

Recombination

Chromosome Separations At Anaphase I And II

Mather (1935 pp. 53-62).

Reductional vs. equational separations

Reductional Division: Sister chromatids go to same pole at anaphase I

Equational Division: Non-sister chromatids to to same pole at anaphase I

- The kinetochore ("spindle attachment") **always** separates reductionally at anaphase I and equationally at anaphase II.

The kinetochore is a granule within the centromere that attaches to the spindles.

- A locus some distance from the centromere will separate reductionally or equationally depending on the **number of cross-over events** between the locus and the centromere.
- Thus, a locus separated by one crossover from the centromere will separate **equationally** at anaphase I and **reductionally** at anaphase II. (Figure 1a)
- The probability that a locus separated by two crossovers will separate **equationally** at anaphase I is $1/2$ and therefore $1/2$ **reductionally**, **if non-sister chromatids, chosen at random, are involved in the cross-over events.** (Figure 1b)
- To determine the proportion of equational separations at a locus after "n" chiasmata:
$$E_n = 2/3[1 - (-1/2)^n]$$
 (Table 1 and page 57)
- $E = 2/3$, if unlimited cross-overs between centromere and locus and only $1/3$ of the time reductionally. (Figure 3)

Genetic Recombination And Physical Crossing-Over

1. Correlation of genetic and cytological crossing-over

Creighton and McClintock (1931)

Requirement to study crossing-over genetically: two heterozygous, linked loci

Cytological stocks:

- Chromosome 9 in maize.
 - Some strains have a “knob” on short arm; transmitted like a normal gene.
 - Some strains have an interchange with chromosome 8.
 - Ch 9-interchange is longer than normal 9, thus easy to identify synapsis between N9 and I9 occurs along homologous parts; therefore crossover should be possible.

Crosses:

(F₁) knob-interchanged/knobless-normal x knobless-normal
(female x male)

[Testcross]

Observations (Figure 1)

- ***Non-crossover types:*** knob-interchange/knobless-normal ; knobless-normal/knobless-normal
- ***Crossover types:*** knob-normal/knobless-normal; knobless-interchange/knobless-normal

(Table 1 for data)

39.6% recombination [= Crossover / (non-crossover + crossover)]

Genetic stocks:

- *wx* (waxy endosperm), *sh* (shrunken endosperm), *C* (colored aleurone)

Gene order: *knob-c-sh-wx-interchange*

$$\begin{array}{cccc}
 & 1 & 2 & 3 \text{ (crossover region)} \\
 \textit{Knob} & \textit{C} & \textit{wx} & \textit{interchanged} \\
 \hline
 \textit{Knobless} & \textit{c} & \textit{Wx} & \textit{normal}
 \end{array}
 \times
 \begin{array}{cccc}
 \textit{Knobless} & \textit{c} & \textit{Wx} & \textit{normal} \\
 \hline
 \textit{Knobless} & \textit{c} & \textit{wx} & \textit{normal}
 \end{array}$$

Table 3:

Male always contributes knobless, *c*, and normal (no interchange)

Class I	3	Knob- <i>C-wx</i> - interchange	----	non-recombinants
Class II	2	Knobless- <i>c-wx</i> -interchanged only)	----	recombinants (crossover in 2
Class III	8	Knob- <i>C-Wx</i> - normal	----	recombinants (crossover in 2 only)
Class IV	12	Knobless- <i>c-Wx</i> -normal	----	non-recombinant (pollen either <i>Wx</i> or <i>wx</i>)
	3	Knobless- <i>c-Wx</i> -interchanged	----	recombinant (crossover in 2 or 3).

- Determine plant's genotype (*Wx/wx*)– with KI staining of pollen.
- Conclusion: They had correlated the cytological and genetic evidence to show that crossing over occurs and that it has genetic consequences.

2. Correlation of chiasmata and crossing-over

Brown and Zohary (1955)

Found 1:1 correspondence between chiasmata and crossing over. Thus, chiasmata provide an indirect measure of crossing over.

Confirmed that only two of four chromatids were involved in any one cross over event.

3. Relationship between chiasma and recombination

Recombination frequency = 1/2 (chiasma frequency)

- If 1 crossover between two loci, A & B, then 50% gametes are recombinant types and 50% non-recombinant or parental types.
- Therefore, 100% chiasma freq = 50% recombination (crossing-over)
= 50 map units (centiMorgans, cM)
= maximum separation of two loci
= UNLINKED
=Random assortment (same as markers on different

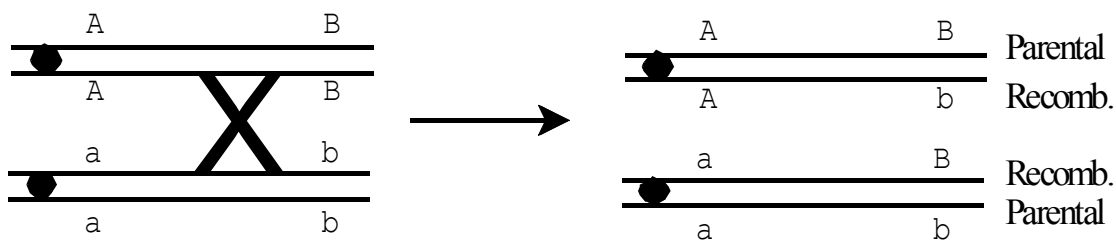
chromosomes)

- Thus, the MAXIMUM recombination between two loci is 50%.
- To determine recombination rate between two loci, we study many meioses – an sampling issue:

- *On average*, how many cross-overs occur between two loci?
- If, on average, a segment of chromosome contains 1 crossover/meiosis, then the segment is 50 cM long
that is, there is 50% recombination between the ends of the segment.

$$50\% \text{ recombination} = 50 \text{ map units} = 50 \text{ cM}$$

- So, between any two loci, the map distance depends on the proportion of meioses with a crossover.
- For the example above: $(1/1 \text{ meioses with recombination})(50 \text{ map units}/1 \text{ recombination/meiosis}) = 50 \text{ cM}$
- If you observe 50 cells, 25 have a cross-over, then



$$(25/50)(50) = 25 \text{ cM i.e. } 25\% \text{ recombination}$$

In Sherman and Stack (1995): they estimate the number of map units per 0.1 μm section of chromosome:

$$\text{e.g. } (3 \text{ RN}/430 \text{ SC})(50) = 0.35 \text{ map units (cM)}$$

4. High-Resolution Recombination Nodule Map:

Sherman and Stack (1995)

A. Introduction: methods to *physically* map locations of markers

- Linkage maps do not provide an estimate of physical distance.
 - Recombination is not equal in all regions.
 - Low recombination, markers appear to be physically closer than they actually are.

- *In situ* (FISH) hybridization is difficult, not precise.
- Chiasma are a poor choice for a physical map.
 - Hard to relate contracted bivalent to specific chromosomes.
 - Hard to count and position chiasmata, even in large chromosomes hard to resolve chiasmata that are close together.
 - Hard to localize-bivalents so contracted.
 - Confusion with twists in longer chromosomes.
 - Chiasmata move-terminalize.
- Recombination nodules (RN) are a solution
 - Individual SCs have been related to specific chromosomes or linkage groups.
 - Easy to count RN even in small chromosomes.
 - Precise measurements of the positions of RN along SCs, because RNs are 10x smaller (0.1 μm) than chiasmata.
 - RNs do not terminalize and are detected when chromosomes are in pachytene stage. Chromosomes are 5-10x larger in pachytene than in diakinesis when chiasmata are visible.
- Problems of RN
 - Mostly procedural; techniques are, however, improved.
 - May not be applicable to all organisms.
 - Can be applicable in tomato at least.

B. Materials and Methods

1. Isolated microsporocytes from greenhouse grown tomatoes 2-3 months old
2. Two-dimensional spreads of SC--(**Fig 1-11**)
 - Burst microsporocytes
 - stain with silver nitrate, lead citrate or uranyl acetate.
 - Localize SC with phase contrast light microscope photographed with electron microscope
 - Mark kinetochores, euchromatin/heterochromatin boundary, RN position- SCs stain more densely in euchromatin than in the heterochromatin.
3. Observed RN position on >400 of each of tomato's 12 SCs, determined position of RN from the centomeres.

C. Results/discussion

- **Environmental variables do not effect frequency or placement of RN on SC.**
- **A chiasma forms at each RN**
 - Counted chiasma as rods (1) or rings (2) at diakinesis
fewer chiasma than RN.
 - Can't see >1 chiasma on an arm with this method; ---- Observed only 17
chiasmata as opposed to 21-22 RNs.
 - Correcting for multiple chiasmata, chiasma number = RN number
- **Every SC has at least one RN--(Table 4)**
 - Average 22 RN/SC set (i.e. 12 chromosome pairs; 12 SCs/set)
- **No RN at ends of SC**
 - No RN in distal 0.4 μm (average) end of chromosomes.
 - Causes clustering of molecular markers near telomeres.
 - Protects telomeric repeats from unequal crossing over.
- **No RN at kinetochore--(Fig 12)**
 - Never in kinetochore, very rare close to kinetochore.
 - Probably protect repeat sequences important for normal spindle attachment.
- **RN more common in euchromatin than heterochromatin --(Table 5)**
 - 20-50 x more likely.
 - Low recombination in heterochromatic regions can lead to tight linkages of
markers that are physically distant. Clustering of markers results.
- **RN numbers strongly correlated with SC length.**
 - Each SC has at least one; subsequent RN occur in proportion to SC length.
 - A strong association between RN frequencies and average arm length of
euchromatic regions (**Figure 15**).
- **Single RN found more often in long arm than expected (Table 8)**
 - Due to synaptic initiation point being the site of crossing over and this occurs
more often in long arm (hypothesis).
- **Crossover interference when multiple RN on an SC**
 - RN in one arm, the next will go to other arm-*interference*.
- **Variation in distance between RNs along chromosome--(Table 9; Fig 16)**
 - Longer arms have larger distances between RN. Observed mean distance
between RN larger than expected- crossover interference (**Table 9**).
 - If only 2 RNs in long arms--both *positive* and *negative interference* (**Fig 17**)
 - positive interference: one cross-over drives another farther away (too
few crossovers). Separated by 0.5-1.0 μm interval.
 - Negative interference: one crossover increases chances of another close
by (too many crossovers; hotspot of recombination??). Separated by 0.1-
0.5 μm interval.
- **RNs are not randomly distributed along SC arms**
 - Can occur anywhere in most euchromatin,
 - Sample size-large will reduce.
 - Crossover hot spots
- **RNs are may occur randomly in euchromatic regions**
 - For most euchromatic length the distribution is random.

- Telomeres and euchromatin-heterchromatin borders are excluded.
- **RN maps are physical maps of recombination**
 - RN represents a crossover event. One crossover/meiosis = 50 map units.
 - Therefore, 1 RN = 50 map units. Set of tomato SC averages 21.89 RN
 $21.89 \times 50 = 1094.5$ map units (cM).
- **Comparison RN map with genetic and molecular maps--(Table 11)**
 - Classical map shortest and molecular map longest.
 - Discrepancies between molecular and RN map.
 - Microsporocytes (RN map) vs. both gametes (molecular map).
 - Scoring errors.
 - Difficulty in resolving multiple crossovers between two markers

D. Conclusion

1. RN maps show physical distribution of crossover events at higher resolution than chiasma maps (**Fig 18**).
2. Will permit estimation of crossover rates between defined molecular markers, correlating RN (physical map), genetic and molecular maps of recombination.

5. Variation in recombination in different genetic backgrounds

(Williams et al., 1995)

- Studied recombination in a number of maize genetic backgrounds by crossing 16 different stocks—including adapted and tropical maize lines and teosinte stocks—to a common parent that had numerous rare alleles at isozyme loci on chromosome 1.
- Recombination varied 2- to 3-fold in some intervals—e.g. the interval between *pgi1-dia2* varied from 7.1 cM to 22.8 cM.
- Recombination distances were different even among germplasm of common origin.

Variation in the recombination rates in the *Rps1-k* region

- Depending upon the crosses the recombination rates vary in a region of ~700 kb region (Bhattacharyya et al. unpublished).



- Soybean cultivars, near isogenic; Williams 82 and Elgin 87 carry *Rps1-k*.
- *Rps1-k* encodes resistance to *Phytophthora sojae*.

Williams x Williams 82: Crossing over between *Tgmr* and TC1

Elgin x Elgin 87: Crossing over between TC1 and CG1.

Conclusion: Physical distance between two markers depends upon the crosses under investigation.

Review: Crossingover And Recombination

Crossingover: Actual chromatid interchange between homologues seen as *chiasma* during meiosis.

Recombination: Observed chromatid interchange, seen as rearrangements in genes or markers after meiosis.

- **During meiosis:**

For each genetically distinct chromosome, e.g. chromosome 1 of corn:

- 2 homologues; 2 chromatids/homologue.
- If 50% recombination, then 100% of meioses have a cross-over between them.
- If 1% recombination, then 2% of meioses have a cross-over event—therefore, need large population sizes.

