Inventing tetraploid breeding for animals using the eastern oyster *Crassostrea virginica* as the model

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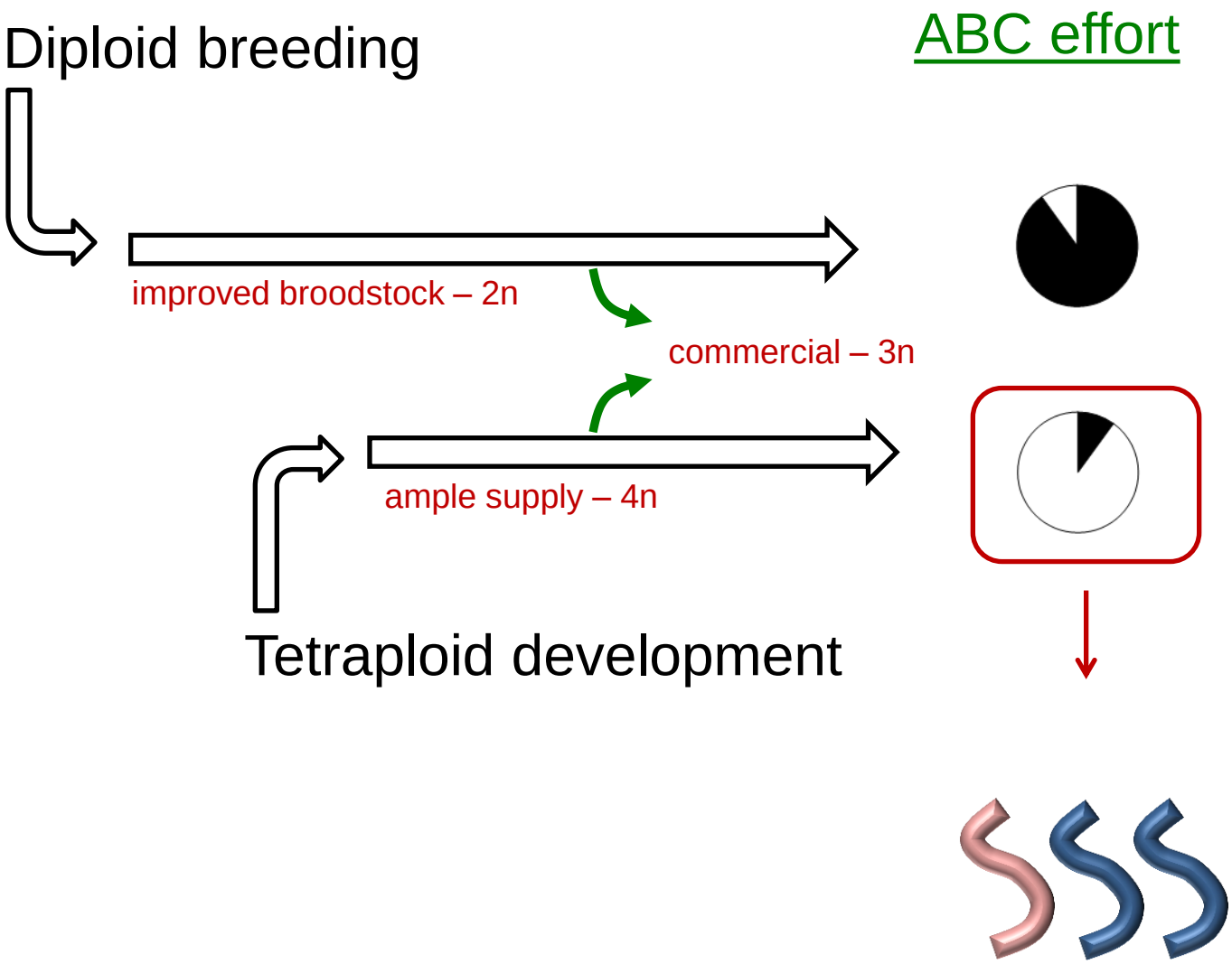
Why do we care so much about tetraploids at ABC?



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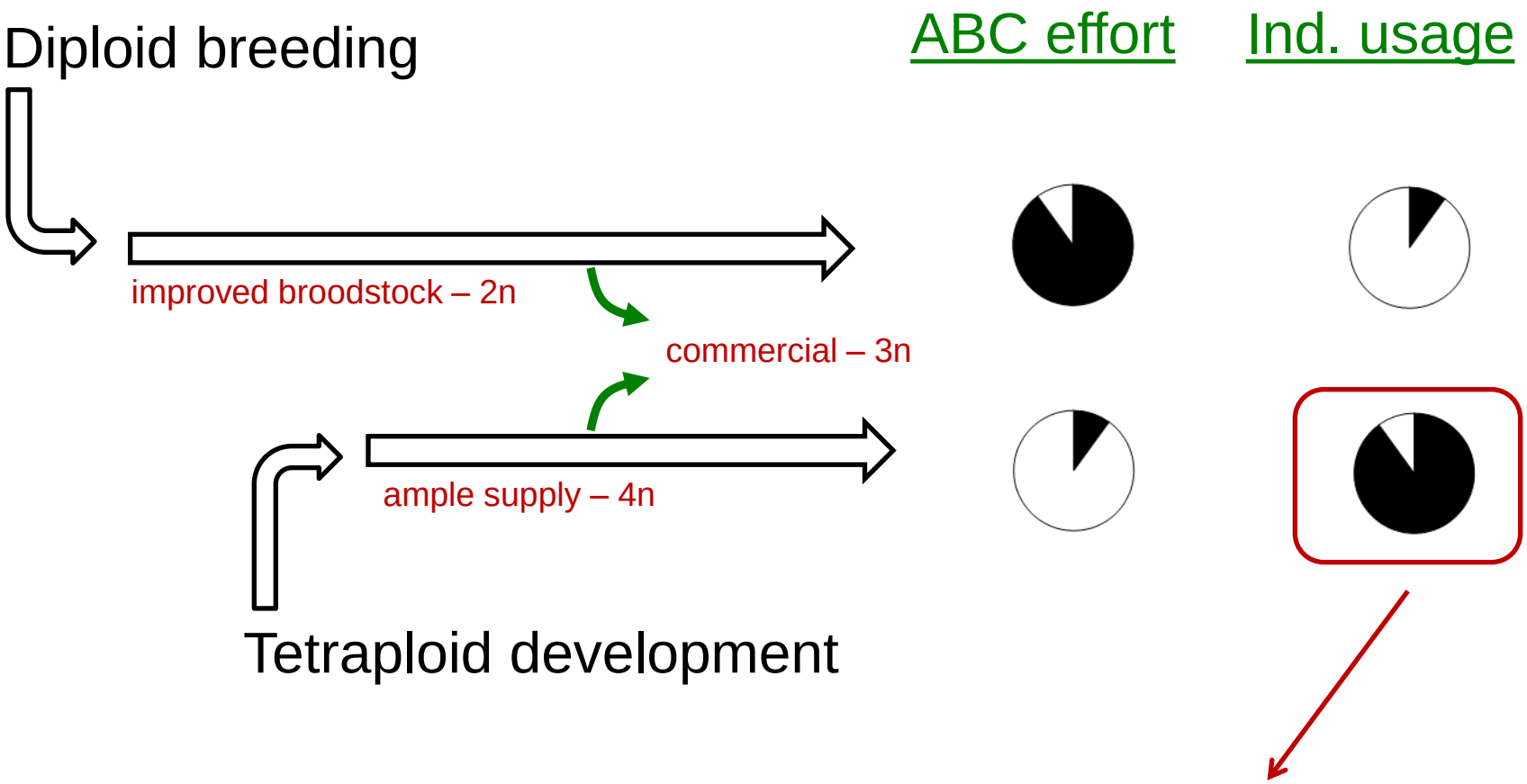
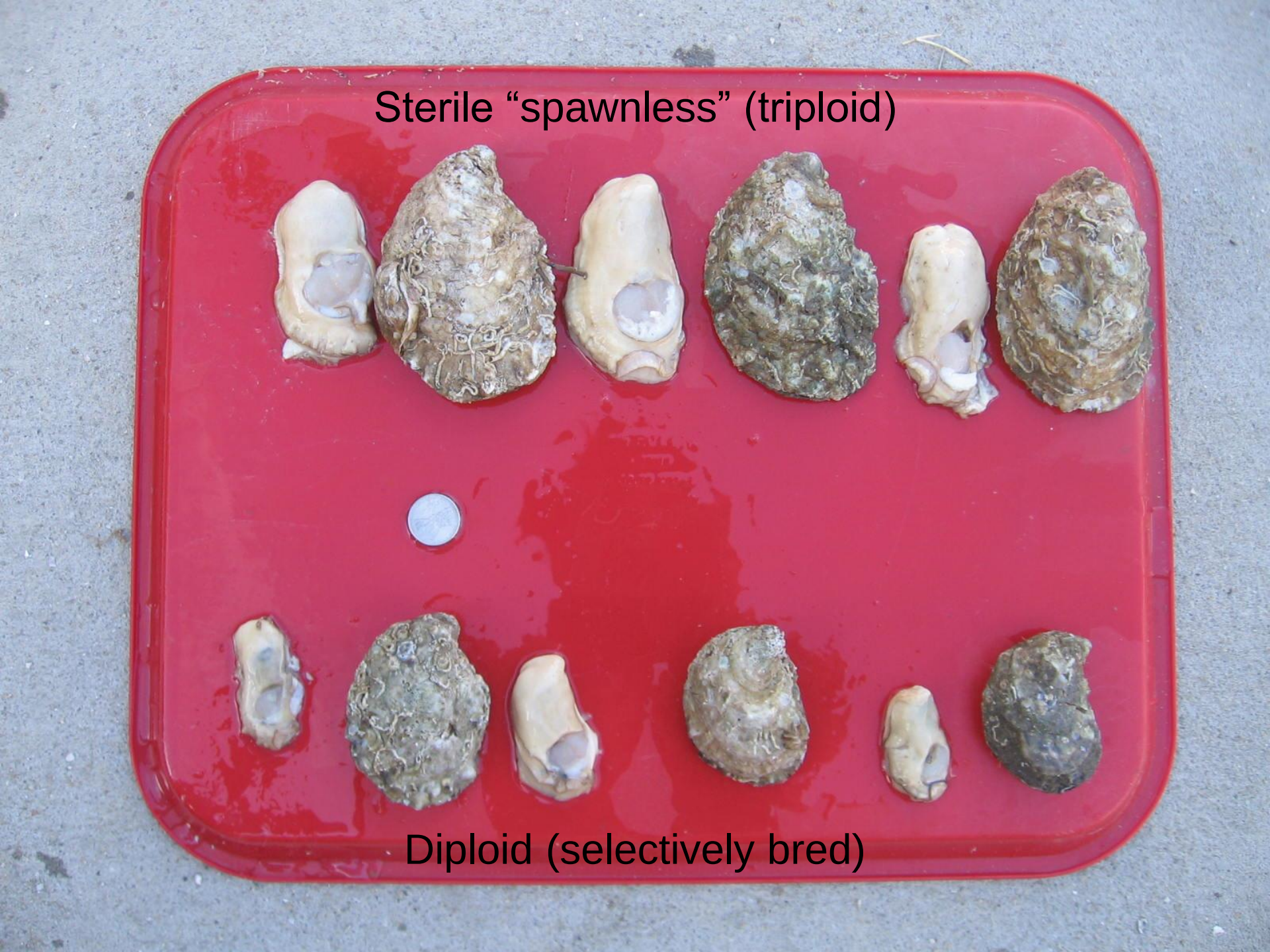
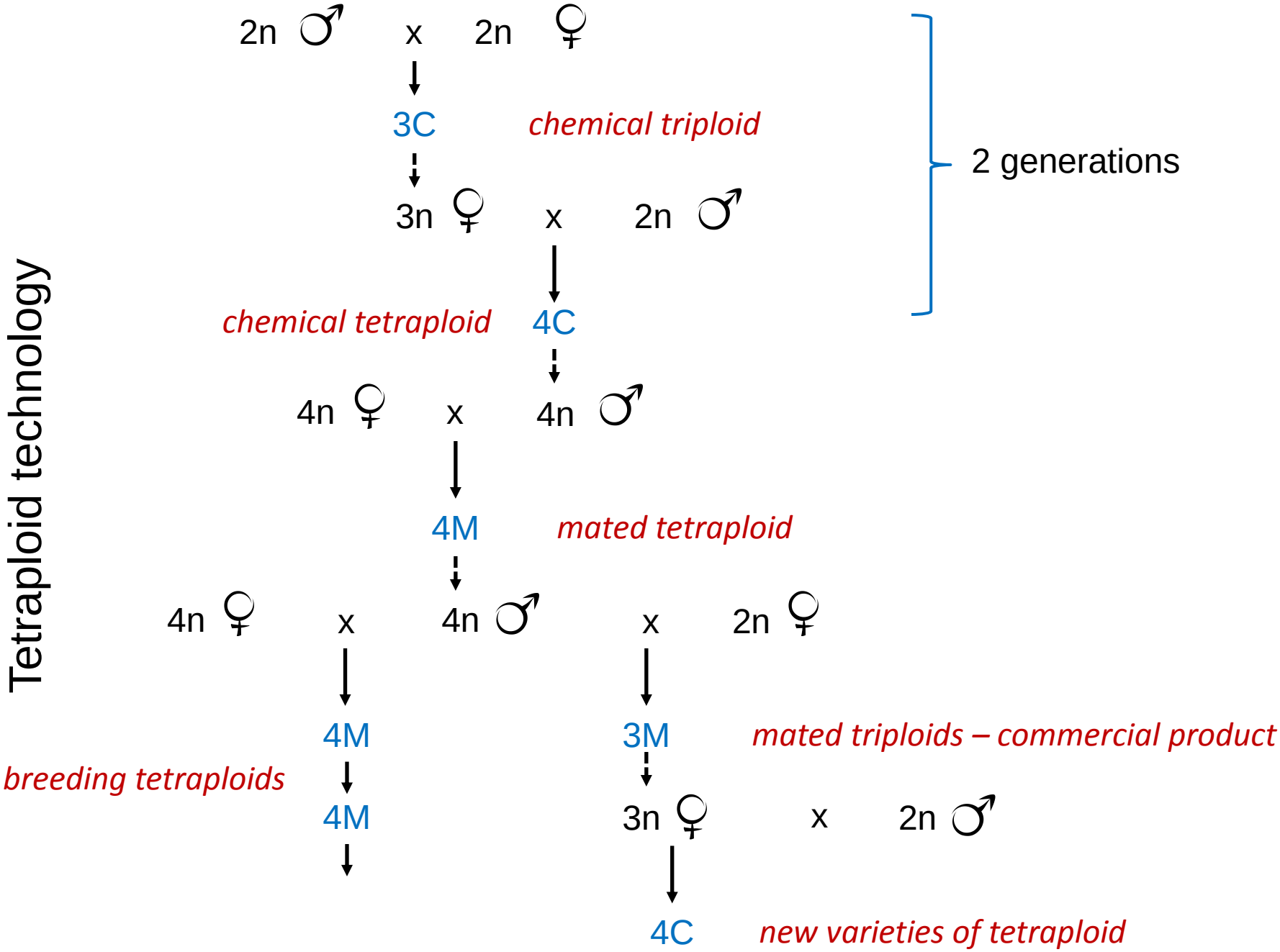
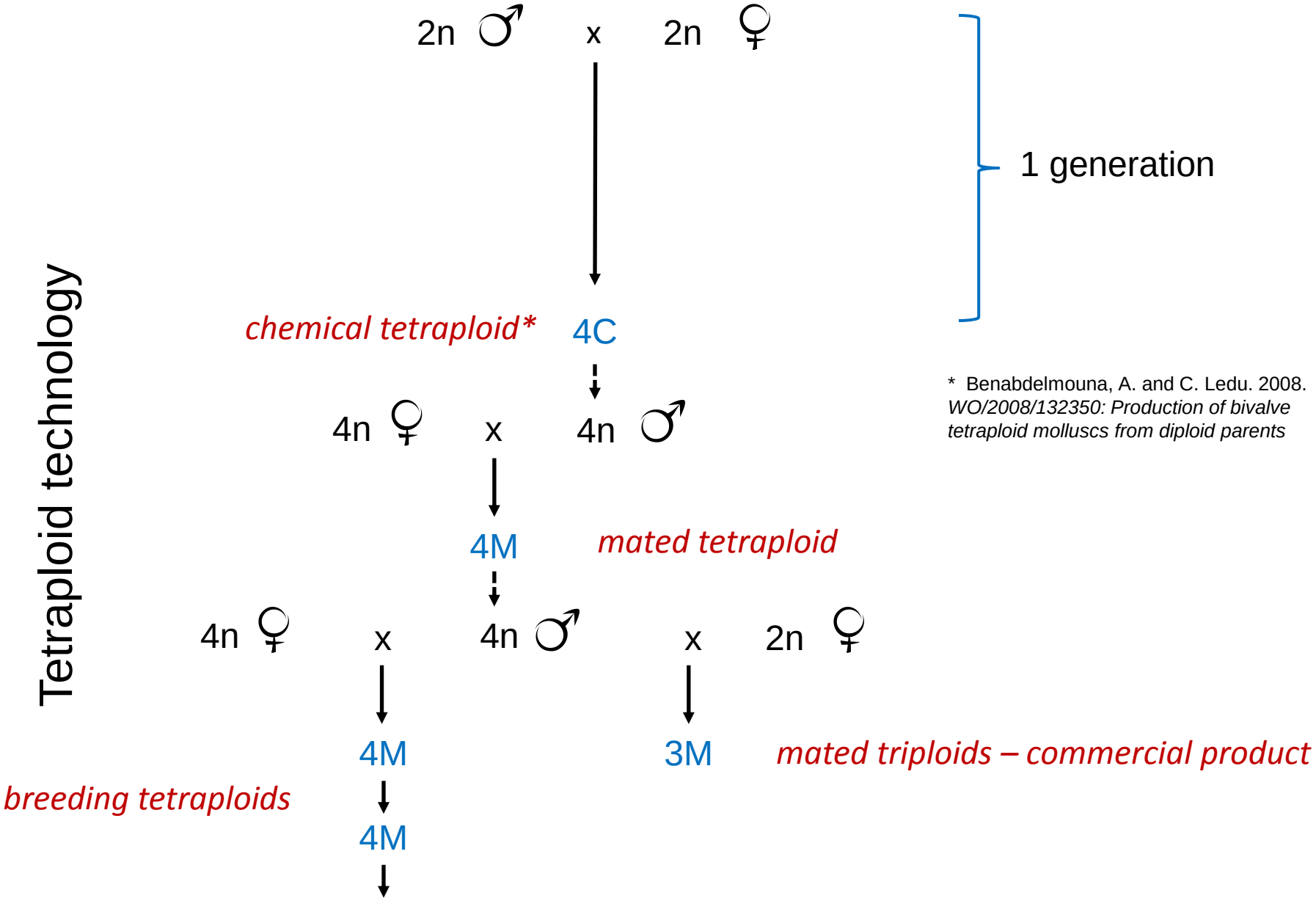



Sterile “spawnless” (triploid)
Diploid (selectively bred)

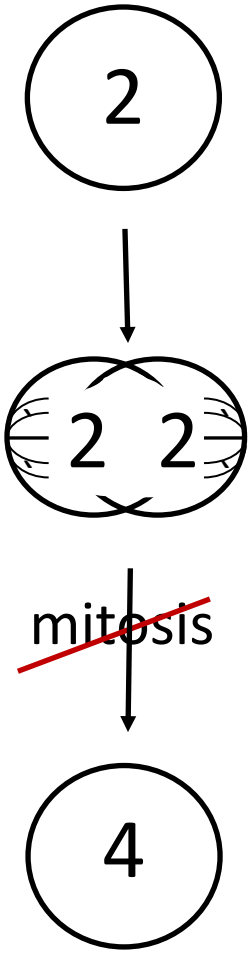

Tetraploid technology
2n ♂ x 2n ♀
3C
chemical triploid
3n ♀ x 2n ♂
2 generations
chemical tetraploid
4C
4n ♀ x 4n ♂
4M
mated tetraploid
4n ♀ x 4n ♂
x 2n ♀
4M
3M
mated triploids – commercial product
breeding tetraploids
4M
3n ♀ x 2n ♂
4C
new varieties of tetraploid



* Benabdelmouna, A. and C. Ledu. 2008. *WO/2008/132350: Production of bivalve tetraploid molluscs from diploid parents*

Neither way of making tetraploids is at all "standard" in tetraploid breeding

plants, fish



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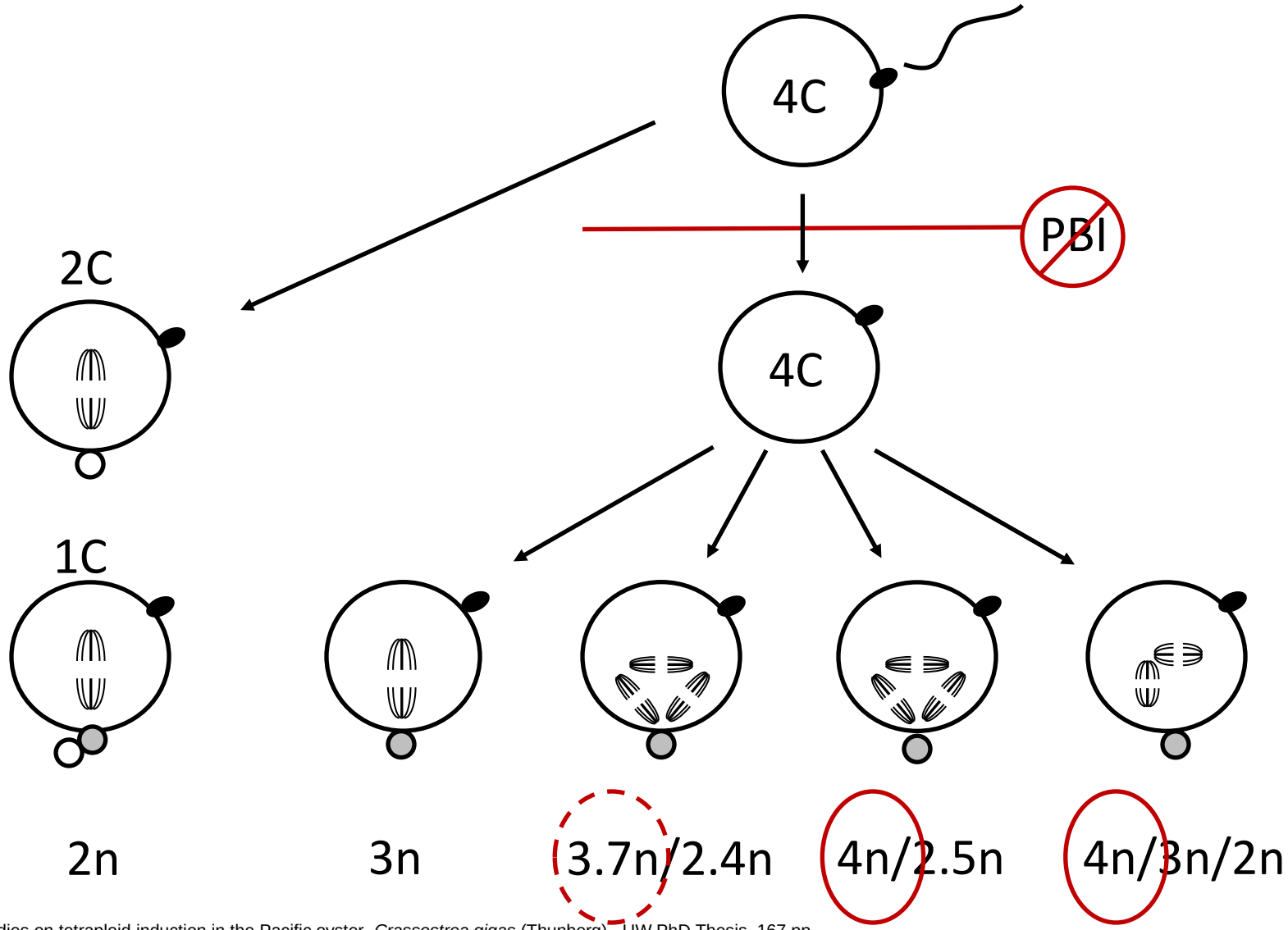

Guo, X. 1991. Studies on tetraploid induction in the Pacific oyster, *Crassostrea gigas* (Thunberg). UW PhD Thesis. 167 pp.

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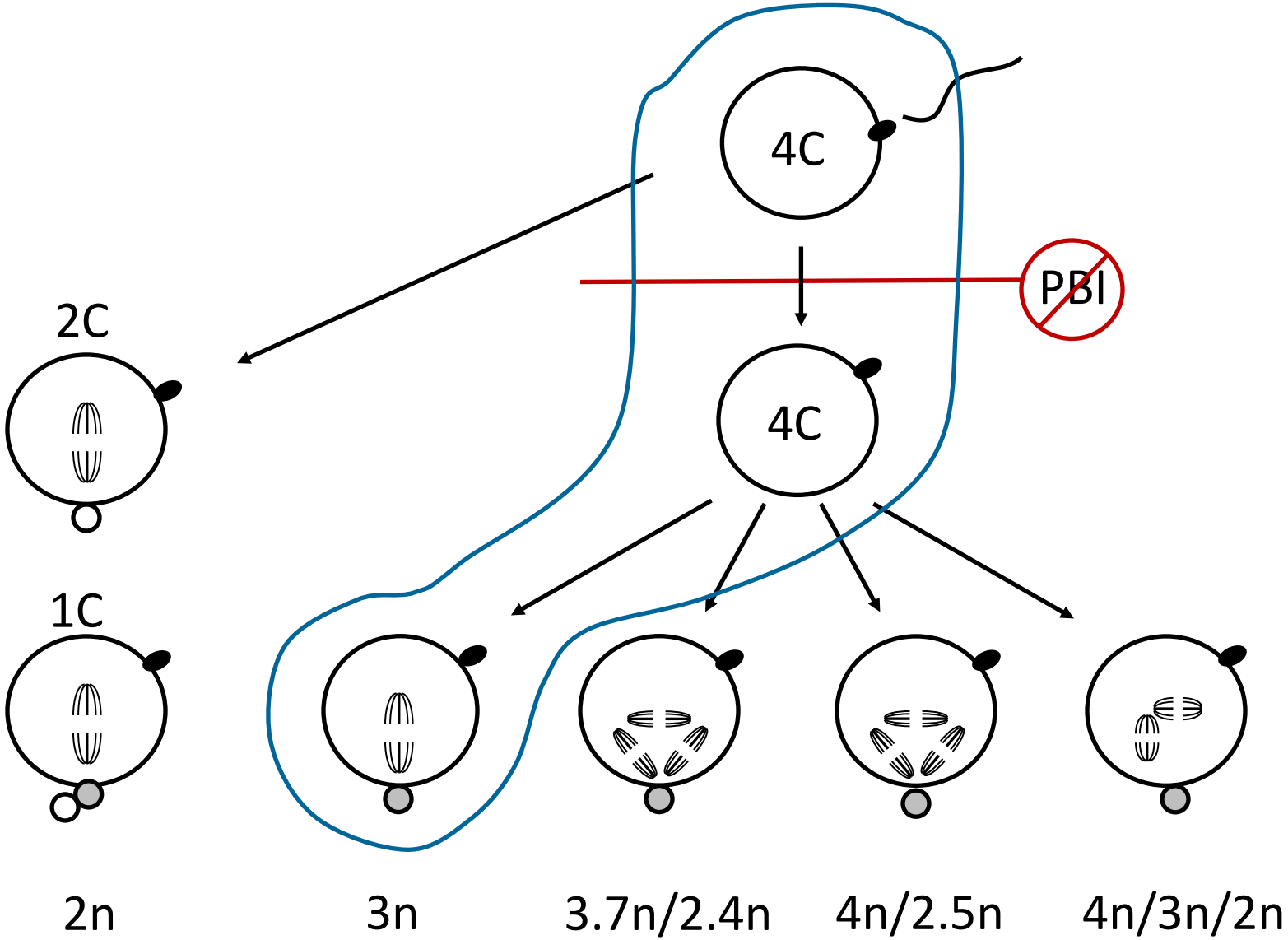

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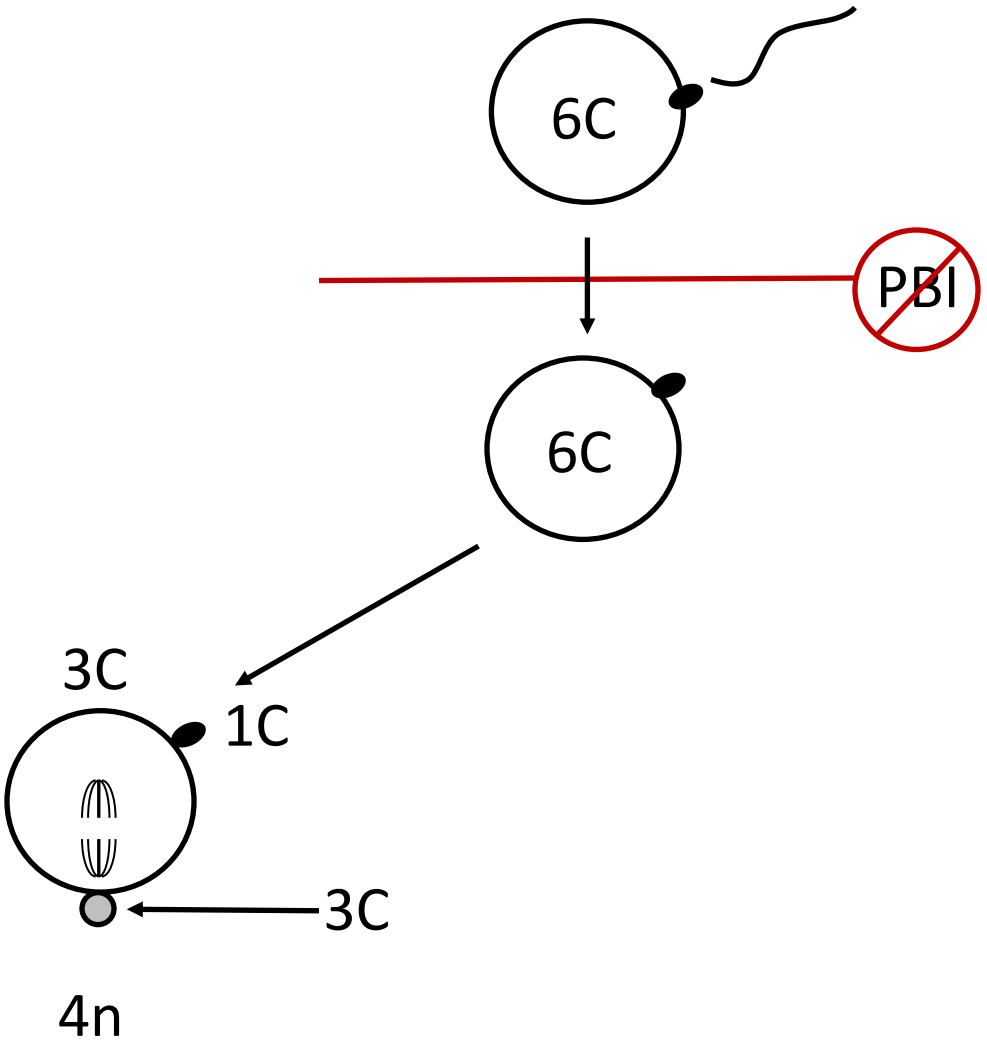
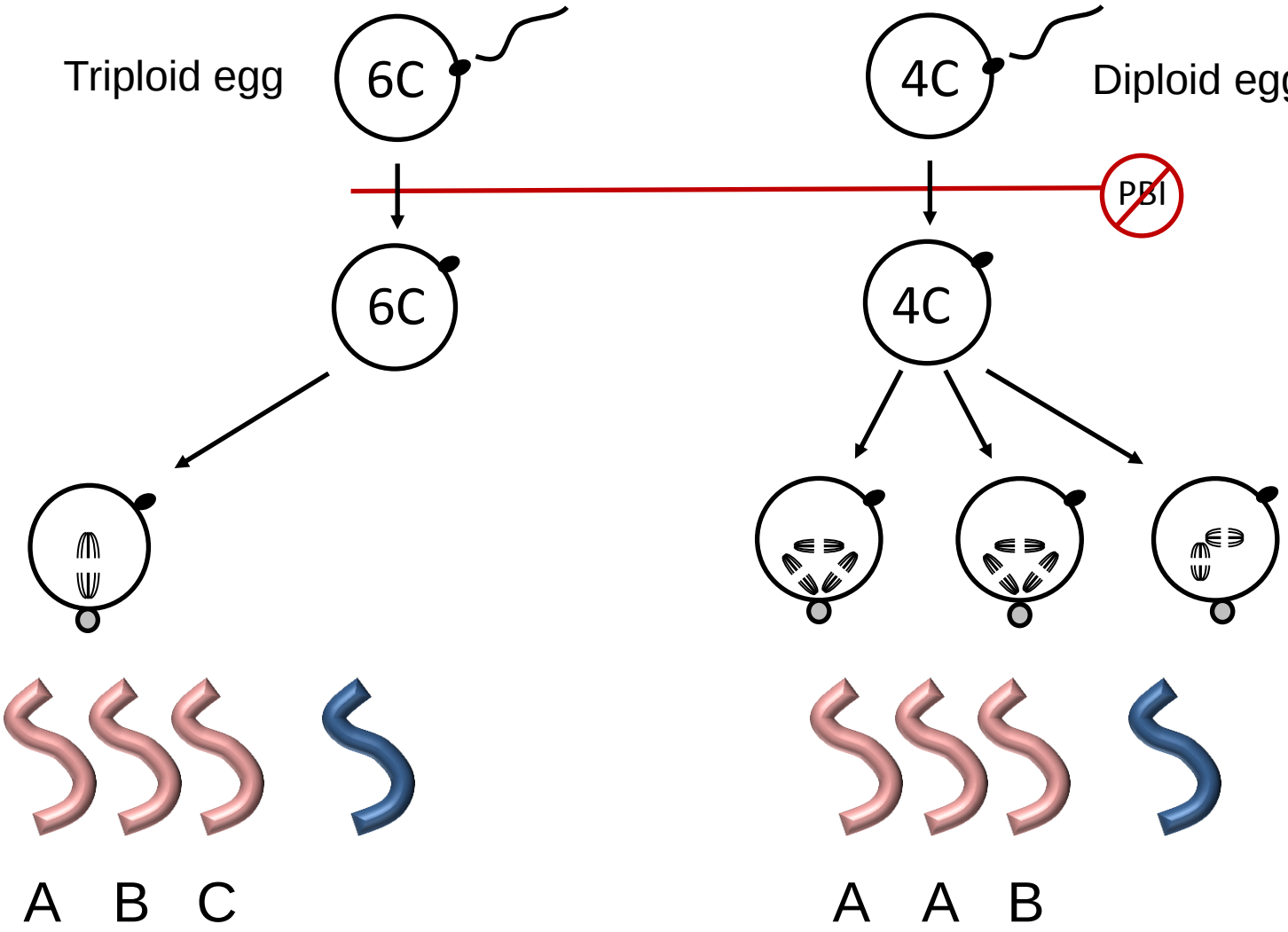
Eggs from ripe triploid and “united bipolar” division



Guo, X. and S.K. Allen, Jr. 1994. Viable tetraploid *C. gigas.* Mol. Mar. Biol. Biotechnol.

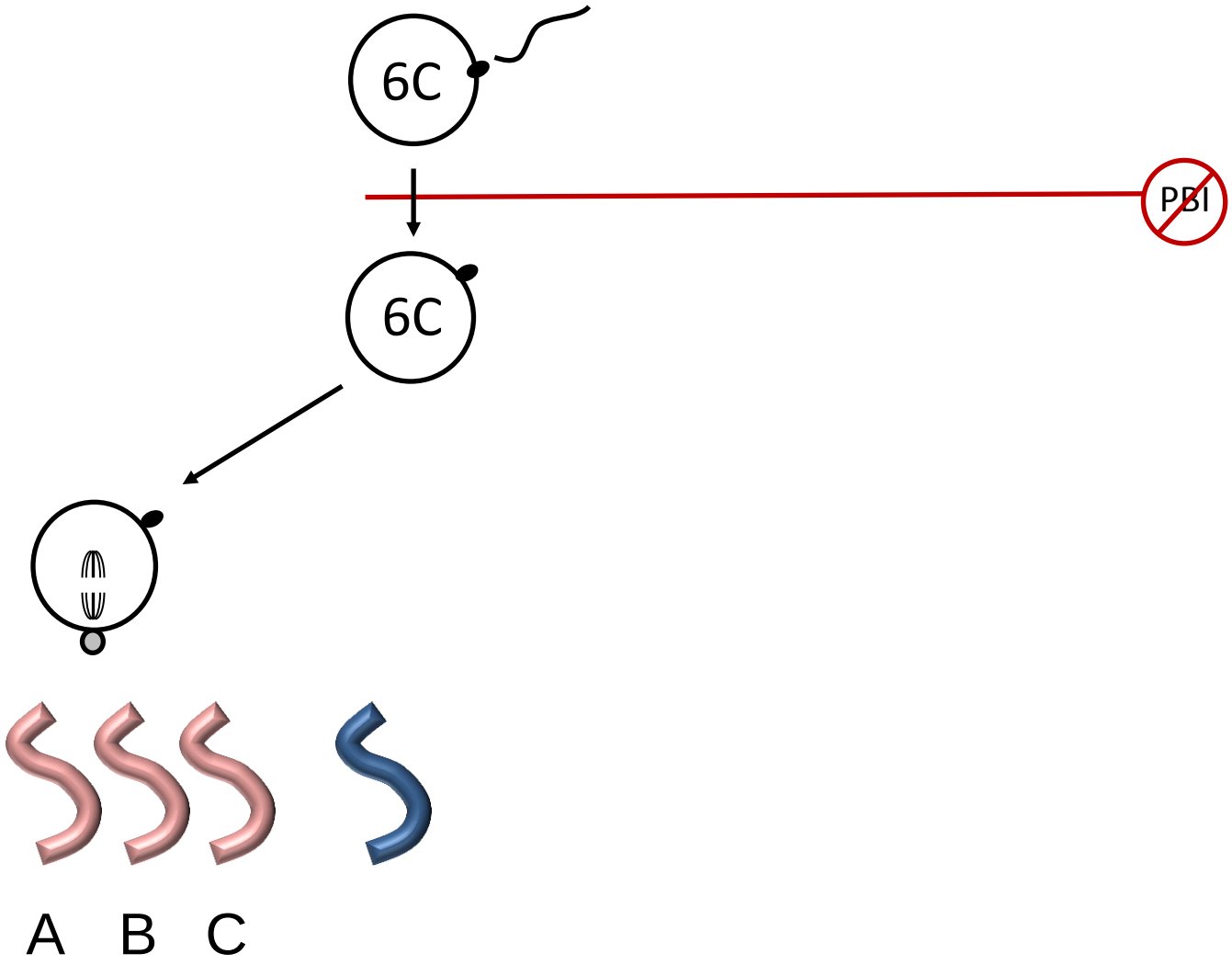


Can be done by mere mortals

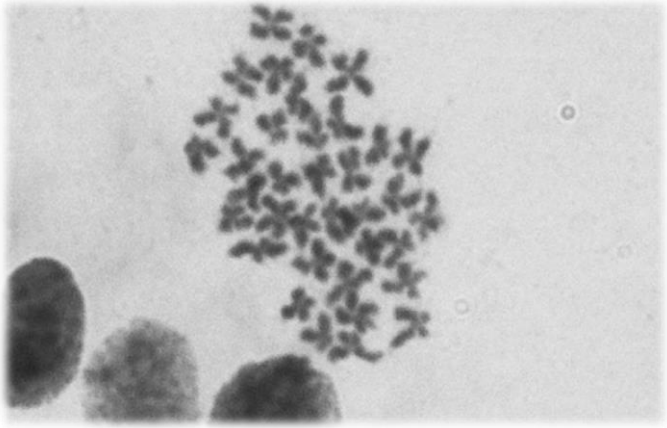
Get “fecundity” genes?

Direct conversion, select material

Lower rate of reversion

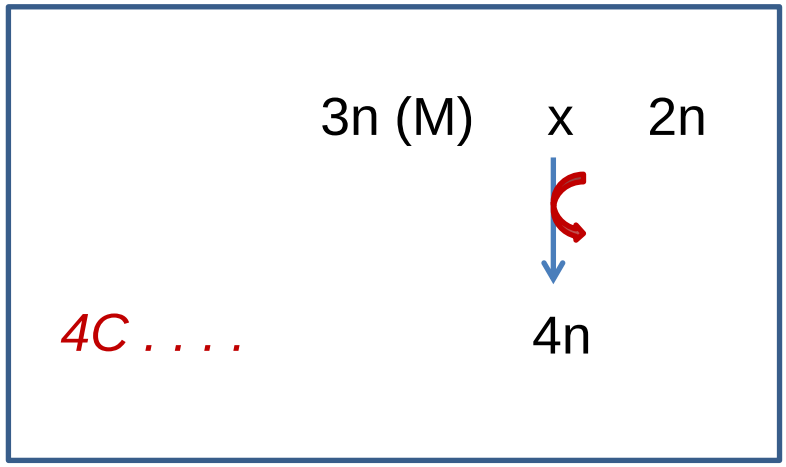

6C
PBI
6C
A
B
C

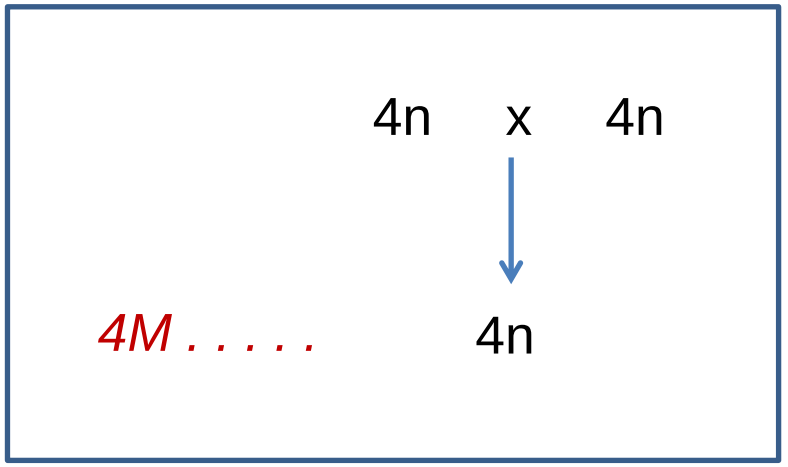
Approaches to tetraploid breeding – A B C type



- Broaden genetic base
- Establish discreet lines
  (as part of genetic base?)
- Evaluate chromosomal consequences of reversion
- Start evaluation of genetic affects in tetraploids with families

Nouveau tetraploids 2013





3M DBSL → 4C DBSL

3M DBY → 4C DBY

3M LGT → 4C LGT

3M Lola → 4C Lola

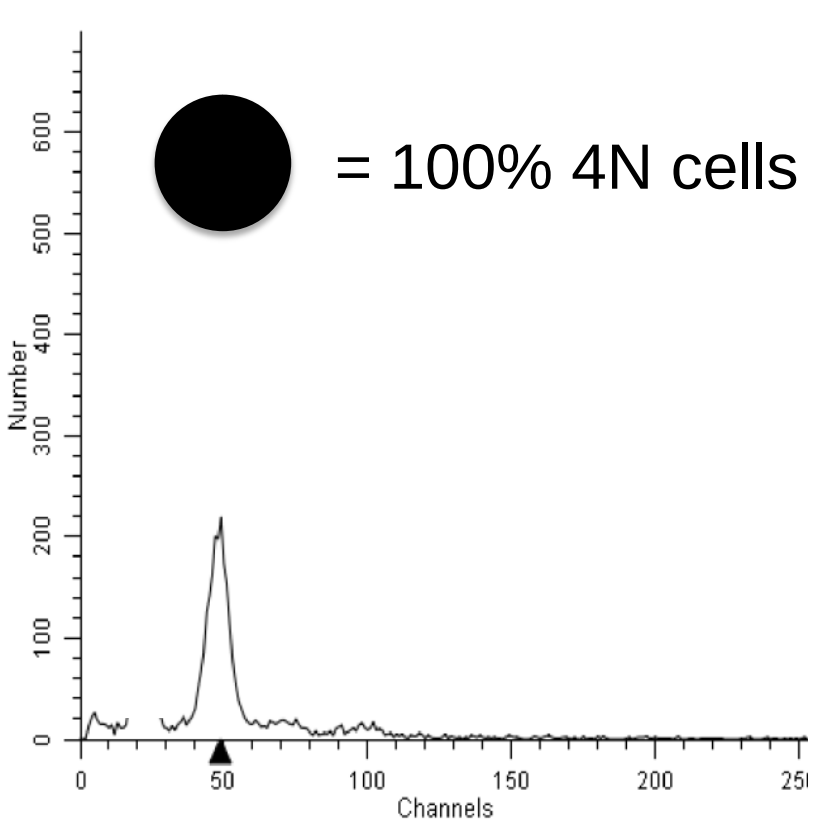
3M XBSL → 4C XBSL

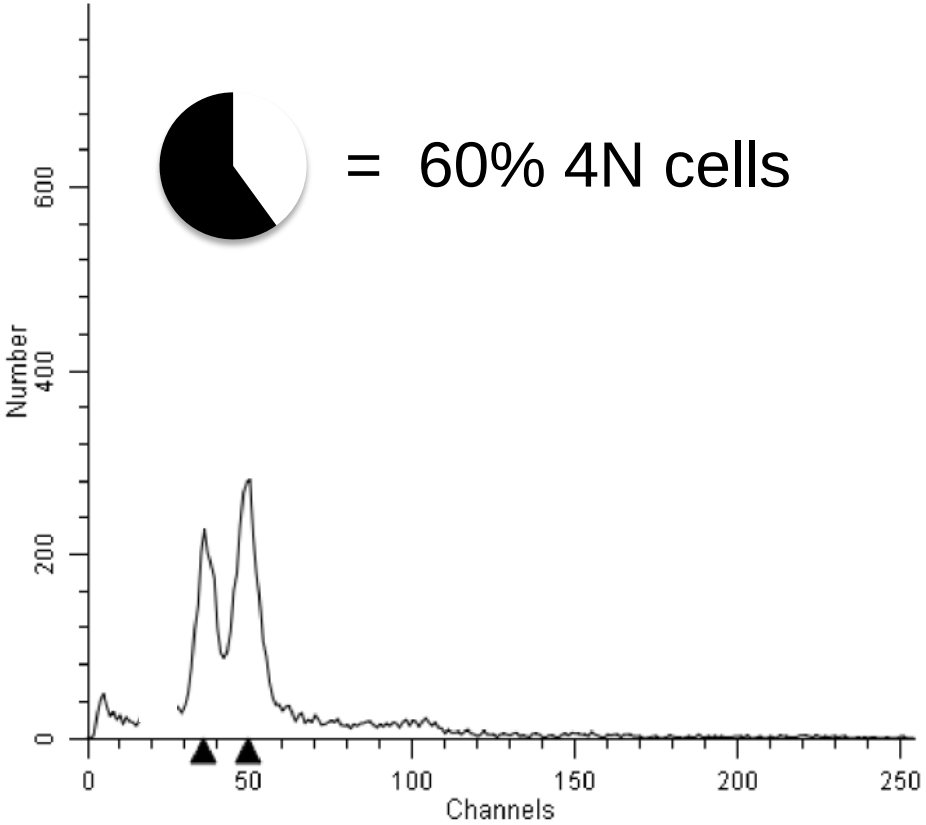
4M GEN x 4M VBOY

↓

4M GNL

Reversion


= 100% 4N cells
Number
0
100
200
300
400
500
600
0
50
100
150
200
Channels


= 60% 4N cells
Number
0
200
400
600
0
50
100
150
200
250
Channels

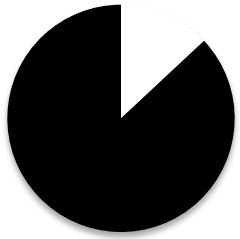
Reversion:
prevalence vs. intensity

prevalence

2/5= 40%

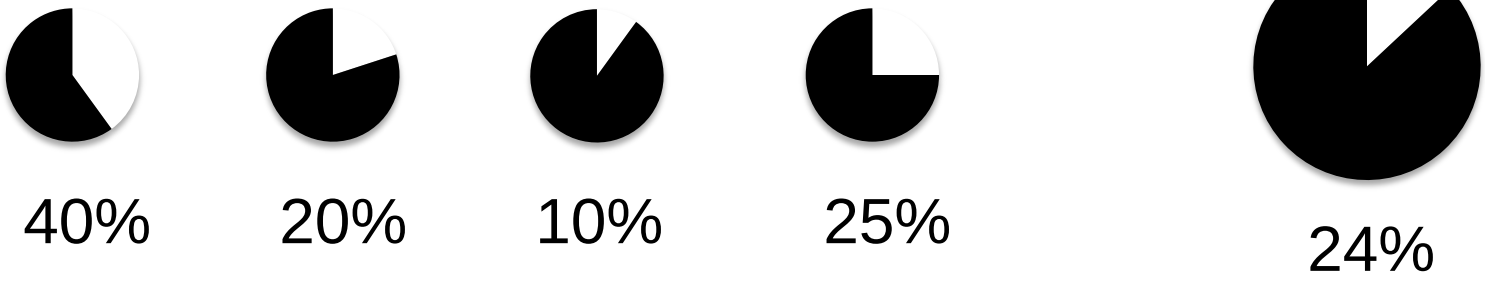
intensity

40% 20% 10% 25%



24%

Reversion: intensity



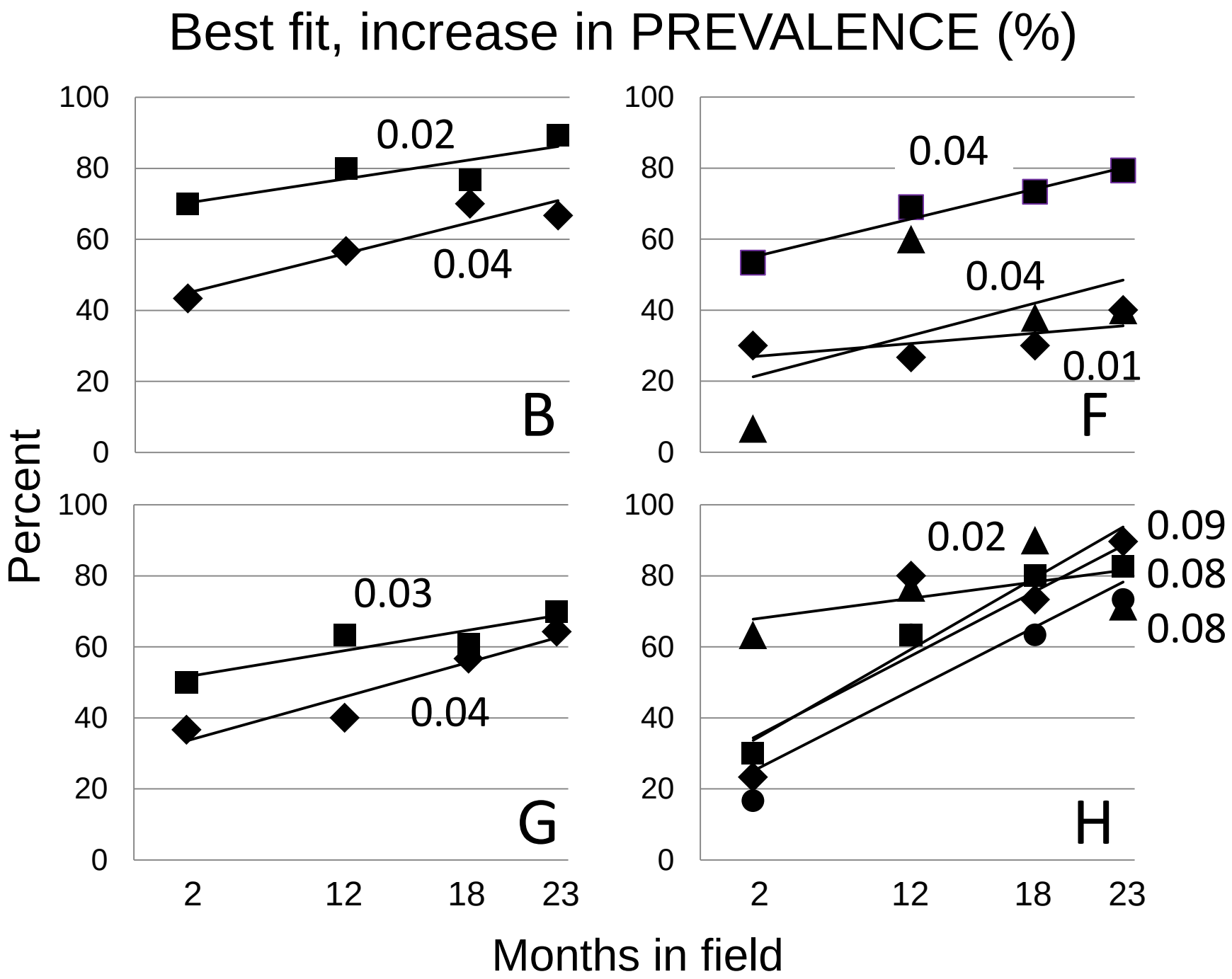
- Measure in gill tissue (high mitotic index)
- Ranges from 0-100%

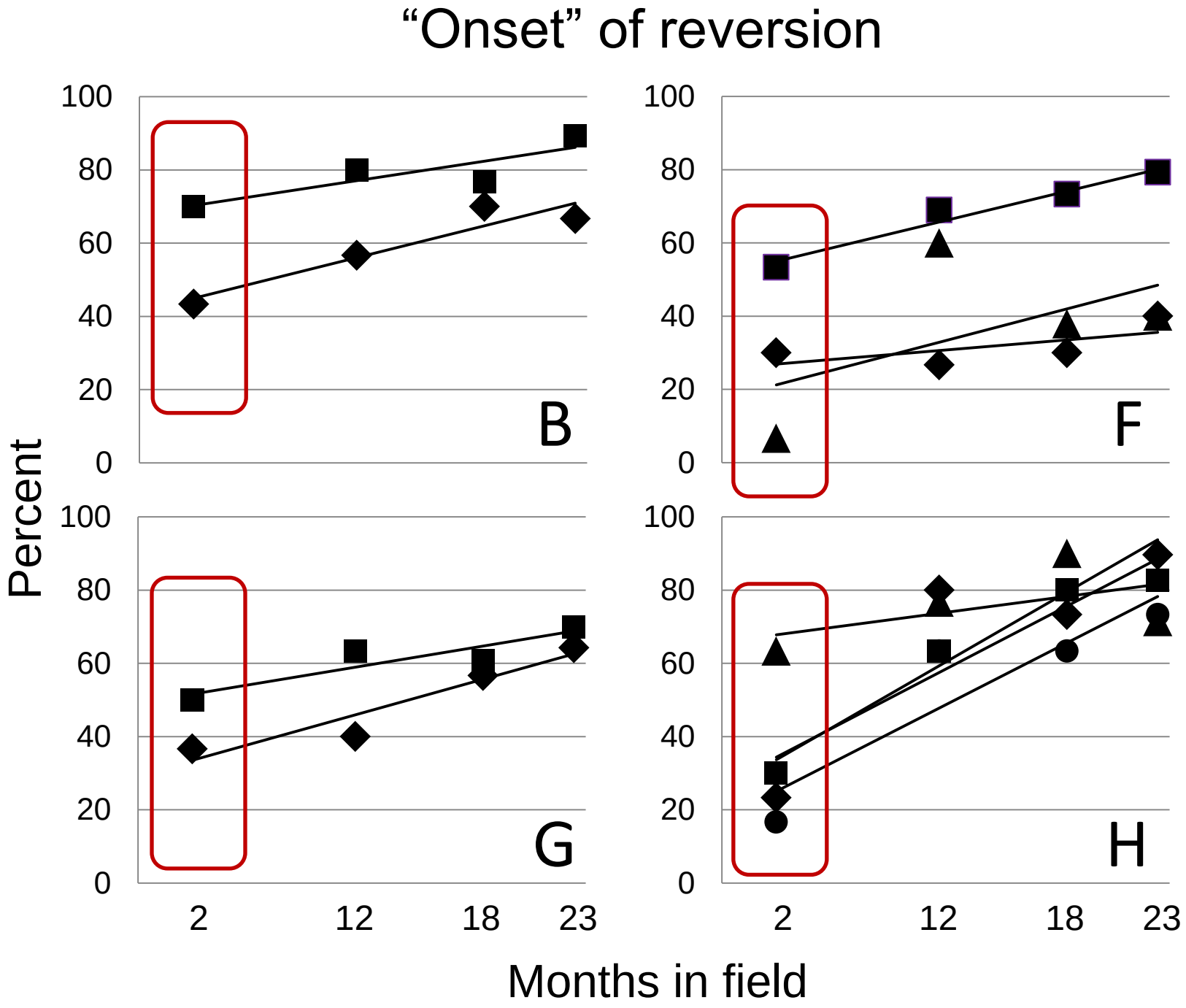
Reversion: prevalence

prevalence

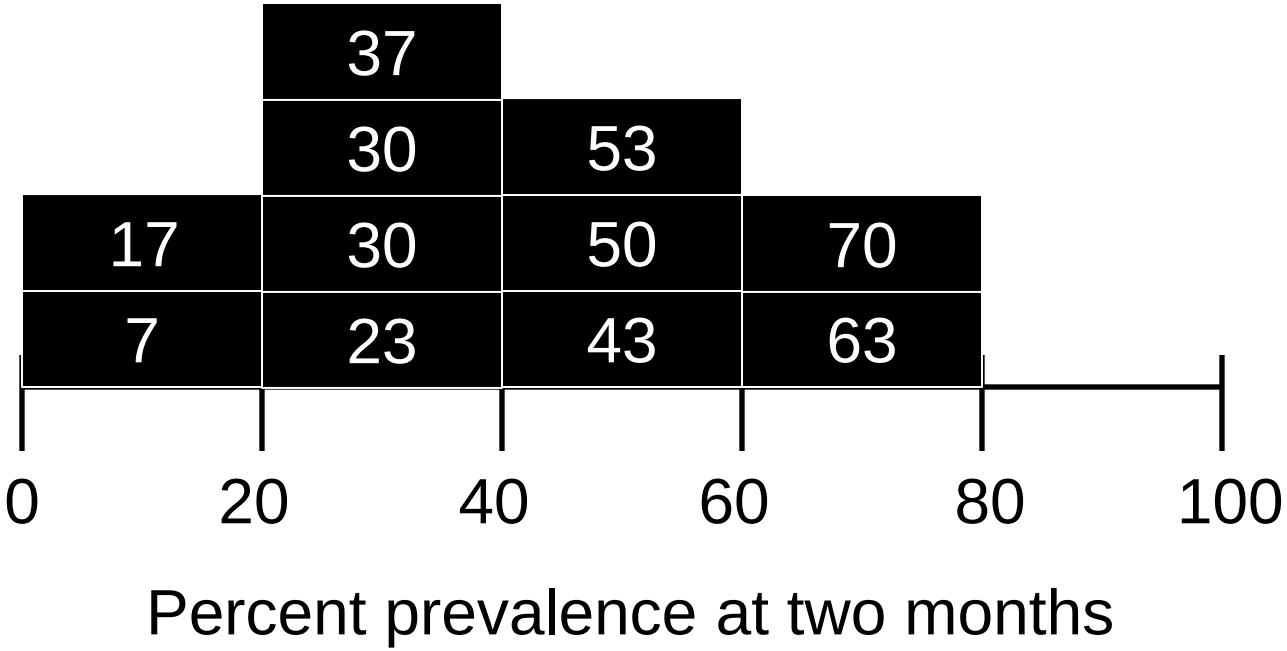
2/5= 40%

- Determined over 2 year period (n=25 ea)
- 11 tetraploid families
- Broad sense heritabilities estimated


Best fit, increase in PREVALENCE (%)
Percent
Months in field
B
F
G
H
0.02
0.04
0.04
0.01
0.03
0.04
0.02
0.09
0.08
0.08
100
80
60
40
20
0
2
12
18
23


“Onset” of reversion
Percent
100
80
60
40
20
0
B
F
G
H
2
12
18
23
Months in field

Frequency of “onset” (apparition)



REVERSION

MONTH	HERITABILITY	SE
2	0.33	0.11
12	0.19	0.09
18	0.23	0.11
23	0.08	0.06
COMBINED	0.19	0.07
COMBINED (adult)	0.17	0.07

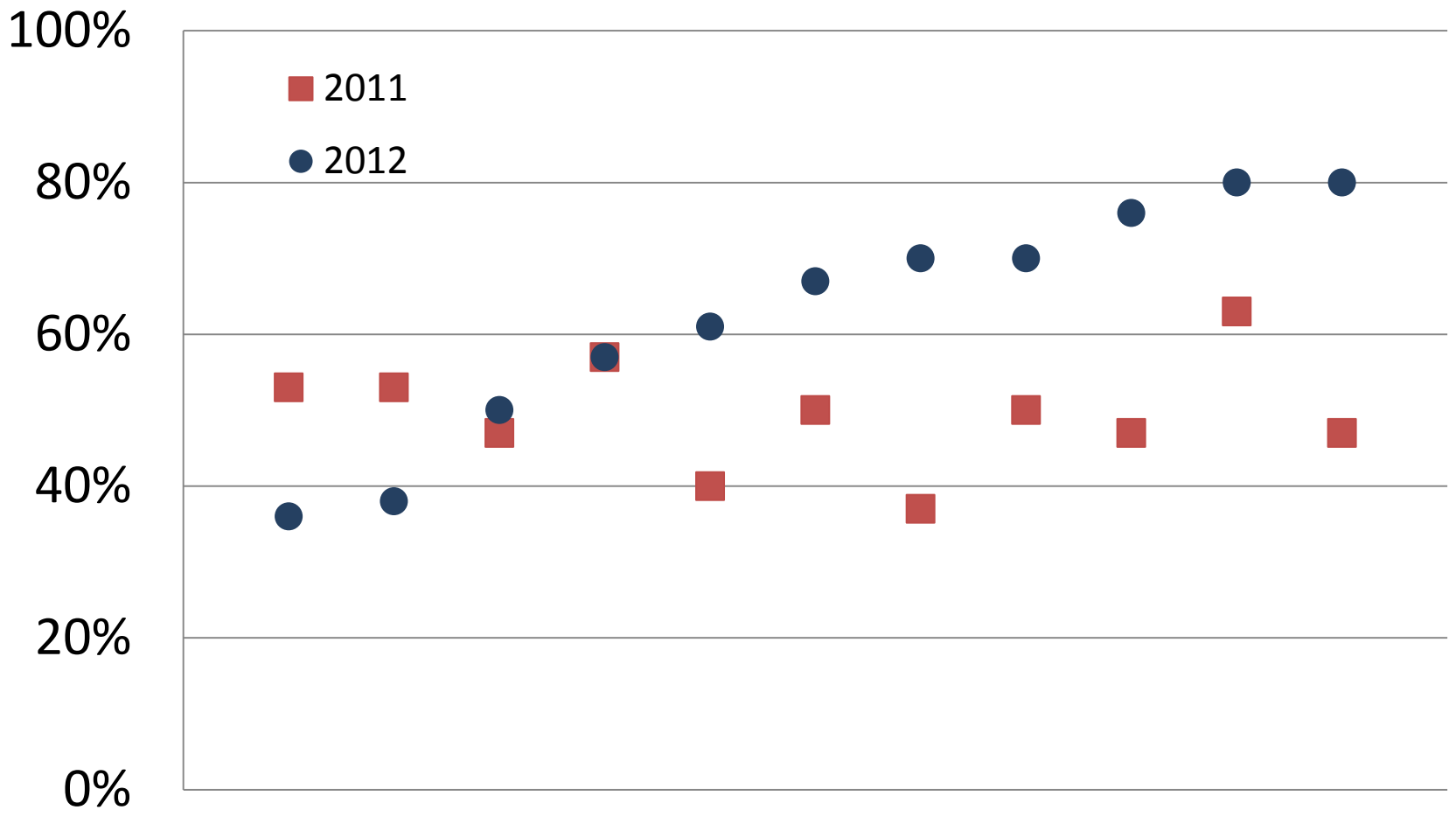
	RG REVERSION		
	2	12	18
12	-0.19 (0.38)		
18	-0.39 (0.33)	0.70 (0.33)	
23	0.28 (0.33)	0.56 (0.22)	0.75 (0.30)

Continuation of family breeding – tetraploids

Traits

- Reversion (onset?)
- The usual suspects (commercial)
  - Axiomatic that trait will show in triploid (?)
- Sex ratio (because we don’t want too many females)
  - Will require an extra 7 months in the field v. 2ns

Difference in male ratio: 2011 – 2012



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- Low salinity tolerance
  - Because we are trying to develop low salinity diploids too

Photo credit: Lionel Dégremont