Status of the Breeding Program of Asian Sebass
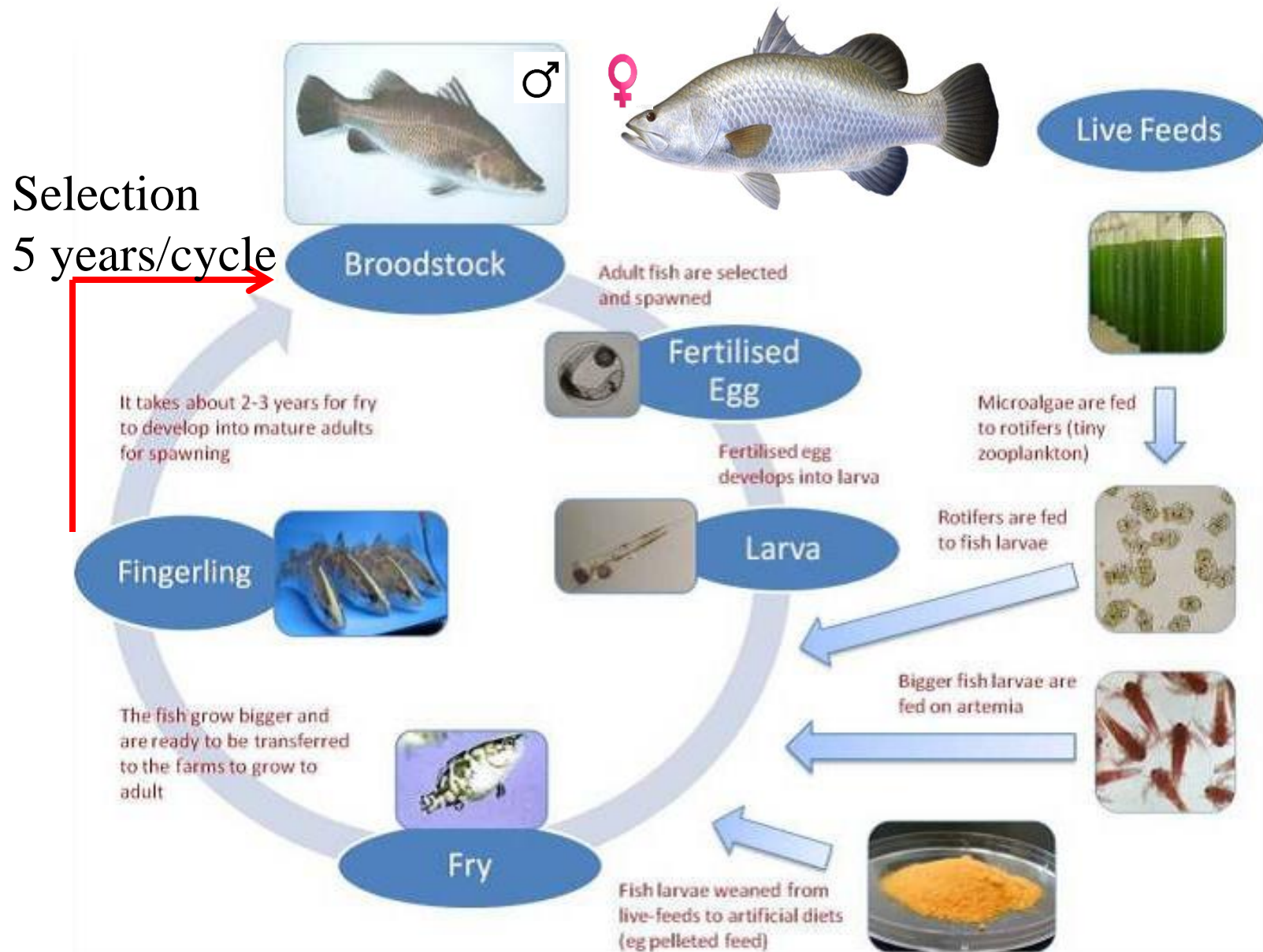
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Breeding Asian Seabass (2004-)

Selection
5 years/cycle

Broodstock

Adult fish are selected and spawned

Live Feeds

Fertilised Egg

It takes about 2-3 years for fry to develop into mature adults for spawning

Fertilised egg develops into larva

Microalgae are fed to rotifers (tiny zooplankton)

Fingerling

Larva

Rotifers are fed to fish larvae

The fish grow bigger and are ready to be transferred to the farms to grow to adult

Bigger fish larvae are fed on artemia

Fry

Fish larvae weaned from live-feeds to artificial diets (eg pelleted feed)

Founder Population and Genetic Parameters

549 brooders
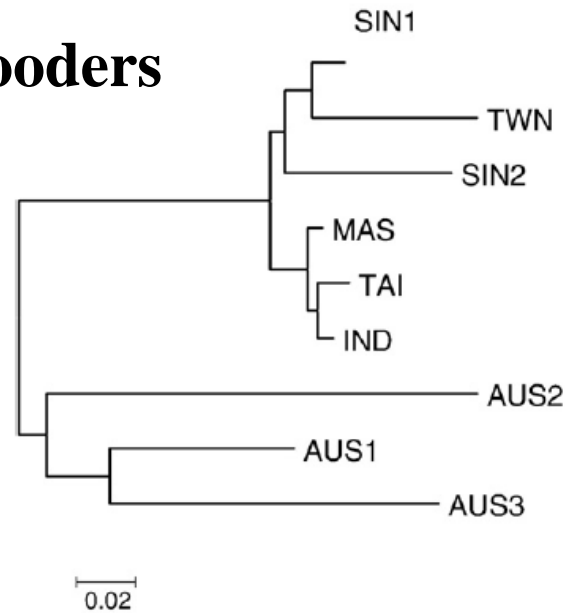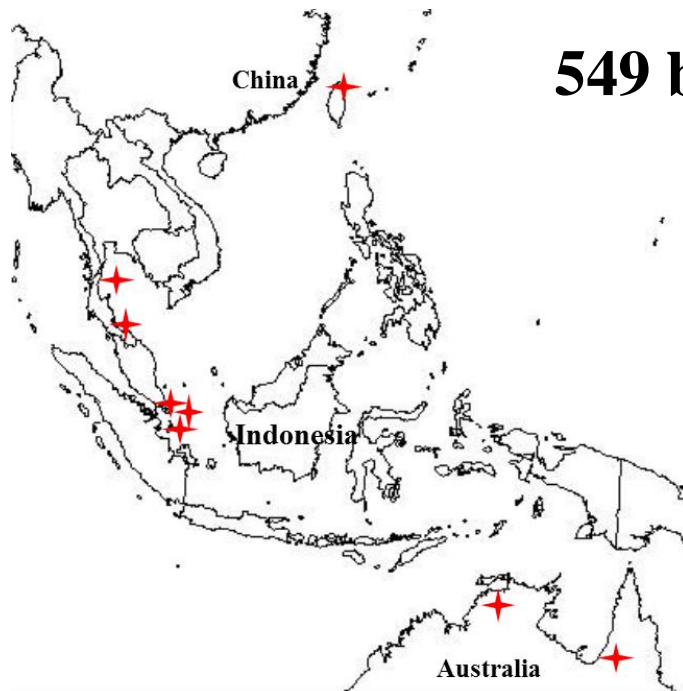

Fig. 3. NJ phylogenetic trees constructed with F_{ST} genetic distances.

h^2 **for growth**

~ 0.24 (BW at 90 dph)

~ 0.39 (BW at 270 dph)

G X D interaction = 0

Genetic diversity in 9 Asian seabass populations

	Malaysia	Singapore	Thailand	Indonesia	Aus1	Singapore	Aus2	Aus3	Taiwan
n	165	104	132	148	32	32	26	71	62
A	10.21	9.14	10.71	10.57	4.93	8.21	3.57	3.79	7.36
AR	7.74	7.6	8.5	8.23	4.8	7.89	3.57	3.72	6.65
He	0.76	0.75	0.78	0.76	0.6	0.74	0.47	0.55	0.68
Ho	0.71	0.74	0.73	0.74	0.55	0.66	0.52	0.65	0.66

Traits Selected

1. Growth (from 1ˢᵗ generation)

7g- 140g at 90 dph

2. Resistance to bacterial and viral diseases (from 2ⁿᵈ generation)

Big belly

NNV

3. Content of omega-3 in meat (from 2ⁿᵈ generation)
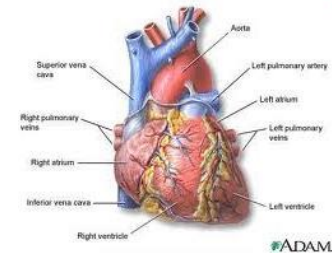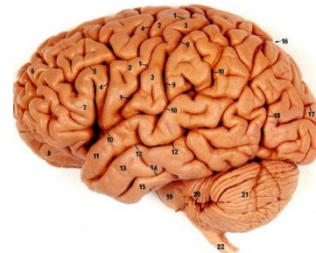
Family-based Selection in Mass-crosses

549 Brooders

15 ♂ x 15 ♀

→ Top 5-6 parents **180**

↓ 60,000 eggs

Offspring 90dph
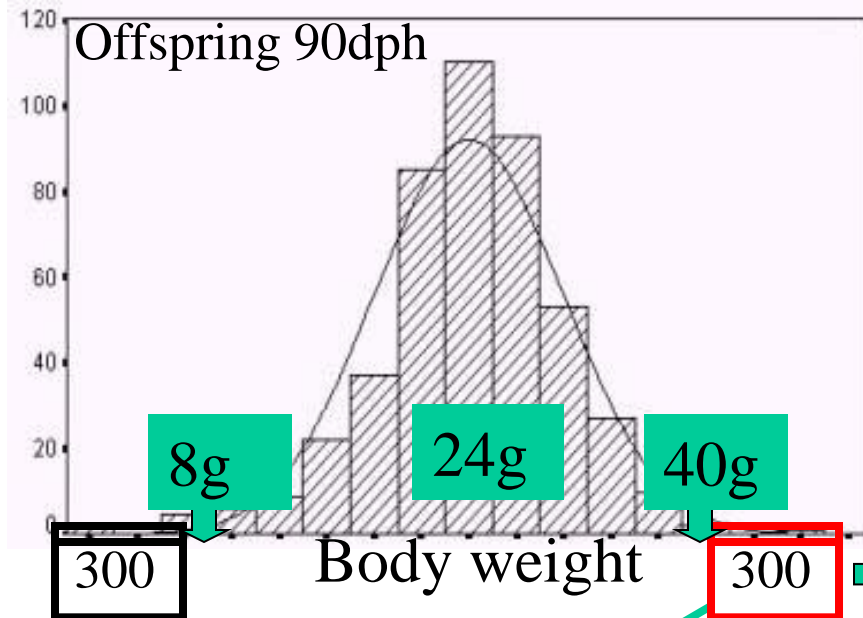
X 40

Advantages:

gain time

improve performance

8g 24g 40g

Body weight

300 300 → Top 95 F1 **~3000**

BLUP in 2 years

400

Parentage with 10 markers
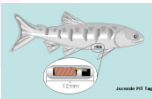
$+ R\% = h^2 (X_1/X_0 - 1), h^2 \sim 0.24$
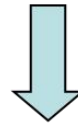
Molecular Parentage Analysis

15 ♂ x 15 ♀

offspring

Collect 600 offspring

10 loci/PCR

```
Offspring
F2(90)-m-012    254254 128128 160186 415415 286290 346350 310310 162168 211219
Parents
Mac013_m        254258 128132 184186 415441 286290 346350 301310 162172 219195
Mac010_f        254254 128132 190160 415419 286288 346350 310310 168158 195211
**********************************************************

Offspring
F2(90)-m-013    254254 132136 160190 415425 286286 346348 310322 168168 195239
Parents
Mac020_m        254254 132136 186190 421425 286286 348348 298322 162168 195239
Mac010_f        254254 128132 190160 415419 286288 346350 310310 168158 195211
**********************************************************

Offspring
F2(90)-m-014    254254 128136 160190 415421 286286 346348 298310 162168 195195
Parents
Mac020_m        254254 132136 186190 421425 286286 348348 298322 162168 195239
Mac010_f        254254 128132 190160 415419 286288 346350 310310 168158 195211
**********************************************************
```
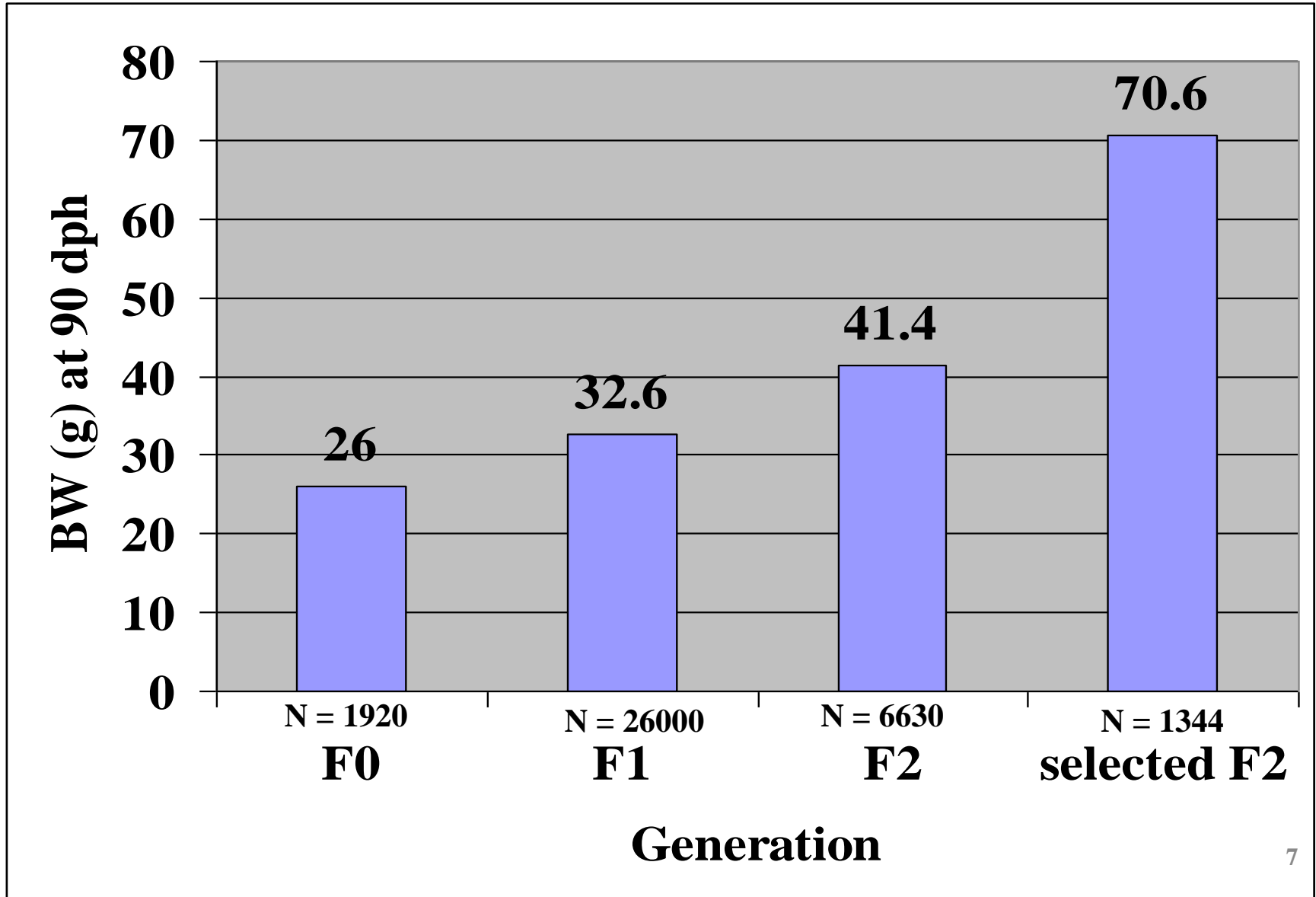
> 96.1% offspring can be assigned

Genotyping > 40,000 fish/year since 2004

Body Weight Improvement of Asian Seabass

Genetic Diversity in Selected F1 Individuals

Population	n	A	He	Ho	f
Brooders	549	10.22	0.745	0.696	0.0650
Selected F1	1200	9.80	0.727	0.716	0.0150
Farm's brooders	104	8.11	0.719	0.740	-0.0290
Farm-F1	26	4.33	0.644	0.748	-0.0160
Farm-F1	105	4.11	0.541	0.603	-0.0114

n: number of brooders; A: allele number; Ho: observed heterozygosity;

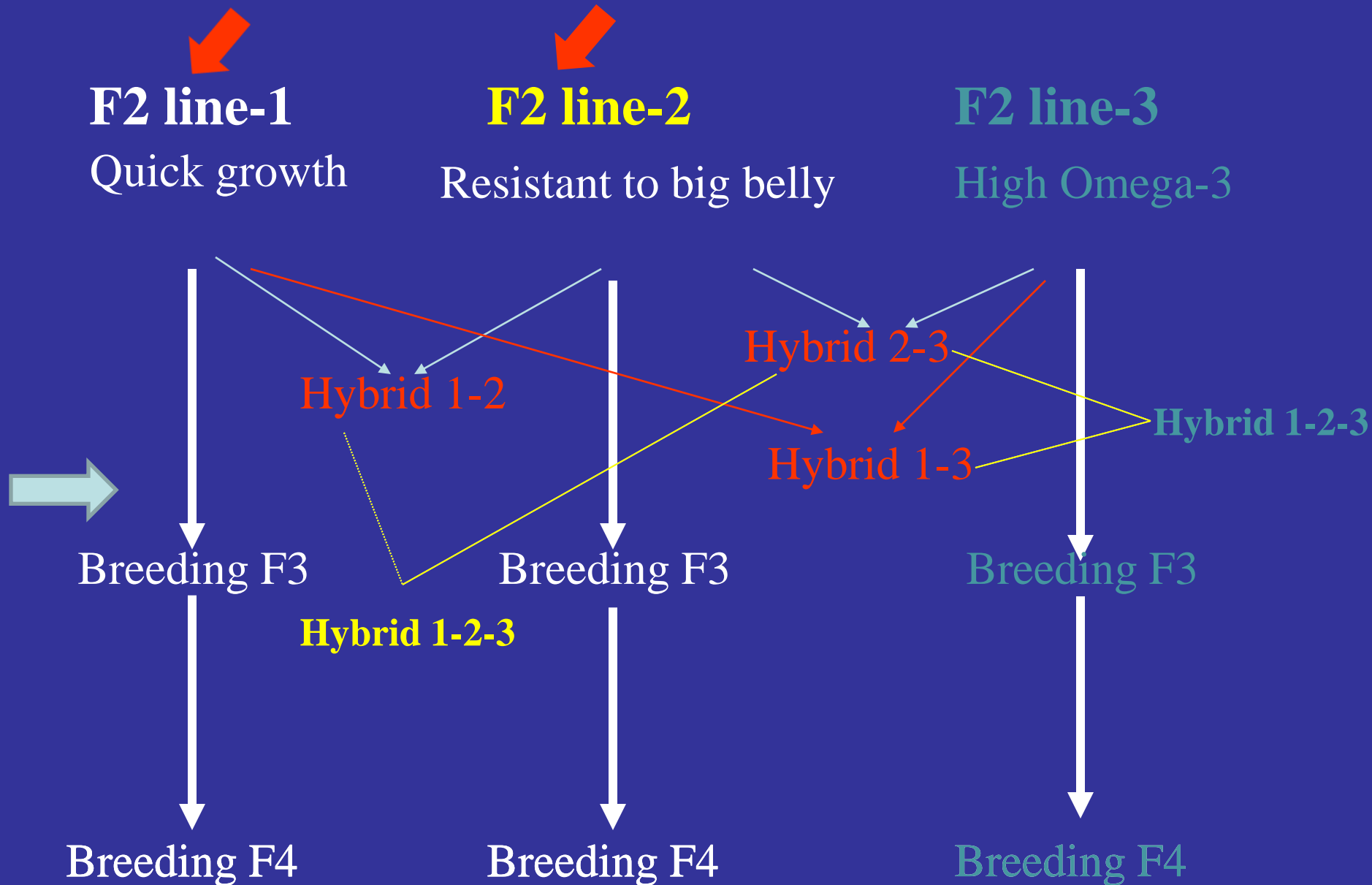He: expected heterozygosity; and f: inbreeding index

Selection for Resistance to Diseases

Big belly

males   X   females

Offspring challenged with a pathogen

Dead fish   Survival fish

Molecular parentage analysis

NNV
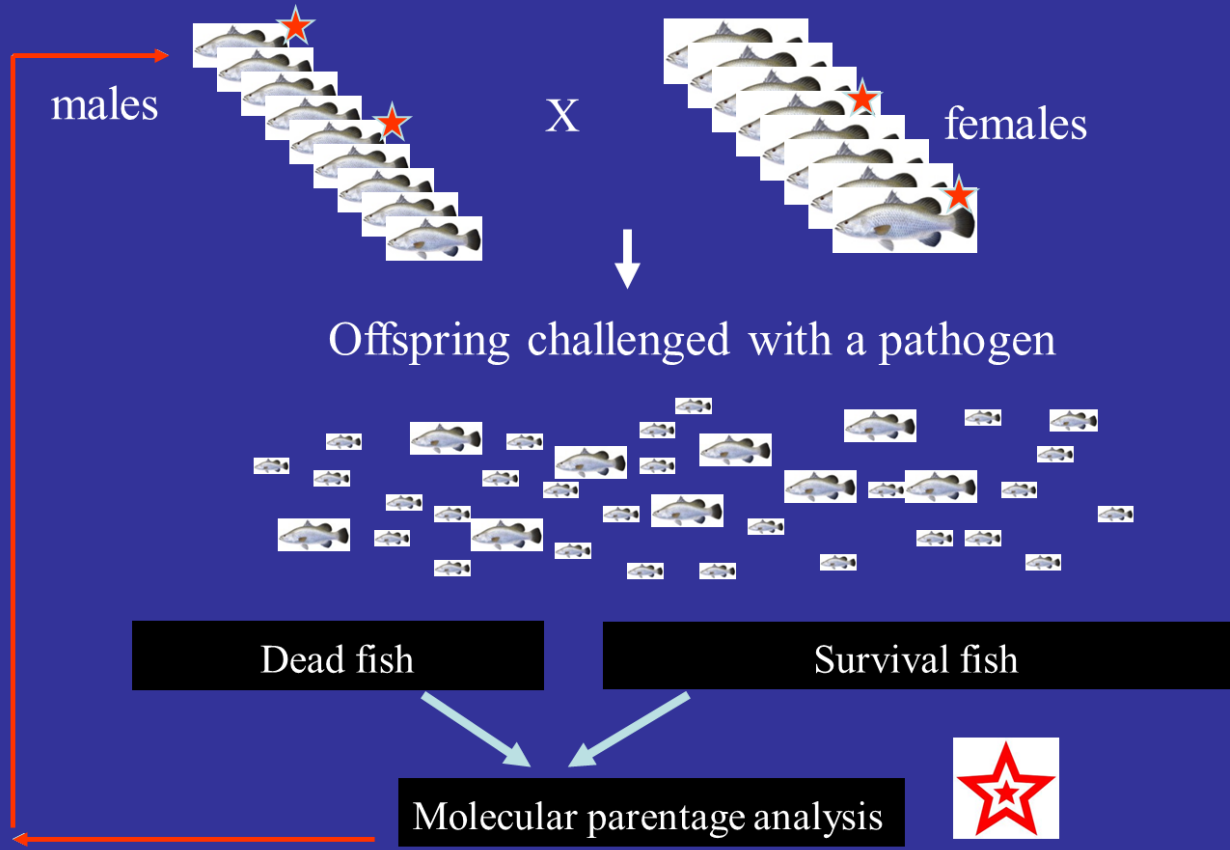
We are working on resistance against nodavirus, iridovirus & cacterial diseases

Establishing lines

Genomic Resources for Asian Seabass

- **DNA markers > 60,000**
- Marine Biotechnology (2001), Molecular Ecology Notes (2002), Aquaculture (2006, 2009), Plos One (2014), Fish and Shellfish Immunology (2014), Fisheries Research (2015), Conservation Genetics (2015)

- **cDNA, BAC Libraries and RNA-seq**
  Animal Genetics (2006) , BMC Genomics (2008), Plos One (2010), DNA Research (2011, 2013)

- **Linkage and physical maps**
  Genetics (2007), BMC Genomics (2011), Plos One (2010, 2012)

- **Candidate genes and MicroRNA**
  Animal Genetics (2006, 2011, 2012), Plos One (2011, 2013), Animal (2012)

- **QTL for important traits**
  BMC Genomics (2006), Animal Genetics (2008), BMC Genomics (2011, 2013), Marine Biotechnology (2011, 2013, 2014)
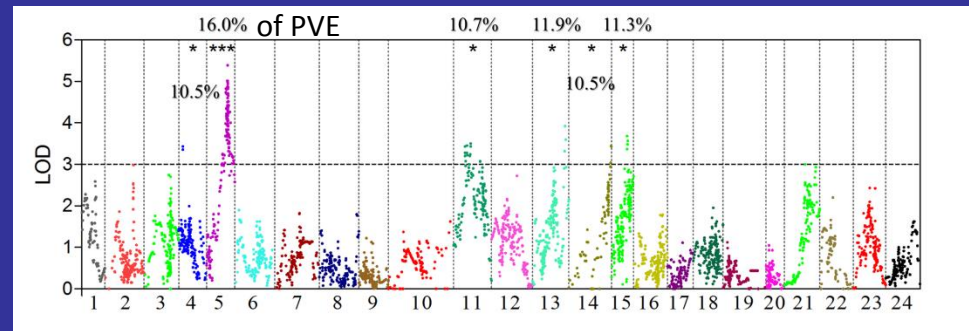
QTL for Growth
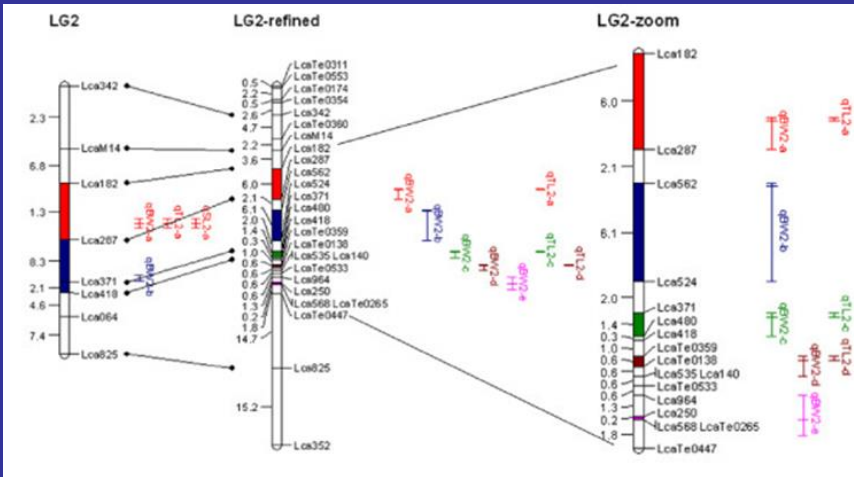
QTL for BW at 90dph
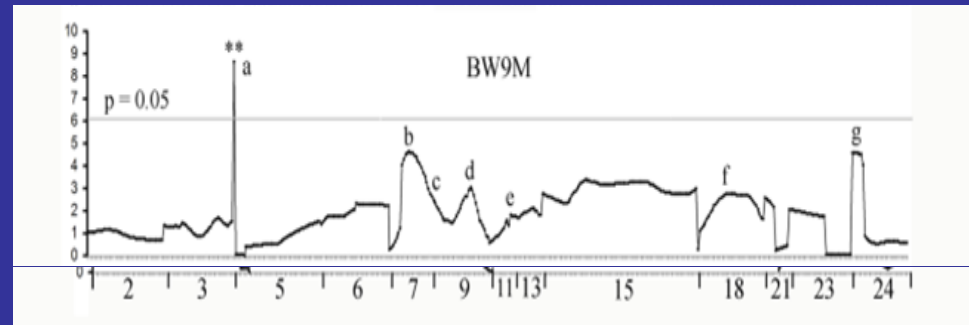
QTL for BW at 270 dph in family 1

QTL for BW at 270 dph in family 2

- Only few QTL for growth were identical at different developmental stages.
- Large number of suggestive QTL and a few significant QTL for growth
- Most QTL are family-specific with small effects (PVE: $\leq$ 16%)

Mapping QTL for Omega-3 in Meat

336 F2 individuals, 123 markers

GC analysis

Traits	QTL ID	Linkage Group	Peak position (cM)	Marker on Peak	LOD on Peak	mu_ac{00}	Estimated mean of the QTL mu_ad{00}	mu_bc{00}	mu_bd{00}
C20:5	C20:5a	LG2	24		5.59	6.9	6.7	8.3	8.4
EPA	C20:5b	LG2	35.595		64.38	6.9	7.0	9.2	9.0
	C20:5c	LG7	6.943	LcaTe0598	3.1	6.8	6.9	7.0	7.0
	C20:5d	LG19	19.795		39.28	6.9	7.0	3.9	3.9
C22:5	C22:5a	LG2	26		9.1	4.3	5.2	5.3	4.2
DPA	C22:5b	LG2	30.595		3.43	4.3	4.5	4.9	5.3
	C22:5c	LG5	1.19	LcaTe0181	3.29	4.4	4.2	4.3	4.2
	C22:5d	LG6	20.067		23.98	4.4	4.4	7.6	6.6
	C22:5e	LG9	1		3.74	4.3	4.4	4.3	4.4
	C22:5f	LG9	15.91		3.81	4.3	4.4	4.4	4.4
	C22:5g	LG14	22.22	Lca633	1.97	4.3	4.4	4.3	4.4
	C22:5h	LG24	1		2.64	4.3	4.3	4.4	4.4
C22:6	C22:6a	LG6	4.662		2.93	14.8	17.2	16.0	16.8
DHA	C22:6b	LG6	16.91	LcaTe0176	3.01	14.8	12.7	15.0	14.6
	C22:6c	LG23	9		2.66	14.9	15.0	13.9	15.7
	C22:6d	LG24	14.47	LcaTe0157	2.51	14.8	15.5	15.9	16.7

Sixty-two suggestive QTL for different fatty acids were detected on the whole genome. Multiple QTL for *n*-3 PUFA fatty acid traits in flesh were identified and their effects are small.

QTL Mapping for Resistance to NNV

Five significant QTL located in four LGs and eight suggestive QTL in seven LGs were identified for resistance to NNV. These QTL explained 2.2 - 4.1% phenotypic variance.
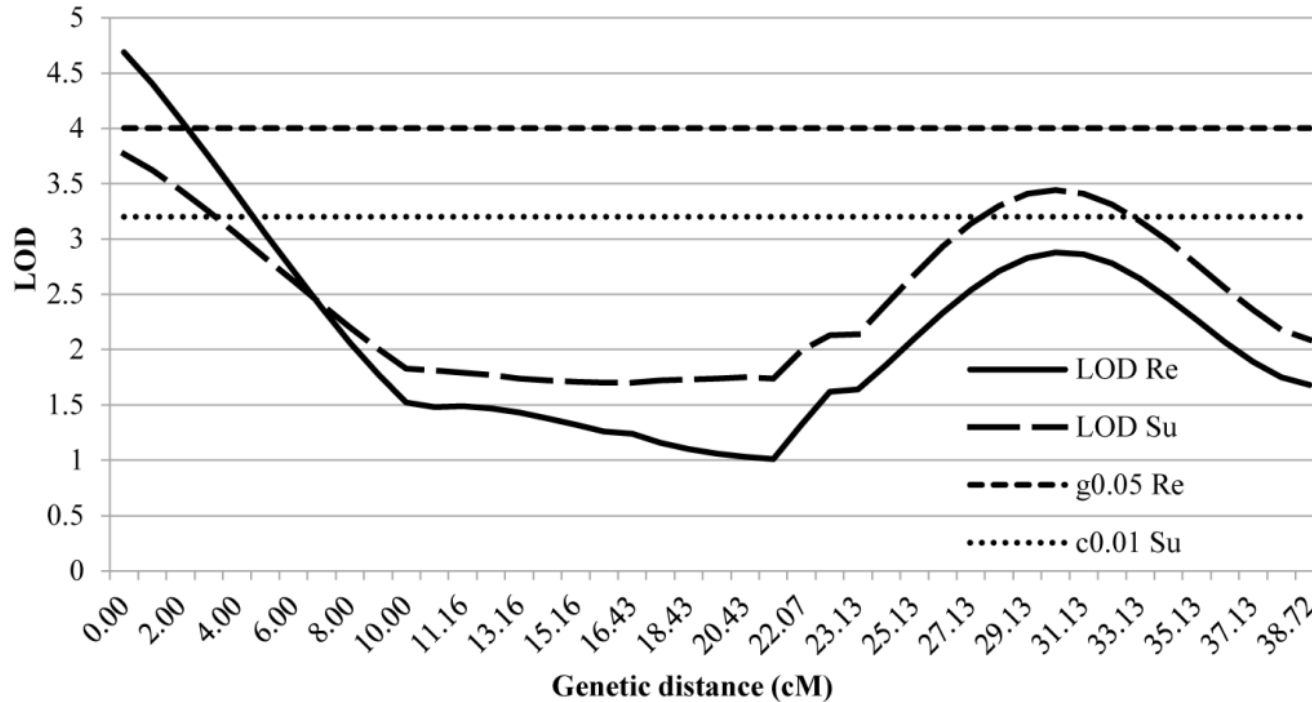

Fig 1. QTL for resistance and survival time in LG 20 of Asian seabass fingerlings.

Summary of QTL Mapping

- Most QTL for growth are family-specific. The effects of QTL are small.

- Only few QTL for growth are identical at different developmental stages.

- Multiple QTL for Omega-3, with small effects were mapped to several chromosomes.

- The effects of QTL for resistance to NNV are small.

Moving from QTL Mapping to GWAS

- Marker-Assisted-Selection (MAS)
  - Only significant markers are used for selection.

Conventional marker aided selection
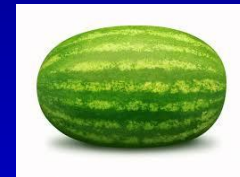
- GWAS to Genomic Selection (GS)
  - Requires genome-wide markers that are used to develop a prediction model for estimating a breeding value for each individual
  - Marker/QTL effects are estimated for individuals in a breeding population without phenotyping

Genomic selection

Genotyping cost is a big issue

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- TLL management

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- Prof. Hew Choy Leong

- Mr. Huan Sein Lim

- Sequencing facility

- All lab members

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Breeding