Chromosome loss in tetraploid families of the Pacific oyster *Crassostrea gigas*

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Ploidy variation in oysters

- All oyster species are diploid (2n = 20)

However…

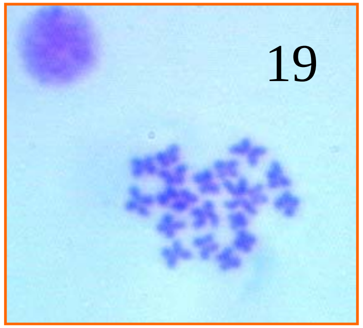
1 - Aneuploidy has been reported in diploid *C. gigas*

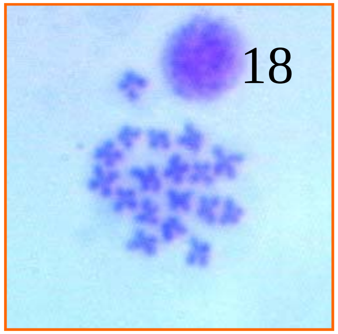
2 - Polyploid *C. gigas* (3n, 4n) can be obtained using various induction methods

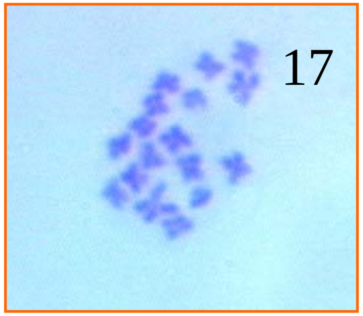
→ Aneuploidy in tetraploid oysters ?

1 - Aneuploidy in Diploids

➢ Loss of 1-3 chromosomes in metaphases of gill cells (*i.e.* within individual variation) (Thiriot *et al* 1992) : **hypodiploidy**





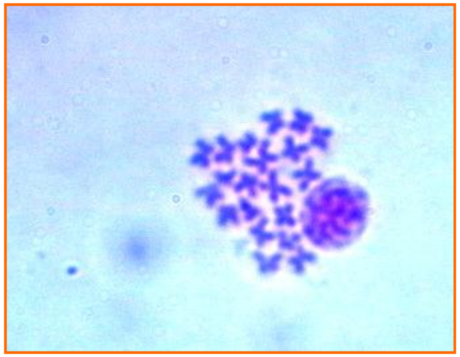


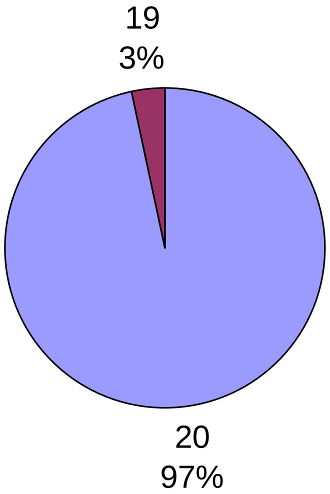
➢Thought to be due to

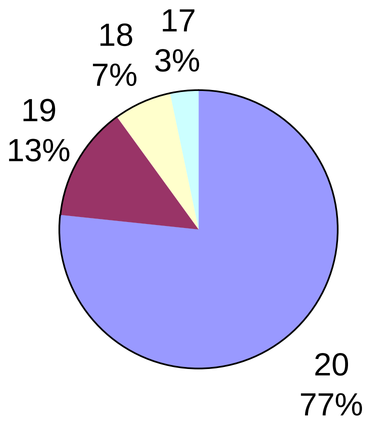
- unequal division at mitosis ? (but 2n > 20 is extremely rare)
- bias related to slide preparation technique ?

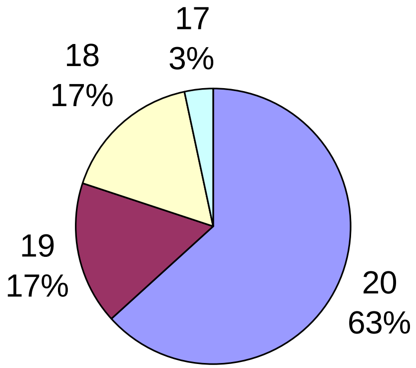
Extent of somatic Chromosome Loss in Diploids

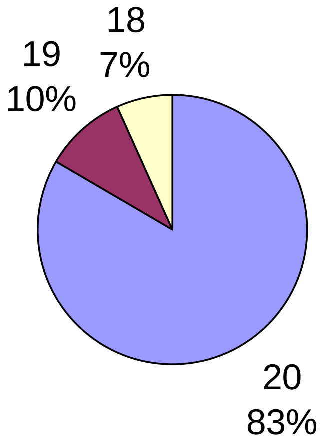
Up to **37 %** of metaphases can be affected





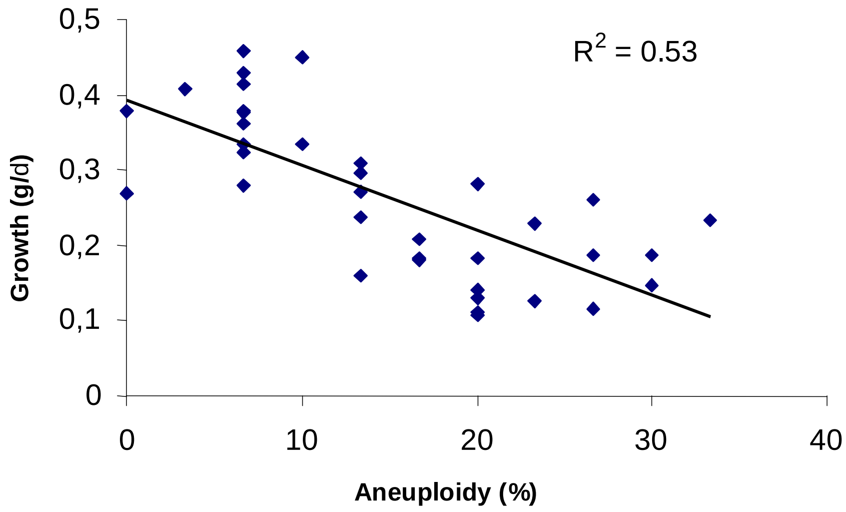






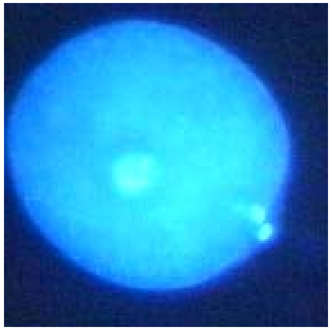
Aneuploidy in diploids :

- probably under genetic influence (Leitao et al 2001a)
- associated with poor growth (Leitao et al 2001b)



- increased by some pollutants (Bouilly et al 2003)

2 - Polyploidy in oysters



- Why triploid oysters ? (Nell, 2002)
  - Sterility
    - Improves taste and saleability
    - Avoids reproduction in the wild
  - Faster Growth

  (heterozygocity, resource allocation, cell size)

- How to produce triploids ?
  - Chemical induction using gametes of diploids
  - Crossing between diploids x tetraploids

  (Guo *et al*, 1996)

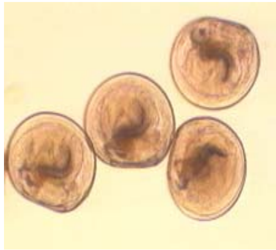
Aneuploidy in tetraploid oysters ?

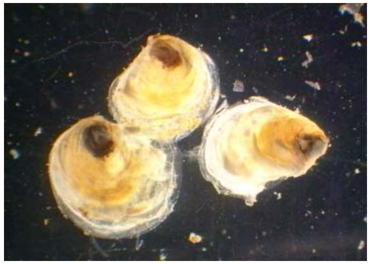
Once tetraploids have been obtained,

1. Do they remain (somatically) tetraploids ? (Zhang *et al*, 2002)

2. Are their gametes always diploid ? (Guo & Allen, 1997)

   - to give triploids in a 2n x 4n crosses (Guo *et al,* 1996)
   - to give tetraploid in 4n x 4n crosses





What would be the possible implications of aneuploidy in tetraploid oysters ?

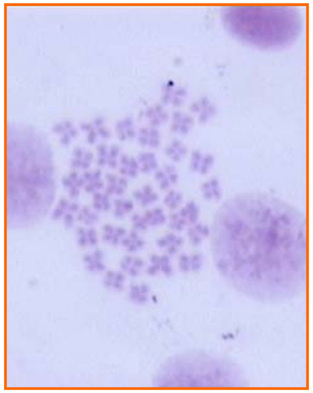
- Loss of quality as 4n genitors (“bad” gametes giving aneuploid offspring)
- Degradation of 4n lines over time or generations
- Conferring tendency to lose chromosomes to triploid offspring (In triploids chromosome loss increase with age (Allen *et al*, 1999))

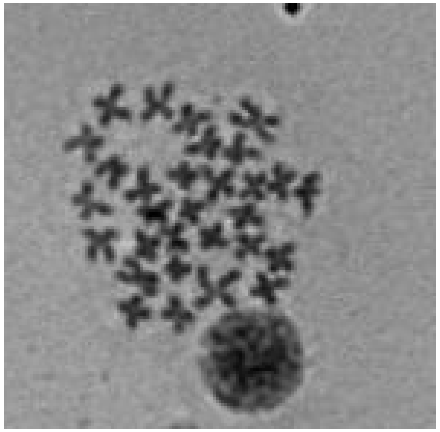
Material: 6 tetraploid families

	Good Quality Female (Fine 4n DNA peak)	Poor Quality Female (Broad 4n DNA peak)	Poor Quality Female (Broad 4n DNA peak)
Good Quality Male (Fine 4n DNA peak)	GG 'Good-Good'	PG1	PG2
Poor Quality Male Double'mosaic' peak	GP	PP1	PP2

Method: Chromosome counts (Thiriot & Ayraud, 1982)

- 30 metaphases per animal
- 10 animals per family per sampling time
- 2 sampling times : 4 and 12 months





Factors studied :

- age (4 and 12 months)
- parental characteristics (“good” and “bad”)
- family (n=1 to 6)
- individual (n = 120)

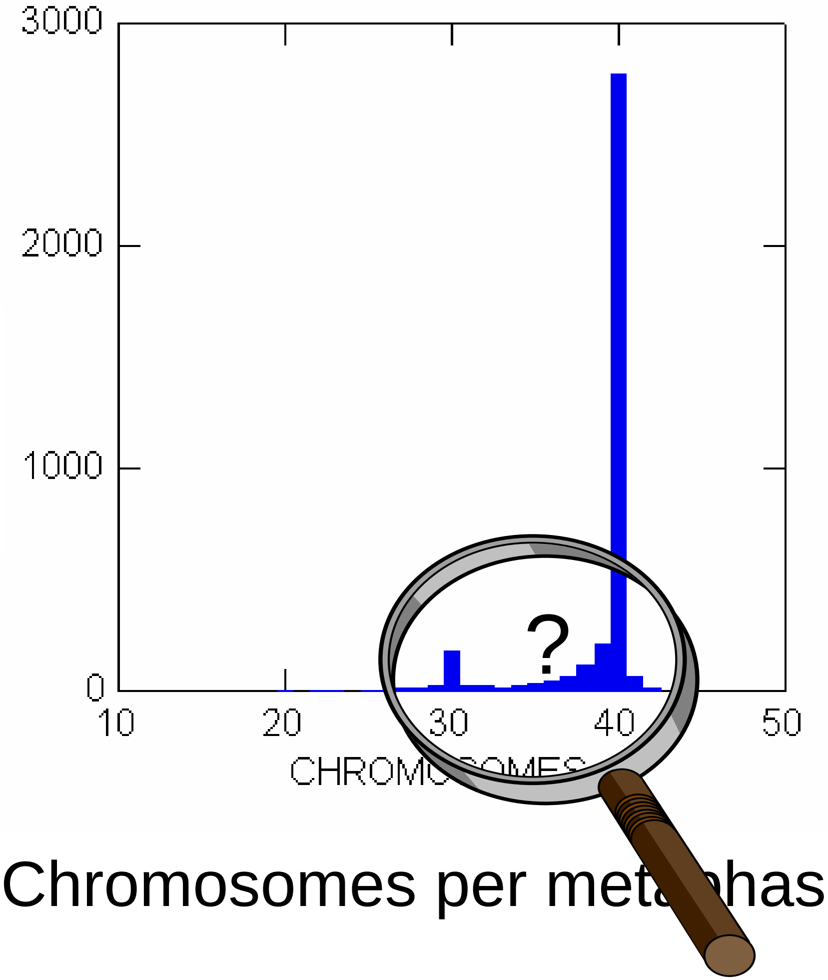
Traits studied :

- percentage of metaphases affected
- extent of loss (no. missing chromosomes)

Results :

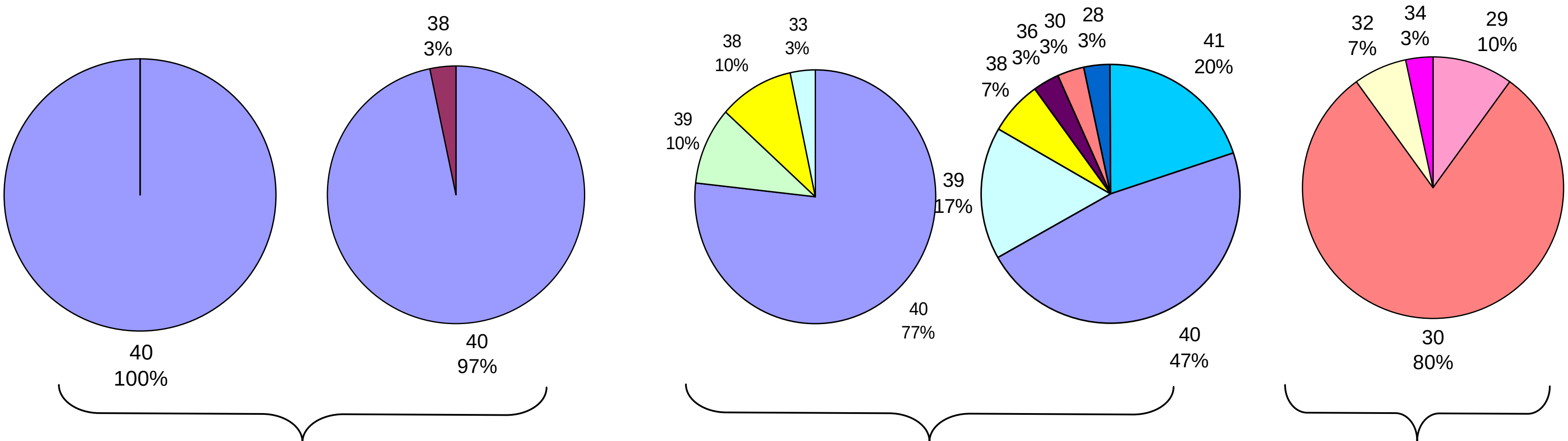
variation among metaphases **(all metaphases together)**

Number of metaphases



Chromosomes per metaphase

Range of aneuploidy among “4n” progenies



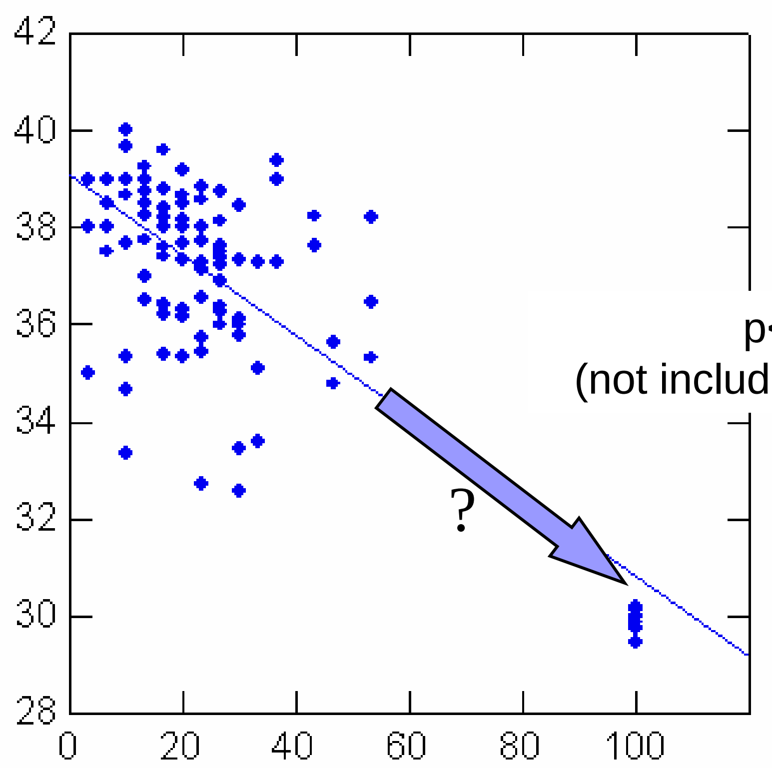
Tetraploids Aneuploids Triploids

This is a two dimensional phenomenon :

1. The percentage of metaphases which are affected
2. The number of chromosomes missing from them

Variability among individuals and relationship between cell chromosome loss and individual aneuploidy (excluding 4n= 40 individuals)

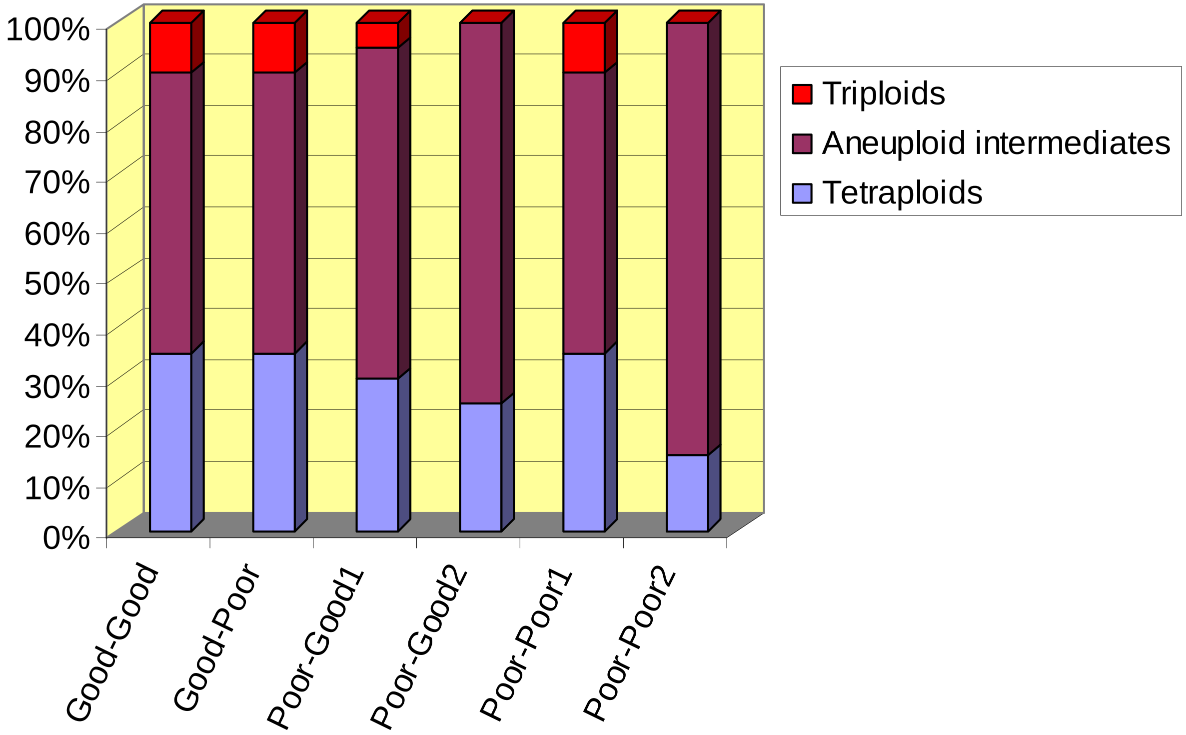
Mean chromosomes per metaphase



$p<0.001$ $r^2=0.12$
(not including “triploid” individuals)

Aneuploid metaphases per animal (%)

Comparison between families



- No significant differences between families or time on number of cells affected (within individual) or number of chromosomes missing.

- Differences between families in the incidence of aneuploids and triploid individuals.

Discussion :

Origin and relationship between aneuploidy and reversion towards triploidy ?

- The lack of effect with time suggests the phenomenon is not progressive within the first year.
- Families where triploids were observed were no more aneuploid than the other families studied, but...
- When aneuploid metaphases were more frequent, the number of chromosomes missing was greater.

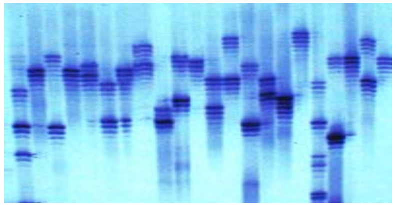
Is this therefore a gradually worsening process ?
(at least at the cell level if not a the individual level)

Do aneuploid cells die more ?

Why triploids in tetraploid progenies ?

- Contamination ?

  excluded according to microsatellite parentage analyses



- “Bad” (non-2n) gametes ?

- Degradation by uneven mitosis division to a point of stability ? (do cells with chromosome numbers multiples of ten survive better ?)

Implication for maintenance of 4n strains ?

- Selection of parents : appear difficult due to the absence of correlation with offspring's ploidy class (?)
- Selection of "good" families (absence of 3n/mosaics/limited aneuploidy)

Conclusions :

- Genetic influence but not direct
- Evidence for increased chromosome loss with aneuploidy frequency but not with time

Future work:

- Further time points
- Study of gametes: relation somatic-gametic aneuploidy ?
- Environmental impact on tetraploid chromosome loss ?
- Identification of missing chromosomes by FISH