



# Research and development for the genetic improvement of the Pacific oyster in France: present and prospects.

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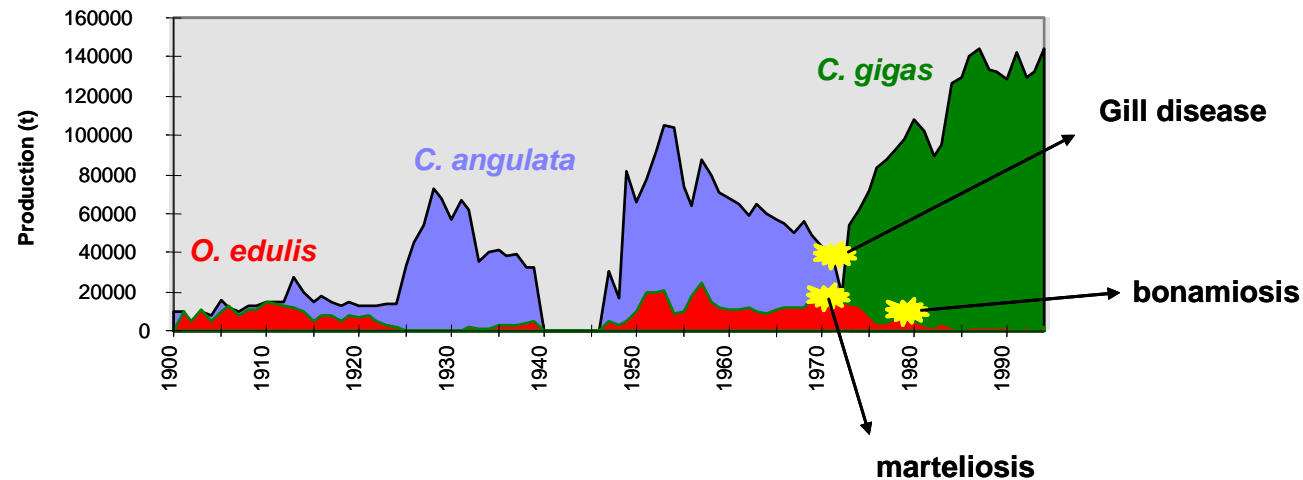
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# Introduction

- Until now, the way to face problems that affected oyster production in France has been through the introduction of non native species



*Ostrea edulis*



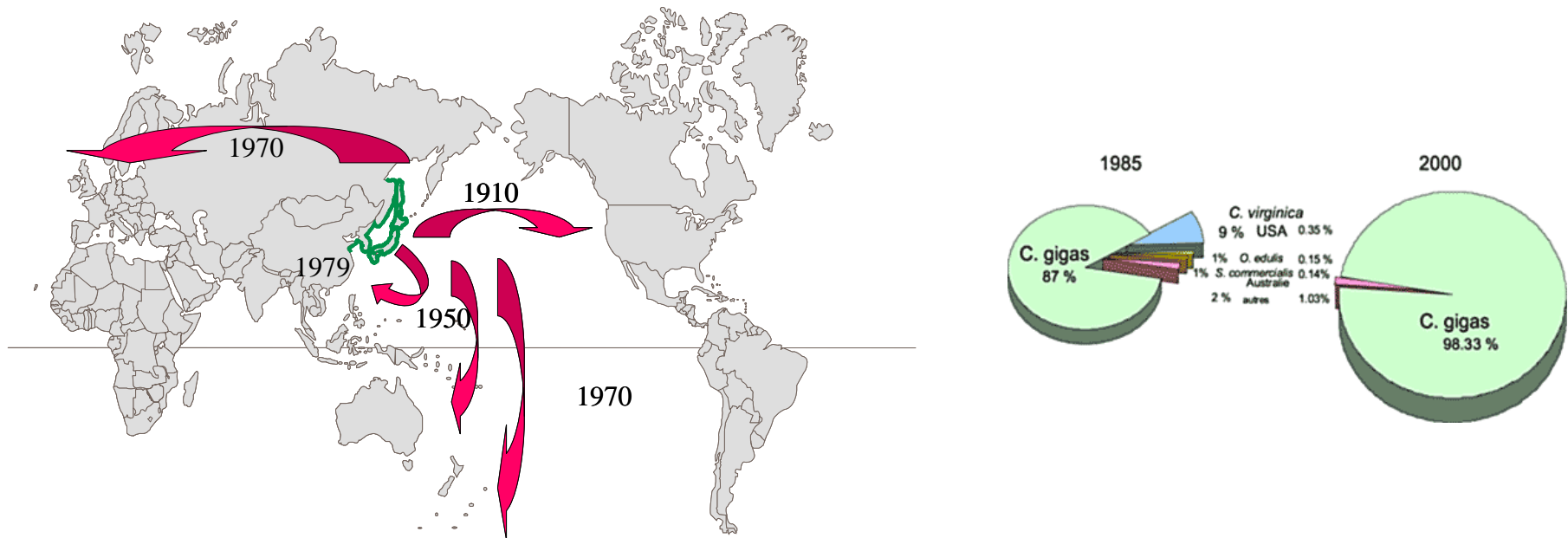
*Crassostrea angulata*



*Crassostrea gigas*

## Introduction

- To date, *Crassostrea gigas* has been introduced into many countries and represents close to 99% of the world's oyster production



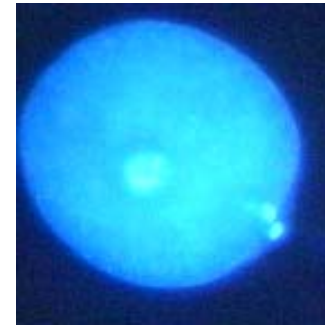
- Similarly to other major aquaculture species, the next step might be domestication and genetic improvement of *C. gigas*

## Genetic improvement of oyster production

### Ploidy manipulations:

- triploidy induction
- tetraploids :

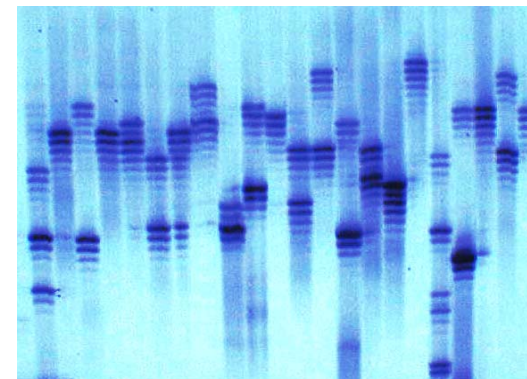
$$4n \times 2n = 3n$$



### Selective breeding:

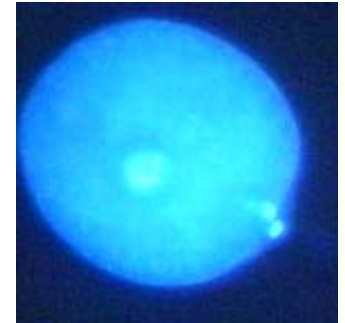
- heritability estimates
- genetic correlations and trade-offs
- family-based or mass selection programs ?
- inbreeding and heterosis
- QTLs and candidate genes

	1	2	3	4	5
1	■				
2	■				
3		■			
4		■			
5			■		
6			■		
7				■	
8				■	
9					■
10					■
11				■	
12				■	
13					■
14					■
15					■

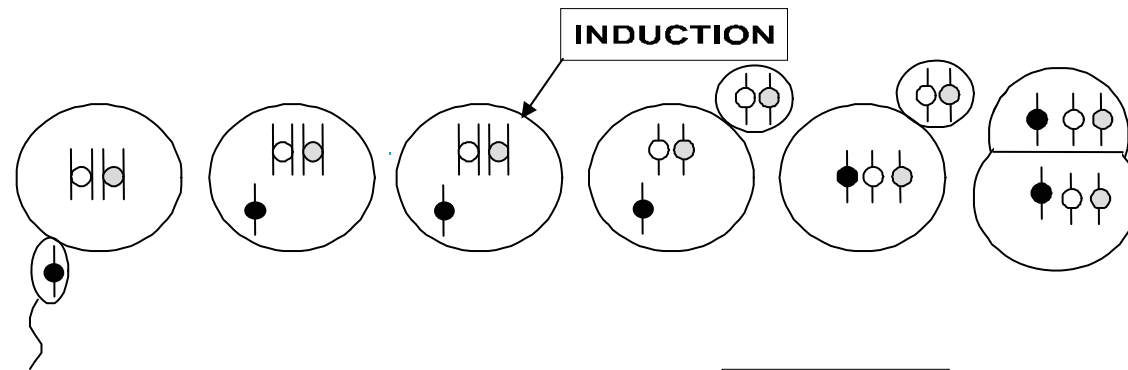


# Methods to produce triploid oysters

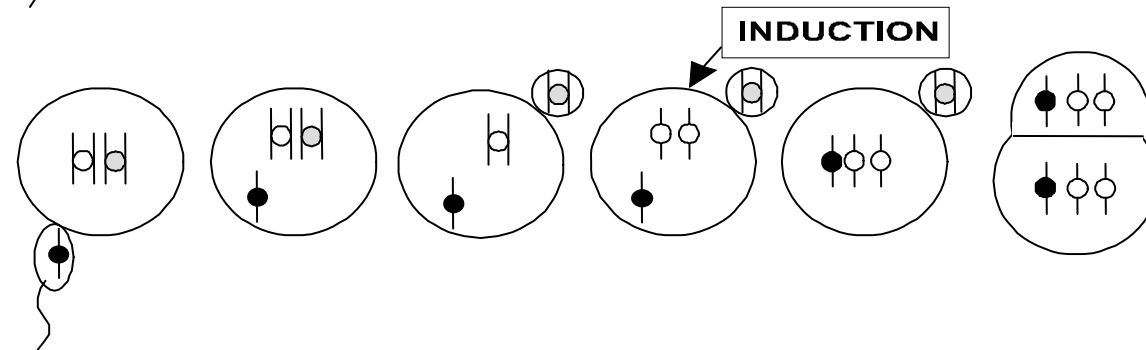
## 1) Chemical treatment of fertilized eggs using Cytochalasine B or 6-DMAP



*Inhibition of the expulsion of the first polar body*



*Inhibition of the expulsion of the second polar body*

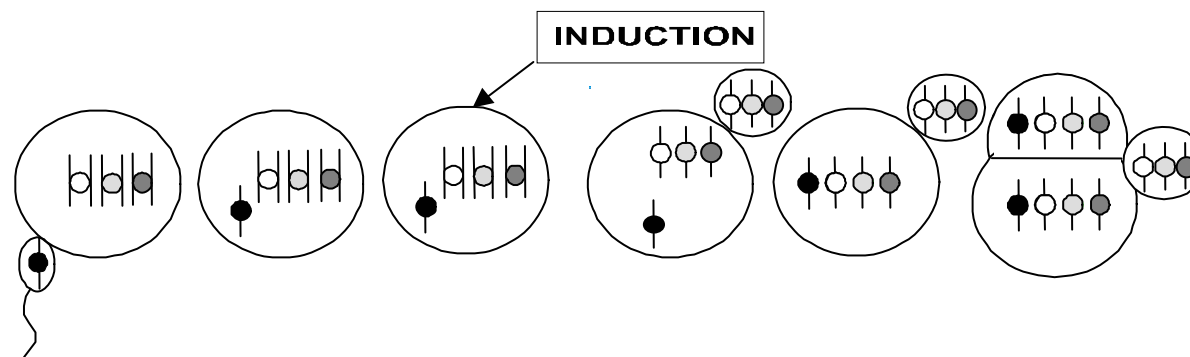


***Successfully applied in oysters, pearl oysters, mussels...  
Dose and timing are key factors for successful production***

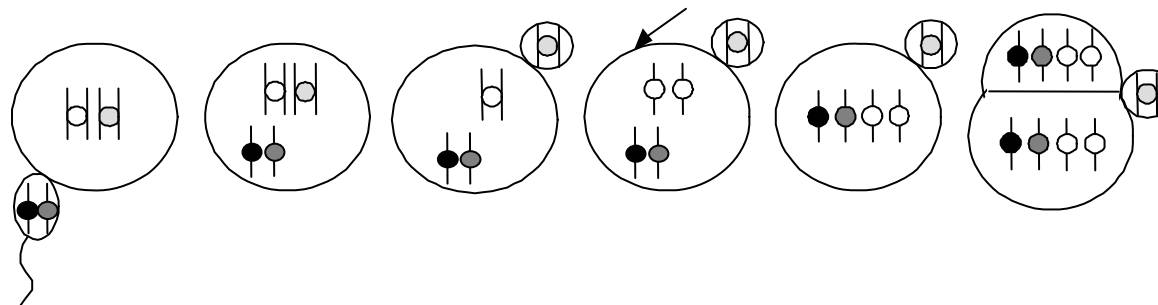
# Methods to produce triploid oysters

## 2) Tetraploid x diploid = 100 % 'natural' triploid

- First method to produce viable tetraploid oysters published in 1994 (Guo & Allen, 1994):  $3n \times 2n + CB = 4n$



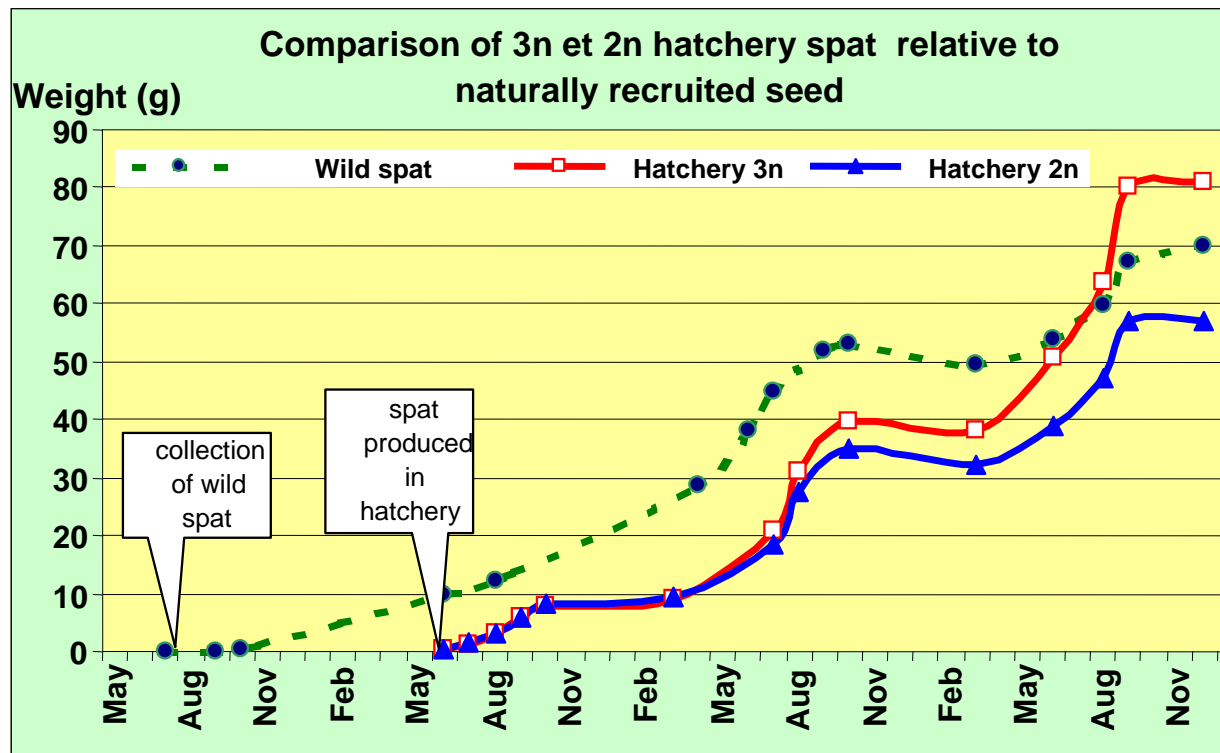
- Complementary method (McCombie et al., 2005):  $2n \times 4n + CB = 4n$





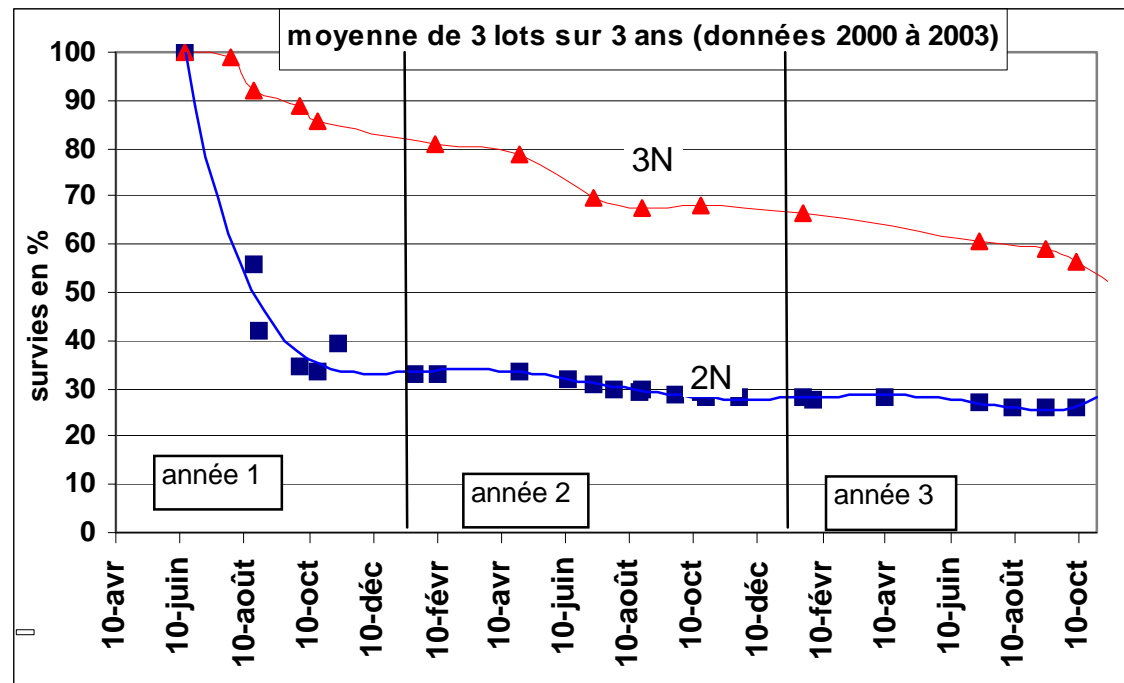
## Triploidy : a “single step” improvement

- Re-allocation of energy from reproduction to growth and survival
- Higher heterozygosity



## Are triploids more resistant to summer mortality ?

- 2002 (G2 - RA) : spat mortality of diploids: 24% , triploids: 7%



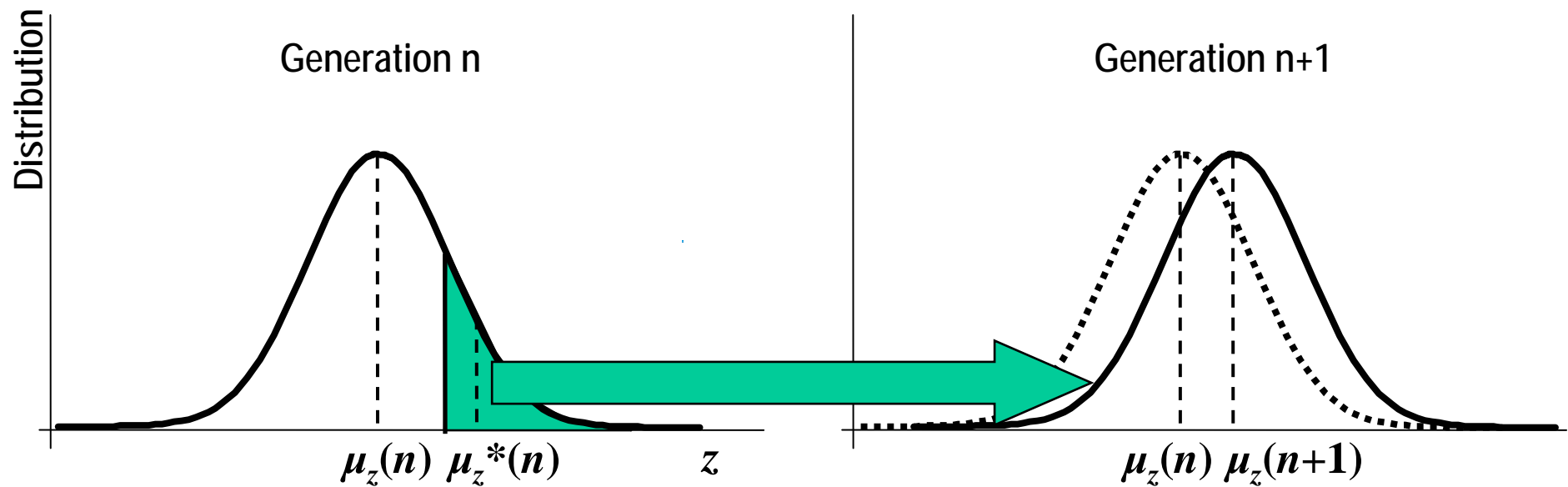
- 2003 (G3 – RA) : : spat mortality of diploids = 48% , triploids : 48%
- We need to better document cases where triploid survival is lower than in diploid controls.





## Selective breeding : a “multi- step” improvement

- Additive genetic variance over successive generations



$$\mu_z(n+1) - \mu_z(n) = \underbrace{\frac{V(g_A)}{V(z)}}_{\text{Heritability, } h^2} \underbrace{(\mu_z^*(n) - \mu_z(n))}_{\text{Selective pressure}}$$

Heritability,  
 $h^2$

Selective  
pressure

$Z$  : phenotypic trait

$V(z)$  : phenotypic variance

$V(g_A)$  : additive genetic variance



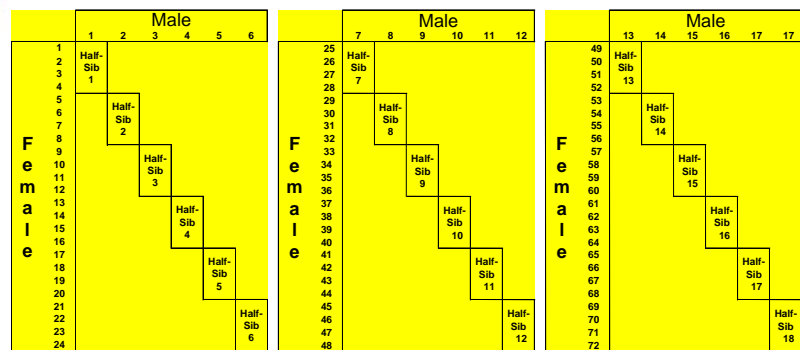
## Mass (*i.e.* individual) selection

- Targeted traits : growth, disease resistance
  - Bonamiosis resistance in *O. edulis* (Naciri-Graven et al., 1998; Culloty et al., 2001)
  - MSX and dermo resistance in *C. virginica*
  - Growth in *S. commercialis* (Nell et al., 2000)
  
- Main advantages :
  - Relatively easy to manage
  - possibility of strong selective pressures
  
- Main constrains :
  - Risk of rapid loss of genetic variability
    - inbreeding ?
  
  - Selection under a single environment
    - genotype x environment interaction ?

# Family-based selective breeding



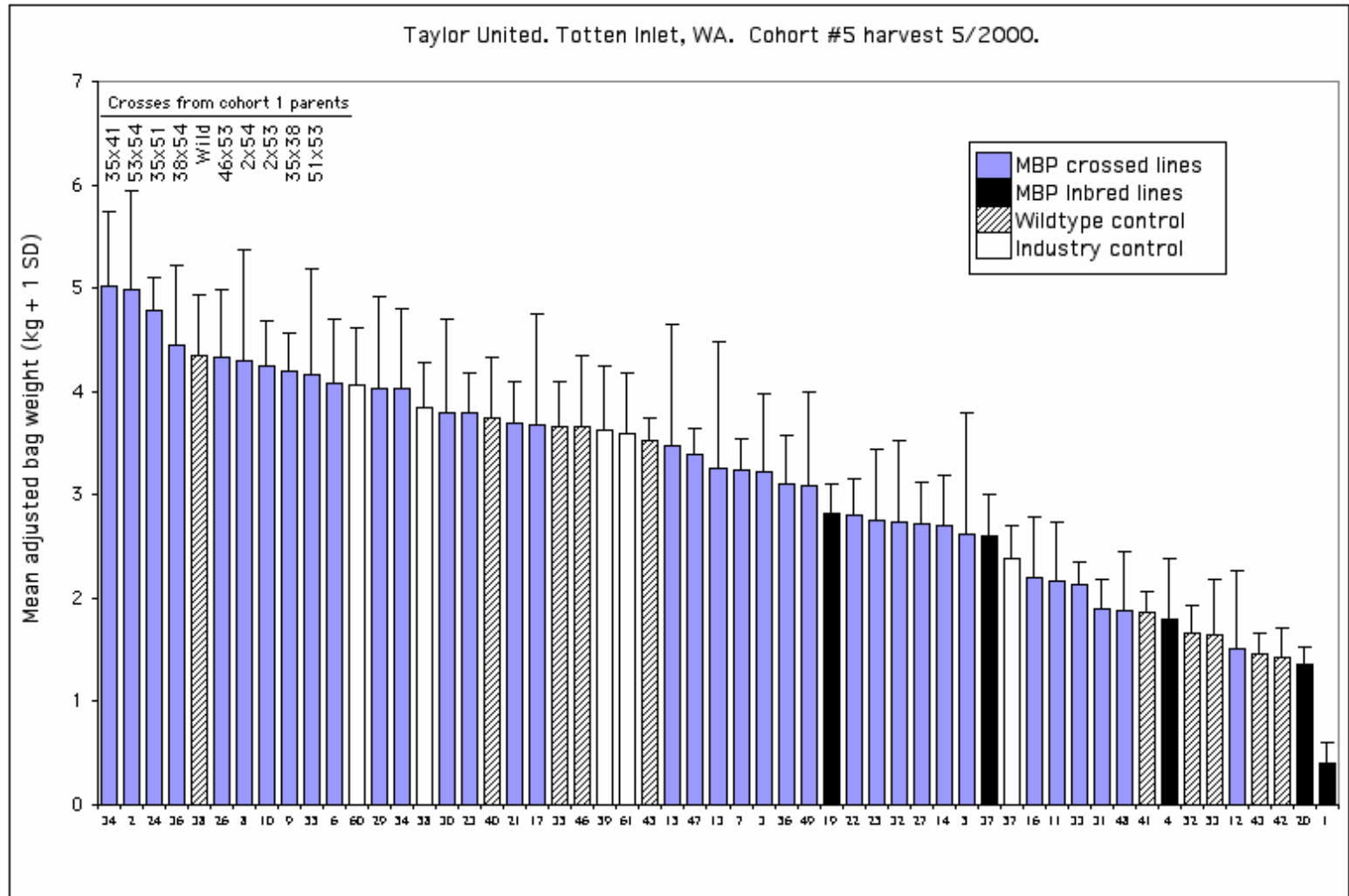
Relative performance of (many) families reared under common condition(s) to estimate their genetic value





# Family-based selective breeding programs

- ◆ Molluscan Broodstock Program (MBP): selection for yield



## Current family-based selective breeding programs

- U.S.A. : **yield**
  - MBP (<http://www.hmsc.orst.edu/projects/mbp>)
  - WRAC: « Crossbreeding » and heterosis
  
- Australia: **Growth**
  - CSIRO, ASI
  
- New Zealand: **Growth**
  - Cawthron Institute





# What about France?

- 7 hatcheries  $\approx$  25 % of the production (i.e. 1 billion seed)
- individual selection + a few families
- Loss of genetic variability in hatchery broodstock:

	Wild population		Hatchery A		Hatchery B	Hatchery C		Hatchery D	
	1	2	Lot 1	Lot 2		Lot 1	Lot 2	Lot 1	Lot 2
N	50	47	49	37	65	50	50	50	50
Nb <sub>allèles</sub>	30	34	12	10	12	30	29	10	15
H <sub>obs</sub>	0,807	0.863	0,660	0,66	0,990	0.691	0.713	0.801	0.768
H <sub>n.b.</sub>	0,954	0.962	0,803	0,77	0,867	0.960	0.958	0.793	0.897
F <sub>is</sub>	0,155	0.104	0,180	0,15	-0,144	0.282	0.257	0.077	0.145
	**	**	**	**	ns	**	**	*	**

(Taris et al., 2006)

**In most hatcheries, broodstock shows  $\approx$  40% of the genetic diversity observed in wild populations**





# How to breed for improved survival ?

- **Survival is a threshold trait ⇒ Selection on family/relatives to estimate their genetic value**
- **150 to 200 families should lead to significant gains and a very slow increase of inbreeding over a relatively high number of generations**
- **Individual tagging is impossible at early stages, so families be reared in separate units**
- **Alternatively, microsatellite markers can trace parentage in 'mixed-family' populations.**
- **In any case, this is likely to be expensive and technically challenging !**



## IFREMER

- 196 families / generation
- 4 sets of 24 families / year



## SYSAAF

- Estimation of genetic values
- selection of the best 50 families

## Gigas+

## Hatcheries

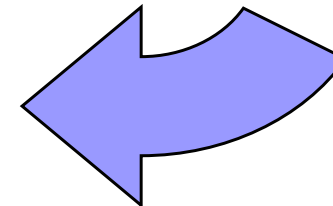
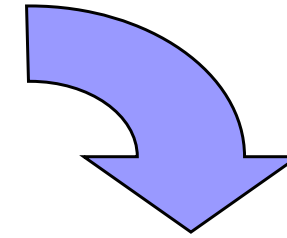
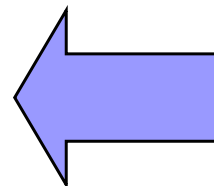
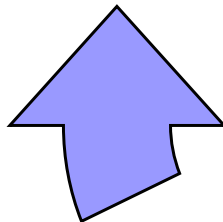
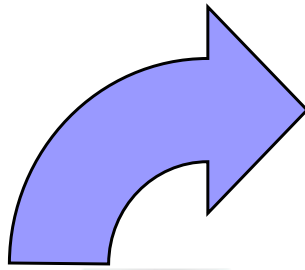
- Nursing of 24 families / set
- transfer of seed in bags
  - 3 1/2 bags / familie / site
  - 150 seed / bag

## Hatcheries

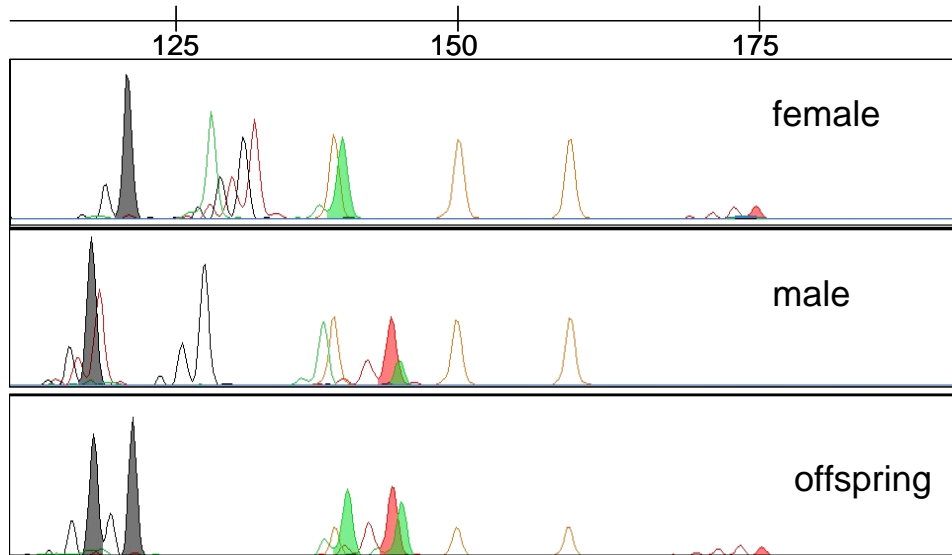
- 6 data recording / year
- for 4 sets:
  - weight of 288 bags
  - number o dead and live oysters / bag
  - individual live weight (50 oysters / bag)

## Three field trials

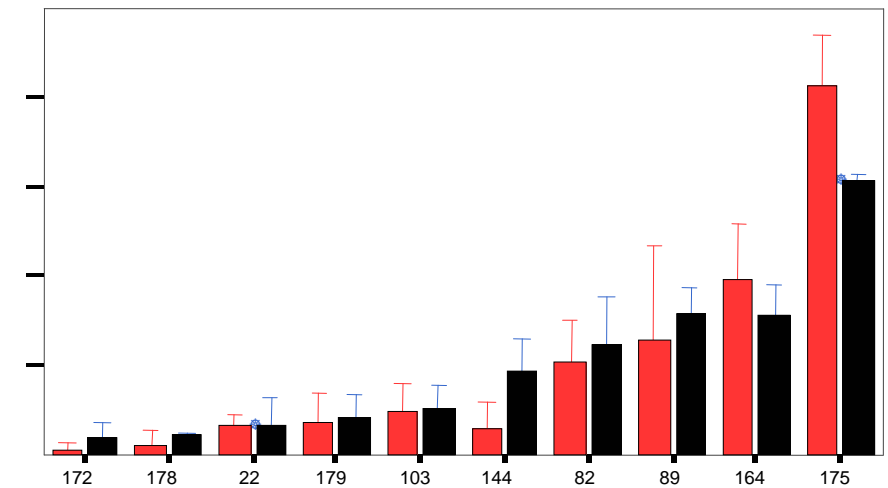
- 24 families / set / year = 72 bags / set / site
- 4 sets of 24 families / year (288 bags / year)



# The « mixed-family approach »



PCR-multiplexed microsatellite loci  
(*Taris et al., 2005*)

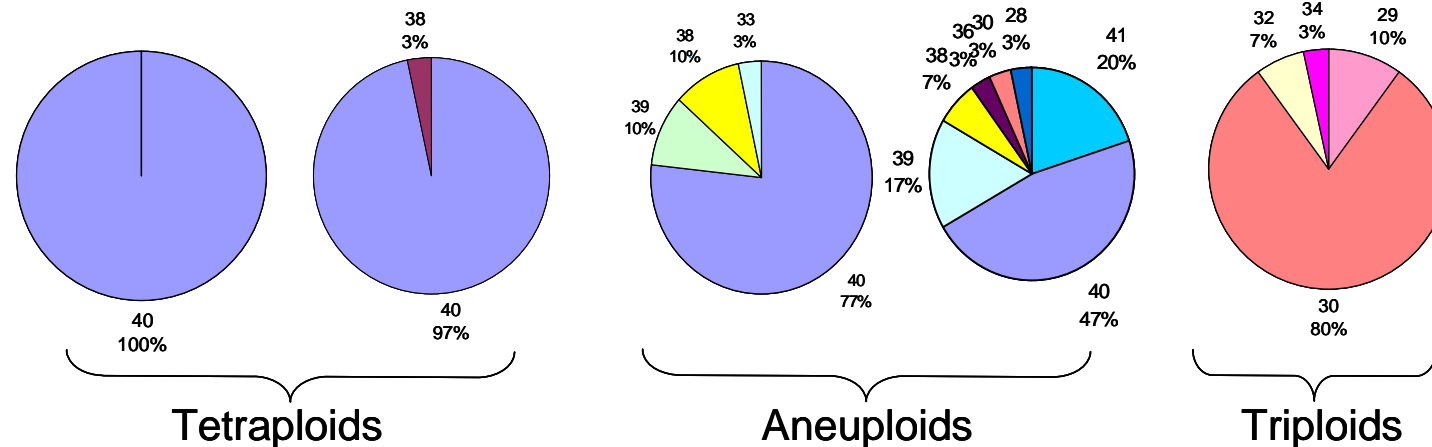


High variance in reproductive success  
between parents at early stages

- **Efficient tool to perform genetic studies** (Boudry et al., 2002 ; *Taris et al., 2006*)
- **Feasibility of the 'mixed-family approach' for a breeding program aiming to improve survival remains to be demonstrated, due to high variance of reproductive success at early stages**

# Toward the integration of selective breeding and polyploidy?

- Production and maintenance tetraploid oysters is rather difficult, notably due to chromosome number instability

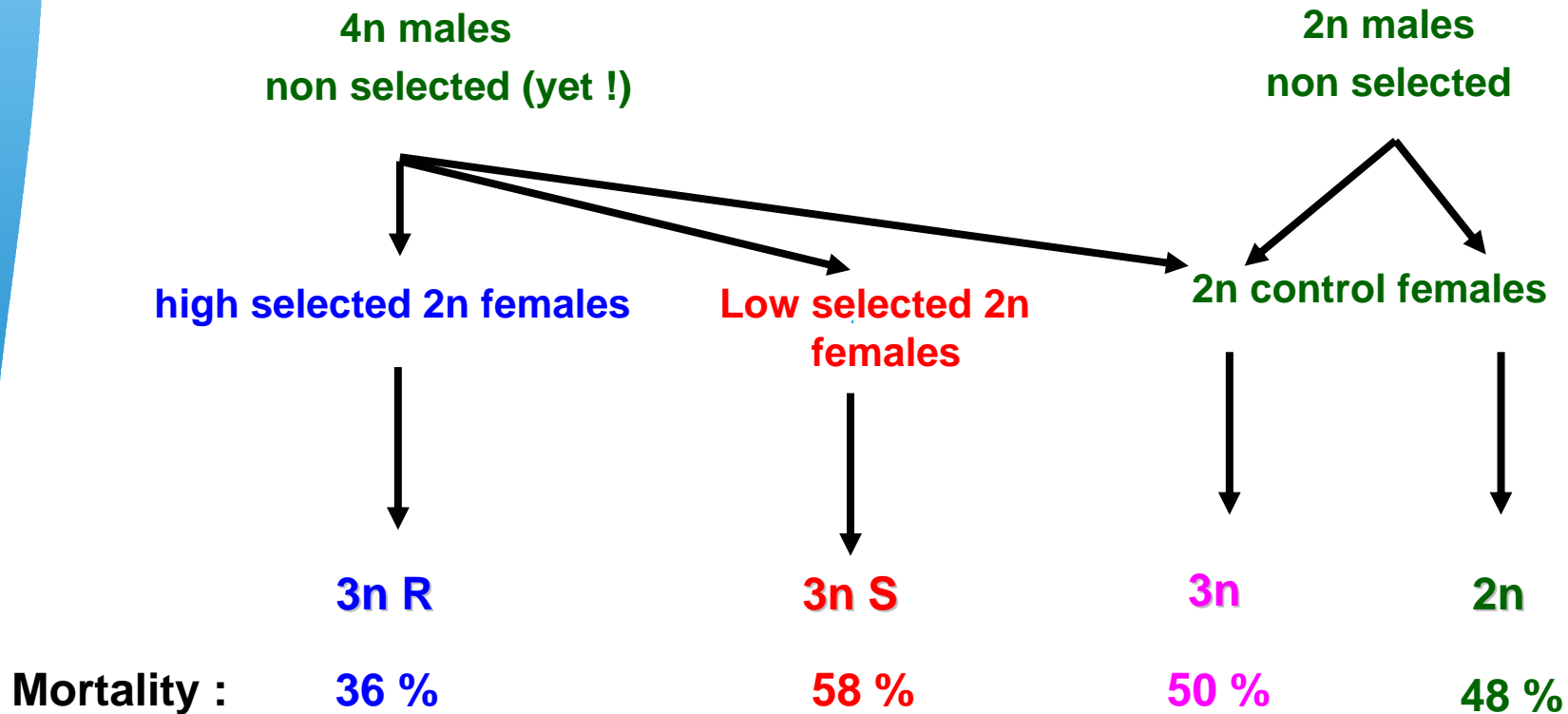


Variation in chromosome number in 4n x 4n progeny (McCombie et al, 2005)

- Selective breeding of tetraploids is known to be slower than in diploids and complex



# Summer mortality in 'selected' triploids



Mortality :

$$3n R < 2n = 3n = 3n S$$

# Conclusions

- **Selective breeding to improve survival can be efficiently implemented. This implies the establishment of long-term programs with proper management of genetic variability of broodstock.**
- **Such programs are most likely to be successfully established by joined efforts between public research and private companies**
- **Marker-assisted selection and integration of polyploidy in selective breeding strategies are challenging but promising perspectives.**





# Acknowledgements:



*La Tremblade, Charente-Maritime, France*