92nd Annual meeting of the National Shellfisheries Association,
Seattle, March 19th – 23rd 2000

Microsatellite Markers as a tool to study reproductive success in the Pacific oyster, *Crassostrea gigas* (Thunberg), under controlled hatchery conditions

P. Boudry [1], B. Collet [1], F. Cornette [1,2], V. Hervouet [1,2], & F. Bonhomme [2]

[1] IFREMER
Laboratoire de
Génétique et Pathologie
La Tremblade, France

[2] CNRS
Laboratoire Génome, Populations, Interactions
Sète, France

EU funded project FAIR PL. 95.421 "GENEPHYS"

Introduction

Oysters, like many marine species, are characterised by a very high fecundity.

Natural or hatchery populations often show low *Ne/N* ratios.

☞ **high variance of their reproductive success ?**

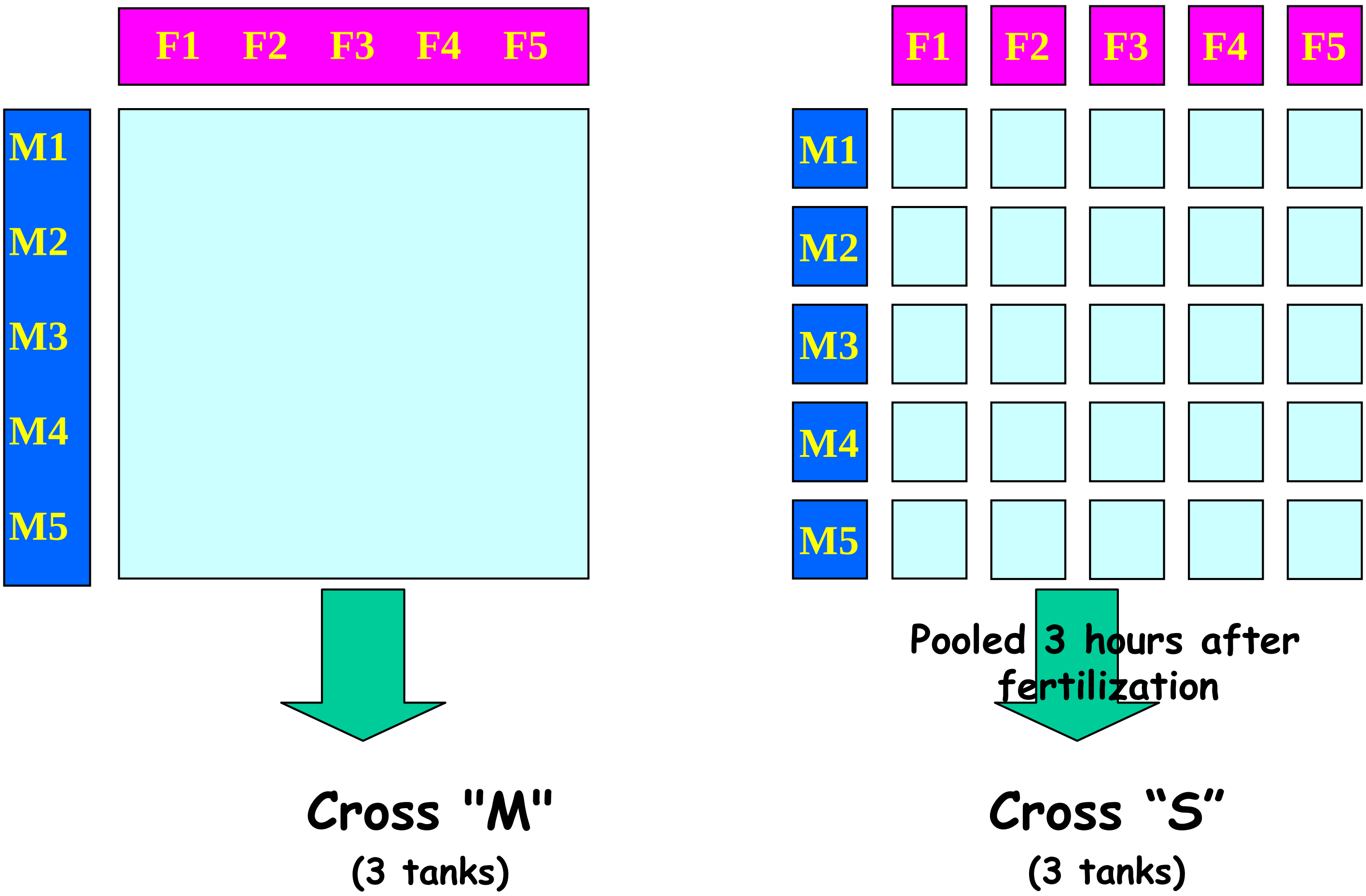
Demonstrated by Li et Hedgecock, (1998) for a wild population of *C. gigas* and by Launey (1998) for a hatchery population of *O. edulis*.

☞ **but why such a variance ?**

Experimental design

Factorial *in vitro* crosses by stripping:
5 males x 5 females.

2 different types of crosses :



Methodes

- ***In vitro* fertilization:**

  Equal number of gametes between males and between females:

  ☞ Balanced gametic contributions

- **Parental oysters:**

  Selected genotypes showing different alleles (biopsies):

  ☞ Ease the parental identification in the progeny

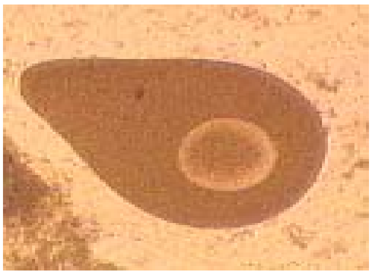
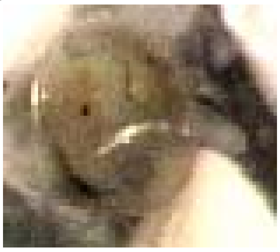
- **Genetic markers:**

  4 highly polymorphic loci (more than 50 alleles / locus).

- **Sampling:**

  Larvae (day 6, day 18) & spat (day 90)

Surval during the experiment

Day	N	stage	Survival (%)
0	9000000	Female gamete	100
			
1	3000000	"D" larvae	30
			
18	300000	Eyed larvae	3
			
90	9000	Spat	0.1
			

Parentage identification

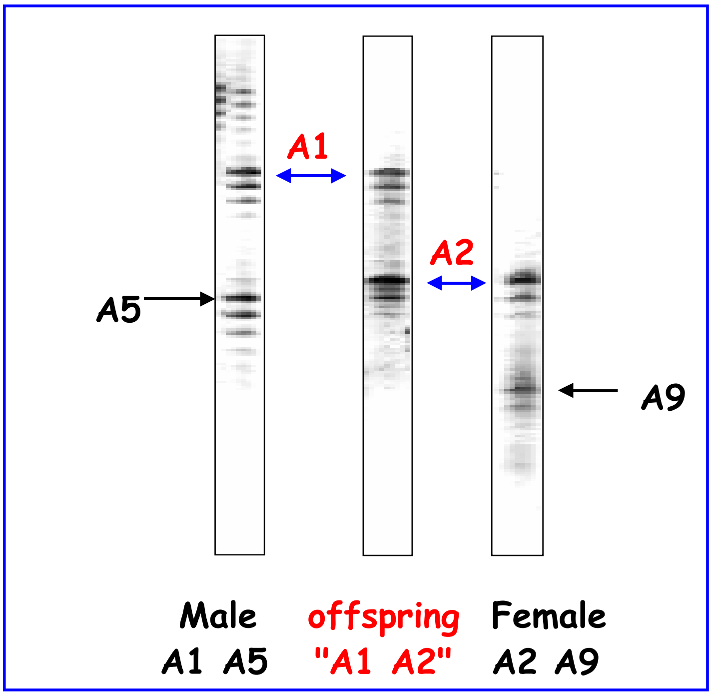
5 males and 5 females, selected to be heterozygous at the L10 locus

☞ no null alleles

18 different alleles

1581 offspring genotyped

☞ Parentage determined for 99,2 % of the genotyped progeny.



Are parental contributions balanced ?

Contributions in Cross "M" at Day 90 (n = 352)

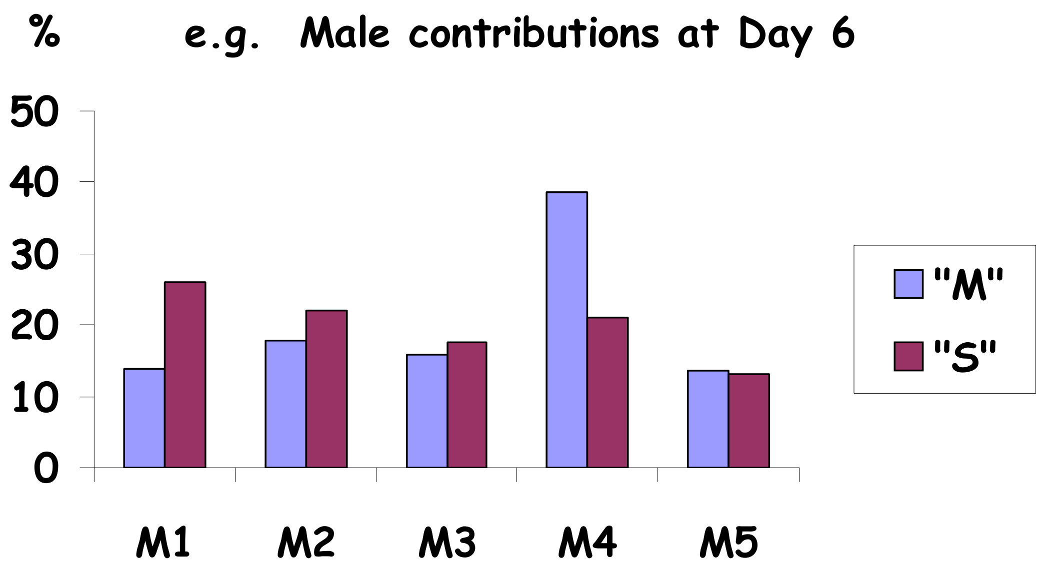
	Males					
Females	M1	M2	M3	M4	M5	
F1	0.6	1.5	1.5	2.1	0.0	**5.7**
F2	0.0	0.9	3.9	1.5	0.9	**7.2**
F3	0.0	0.3	0.6	0.0	0.9	**1.8**
F4	3.0	11.4	21.4	21.1	8.7	**65.7**
F5	1.8	2.7	7.8	5.7	1.5	**19.6**
	5.4	**16.9**	**35.2**	**30.4**	**12.0**	**100.0**

G Tests

Cross	stage	Males	Females
"M"	Day 6	***	***
	Day 18	***	***
	J90	***	***
"S"	Day 6	**	***
	J18	***	***
	Day 90	***	***

☞ Differences in gamete quality or genetic differences ?

Are parental contributions different in the crosses “S” and “M” ?

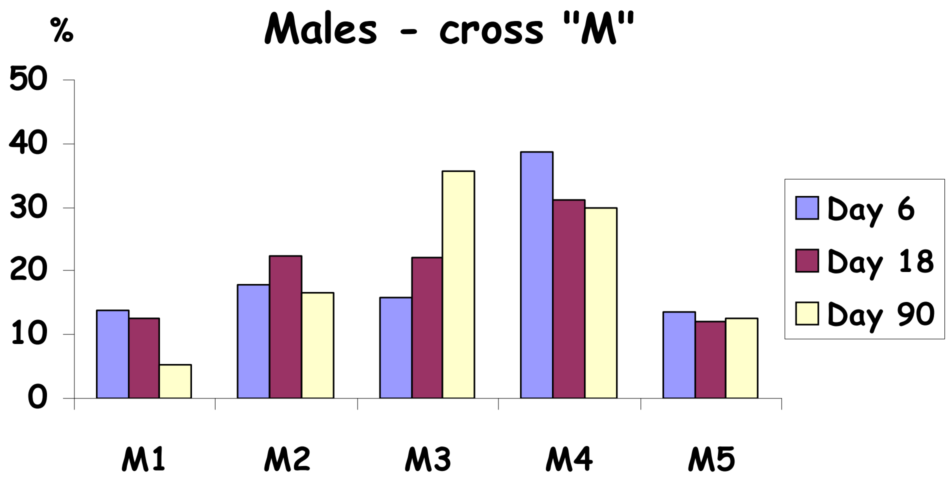


stage	males	Females
J6	***	ns
J18	***	***
J90	***	***

(G Tests)

☞ Gametic competition between males

Are parental contributions stable over time ?



From Day 6 to Day 18 (G tests)

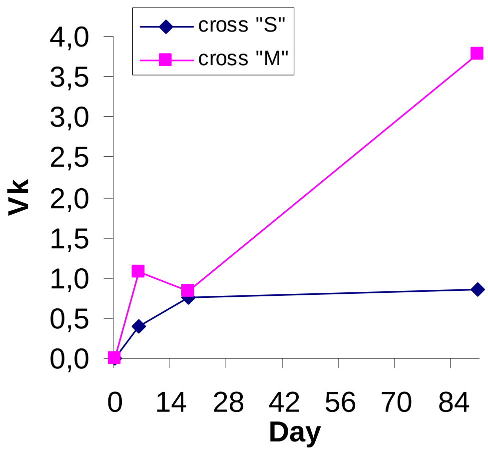
Cross	males	females
"M"	ns	***
"S"	***	ns

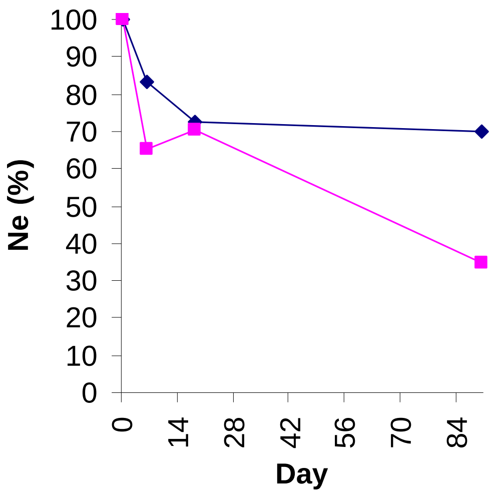
From Day 18 to Day 90 (G tests)

Cross	males	females
"M"	***	***
"S"	***	***

Parental effects on survival

Do the variance in reproductive success (Vk) and the effective population size (*Ne*) decrease over time ?





☞ Reduction of the effective population size due to an increasing variance in reproductive success

How much does the variance in reproductive success decrease the effective population size *Ne* ?

Wright (1938):

$$Ne = \frac{4N - 2}{Vk + 2}$$

Robertson (1961):

$$Ne = \frac{(\sum n_{ij})^2}{\sum n_{ij}^2}$$

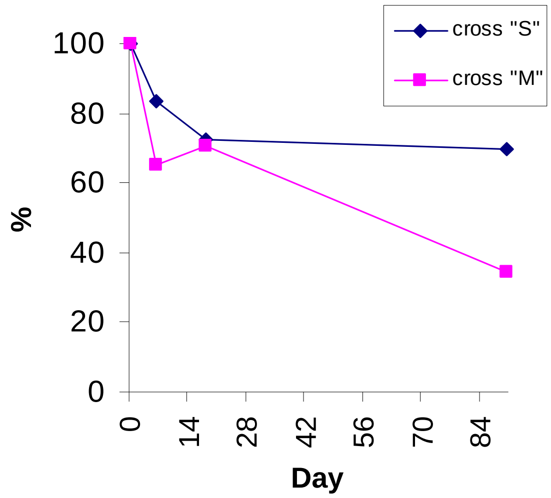
Maximum possible value in our crosses :

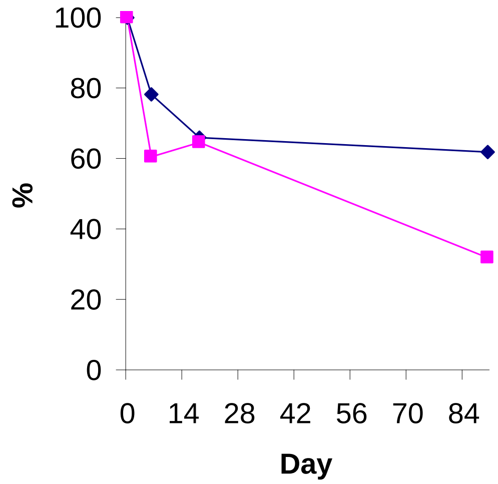
$Ne_{max} = 19$

(assuming a stable population size)

$Ne_{max} = 25$

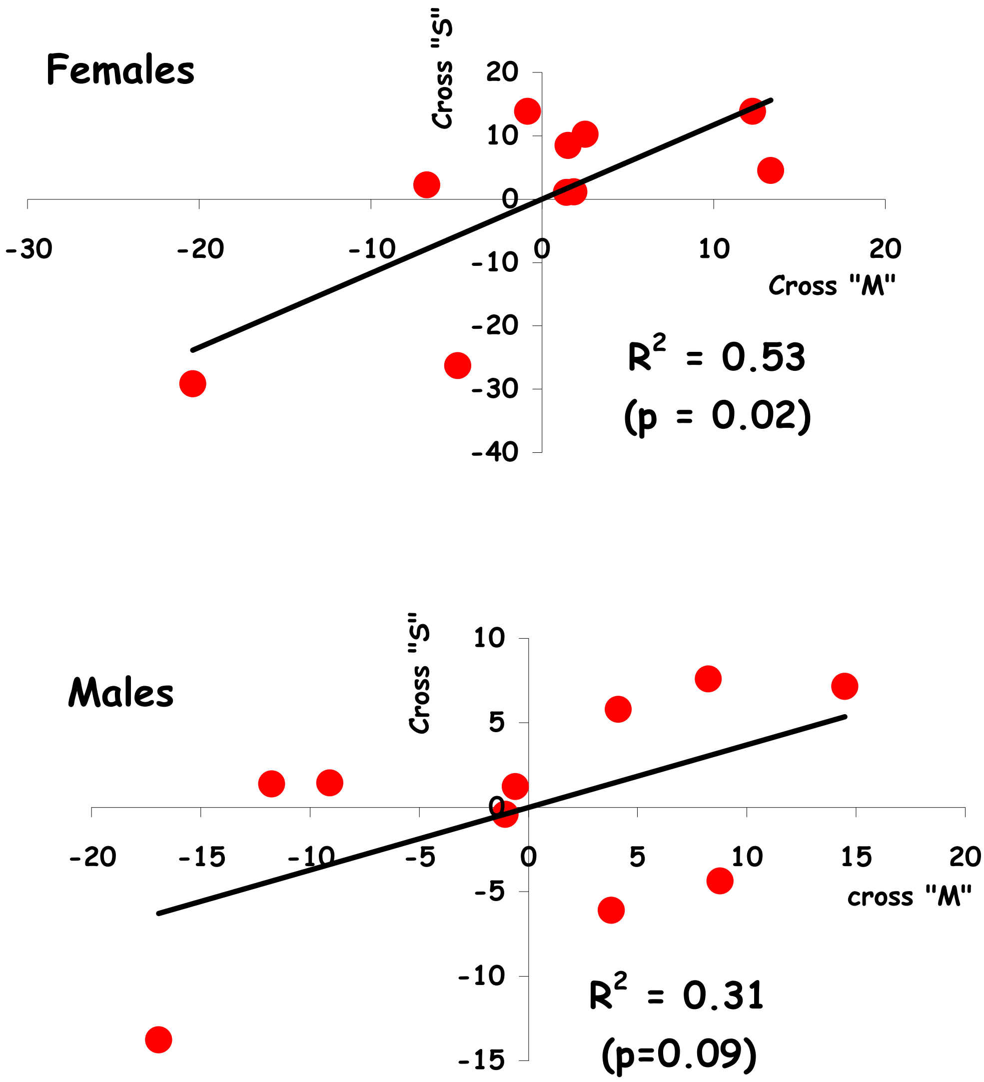
Observed decrease of *Ne* in the two crosses:





Are "good parents" the same in the two crosses ?

(% at Day 6 - % at day 18) and (% at day18 - % à Day 90)



☞ Changes over time of parental contributions (from Day 6 to Day 18 and from Day 18 to Day 90) are similar in the two crosses.

Is reproductive success heritable ?

Percentage of the variance and significance of effects (anovas on arcsin√ transformed data):

Day	Cross "M"			Cross "S"		
	males	females	m x f	males	females	m x f
6	19 ***	28 ***	24 **	12 *	19 **	16 ns
18	19 ***	43 ***	20 ***	34 ***	20 ***	17 ns
90	15 ***	64 ***	9 **	22 ***	25 ***	26 **

Heritability estimates from arcsin√ transformed data (Bogyo and Becker, 1965):

Day	Cross "M"		Cross "S"	
	males	females	males	females
6	0.03	0.05	0.02	0.03
18	0.02	0.06	0.04	0.02
90	0.05	0.23	0.03	0.04

☞ Most heritability estimates are low.

Conclusions

- ☞ Despite the initial balanced gametic contributions, parental contributions in the progeny are unbalanced. The effective population size is therefore reduced.
- ☞ These unbalanced contributions change over time due to differential mortality rates. These changes are similar between the two crosses.
- ☞ The observed variance in reproductive success increases over time, leading to a decreasing effective population size.
- ☞ Heritability estimates of reproductive success (transformed percentage data) are low, but male, female and male x female effects are significant.