FINDING THE PAIN GENE
HOW DO GENETICISTS CONNECT A SPECIFIC GENE WITH A SPECIFIC PHENOTYPE?

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The Primary Erythermalgia–Susceptibility Gene Is Located on Chromosome 2q31-32

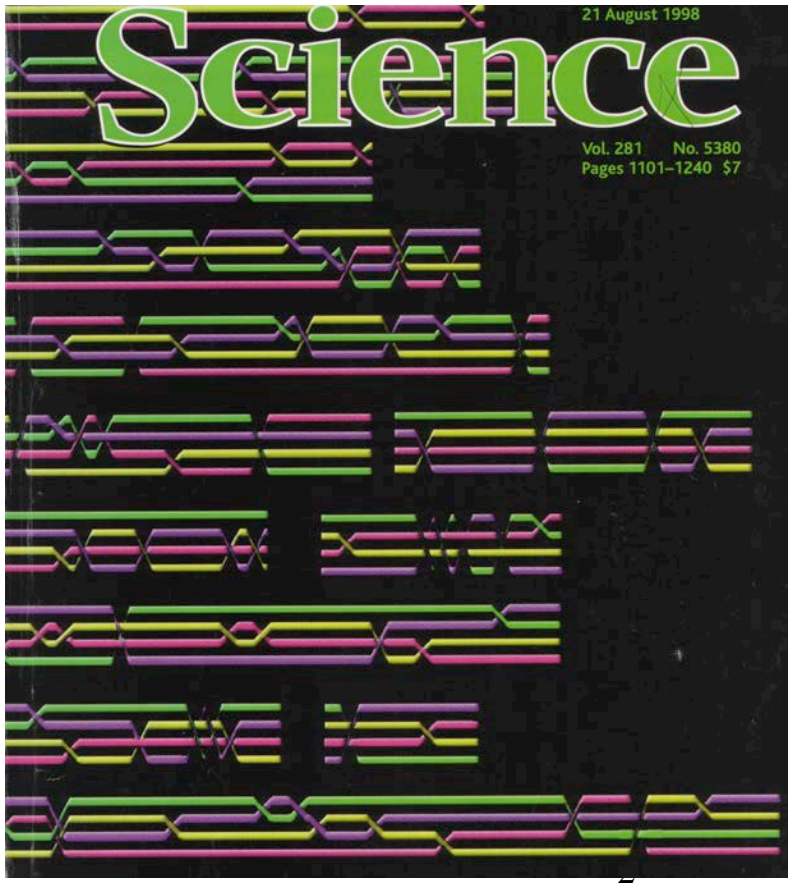
Joost P. H. Drenth,[1] Wayne H. Finley,[2] Guido J. Breedveld,[3] Leon Testers,[4] Jan J. Michiels,[5,6] G. Guillet,[7] Alain Taieb,[8] R. Lee Kirby,[9] and Peter Heutink[3]

Abstract

Primary erythermalgia is a rare disorder characterized by recurrent attacks of red, warm, and painful hands and/or feet. The symptoms are generally refractory to treatment and persist throughout life. Five kindreds with multiple cases of primary erythermalgia were identified, and the largest was subjected to a genomewide search. We detected strong evidence for linkage of the primary erythermalgia locus to markers from chromosome 2q. The highest LOD score (Z) was obtained with D2S2330 (Z_{max} = 6.51). Analysis of recombination events identified D2S2370 and D2S1776 as flanking markers, on chromosome 2q31-32. This defines a critical interval of 7.94 cM that harbors the primary erythermalgia gene. Affected members within the additional families also shared a common haplotype on chromosome 2q31-32, supporting our linkage results. Identification of the primary erythermalgia gene will allow a better clinical classification of this pleomorphic group of disorders.

Linkage & Recombination

HUH? What? Why? Who cares? How?



Multiple choice question. Each colored line represents a:

a. *double-stranded DNA polymer*

b. *one strand of a d-s DNA polymer*

c. *a chromatid*

d. homolog

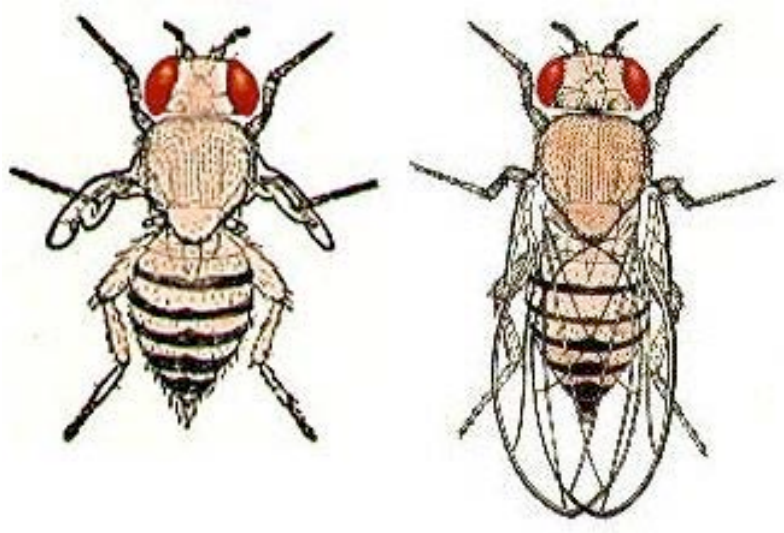
e. a & c

The phenomenon of linkage was first described by the Drosophila geneticist Thomas Hunt Morgan (of sex-linkage fame)

Morgan was investigating the inheritance of two autosomal traits in Drosophila

pr^+ = wild-type red eye *vg^+ = wild-type long wings*

pr = purple eye *vg = short, vestigial wings*



P purple, vestigial X wildtype

pr pr vg vg $\quad$ *pr*$^+$ *pr*$^+$ *vg*$^+$ *vg*$^+$

↓

F1 wildtype *pr*$^+$ *pr vg*$^+$ *vg*

F1 □ ↓ test cross *pr pr vg vg* □

Phenotypic class	number of flies	genotype of male gamete	genotype of female gamete
wildtype red, long	1339	*pr vg*	
purple, vestigial	1195	*pr vg*	
red, vestigial	151	*pr vg*	
purple, long	154	*pr vg*	

Fill in genotype of gametes

If traits were segregating independently expect to see what phenotypic ratios would we expect to see?

Morgan Did another set of experiments with this markers

P red, vestigial X purple, long

pr^+ pr vg vg pr pr vg^+ vg^+

↓

F1 wildtype pr^+ pr vg^+ vg

F1 ♀ ↓ test cross pr pr vg vg ♂

Phenotypic class	# of flies	genotype of male gamete	genotype of female gamete
wildtype red, long	157		
purple, vestigial	146		
red, vestigial	965		
purple, long	1067		

Explain why the data look so different for the two experiments

pr^+ = wild-type red eye *vg^+ = wild-type long wings*

pr = purple eye *vg = short, vestigial wings*

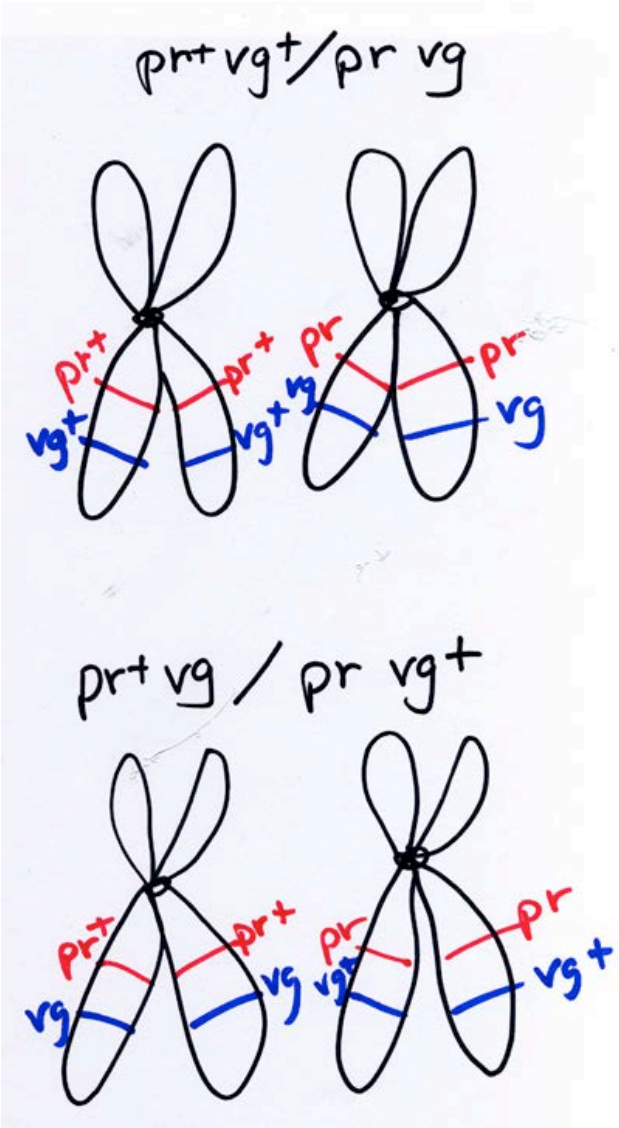
	Expt #1	*Expt #2*
Phenotypic class	number of flies	number of flies
wildtype red, long	1339	157
purple, vestigial	1195	*146*
red, vestigial	151	965
purple, long	154	*1067*

The largest phenotypic classes resulting from the testcross always reflects the allele combinations of the parental flies (known as the parental types)

Linkage: **the association of genes on the same chromosome**

Linked genes **are physically tied to each other on the same chromosome (that is, on the same DNA molecule). Therefore they will segregate with each other during meiosis**

Rewrite genotypes using nomenclature for linked genes



Top panel shows F1 genotype produced by mating wild-type and doubly mutant (purple, vestigial) flies. One homolog carries both wild-type alleles; the other homolog carries the mutant alleles of each gene.

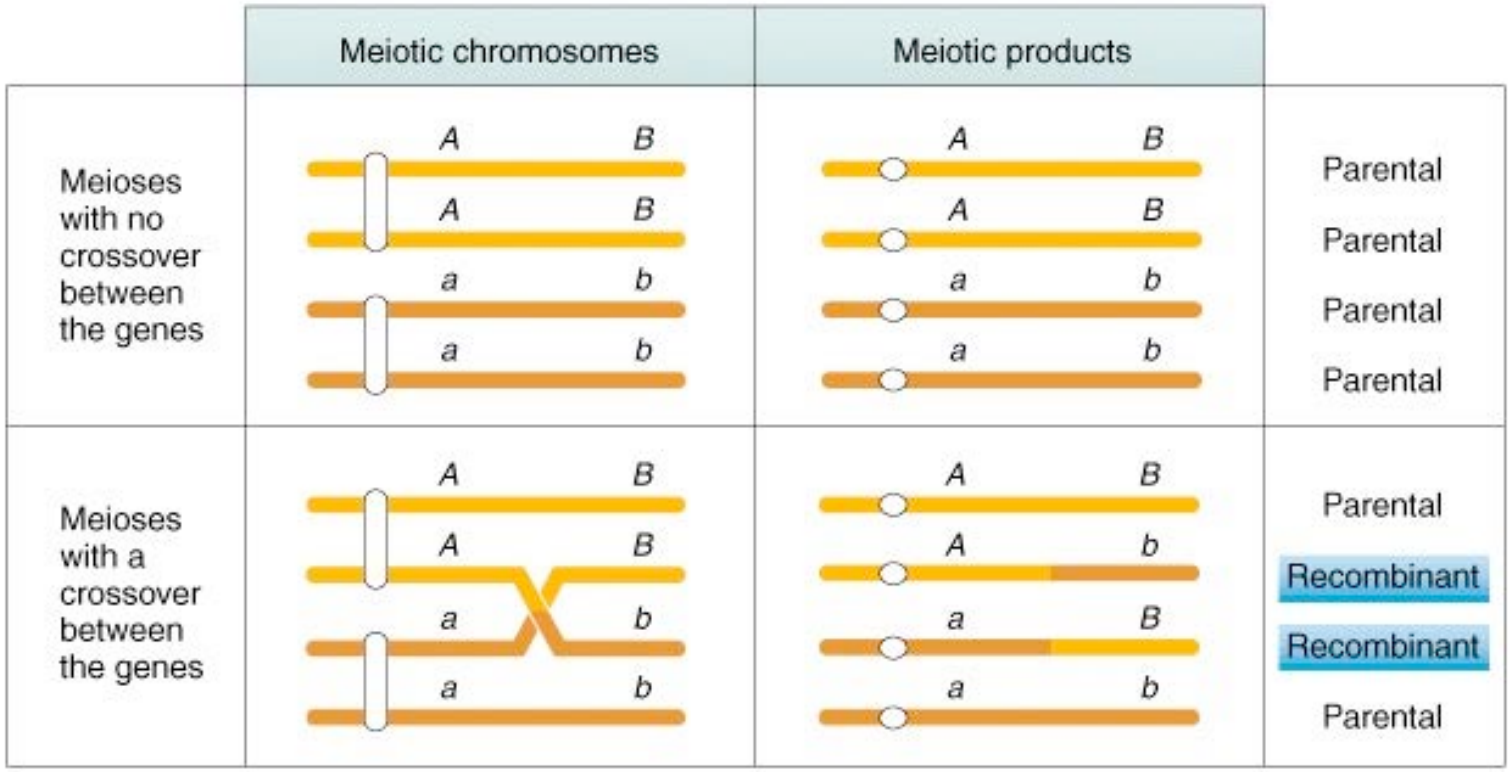
Bottom panel shows F1 genotype produced by mating red, vestigial with purple, long flies.

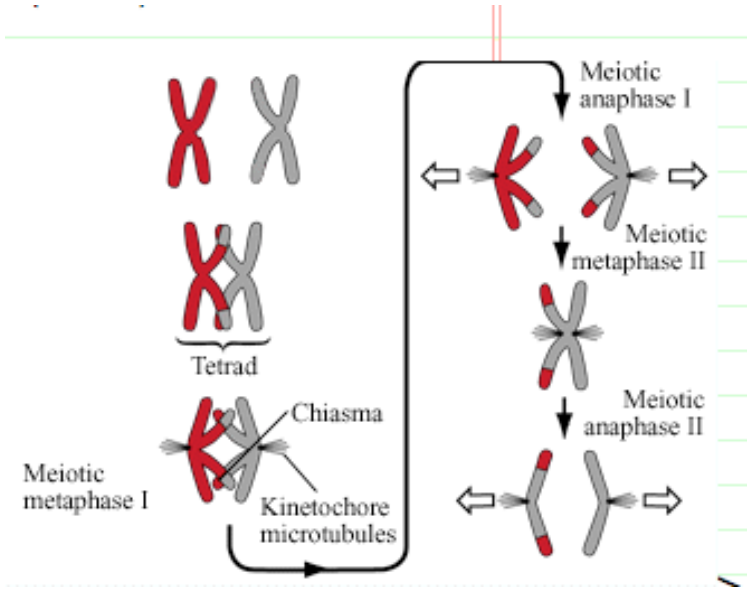
How to explain the *non-parental types?*

pr^+ = wild-type red eye vg^+ = wild-type long wings
pr = purple eye vg = short, vestigial wings

	Expt #1	*Expt #2*
Phenotypic class	number of flies	number of flies
wildtype red, long	1339	157
purple, vestigial	1195	*146*
red, vestigial	151	965
purple, long	154	*1067*

Crossing over during meiosis generates *recombinant* chromatids





Why is crossing over important?

- *Recombination has an important role in evolutionary dynamics* in generating genetic diversity that is acted on by natural selection
- *Recombination can purge DNA of mutations* which would otherwise accumulate in an asexual population

(see pg 28 &29 for example & google Muller's rachet & recall our discussion of the evolution of the mammalian Y chromosome)

- In many organisms, *recombination* is fundamental to the successful completion of meiosis, helping to align homologous chromosomes and to ensure their proper disjunction in anaphase of Meiosis I
- *Unequal crossing-over events* result in deletion and duplication of genes or segments of a chromosome: duplicated genes are a rich source of raw material for the evolution of new gene function

Why is crossing over so important?

The rate of ***recombination*** *between genes can be indirectly measured by geneticists and used to generate a meiotic linkage map*

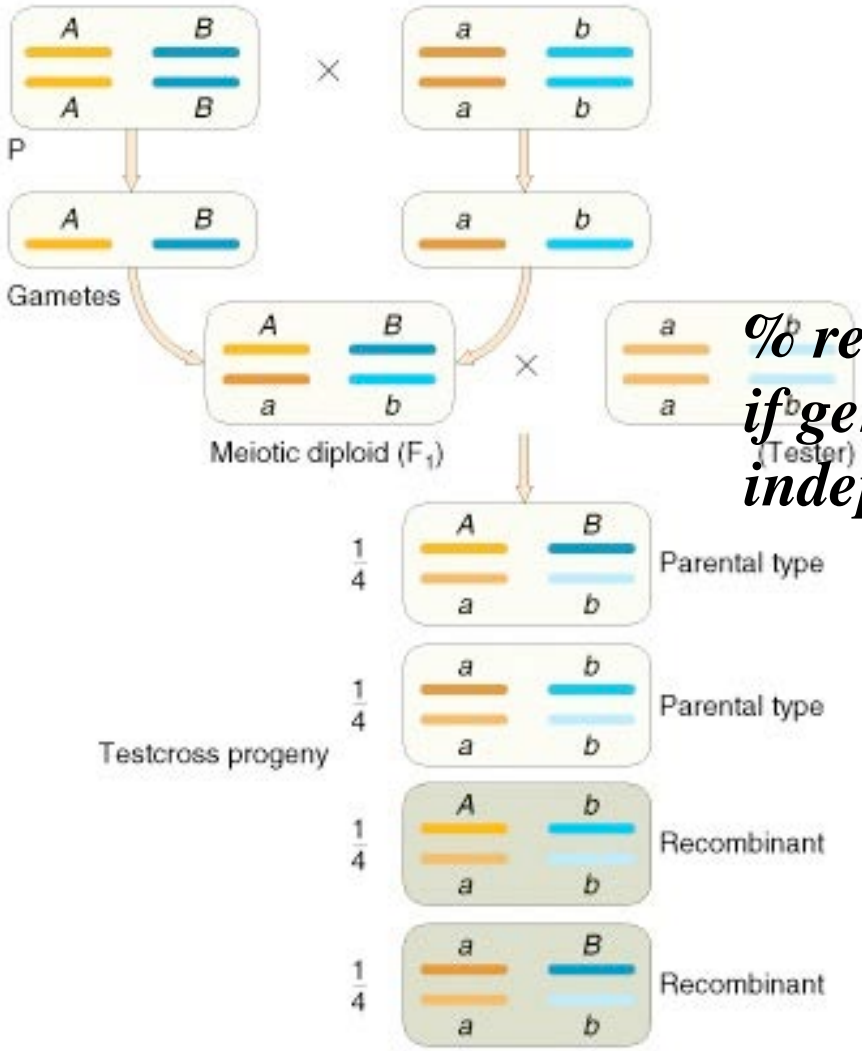
Recombination: term is used to describe many different situations in which genetic material is shuffled or rearranged

Meiotic recombination: any meiotic process that generates a haploid product with a genotype that differs from the two haploid genotypes that produced the meiotic diploid

Includes:

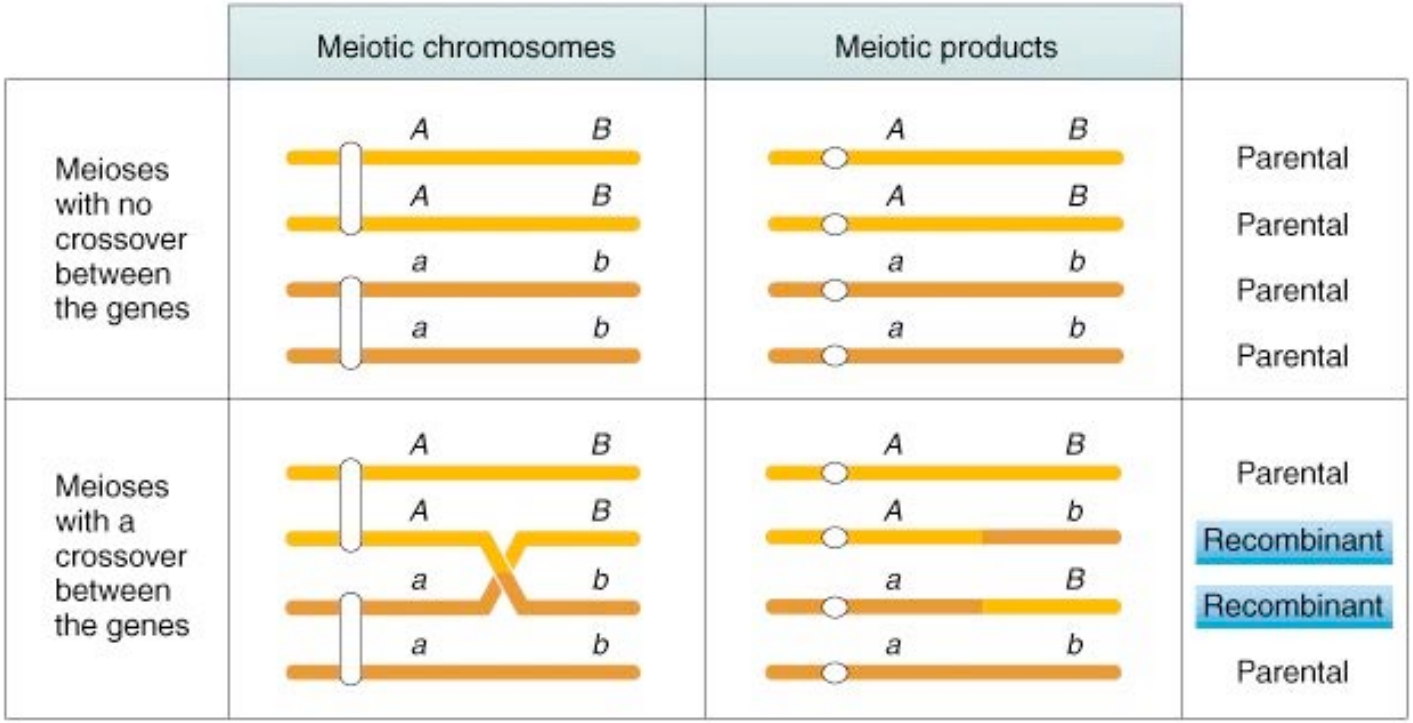
- independent assortment
- crossing over

Independent Assortment results in 50% recombinant (non-parental) progeny



% recombinant gametes = 50% if genes are assorting independently

Two genes are said to be linked if the % of recombinant gametes produced by a AaBb organism is less than 50%



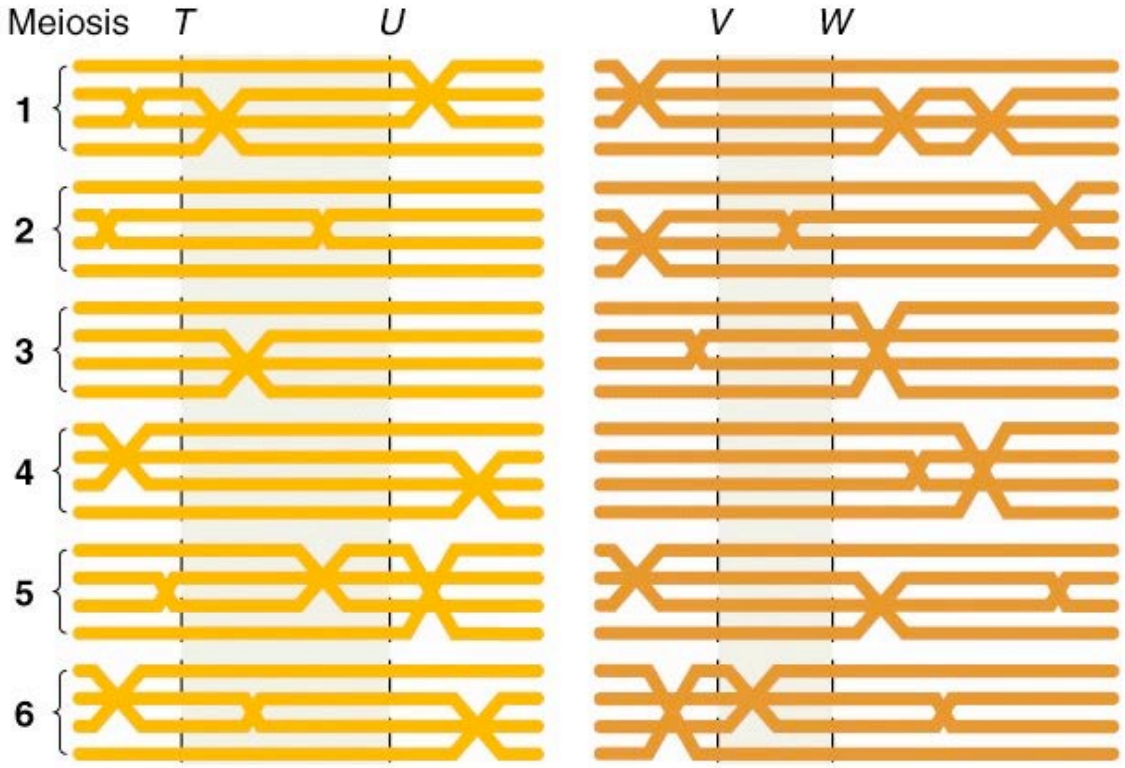
Why does % recombination max out at 50% for linked genes at the distal ends of a chromosome? SEE end of lecture

Morgan observed that the proportion of recombinant progeny varied widely depending on the particular pair of linked genes being studied:

% recombination between linked genes varies depending on what genes are being examined

What would this variation reflect?

Crossovers can occur between any two nonsister chromatids within a homologous pairing



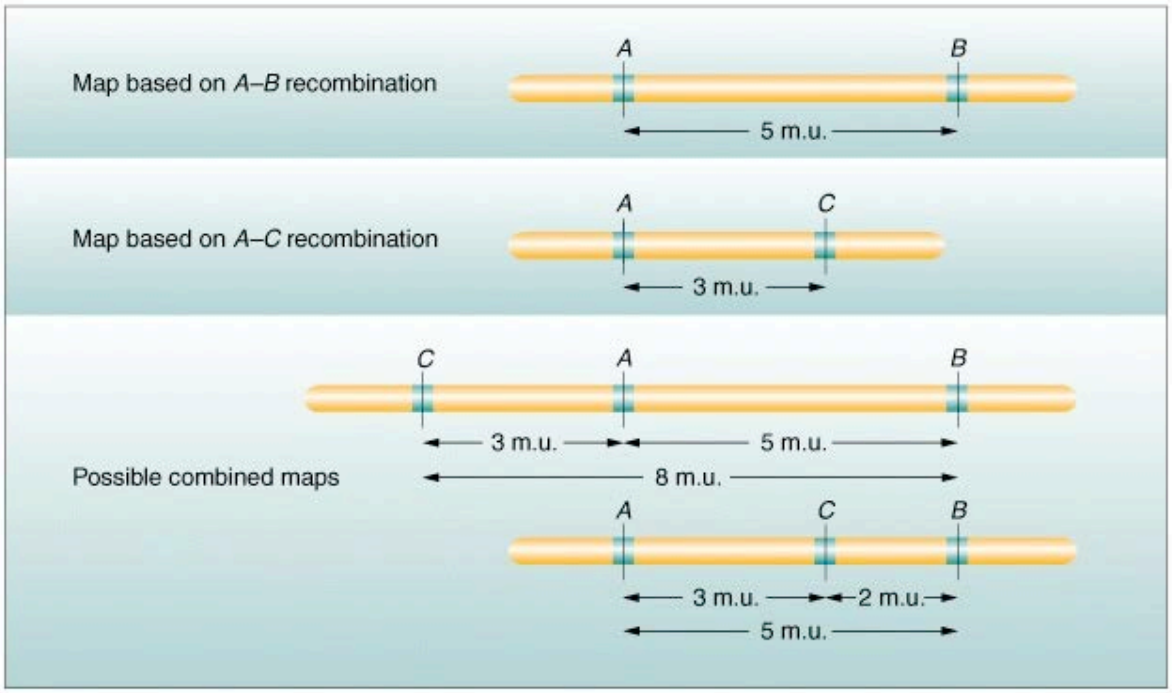
Proportionality between chromosome distance and recombinant frequency. In every meiosis chromatids cross over at random along the chromosome.

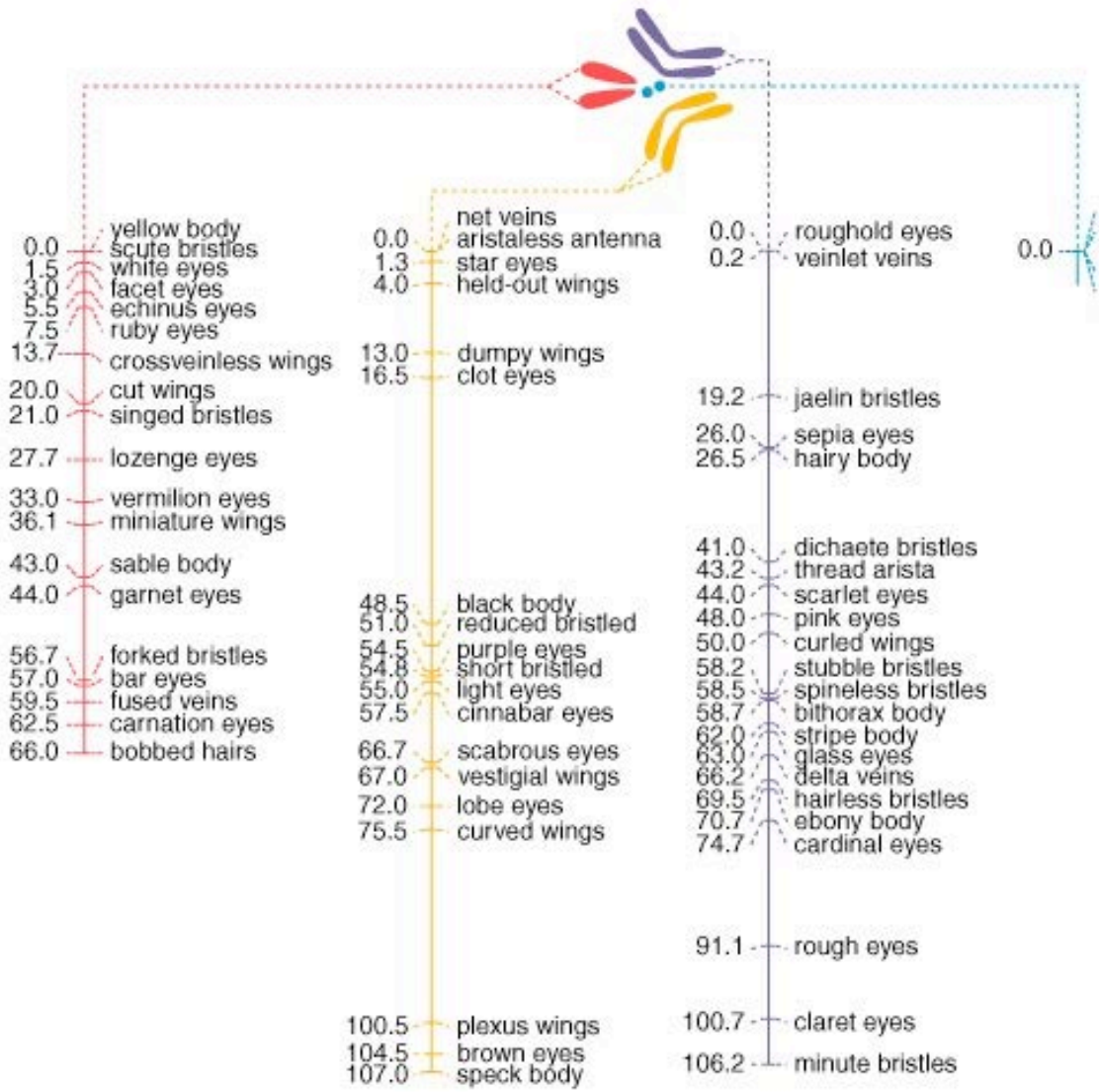
The distance between two genes is defined in map units:

1 map unit = 1% recombination

Linkage data can be used to determine

- *the chromosomal location of a newly identified gene*
- *relative distances between genes on a chromosome*
- *the order of genes on a chromosome*





The end product is a linkage map.

Genetic Map: a map showing the relative location of specific genes on a chromosome

Linkage Map: a genetic map based on linkage and recombination studies

New mutant strain

OSW = oddly shaped wings

OSWT = wildtype

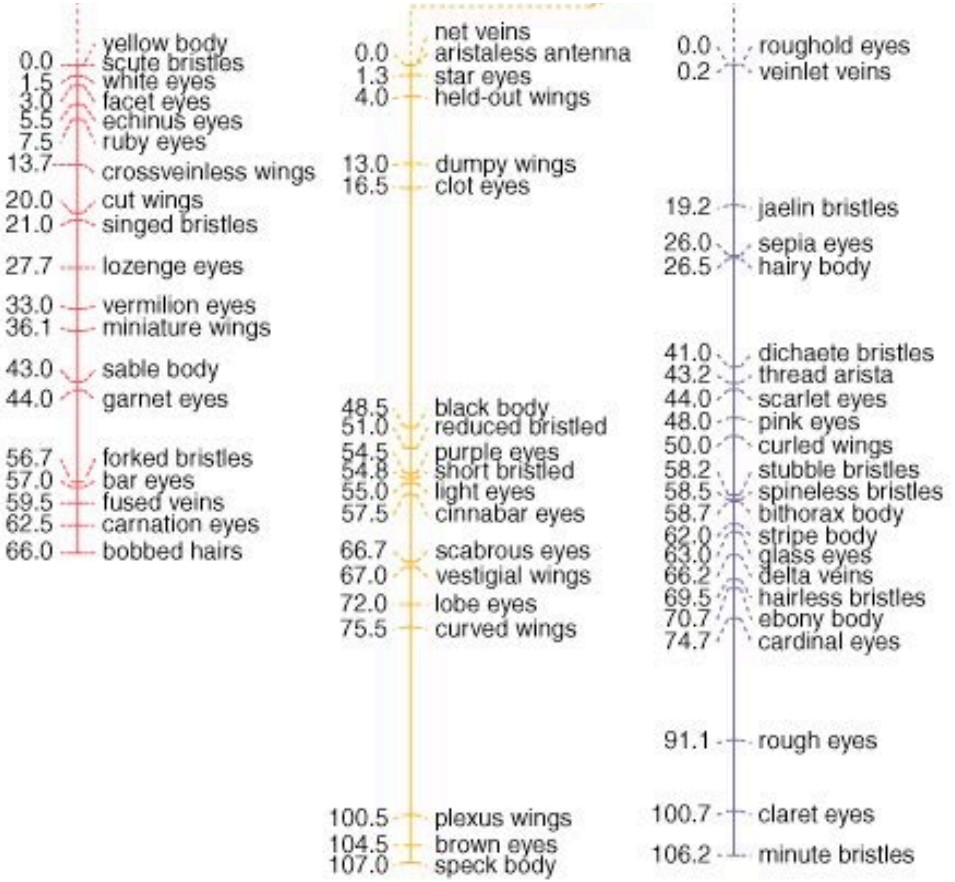
4 chromosomes/haploid set

I (X)

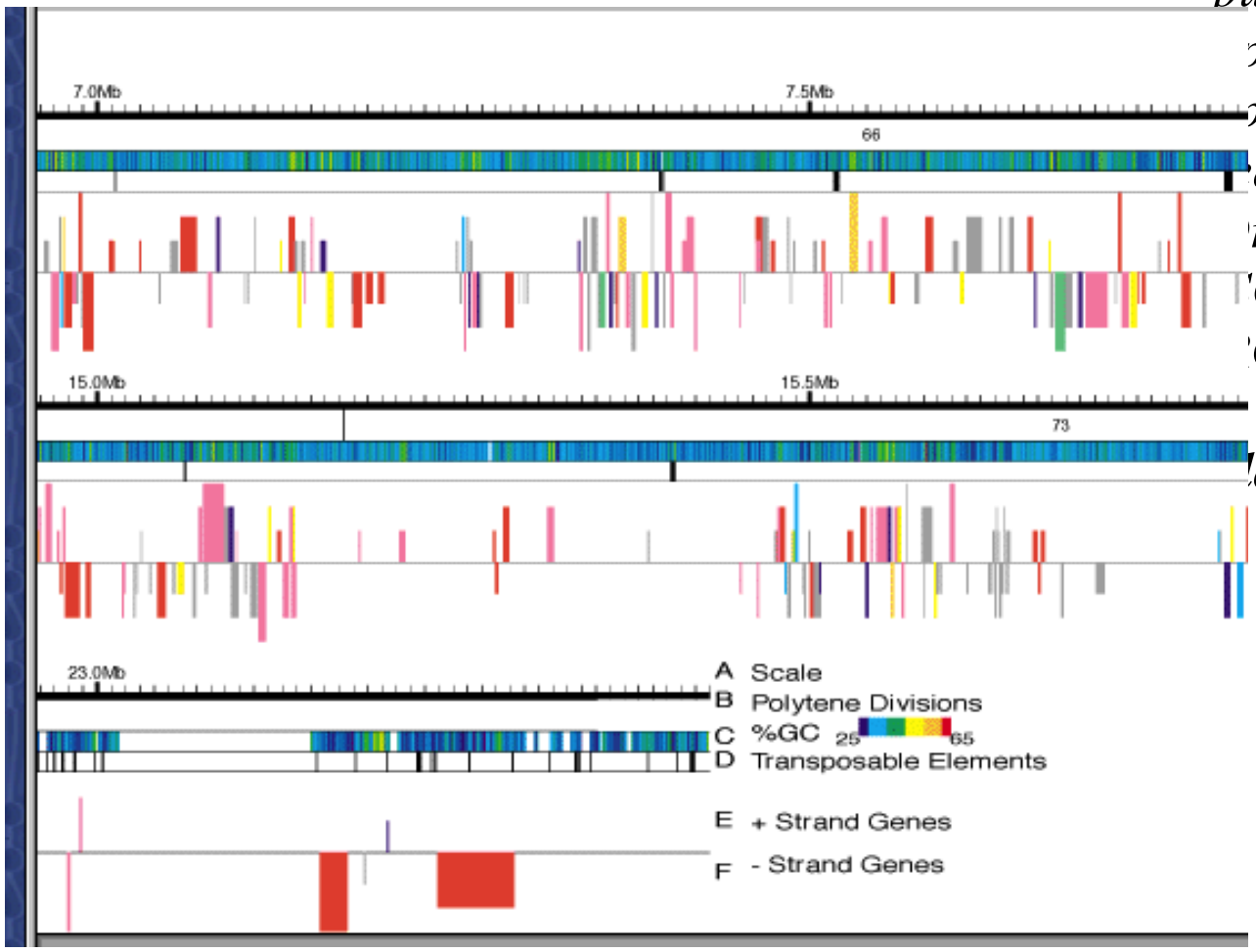
II

III

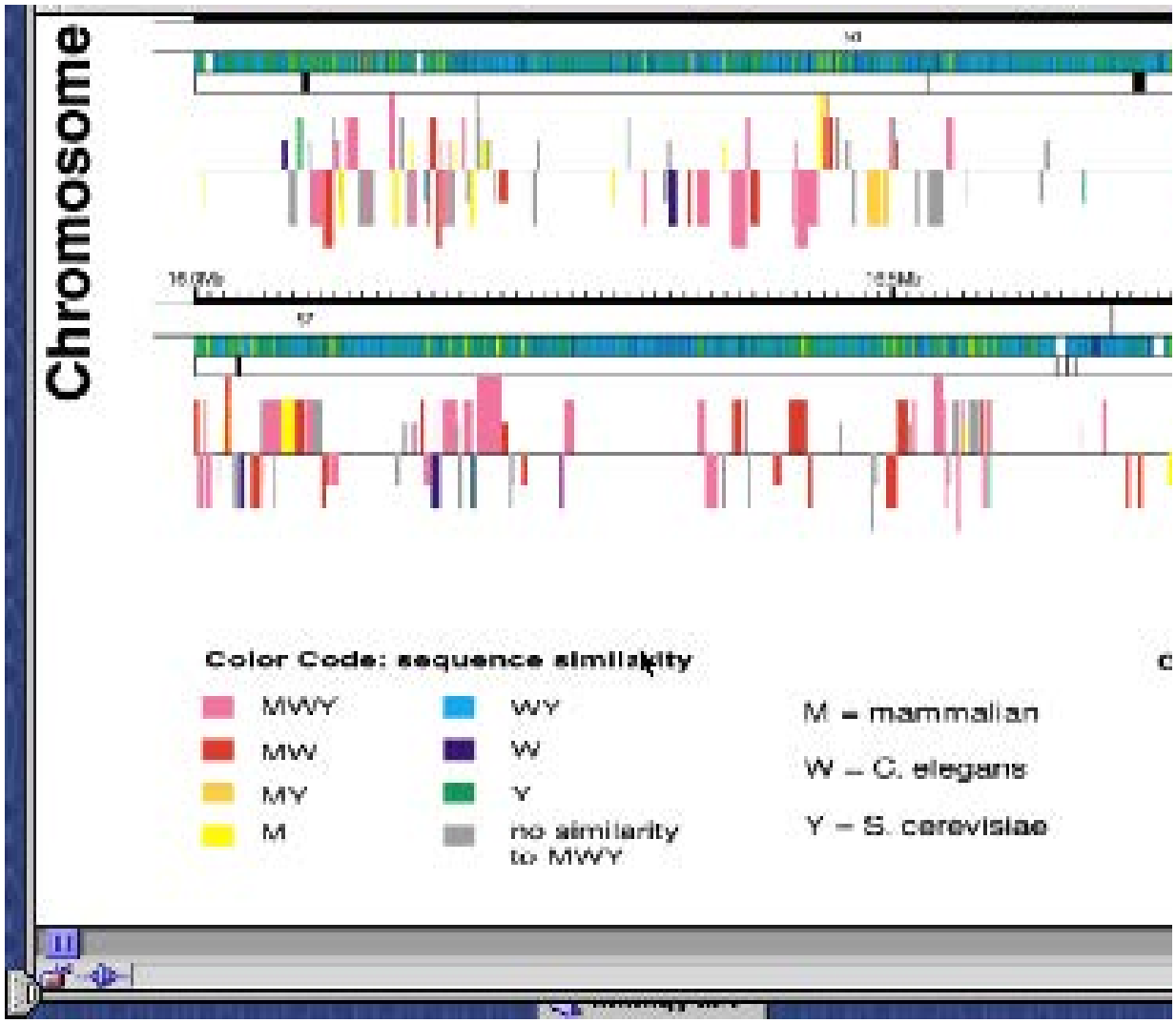
IV



Presentation of the Drosophila genetic map in the publication porting the plete genome quence of rosophila CIENCE CH 24, 2000



egend on pg 21



See legend on pg 21

Coding content of the fly genome. Each predicted gene in the genome is depicted as a box color-coded by similarity to genes from mammals, C. elegans, and S. cerevisiae. A legend appears at the end of each chromosome arm describing the components of each panel. In order from the top, they are
(A) scale in megabases,
(B) polytene chromosome divisions,
(C) GC content in a range from 25 to 65%,
(D) transposable elements, and genes on the
(E) plus and (F) minus strands.
The width of each gene element represents the total genomic length of the transcription unit. The height of each gene element represents EST coverage: The shortest boxes have no EST matches, medium-size boxes have 1 to 12 EST matches, and the tallest boxes have 13 or more EST matches.

The color code for sequence similarity appears on each side of the fold-out figure. The graphics for this figure were prepared using gff2ps (68). Each gene has been assigned a FlyBase identifier (FBgn) in addition to the Celera identifier (CT#).
EST = expressed sequence tag

Are Linkage Studies Boring?

by Thomas D. Bird Nature Genetics July 1993

" A colleague told me recently that linkage studies are boring. By this he meant they are easy to do, tedious and produce little information. ***I disagree. I find genetic linkage studies to be challenging, fascinating and valuable****. The two linkage studies concerning the hereditary ataxias in the present issue of Nature Genetics are a case in point.*

Some historical perspective is helpful. As recently as 1980, only foolish faculty and fellows with extra time on their hands dabbled in (human) linkage studies. There were so few genetic markers that the likelihood of a "hit" was very small…… Then Botstein and colleagues announced the potential use of restriction fragment length polymorphisms for linkage.."

Why map genes? Some reasons:

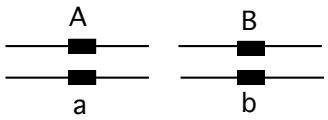
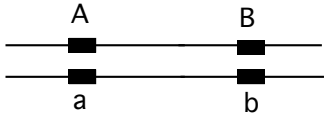
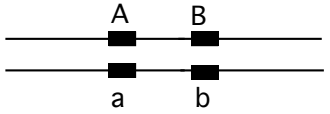
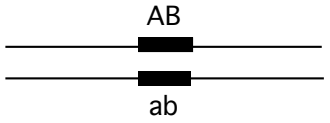
- Genetic mapping provides basic information about the arrangement of the genome that is critical for the study of gene and genome evolution and to understand factors that influence the expression pattern of a gene

- Genetic mapping provides basic information about the organization of a chromosome and the "physical" context of a gene. Who are its neighbors? *This is important basic information useful in strain construction and for designing good experiments*

- ***Genetic mapping has been a critical first step in the cloning of many genes (such as the cystic fibrosis gene and the Huntington gene) by a process called positional cloning***

How do we know if genes are linked or not?

Gametes formed by a organism who developed from the joining of gametes (AB) and (ab)

Location of genes A and B with respect to each other	PERCENTAGE OF EACH GAMETE TYPE				Percentage of recombination	Description of double heterozygote
	Parental		Recombinant			
	(AB)	(ab)	(Ab)	(Ba)		
I. On different (nonhomologous) chromosomes	25	25	25	25	50	
II. On the same chromosome, but very far apart	25	25	25	25	50	
III. On the same chromosome, neither very far apart nor very close[a]	$\frac{100-x}{2}$	$\frac{100-x}{2}$	$\frac{x}{2}$	$\frac{x}{2}$	x=a number between 50 and 0	
IV. On the same chromosome, very close together	50	50	0	0	0	

a. Only in Cases III and IV do we refer to A and B as linked genes.

Map distance between genes is measured in map units.
1 map unit = 1% recombination, x =# of map units

No crossovers

RF = $\frac{0}{4}$ = 0%

One crossover
(Can be between any nonsister pair.)

RF = $\frac{2}{4}$ = 50%

Two crossovers
(Holding one crossover constant and varying the position of the second produces four equally frequent two-crossover meioses.)

RF = $\frac{0}{4}$ = 0% — Two-strand double crossover

RF = $\frac{2}{4}$ = 50% — Three-strand double crossover

RF = $\frac{2}{4}$ = 50% — Three-strand double crossover

RF = $\frac{4}{4}$ = 100% — Four-strand double crossover

Average two-crossover RF = $\frac{8}{16}$ = 50%

Why does the loss of recombination cause "genetic decay"? (recall the human Y chromosome)

Recombination events between homologous chromosomes can purge harmful mutations from a chromosome:

a^+ = wild-type a = mutant deleterious allele

b^+ = wild-type b = mutant deleterious allele

These genes are linked together on the same chromosome:

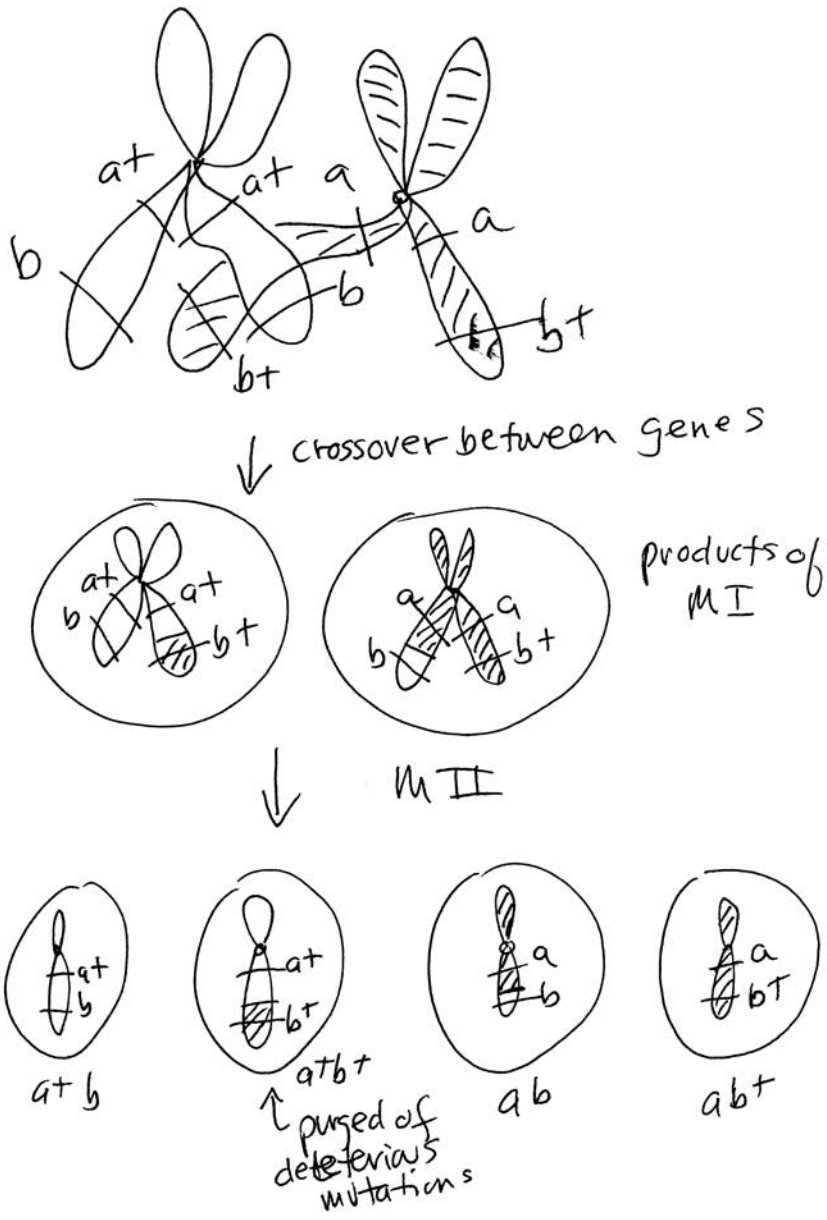
Two individuals mate, each of which is homozygous for a deleterious mutation. They produce an offspring who is heterozygous for both genes,

$$a^+\ b/a^+\ b \quad X \quad a\ b^+/a\ b^+$$

$$\downarrow$$

$$a^+\ b\ /\ a\ b^+$$

The diagram on the next page shows a meiotic division in the germ-line of the $a^+\ b\ /\ a\ b^+$ progeny. A cross-over has occurred between the two genes resulting in recombinant chromatids. One of the gametes is wild-type for both genes -- in other words, the deleterious mutations have been purged from this chromosome.



If a chromosome can't recombine with a homolog in this manner, then deleterious mutations will accumulate -- as has happened over millions of years in the differential region of the Y chromosome -- as it lost the ability to recombine with the ancestral X homolog.

[The block to recombination occurred in stages -- each stage caused by a new chromosomal rearrangement (called an inversion) which suppresses recombination.]

Homologous recombination: a molecular perspective

The first step in crossing over involves single strand exchange (complementary base-pairing) between homologous regions of the paired chromosomes:

http://www.sci.sdsu.edu/~smaloy/MicrobialGenetics/topics/genetic-analysis/recombination/rec-molecular.html

http://www.wisc.edu/genetics/Holliday/index.html

http://www.wisc.edu/genetics/Holliday/holliday3D.html