 8 haplotypes were common
- Grouped in 6 clades
- Distribution patterns were different between even- and odd-year broodlines

2. Gene diversity

- Haplotype diversity: even-year > odd-year

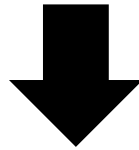
3. Genetic structure

- Strong genetic differentiation between even- and odd-year broodlines
- No genetic structure among populations within same year broodline

Summery: What's the cause of these results?

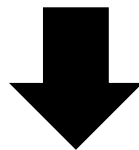
Biological features of pink salmon:

- A rigid **2-year life cycle**
- **Reproductive isolation** of the broodline



Reflected in strong genetic differentiation between broodlines

- **Low rate of homing migration** to a natal river
- **High straying rate** among populations



Relate to no genetic structure among populations within broodline