SELECTIVE BREEDING TO IMPROVE RESISTANCE AGAINST SUMMER MORTALITY IN THE PACIFIC OYSTER *Crassostrea Gigas*: RESULTS AFTER 3 GENERATIONS.

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Summer mortality of adults and juveniles has been reported in the Pacific oyster, *Crassostrea gigas*, for many years and in several countries. The French multidisciplinary program "Morest" aims to investigate the causes of the summer mortality in *Crassostrea gigas*. Within this program, we designed multi-site field experiments to assess to what extent genetic variability exists for summer mortality in French populations of *C. gigas* and to determine whether selective breeding could improve survival.

The first generation was based on 3 sets of bi-parental families, following a nested half-sib crossing design. Progenies (G1: 17 half-sib families (HSF)) were placed in three sites along French coasts in February, March and April 2001 and reared following usual practices until October 2001. A large variation among HSF was observed (Fig. 1). Heritability estimates for survival were very high (mean $h^2 = 0.81 \pm 0.29$). In 2002, 3 "good" HSF and 3 "bad" HSF were selected on their survival performances to constitute a second generation (G2) by divergent selection. Additionally, these were also crossed to make inbred families. Both inbred and outbred families, reared similarly to the first generation, showed a good response to selection (realized heritabilities > 0.7) (Fig. 2). No significant effect was observed on growth performance of the selected families. In 2003, a third generation of inbred and outbred families was produced to confirm the results obtained in 2002. Globally, our results indicate that selective breeding programs can efficiently improve survival of juvenile oysters.

FIGURE 1: variation of mean mortality among HSF in the G1 (*P* <0.001).

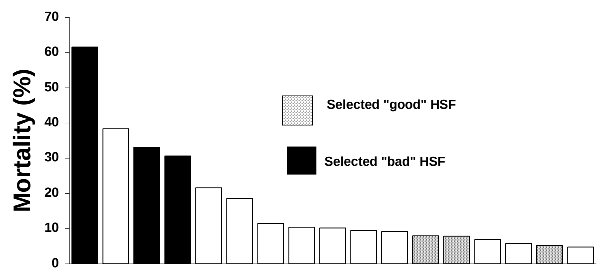
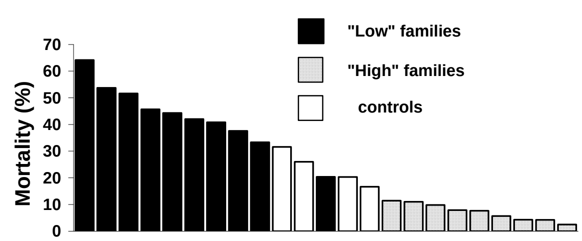


FIGURE 2: variation of mean mortality the families in the G2 (divergent selection) in Brittany (*P* <0.001).



Genetic basis of summer mortality in juvenile cupped oysters (*Crassostrea gigas*) : Results after 3 generations

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Summer mortality in Pacific oysters

- **Reported for many years and in several countries** (Koganezawa, 1975 ; Glude, 1975 ; Goulletquer *et al.,* 1998, Cheney *et al.,* 2000)

- **Affects spat and adults** (Glude, 1975 ; Koganezawa, 1975 ; Maurer *et al.,* 1986 ; Cheney *et al.,* 2000)**,**

- **Affects wild and hatchery-propagated oysters**

- **Affects diploid and triploid oysters** (Calvo *et al.,* 1999 ; Cheney *et al.,* 2000)

Causes of summer mortality remain uncertain

"MOREST" :

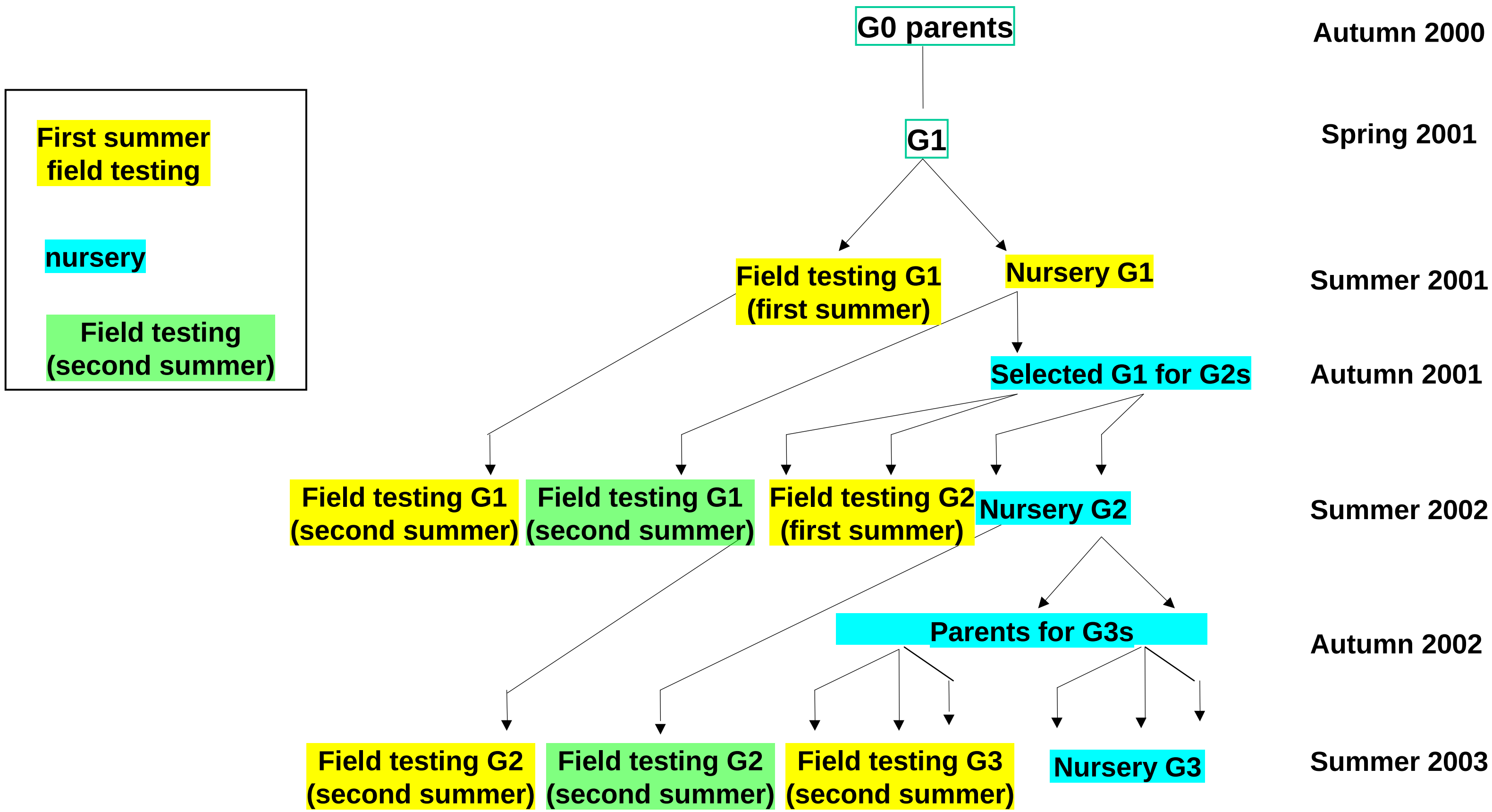
Etude des MORtalités ESTivales chez *C. gigas*

Study of summer mortality in C. gigas

- a French multidisciplinary program to better understand and face summer mortality : physiology, genetics, ecology, pathology, immunology, ecotoxicology, genomics…

- Main objectif in genetics: Determine if selective breeding programs could improve survival
  - Heritability of survival to summer mortality,
  - Response to selection,
  - Correlations between survival, growth and yield.

Selected trait : survival during the first summer



- **Recorded traits:** Survival (1st and second year), growth, yield
- **One generation / year**
- **inbred and outbred families**

First generation (G1):

- 3 sets of 24 nested half-sib crosses = 72 full-sib families

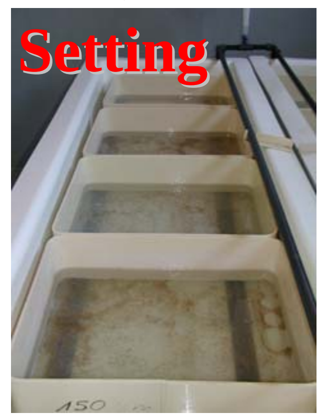
Female	Male 1	2	3	4	5	6
1	Half-Sib 1					
2						
3						
4						
5		Half-Sib 2				
6						
7						
8						
9			Half-Sib 3			
10						
11						
12						
13				Half-Sib 4		
14						
15						
16						
17					Half-Sib 5	
18						
19						
20						
21						Half-Sib 6
22						
23						
24						

Female	Male 7	8	9	10	11	12
25	Half-Sib 7					
26						
27						
28						
29		Half-Sib 8				
30						
31						
32						
33			Half-Sib 9			
34						
35						
36						
37				Half-Sib 10		
38						
39						
40						
41					Half-Sib 11	
42						
43						
44						
45						Half-Sib 12
46						
47						
48						

Female	Male 13	14	15	16	17	17
49	Half-Sib 13					
50						
51						
52						
53		Half-Sib 14				
54						
55						
56						
57			Half-Sib 15			
58						
59						
60						
61				Half-Sib 16		
62						
63						
64						
65					Half-Sib 17	
66						
67						
68						
69						Half-Sib 18
70						
71						
72						

First generation (G1)

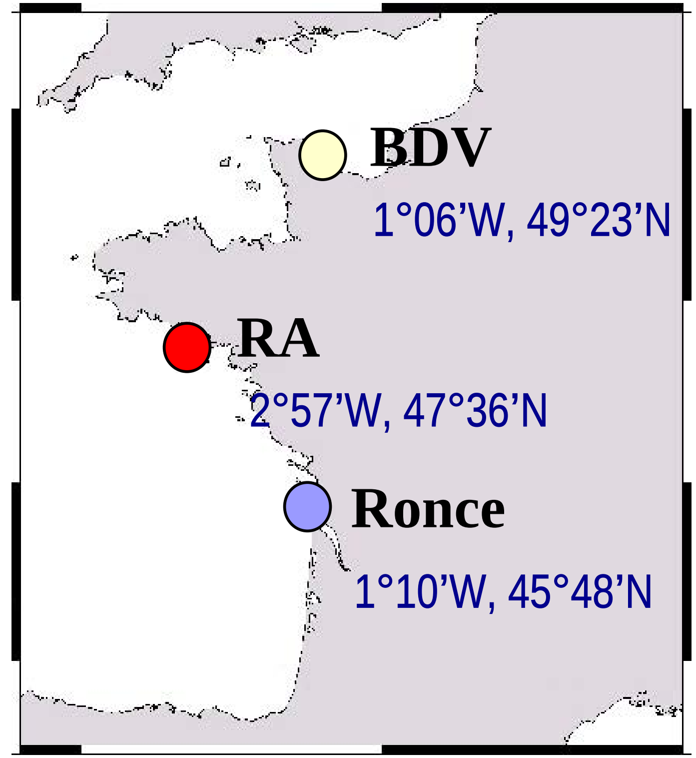
Série	déc-00	janv-01	févr-01	mars-01	avr-01	mai-01	juin-01	juil-01	août-01	sept-01	oct-01
1	maturation		EL (0-16) micro nurserie (16-44)	nurserie (44-134)			site (135-239)				
2		maturation		EL (0-16) micro nurserie (16-44)	nurserie (44-121)			site (122-211)			
3			maturation		EL (0-16)	micro nurserie (16-42)	nurserie (42-107)		site (108-164)		



First generation (G1)

- **15 FS families / set**

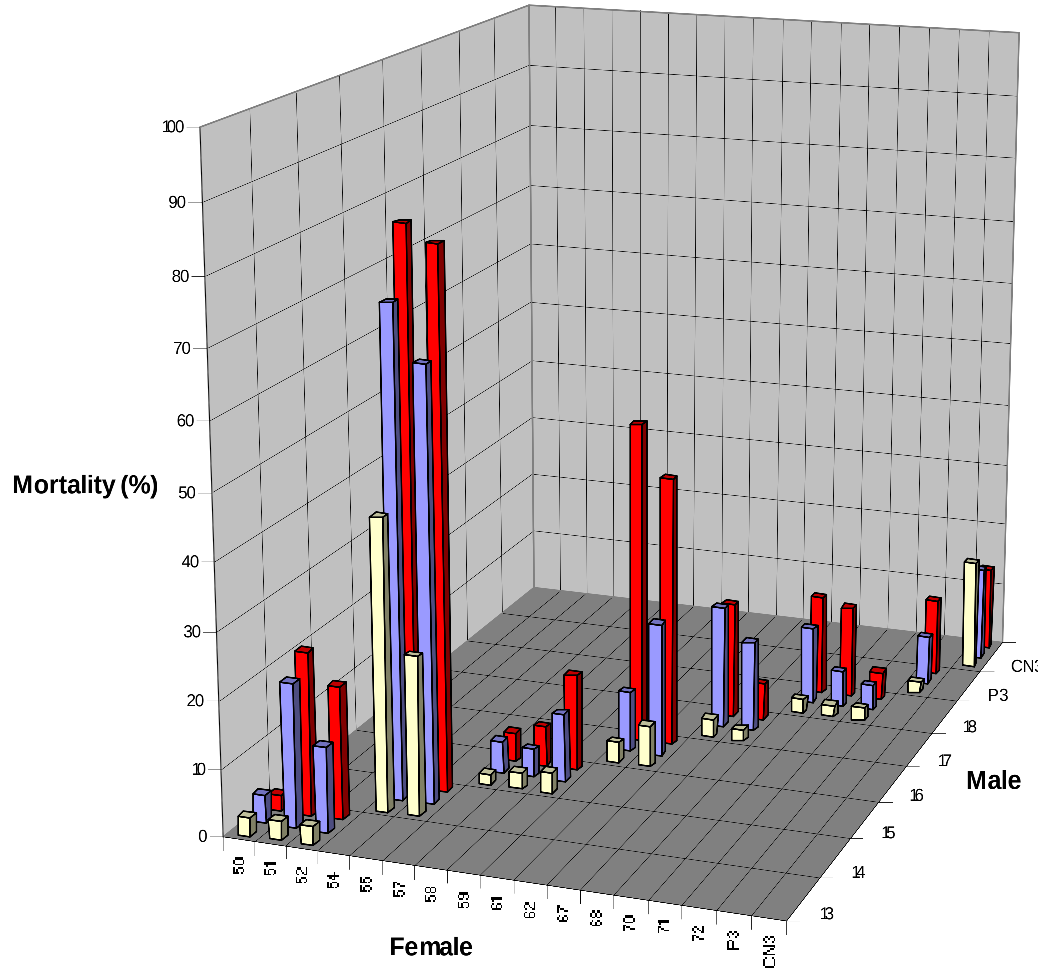
- **3 sites :**
  - Ronce Perquis (Marennes-Oleron bassin) : Ronce
  - Rivière d 'Auray (South Britany) : RA
  - Baie des Veys (Normandy) : BDV



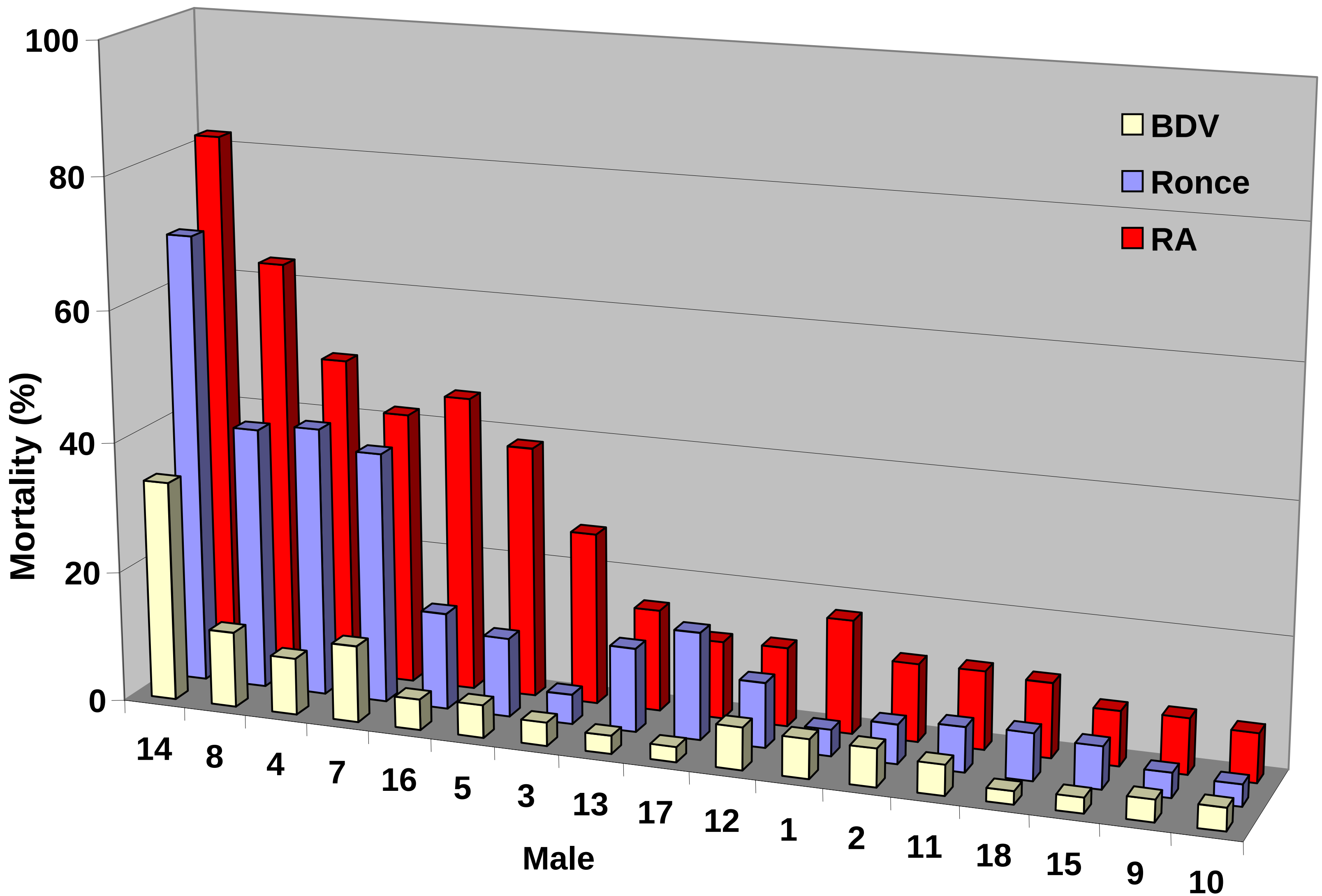
- **3 sampling times :**
  - 2 and 4 weeks after placement in the field
  - at the end of the summer period (October 2001)

3 bags / family / site / sample =
1215 bags of 150 oysters

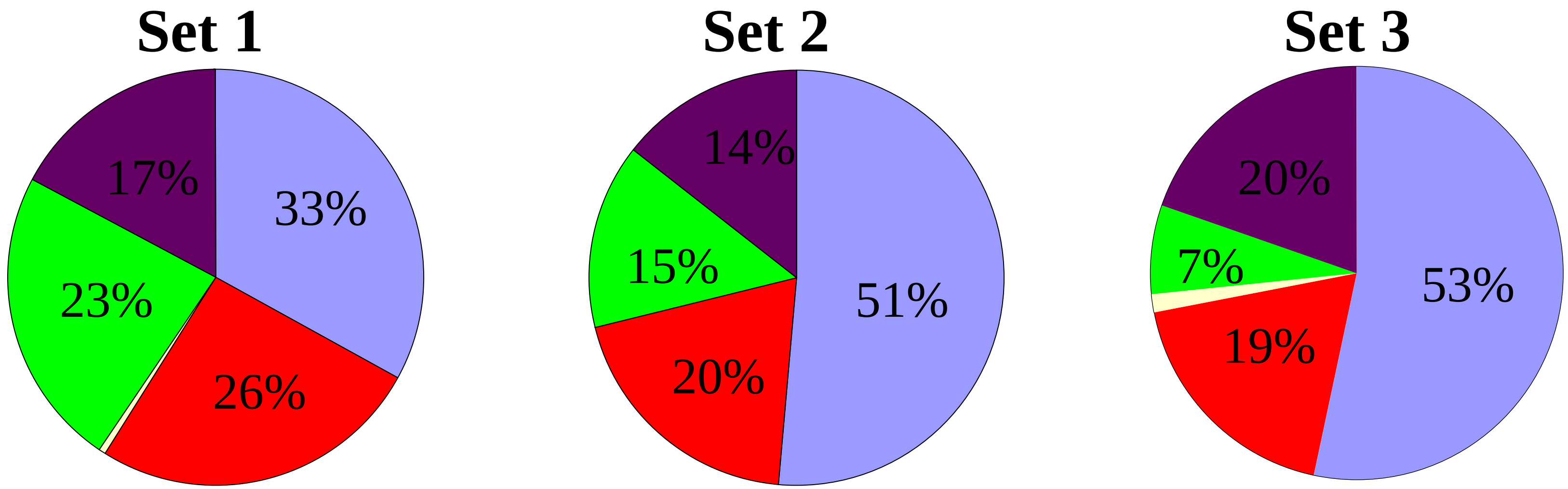
First generation (G1): mortality of set 3 over time



G1 : mortality in the field (summer 2001)



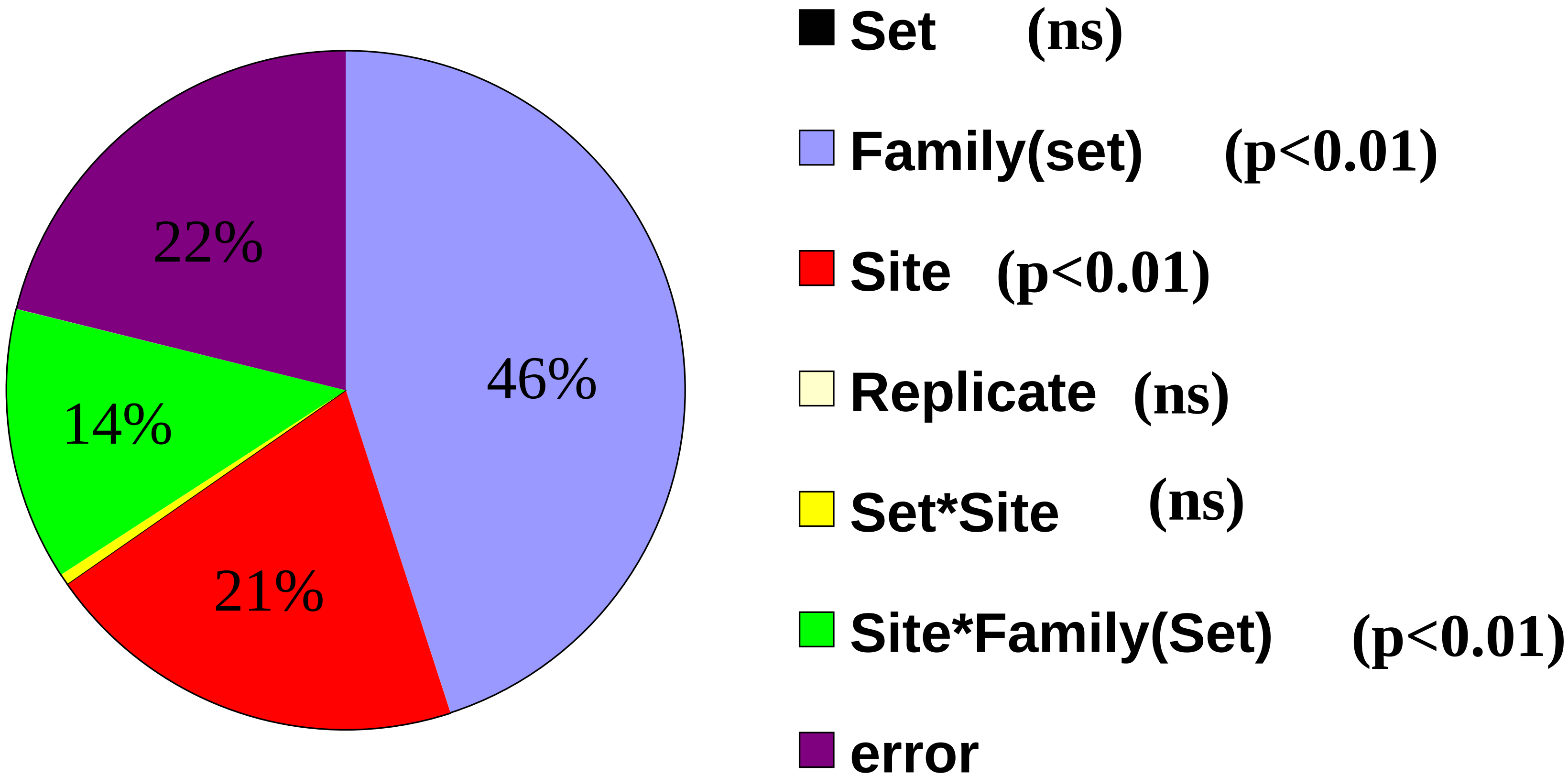
G1 : variance components for survival



$h^2 = 0.23 \pm 0.35$ **$h^2 = 0.85 \pm 0.46$** **$h^2 = 1.21 \pm 0.57$**

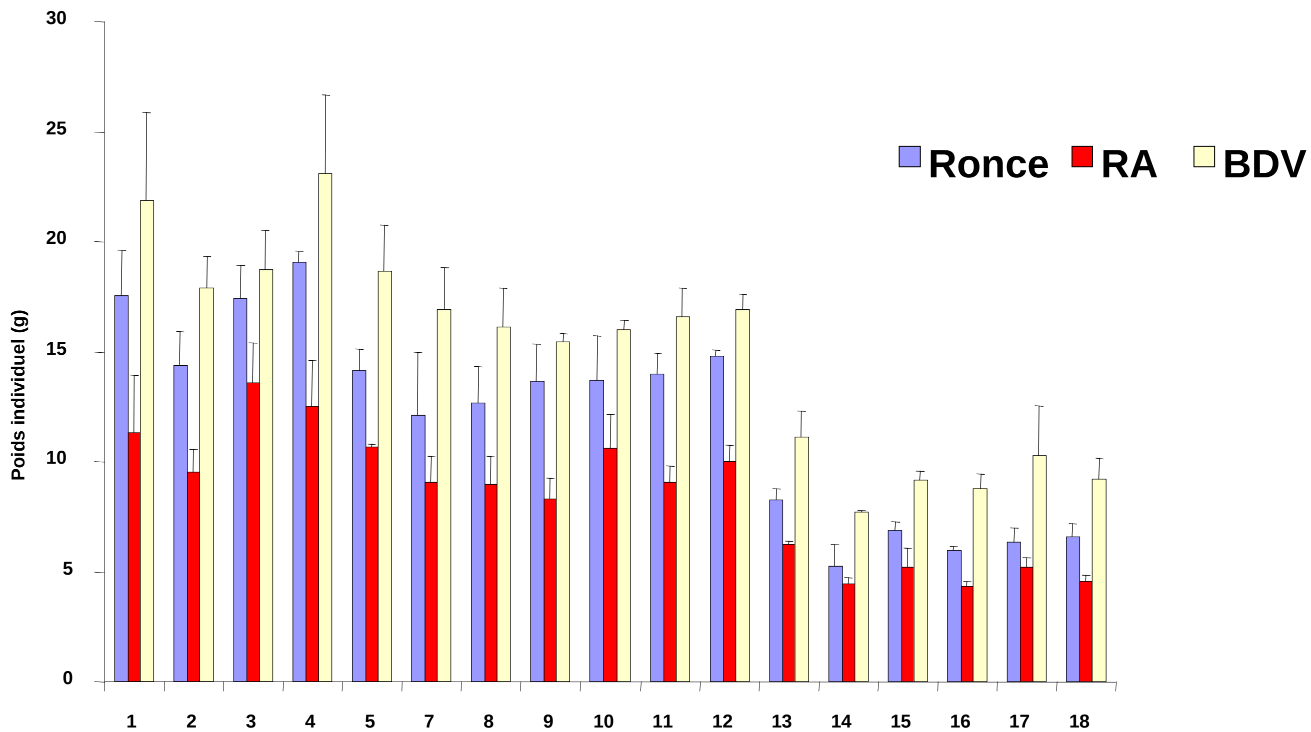
- Family (p<0.01)
- Site (p<0.01)
- Replicate ns
- Family*Site (p<0.01)
- error

G1 : variance components for survival



$h^2 = 0.81 \pm 0.29$

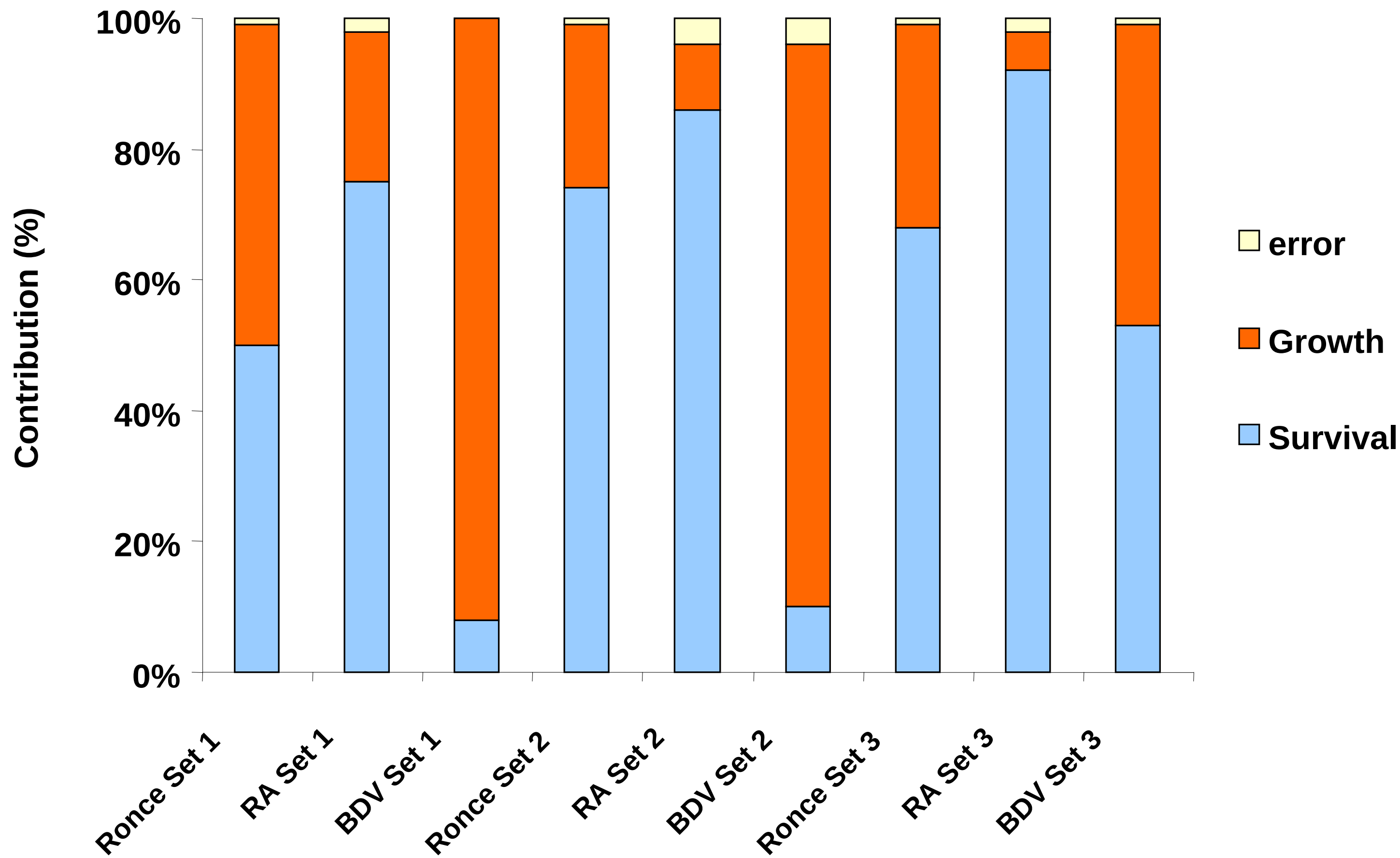
G1 : Growth performance among HF families



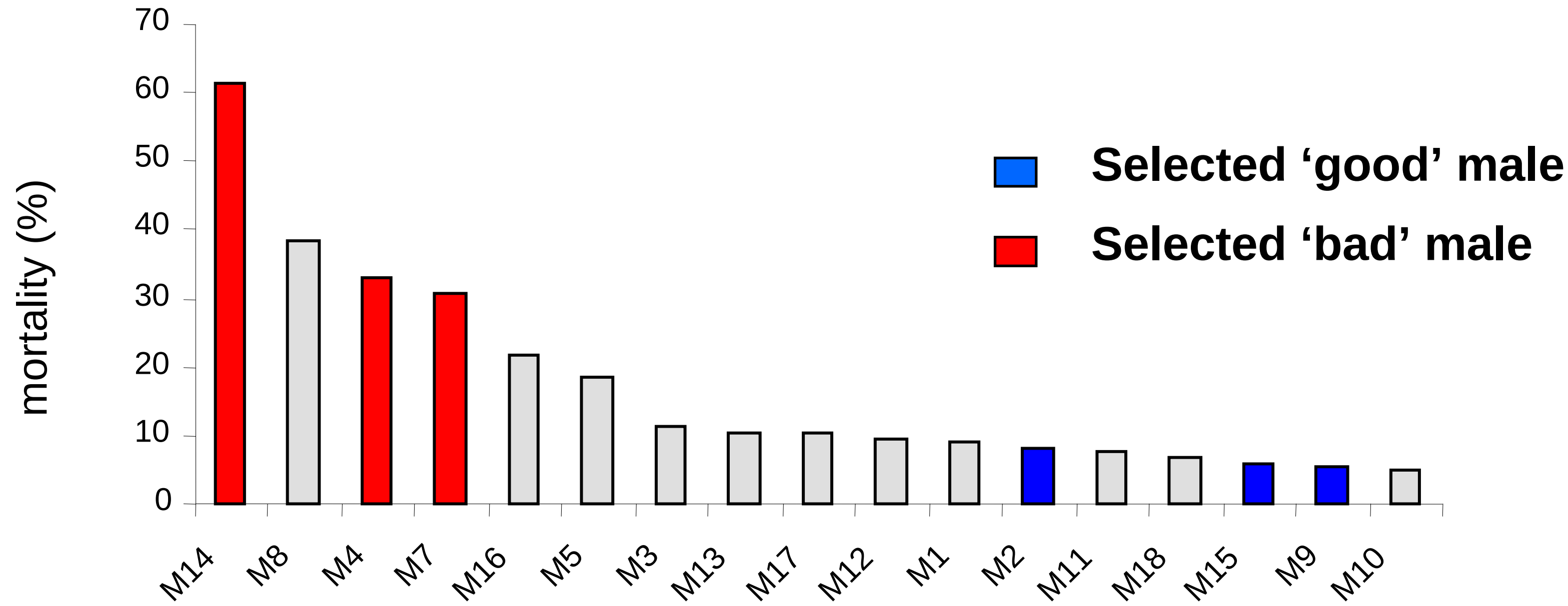
$h^2 = 0.31 \pm 0.15$

G1 : Relative importance of survival and growth variation on yield per set and per site

(based on multiple regression)



Second generation (1) : G2SD



Low selected group 'S'

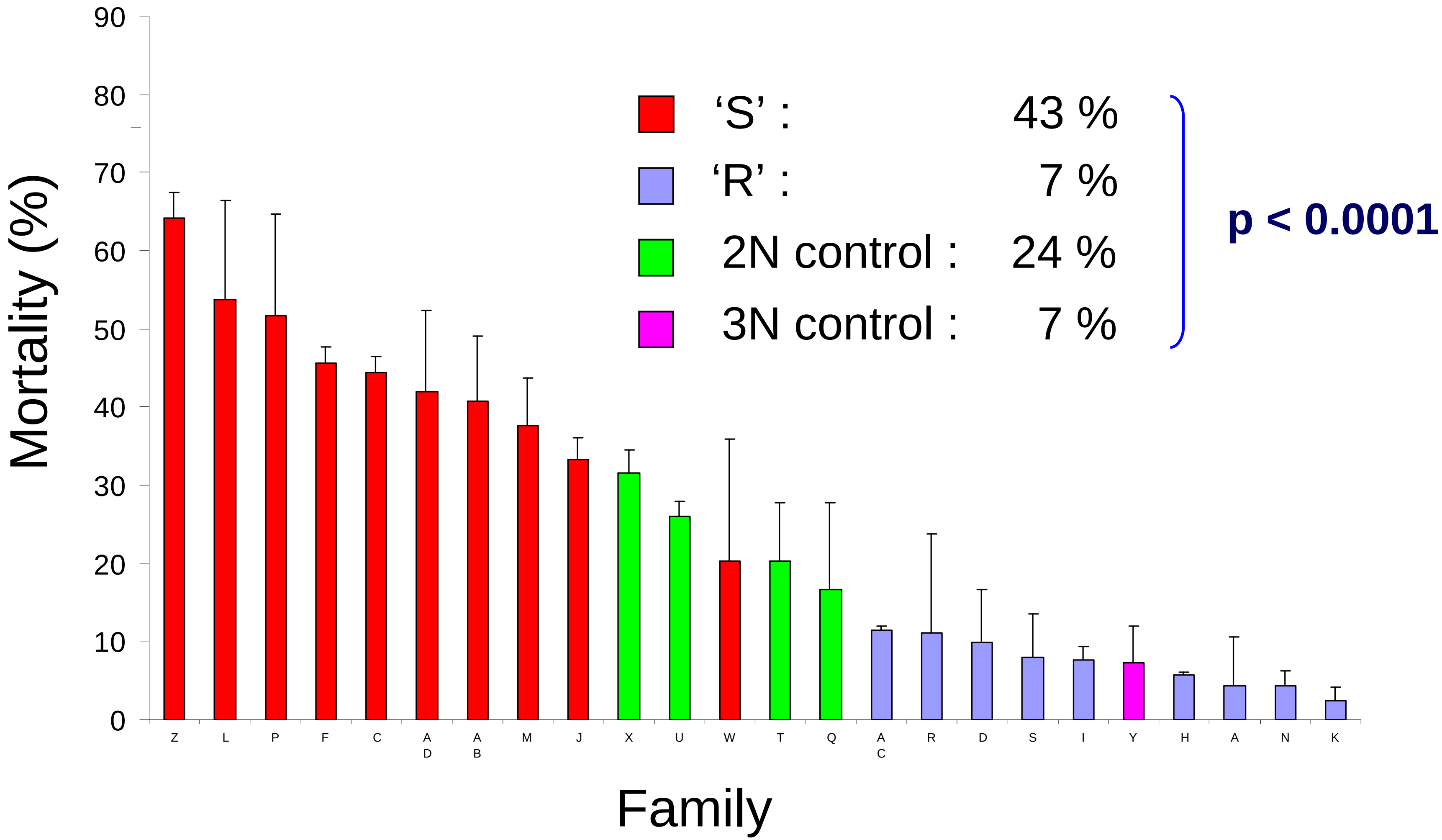
Male	Family	F4-15	F4-16	F7-25	F7-26	F14-54	F14-55
		4		*7*		*14*	
4	F4-15			13	14	17	18
	F4-16			15	16	19	20
7	F7-25					21	22
	F7-26					23	24
14	F14-54						
	F14-55						

High selected group 'R'

Male	Family	F2-5	F2-8	F9-35	F9-36	F15-57	F15-58
		2		*9*		*15*	
2	F2-5			1	2	5	6
	F2-8			3	4	7	8
9	F9-35					9	10
	F9-36					11	12
15	F15-57						
	F15-58						

+ Controls : 2N and 3N

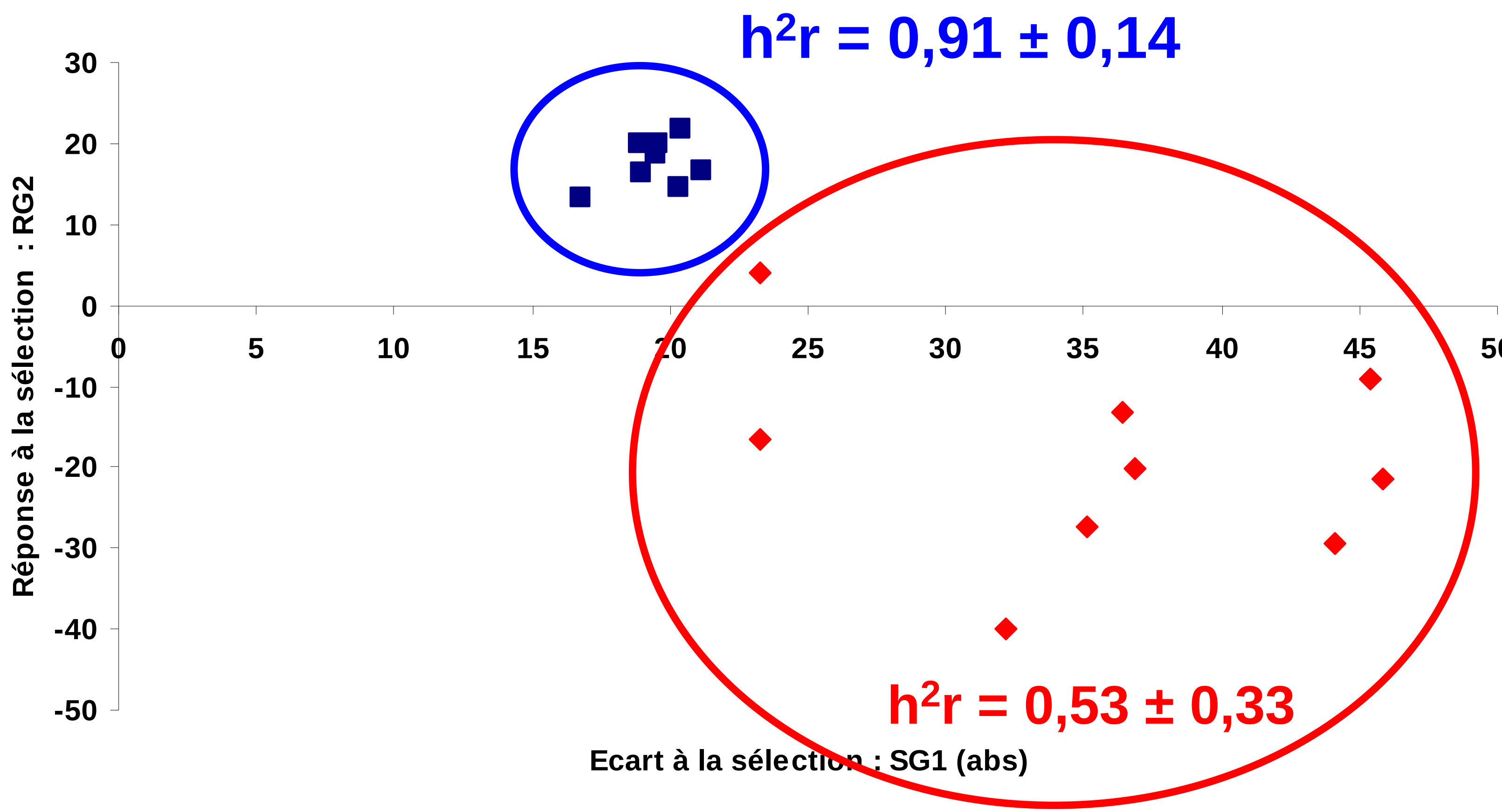
G2SD: Survival in « RA » (summer 2002)



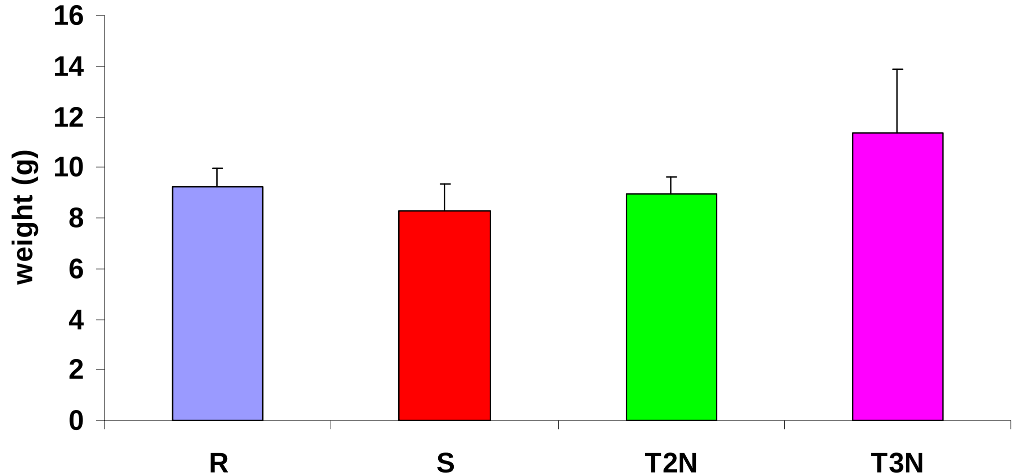
S > T2n > T3n = R

G2SD: Response to selection on survival

(all sites together)

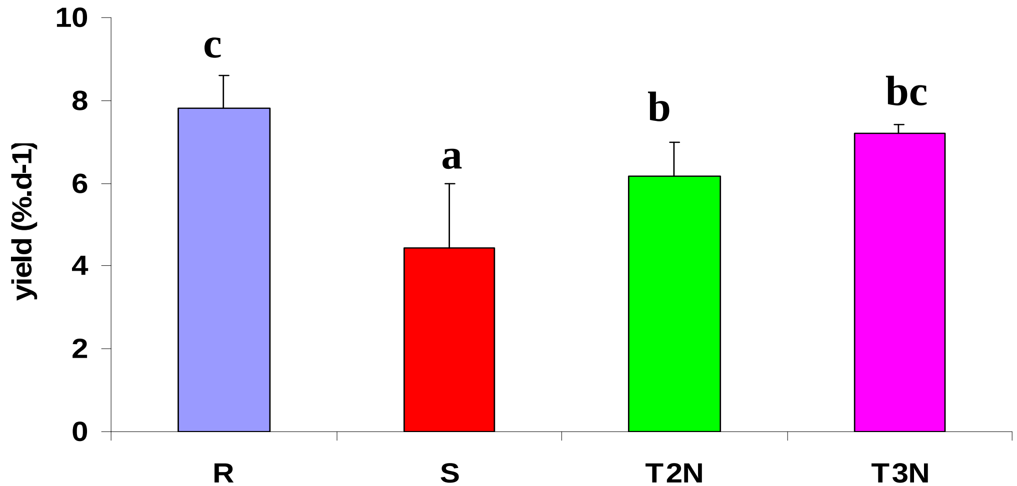


G2SD: Response to selection for survival on growth and yield



Growth

ns



Yield

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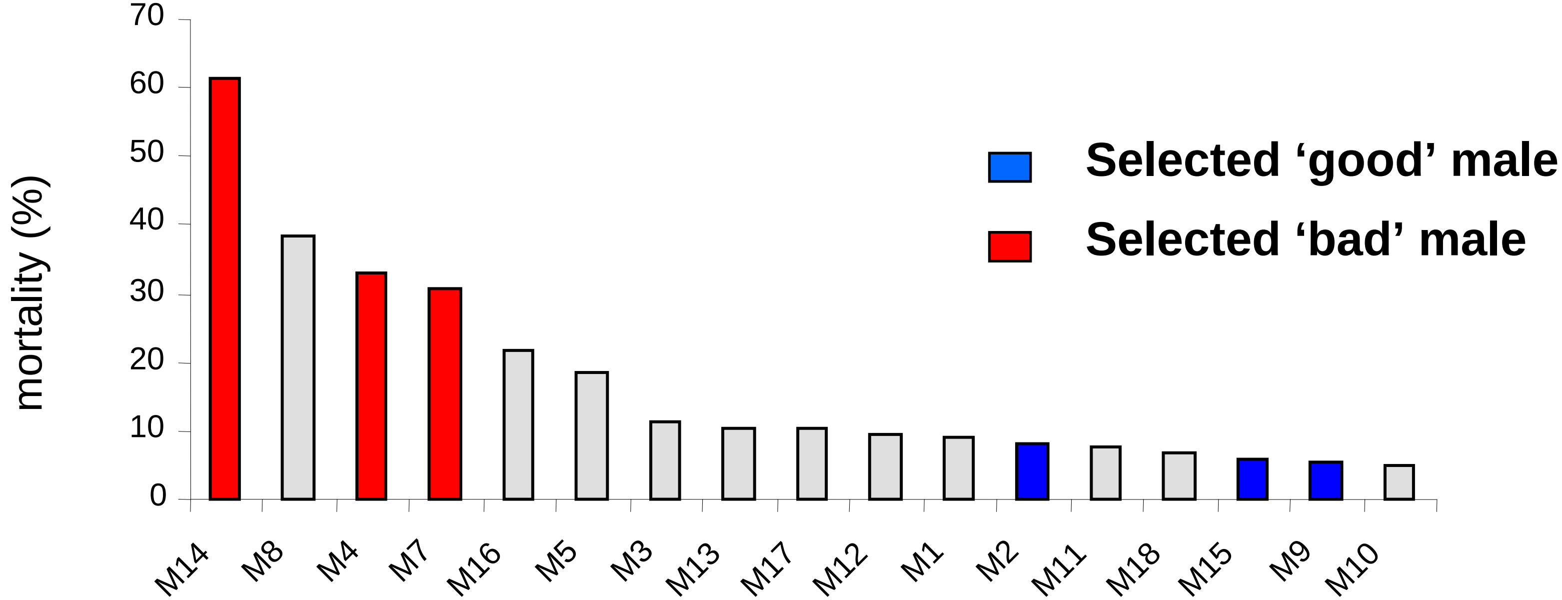
$R^2 = 0.94$

Second generation (2) : G2C (inbred)



Low selected group 'S'

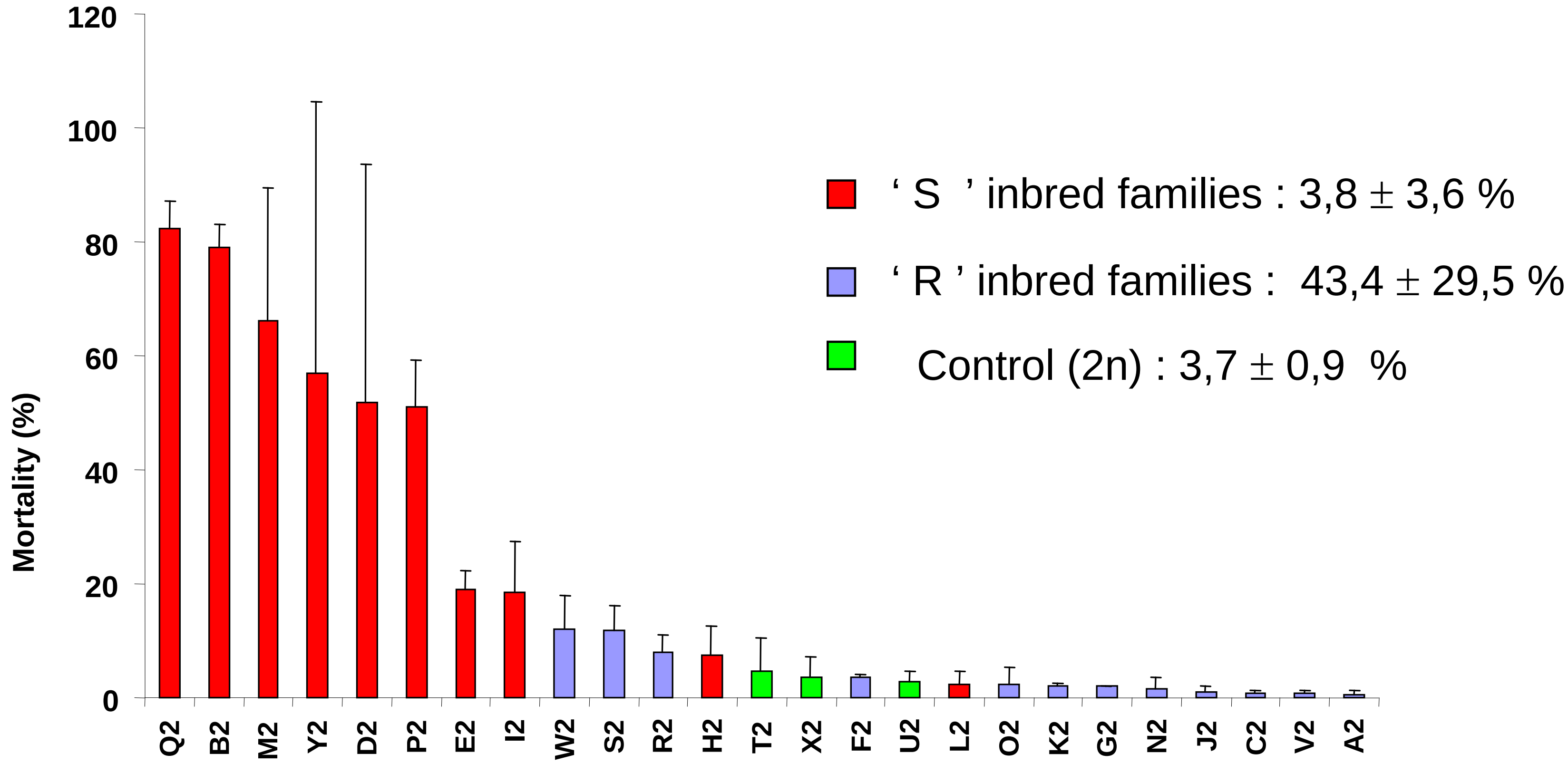
Male		*4*		*7*		*14*	
	Family	F4-15	F4-16	F7-25	F7-26	F14-54	F14-55
4	F4-15	13	14				
	F4-16	15	16				
7	F7-25			17	18		
	F7-26			19	20		
14	F14-54					21	22
	F14-55					23	24

High selected group 'R'

Male		*2*		*9*		*15*	
	Family	F2-5	F2-8	F9-35	F9-36	F15-57	F15-58
2	F2-5	1	2				
	F2-8	3	4				
9	F9-35			5	6		
	F9-36			7	8		
15	F15-57					9	10
	F15-58					11	12

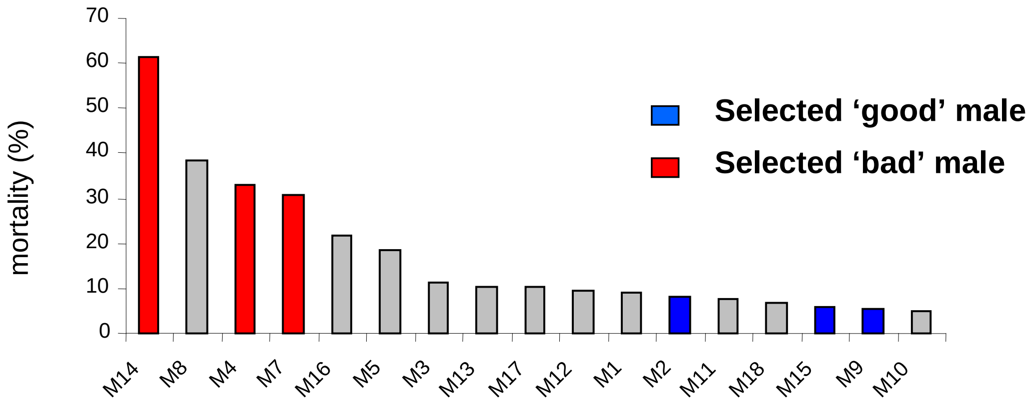
+ Controls : 2N

G2C: Survival in « Ronce » (summer 2002)



S > T2n = R

Third generation (1) : G3SD (outbred)



G1

Low selected group 'S'

Male	Family	4		7		14	
		F4-15	F4-16	F7-25	F7-26	F14-54	F14-55
4	F4-15			13	14	17	18
	F4-16			15	16	19	20
7	F7-25					21	22
	F7-26					23	24
14	F14-54						
	F14-55						

High selected group 'R'

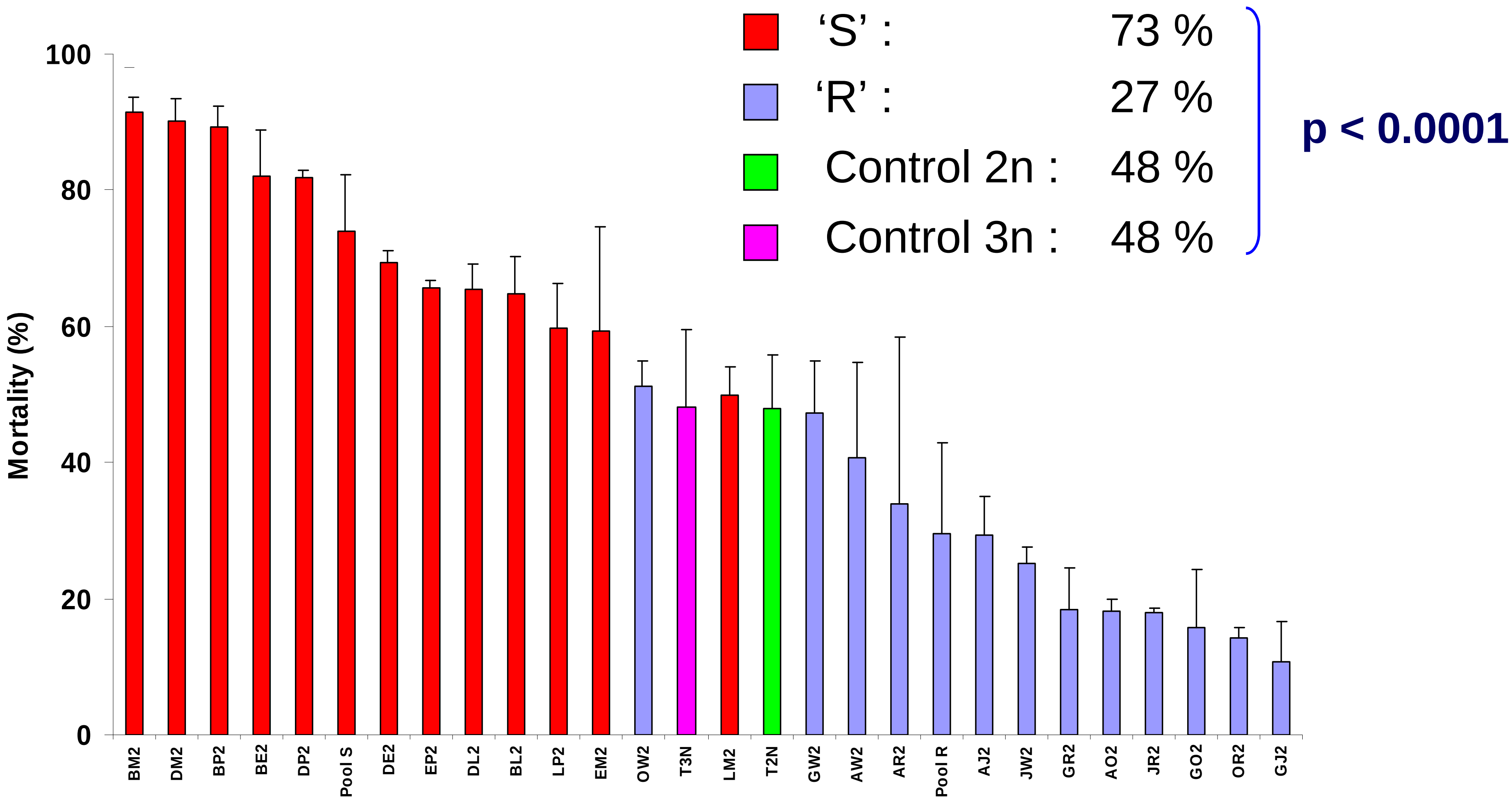
Male	Family	2		9		15	
		F2-5	F2-8	F9-35	F9-36	F15-57	F15-58
2	F2-5			1	2	5	6
	F2-8			3	4	7	8
9	F9-35					9	10
	F9-36					11	12
15	F15-57						
	F15-58						

G2C

G0		7		14	
	G2C	E2	L2	M2	P2
4	B2	BE2	BL2	BM2	BP2
	D2	DE2	DL2	DM2	DP2
7	E2			EM2	EP2
	L2			LM2	LP2

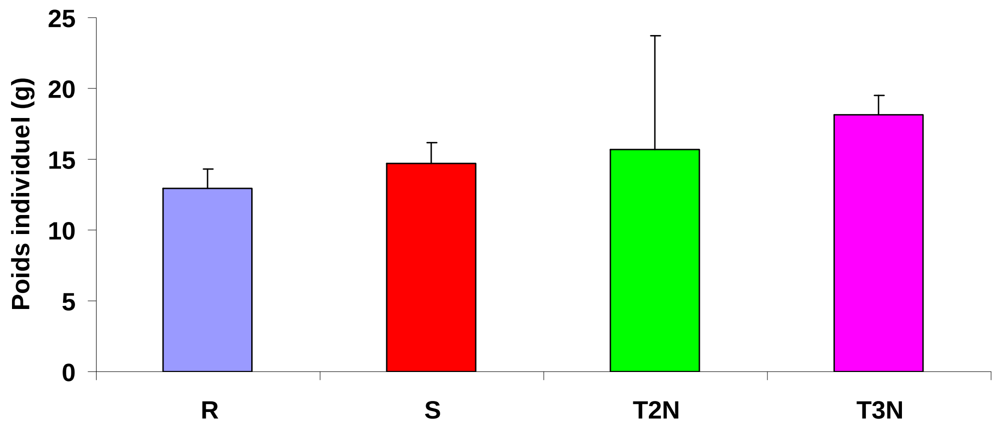
G0		9		15	
	G2C	J2	O2	R2	W2
2	A2	AJ2	AO2	AR2	AW2
	G2	GJ2	GO2	GR2	GW2
9	J2			JR2	JW2
	O2			OR2	OW2

G3SD (outbred): survival in « RA » (2003)



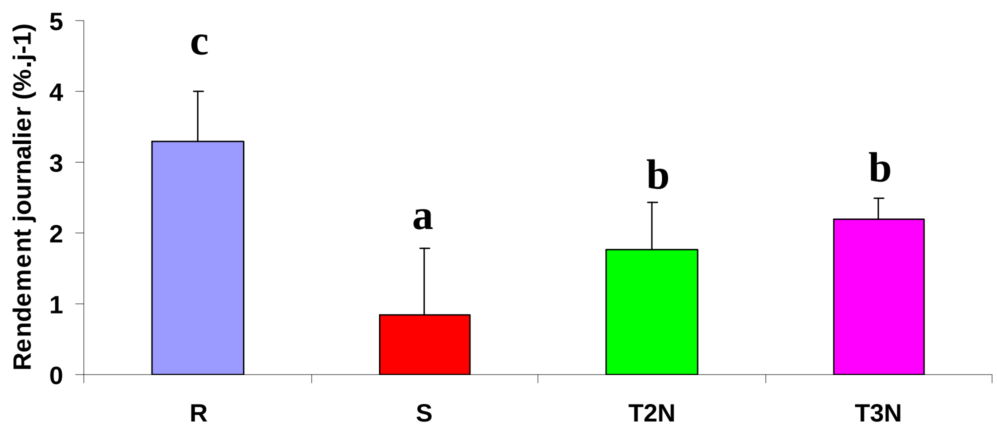
S > control 2n = control 3n > R

G3SD: Response to selection for survival on growth and yield



Growth

ns



Yield

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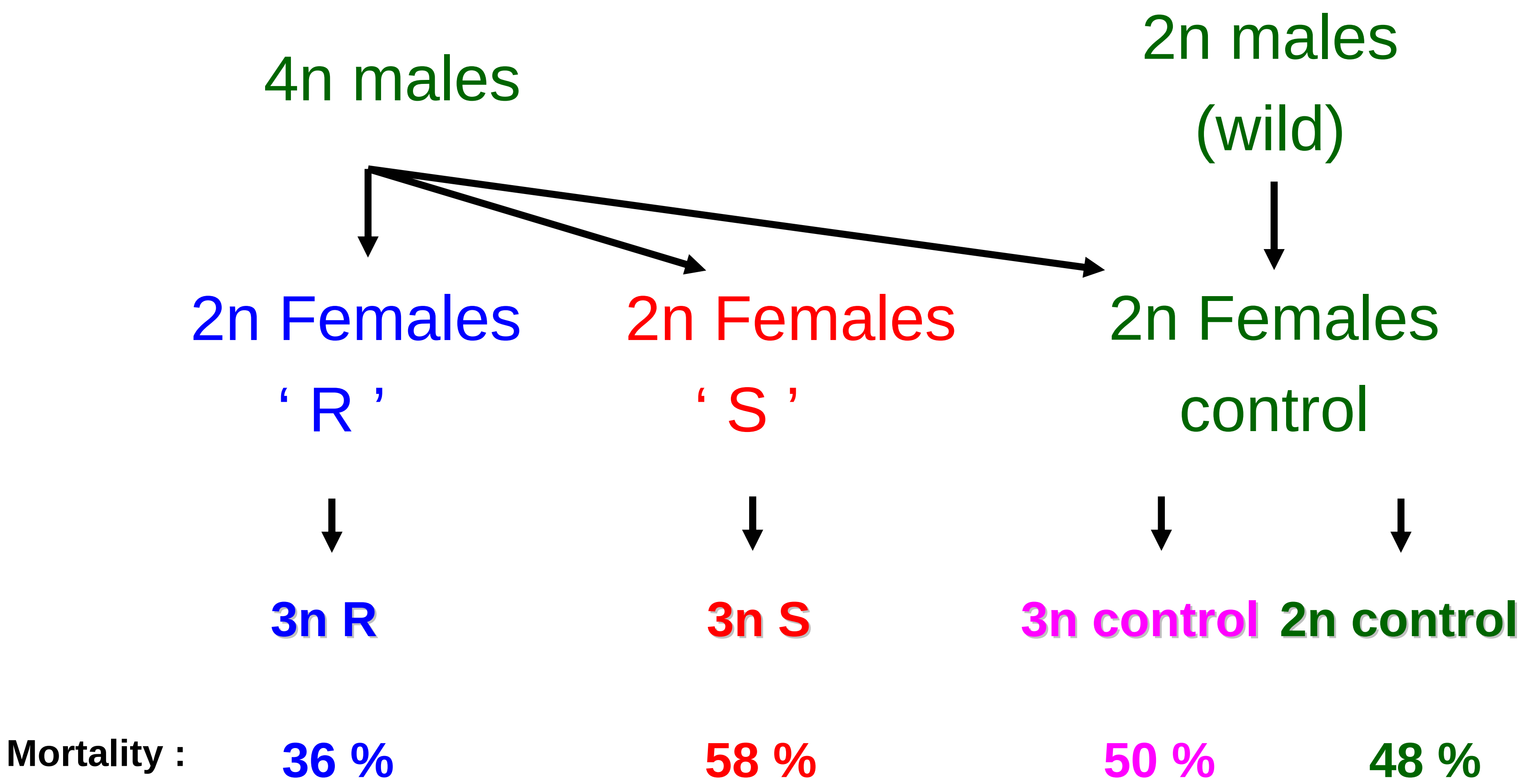
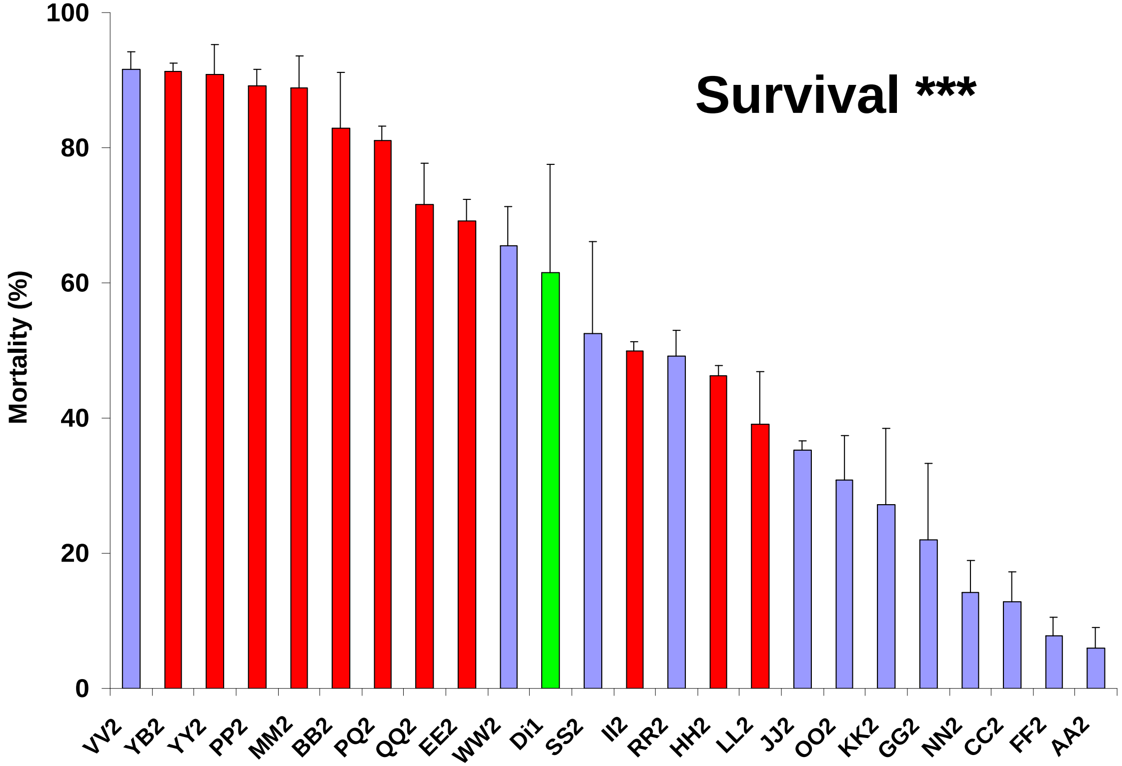
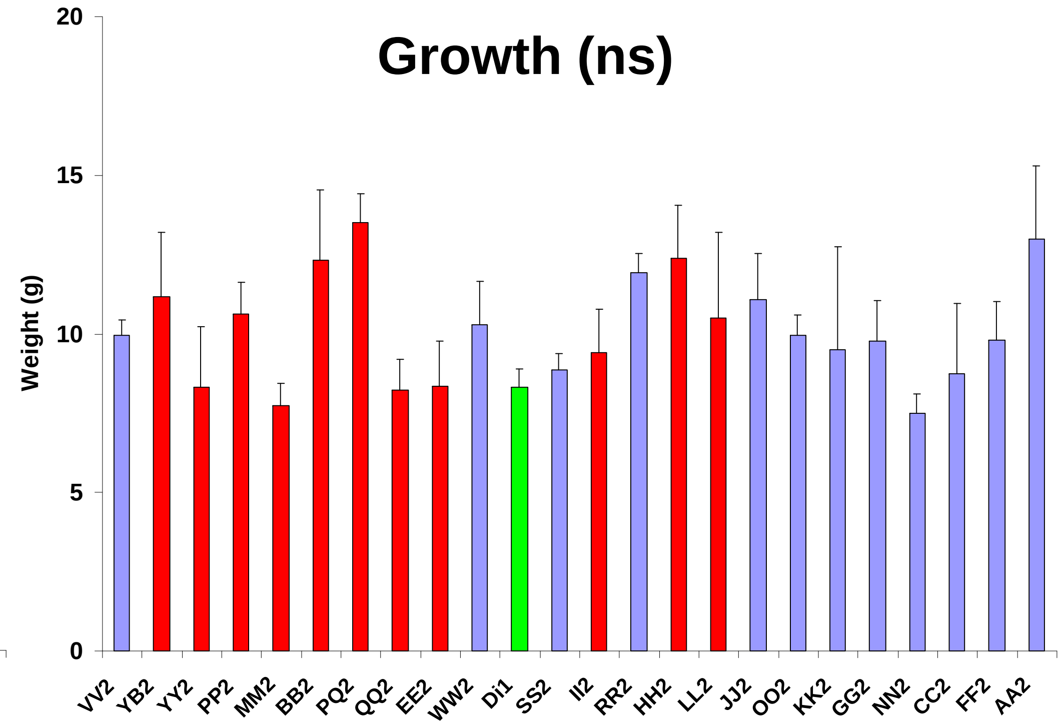
G3SD: Response to selection in triploids

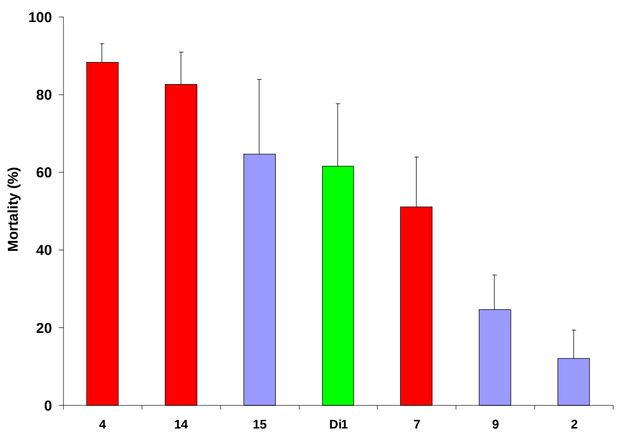


3n R < 2n = 3n = 3n S

Third generation (3) : G3C1 = G2C x G2C

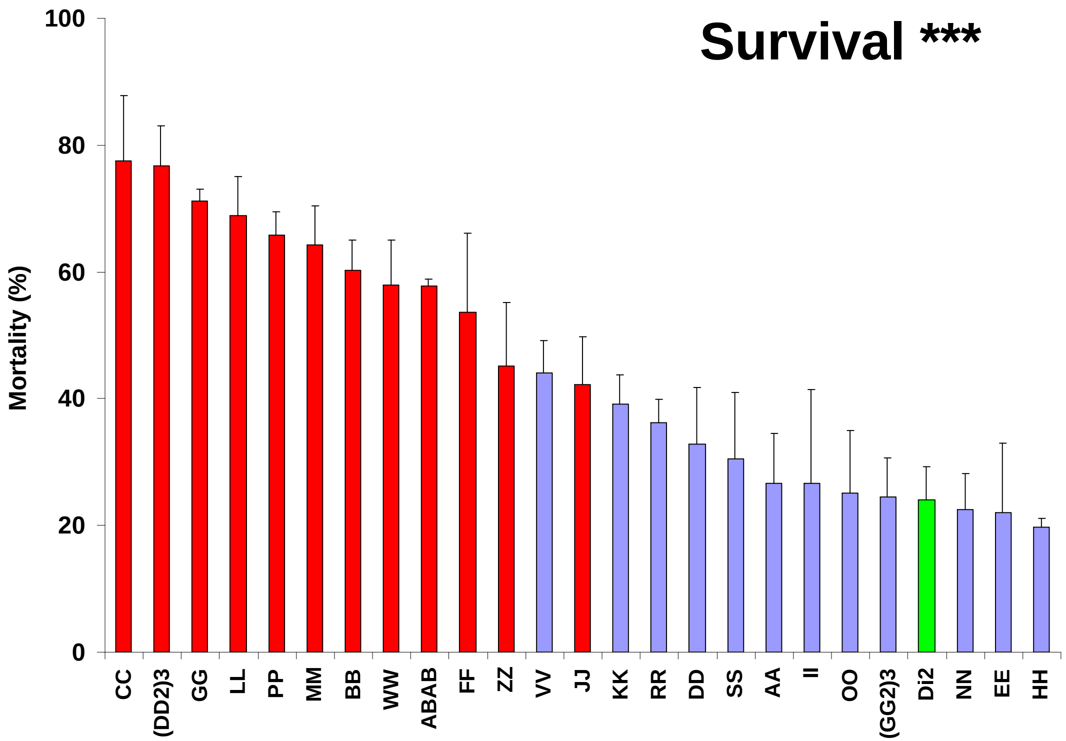


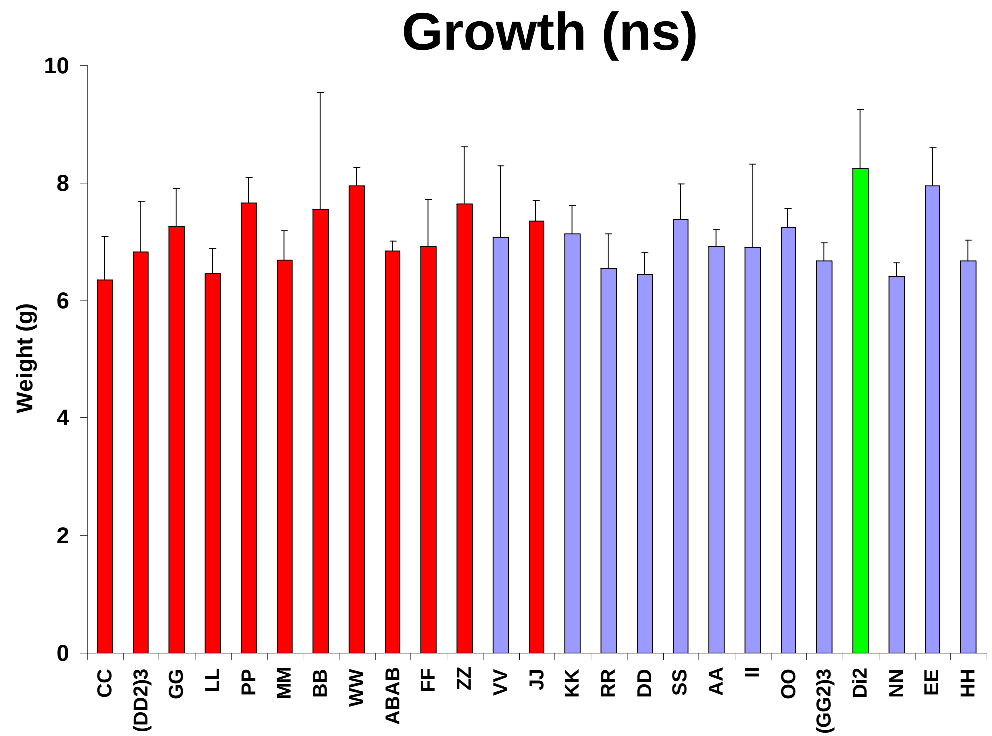




G0 males

Third generation (3) : G3C2 = G2SD x G2SD





What do we know about later survival ? (1)

		'R'		'S	Control	Site	
Summer 2001	6 months	7	<	52	21	*RA*	
Summer 2002	18 months	8	=	7		*RA*	G1
Global mortality	18 months	14	<	55		*RA*	
Summer 2002	6 months	6	<	48	24	*RA*	
Summer 2003	18 months	6	=	7		*RA*	G2
Global mortality	18 months	12	<	52		*RA*	

- Mortality occurs mostly during the first summer in « RA »
- ' S ' and ' R ' oysters show similar performance during their second summer
- Global survival of R oysters is much higher than ' S ' oysters

What do we know about later survival ? (2)

		'R'		'S	Control	Site	
Summer 2001	6 months	7	<	52	21	*RA*	
Summer 2002	18 months	8	=	7		*RA*	G1
Global mortality	18 months	14	<	55		*RA*	
Summer 2001	6 months	0		5		*nurserie*	
Summer 2002	18 months	4		14		*RA*	G1
Global mortality	18 months	4		19		*RA*	

⇨ **Keeping ' S ' oysters in nursery during the first summer reduces global mortality**

⇨ **First summer is the sensitive period in the field**

Why so much additive variance for survival ?

- Trade-off between survival and another fitness-related trait ?

- Impact of hatchery propagation on life cycle / ressource allocation ?

Why so much additive variance for survival ?

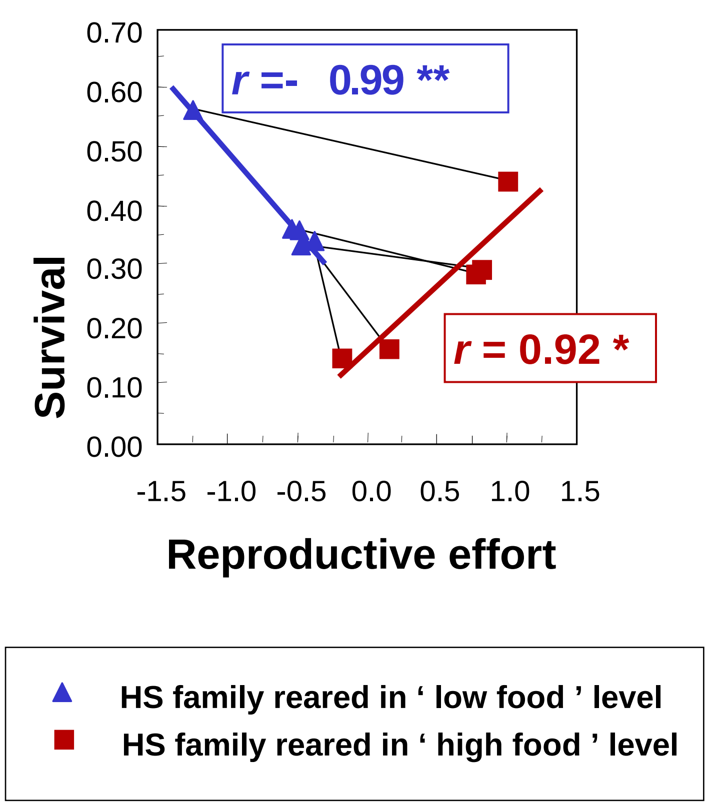
- Trade-off between survival and another fitness-related trait ?

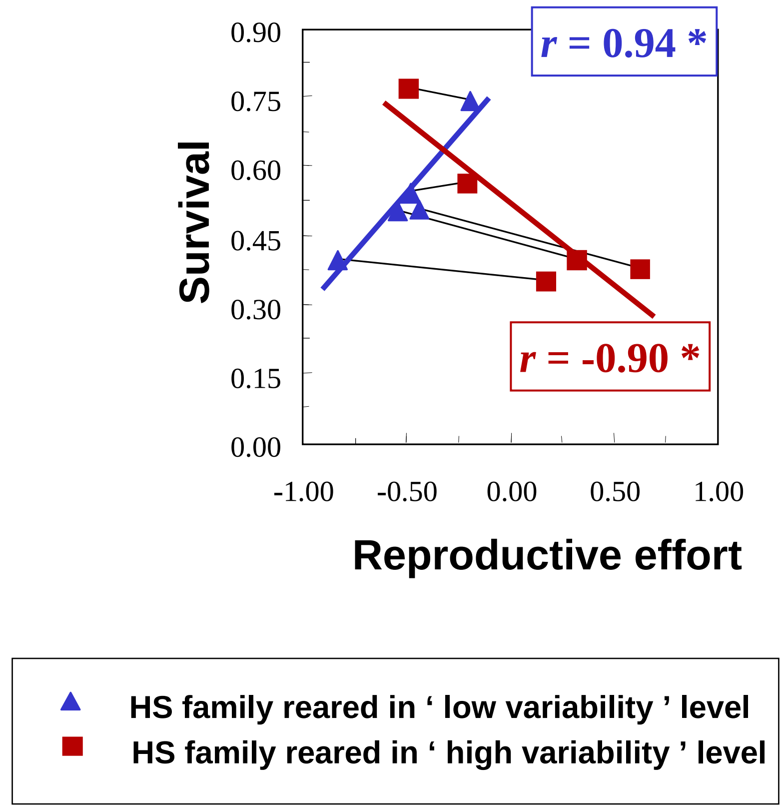
- Maintenance of genetic polymorphism due to spatial and temporal environnementally-induced selective pressures ?

- Impact of hatchery propagation on life cycle / ressource allocation ?

→ **Phenotypic caracterization of ‘ S ’ and ‘ R ’ oysters**

Trade-offs ? Evidence in one-year old oysters





+ Significant positive genetic correlation between plasticity of reproductive effort and survival

Ernande et al., 2004

Ernande et al., en prep.

Conclusion

- High heritability of survival of spat during its first summer
- Significant response to selection on global survival
- No correlated response on growth
- Highly correlated response on yield
- Trade-off between survival and reproduction suspected

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