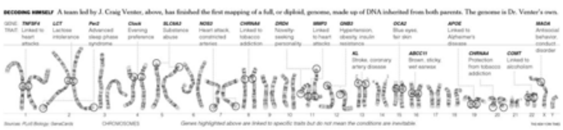
J. Craig Venter's genome
PLOS Biology 2007



"mapping by linkage"

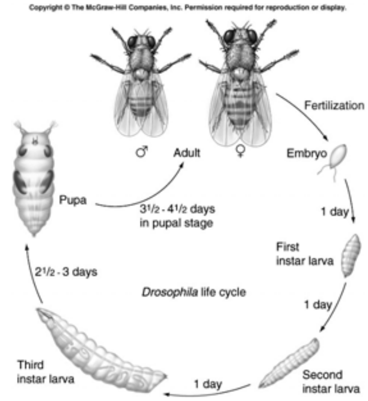
Thomas Hunt Morgan, the first native-born American to win the Nobel Prize, founder of modern genetics

A problem and a solution

"What was needed to open up genetics to new phenomena was an organism that bred rapidly, produced lots of progeny, and was inexpensive to maintain" (Carlson)

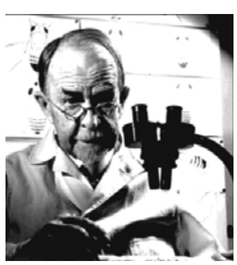
"The value and utility of any experiment…" (Mendel)

"Fruit flies can be raised on a mixture of corn meal, yeast, sugar, and agar. Flies complete their life cycle from fertilization to emergence of the adult fly in 10 days. A female can produce 3,000 progeny in her lifetime. A single male can sire well over 10,000 offspring." (Hartwell)



Tough early going

"… For this new approach, Morgan was his own first student. He bred the flies for two years without assistance. … He pointed to the shelves with flies and [said] that he had wasted two years and had gotten nothing for his work."



"May 1910 was when the revolution began. Morgan found a white-eyed male running around in one bottle."

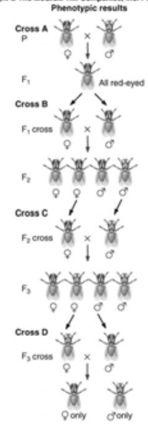
Morgan, T. H. 1910. Sex-limited inheritance in Drosophila, *Science*, 32: 120-122.

SEX LIMITED INHERITANCE IN DROSOPHILA

T. H. MORGAN

Woods Hole, Massachusetts

In a pedigree culture of *Drosophila* which had been running for nearly a year through a considerable number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes.

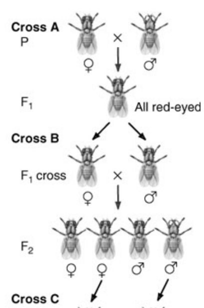


The white-eyed male, bred to his red-eyed sisters, produced 1,237 red-eyed offspring, (F_1), and 3 white-eyed males. The occurrence of these three white-eyed males (F_1) (due evidently to further sporting) will, in the present communication, be ignored.

The F_1 hybrids, inbred, produced:

2,459	red-eyed females,
1,011	red-eyed males,
782	white-eyed males.

No white-eyed females appeared. The new character showed itself therefore to be sex limited in the sense that it was transmitted only to the grandsons.



Nothing special here.

Just like seed color in peas.

Normal Mendelian ratio (3:1) – **but where are the white-eyed females?!!**

No white-eyed females appeared. The new character showed itself therefore to be sex limited in the sense that it was transmitted only to the grandsons. But that the character is not incompatible with femaleness is shown by the following experiment.

The white-eyed male (mutant) was later crossed with some of his daughters (F_1), and produced:

129	red-eyed females,
132	red-eyed males,
88	white-eyed females,
86	white-eyed males.

The results show that the new character, white eyes, can be carried over to the females by a suitable cross, and is in consequence in this sense not limited to one sex. It will be noted that the four classes of individuals occur in approximately equal numbers (25 per cent.).

Nettie Stevens, discoverer of the sex chromosomes

Nettie Stevens was one of the first female scientists to make a name for herself in the biological sciences. She was born in Cavendish, Vermont. Her family settled in Westford, Vermont. **Stevens' father was a carpenter and handyman.** He did well enough to own quite a bit of Westford property, and could afford to send his children to school.

Stevens was a brilliant student, consistently scoring the highest in her classes. In 1896, Stevens went to California to attend Leland Stanford University. She graduated with a masters in biology. Her thesis involved a lot of microscopic work and precise, careful detailing of new species of marine life. This training was a factor in her success with later investigations of chromosomal behavior.

After Stanford, Stevens went to Bryn Mawr College for more graduate work. Thomas Hunt Morgan was still teaching at Bryn Mawr, and was one of her professors. Stevens again did so well that she was awarded a fellowship to study abroad. She traveled to Europe and spent time in Theodor Boveri's lab at the Zoological Institute at Wurzburg, Germany. Boveri was working on the problem of the role of chromosomes in heredity. Stevens likely developed an interest in the subject from her stay.

In 1903, Stevens got her Ph.D. from Bryn Mawr, and started looking for a research position. She was eventually given an assistantship by the Carnegie Institute after glowing recommendations from Thomas Hunt Morgan, Edmund Wilson and M. Carey Thomas, the president of Bryn Mawr. Her work on sex determination was published as a Carnegie Institute report in 1905.

Nettie Maria Stevens, 1904. Photo courtesy of Carnegie Institute of Washington.

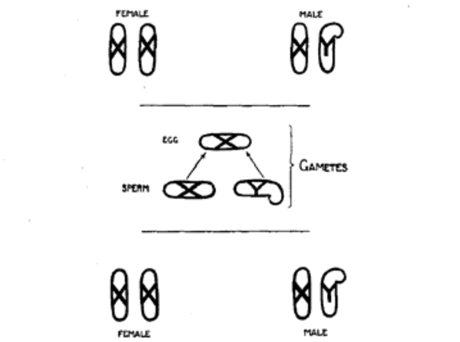
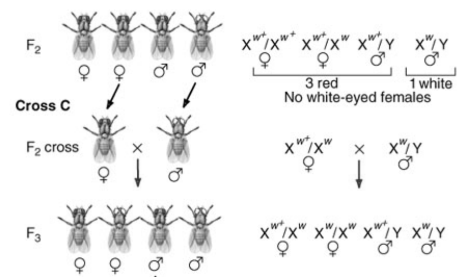


FIG. 8.—Diagram to show the history of the sex chromosomes from one generation to the next.

The white-eyed male (mutant) was later crossed with some of his daughters (F_1), and produced:

129 red-eyed females,
132 red-eyed males,
88 white-eyed females,
86 white-eyed males.



VERIFICATION OF HYPOTHESIS

First Verification.—If the symbol for the white male is WWX, and for the white female WWXX, the germ cells will be WX–W (male) and WX–WX (female), respectively. Mated, these individuals should give

WX — W (male)
WX — WX (female)

WWXX (50%) — WWX (50%)
White female — White male

All of the offspring should be white, and male and female in equal numbers; this in fact is the case.

Second Verification.—As stated there should be two classes of female in the F_2 generation, namely, RRXX and RWXX. This can be tested by pairing individual females with white males. In the one instance (RRXX) all the offspring should be red—

RX — RX (female)
WX — W (male)

RWXX — RWX

and in the other instance (RWXX) there should be four classes of individuals in equal numbers, thus:

RX — WX (female)
WX — W (male)

RWXX — WWXX — RWX — WWX

MENDELIAN SEGREGATION 19

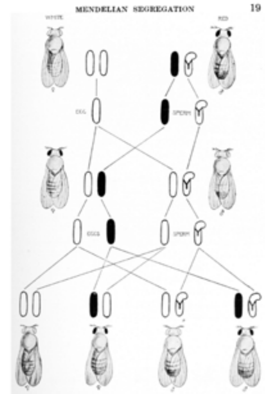


FIG. 10.—White-eyed female by red-eyed male (D. ampelophila). The factors for these characters are carried by the X chromosomes, the factor for red by the black X, and the factor for the white by the plain X. The history of the chromosomes is shown in the middle of the diagram.

It now becomes evident why we found it necessary to assume a coupling of R and X in one of the spermatozoa of the red-eyed F_1 hybrid (RXO). The fact is that this R and X are combined, and have never existed apart.

It has been assumed that the white eyed mutant arose by a male

Morgan et al. 1915

"Certain factors follow the distribution of the X chromosome and are therefore supposed to be **contained in them**."

Emphasis mine – fdu.

↓

Genes lie on chromosomes

Discovered linkage.

Invented the terms "allele, heterozygous, homozygous, homeotic."

"The supposition that particles of chromatin, indistinguishable from each other and **indeed almost homogeneous under any known test**, can by their material nature confer all the properties of life surpasses the range of even the most convinced materialism."

Bateson, W. (1916) The mechanism of Mendelian heredity (a review). *Science*, **44**, 536–5[4]3.

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Calvin Bridges

… "raised by his grandparents in upstate New York, both of his parents dying young. He was a talented student but his grandparents were poor and Bridges had to make do with clothing that was constantly mended. He was too ashamed to go to social activities in high school because of his ragged appearance. He received a scholarship to attend Columbia University, but he had to support himself with part-time work. Bridges took the same introductory biology course as Sturtevant, and Morgan, who learned of Bridges' circumstances, asked him to be a part-time bottle-washer and food preparator for the fly work that was gaining momentum in Morgan's laboratory." Carlson *Mendel's Legacy*

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vermilion

"… Bridges' circumstances changed approximately a year after he began working for Morgan. He showed Morgan a bottle that contained a fly whose eye color seemed to be brighter than usual. Morgan isolated the fly, showed that it carried another X-linked trait, and called that trait *vermilion*. He also assigned Bridges to a desk and told him to look for more mutations."

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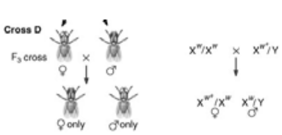
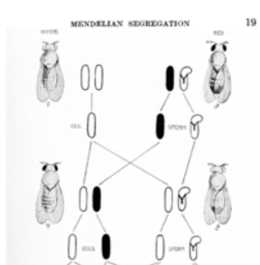
Criss-cross inheritance (what normally happens): white-eyed sons and red-eyed daughters of white-eyed mothers and red-eyed fathers





Therefore, a white-eyed mother and a red-eyed father cannot have a white-eyed daughter!

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The "exceptional female" appears

The advantage of the chromosomal interpretation as applied to the sex chromosomes is nowhere better illustrated than in the history of a process called non-disjunction, which was discovered by Bridges. Furthermore this case, supported on the one hand by extensive and definite experimental breeding and on the other hand by cytological investigation, offers the most direct evidence yet obtained concerning the relations of particular characters and particular chromosomes, for in this case an abnormal distribution of the sex chromosomes goes hand in hand with an identical abnormal distribution of all sex linked factors. It was found that females from a certain strain of white-eyed flies gave, on out-crossing, about 5 per cent. of unexpected classes. For instance, one of the white females crossed to a red-eyed male (wild type) produced not only red-eyed daughters and white-eyed sons, as expected, but also a few white-eyed daughters and a corresponding number of red-eyed sons. The approximate percentage in which these classes appeared is as follows:

Red ♀	White ♂	White ♀	Red ♂
47.5%	47.5%	2.5%	2.5%

In general, therefore, there were 95 per cent. of expected forms and 5 per cent. of offspring that were apparently inconsistent with expectation on the chromosome theory. Closer inspection of these

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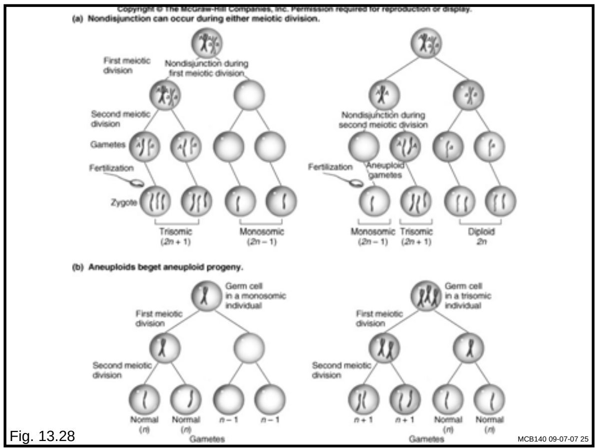
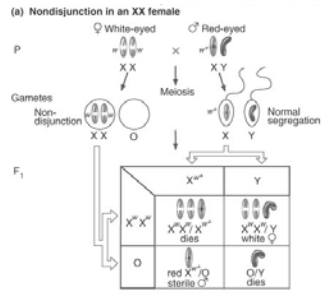
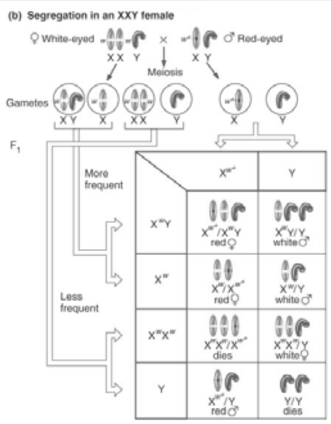


Fig. 13.28

How could a white-eyed mother have a white-eyed daughter?



Note:
An XXY *Drosophila* is female.
An XXY human is male.



Genetic evidence:

Bridges crossed those “exceptional females” to red-eyed males and showed that all the classes of individuals expected from the outcome of a meiosis in this XXY female do, in fact, appear.

He also provided **cytological** evidence that the exceptional female is, in fact, exceptional.

The results bear out to a remarkable degree the hypothesis that they are due to a non-disjunction of the sex chromosomes caused by the presence of a Y chromosome in the females.

The hypothesis is capable of verification and Bridges has made a study of the chromosomes of the non-disjunctional females. He finds that such

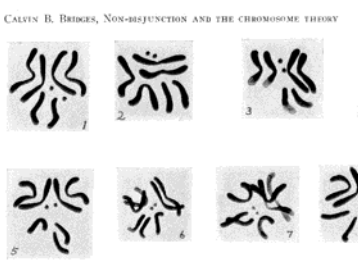
FIG. 49.—Group of chromosomes of an XXY female of a non-disjunctional "line."

females contain an extra chromosome whose size and position show that it is a supernumerary sex chromosome. The normal group of chromosomes of the female of Drosophila ampelophila is shown in Fig. 2, and a group from a non-disjunction female in Fig. 49. They differ by one chromosome, namely, the extra Y.

CALVIN B. BRIDGES, NON-DISJUNCTION AND THE CHROMOSOME THEORY

Normal female

White-eyed daughters
of an “exceptional” mother



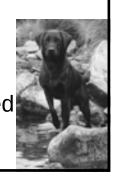
Extraordinarily precise concordance between the inheritance of chromosomes and particular traits argues that Mendel’s “factors of inheritance” (the genes) lie on chromosomes.

Genes lie on chromosomes. What else is there to be found out?

The next two major advances in genetics both came from the study of apparent exceptions to Mendel’s laws.

#1. Strong deviations from a 1:1:1:1 phenotyping ratio in a AaBb x aabb cross → “coupling and repulsion” → linkage → genetic map

#2. Highly aberrant phenotypic ratios (e.g., 9:3:4) when – for example – brother-sister mating black Labrador retrievers fathered by a black Dad and yellow Mom → epistasis



Hmmmmm

"It was not long from the time that Mendel's work was rediscovered that new anomalous ratio began appearing. One such experiment was performed by Bateson and Punnett with sweet peas. They performed a typical dihybrid cross between one pure line with purple flowers and long pollen grains and a second pure line with red flowers and round pollen grains. Because they knew that purple flowers and long pollen grains were both dominant, they expected a typical 9:3:3:1 ratio when the F1 plants were crossed. The table shows the ratios that they observed. Specifically, the two parental classes, purple, long and red, round, were overrepresented in the progeny."

http://www.ndsu.edu/instruct/mcclean/plsc431/linkage/linkage1.htm

What happened to Mendel's 2^{nd} law?!

	Observed	Expected
Purple, long (*P_L_*)	284	215
Purple, round (*P_ll*)	21	71
Red, long (*ppL_*)	21	71
Red, round (*ppll*)	55	24
Total	**381**	**381**

http://www.ndsu.edu/instruct/mcclean/plsc431/linkage/linkage1.htm

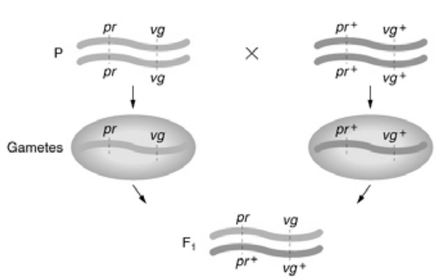
Morgan's observation of linkage

One of these genes affects eye color (*pr*, purple, and pr^+, red), and the other affects wing length (*vg*, vestigial, and vg^+, normal). The wild-type alleles of both genes are dominant. Morgan crossed *pr*/*pr* · *vg*/*vg* flies with $pr^+/pr^+ \cdot vg^+/vg^+$ and then testcrossed the doubly heterozygous F_1 females: $pr^+/pr \cdot vg^+/vg \times pr/pr \cdot vg/vg$.

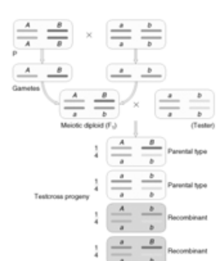
Clearly not 1:1:1:1

$pr^+ \cdot vg^+$	1339
$pr \cdot vg$	1195
$pr^+ \cdot vg$	151
$pr \cdot vg^+$	154
	2839

These two loci do not follow Mendel's second law because they are linked (=lie relatively close to each other on the same chromosome)

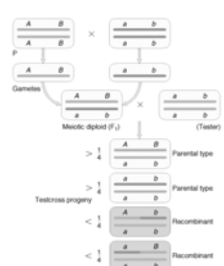


Two unlinked genes



1:1:1:1

Two linked genes



1:1:<<1:<<1

In place of attractions, repulsions and orders of precedence, and the elaborate systems of coupling, I venture to suggest a comparatively simple explanation based on results of inheritance of eye color, body color, wing mutations and the sex factor for femaleness in *Drosophila*. If the materials that represent these factors are contained in the chromosomes, and if those factors that "couple" be near together in a linear series, then when the parental pairs (in the heterozygote) conjugate like regions will stand opposed. There is good evidence to support the view that during the strepsinema stage homologous chromosomes twist around each other, but when the chromosomes separate (split) the split is in a single plane, as maintained by Janssens. In consequence, the original materials will, for short distances, be more likely to fall on the same side of the split, while remoter regions will be as likely to fall on the same side as the last, as on the opposite side. In consequence, we find coupling in certain characters, and little or no evidence at all of coupling in other characters; the difference depending on the linear distance apart of the chromosomal materials that represent the factors. Such an explanation will account for all of the many phenomena that I have observed and will explain equally, I think, the other cases so far described. The results are a simple mechanical result of the location of the materials in the chromosomes, and of the method of union of homologous chromosomes, and the proportions that result are not so much the expression of a numerical system as of the relative location of the factors in the chromosomes. *Instead of random segregation in Mendel's sense we find "associations of factors" that are located near together in the chromosomes. Cytology furnishes the mechanism that the experimental evidence demands.*

T. H. Morgan

September 10, 1911.

Morgan *Science* 1911

Batrachoseps attenuatus
California Slender Salamander

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F.A. Janssens

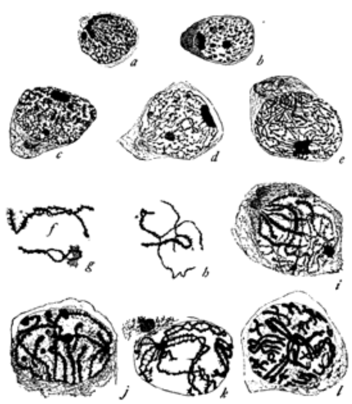

Fig. 43.—Spermatogenesis of Batracoseps attenuatus. *a*, late telophase

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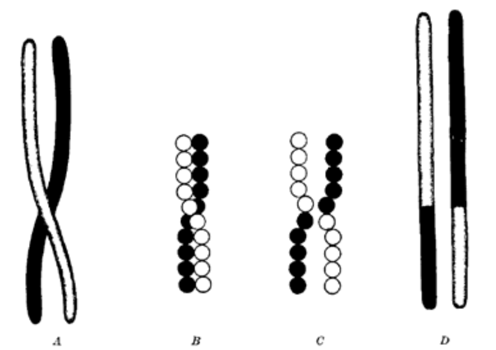

Fig. 24.—Diagram to represent crossing over. At the level where the black and the white rod cross in *A*, they fuse and unite as shown in *D*. The details of the crossing over are shown in *B* and *C*.

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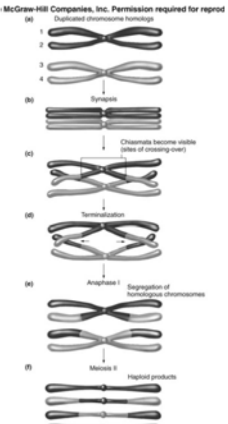

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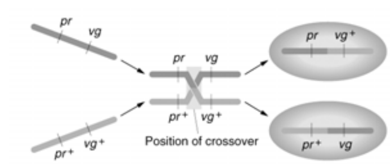
$pr^+ \cdot vg^+$	1339
$pr \cdot vg$	1195
$pr^+ \cdot vg$	151
$pr \cdot vg^+$	154
	2839



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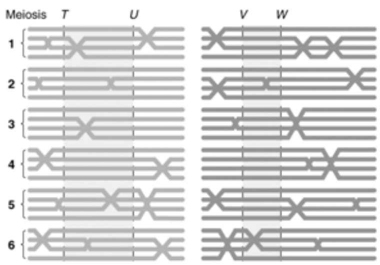
A concept that brings "simple" and "influential" to new shades of meaning



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Recombination Frequency (Morgan's data)

1339 red, normal
1195 vermillion, vestigial
151 red, vestigial
154 vermillion, normal

2839 total progeny.
305 recombinant individuals.

305 / 2839 = 0.107

Recombination frequency is 10%.

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Recombination frequency → a genetic map (Sturtevant's data)

pr vg/pr vg	165	parental
$pr^+\ vg^+/pr\ vg$	191	
$pr\ vg^+/pr\ vg$	23	recombinant
$pr^+\ vg/pr\ vg$	21	
	400	

pr^+ 11.0 vg^+

1% recombinant progeny =
1 map unit =
1 centimorgan (cM)
~ 1 Mb in humans.

→ If two genes lie on the same chromosome and are 1,000,000 bp apart, then, on average, 1% of the gametes made by any given individual will have a recombination event occur between these two genes.

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Calvin Bridges (left) and Alfred Sturtevant in 1920.

G. Rubin and E. Lewis *Science* **287:** *2216.*

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26 *GENETICS: W. E. CASTLE*

arrangement to be linear and in the group of genes most exhaustively studied, that of the 'sex chromosome' has represented them in a 'chromosome map, as shown in Diagram I.

That the arrangement of the genes within a linkage system is strictly linear seems for a variety of reasons doubtful. It is doubtful, for example, whether an elaborate organic molecule ever has a simple string-like form. Let us, therefore, examine briefly the evidence for or against the idea of linear arrangement of the genes. It is supposed by Morgan that two genes lying in the same chromosome show close linkage if they lie close together, but less linkage if they lie farther apart, and that the farther apart they are the less will be their linkage. As a measure of the distance apart of two genes he

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GENETICS: W. E. CASTLE 29

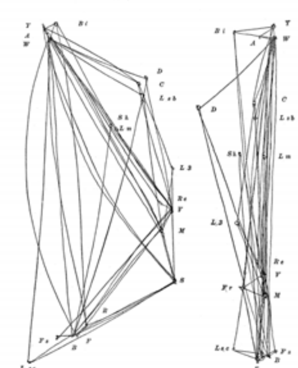

FIG. 1. SIDE VIEW OF MODEL

FIG. 2. EDGE VIEW OF MODEL
Seen at right angles to figure 1.

Showing relative positions of genes of 20 sex-linked characters of Drosophila, linear arrangement not being assumed. For significance of letters, compare Diagram I.

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There was an atmosphere of excitement in the laboratory, and a great deal of discussion and argument about each new result as the work rapidly developed.

In 1909 Castle published diagrams to show the interrelations of genes affecting the color of rabbits. It seems possible now that these diagrams were intended to represent developmental interactions, but they were taken (at Columbia) as an attempt to show the spatial relations in the nucleus. In the latter part of 1911, in conversation with Morgan about this attempt—which we agreed had nothing in its favor—I suddenly realized that the variations in strength of linkage, already attributed by Morgan to differences in the spatial separation of the genes, offered the possibility of determining sequences in the linear dimension of a chromosome. I went home and spent most of the night (to the neglect of my undergraduate homework) in producing the first chromosome map, which included the sex-linked genes *y*, *w*, *v*, *m*, and *r*, in the order and approximately the relative spacing that they still appear on the standard maps (Sturtevant, 1913).

Sturtevant 1961

The three-point testcross

From my perspective, the single most majestic epistemological accomplishment of "classical" genetics.

Let us consider three linked (=on the same chromosome) genes.

1. Determine the genetic distance between each one.
2. Show, that the genes are in **linear** order.

Sturtevant, A. H. 1913. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. *Journal of Experimental Zoology*. 14: 43-59.

THE LINEAR ARRANGEMENT OF SIX SEX-LINKED FACTORS IN DROSOPHILA, AS SHOWN BY THEIR MODE OF ASSOCIATION

A. H. STURTEVANT

HISTORICAL

The parallel between the behavior of the chromosomes in reduction and that of Mendelian factors in segregation was first pointed out by Sutton (1902) though earlier in the same year Boveri (1902) had referred to a possible connection. In this paper and others Boveri brought forward considerable evidence from the field of experimental embryology indicating that the chromosomes play an important role in development and inheritance. The first attempt at connecting any given somatic character with a definite chromosome came with McClung's (1902) suggestion that the accessory chromosome is a sex-determiner. Stevens (1905) and Wilson (1905) verified this by showing that in numerous forms there is a sex chromosome, present in all the eggs and in the female-producing sperm, but absent, or represented by a smaller homologue, in the male-producing sperm. A further step was made when Morgan (1910) showed that the factor for color in the eyes of the fly *Drosophila ampelophila* follows the distribution of the sex chromosome already found in the same species by Stevens (1908). Later, on the appearance of a sex-linked wing mutation in *Drosophila*, Morgan (1910a, 1911) was able to make clear a new point. By crossing white-eyed, long-winged flies to those with red eyes and rudimentary wings (the new sex-linked character) he obtained, in F_2, white-eyed,

Sturtevant's remarkably simple and elegant argument

Let's consider three genes, A, B, and C.

If the distance from A to B is "x"

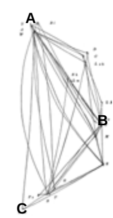
… and the distance from B to C is "y"

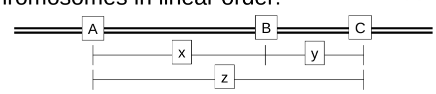
… and the distance from A to C is "z"

… then (drum roll), we find that:

$$x + y \approx z$$

This means that genes are arranged on chromosomes in linear order:





How to Map Genes Using a Three-Point Testcross

1. Cross two pure lines.
2. Obtain large number of progeny from F1.
3. Testcross to homozygous recessive tester.
4. Analyze large number of progeny from F2.

P *v+/v+ · cv/cv · ct/ct* × *v/v · cv+/cv+ · ct+/ct+.*

↓

F1 *v/v+ · cv/cv+ · ct/ct+* × *v/v · cv/cv · ct/ct.*

↓

Two *Drosophila* were mated: a red-eyed fly that lacked a cross-vein on the wings and had snipped wing edges to a vermilion-eyed, normally veined fly with regular wings. All the progeny were wild type. These were testcrossed to a fly with vermilion eyes, no cross-vein and snipped wings. 1448 progeny in 8 phenotypic classes were observed.

Map the genes.

$v \cdot cv^{+} \cdot ct^{+}$	580
$v^{+} \cdot cv \cdot ct$	592
$v \cdot cv \cdot ct^{+}$	45
$v^{+} \cdot cv^{+} \cdot ct$	40
$v \cdot cv \cdot ct$	89
$v^{+} \cdot cv^{+} \cdot ct^{+}$	94
$v \cdot cv^{+} \cdot ct$	3
$v^{+} \cdot cv \cdot ct^{+}$	5
	1448

1. Rename and rewrite cross

For data like these, no need to calculate χ^2. Begin (you don't *have* to, but it helps) by designating the genes with letters that look different in UPPER and lowercase (e.g., not "W/w" but "Q/q" or "I/i"):

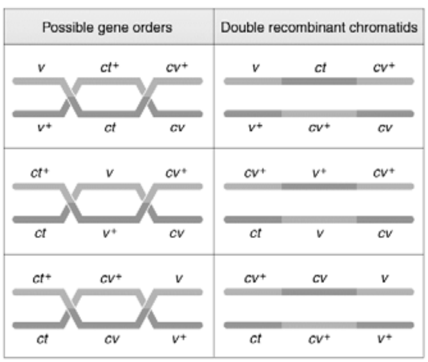
eye color: v^+/v = E/e
vein on wings: cv^+/cv = N/n
shape of wing: ct^+/ct = F/f (you fly using wings)

P:	EE nn ff	x	ee NN FF
test-cross:	Ee Nn Ff	x	ee nn ff

2. Rewrite data

Arrange in descending order, by frequency.

NCOs	e	N	F	580
	E	n	f	592
	e	n	F	45
	E	N	f	40
	e	n	f	89
	E	N	F	94
DCOs	E	n	F	5
	e	N	f	3



3. Determine gene order

e	N	F	580
E	n	f	592

With the confusion cleared away, determine gene order by comparing most abundant classes (non-recombinant, NCO) with double-recombinant (least abundant, DCO), and figuring out, which *one allele pair needs to be swapped between the parental chromosomes in order to get the DCO configuration.* This one allele pair will be of the gene that is in the middle.

E	n	F	5
e	N	f	3

3b. Determine gene order

NCOs: Enf, eNF → DCOs: EnF, eNf

Gene order: E F N (or N F E).

4. E and F

Next, map distance between genes E and F by comparing the number of single recombinants (COs) for those two genes with the number of NCOs.

e	N	F	580
E	n	f	592
e	n	F	45
E	N	f	40
e	n	f	89
E	N	F	94
e	N	f	3
E	n	F	5

RF=(89+94+3+5)/1448=0.132

The E and F genes are separated by 13.2 m.u.

4b. F and N

Now, map distance between genes F and N by comparing the number of single recombinants (COs) for those two genes with the number of NCOs.

e	N	F	580
E	n	f	592
e	n	F	45
E	N	f	40
e	n	f	89
E	N	F	94
e	N	f	3
E	n	F	5

RF=(45+40+3+5)/1448=0.064

The F and N genes are separated by 6.4 m.u.

4c. E and N

Finally, map distance between genes E and N by comparing the number of single recombinants (COs) for those two genes and the number of DCOs for those two genes with the number of NCOs. Count DCOs twice because they represent *two* recombination events, and to calculate the correct RF we must, by definition, count every recombination event that occurred between those two genes (even if it doesn't result in a recombinant genotype for those two genes!).

e	N	F	580
E	n	f	592
e	n	F	45
E	N	f	40
e	n	f	89
E	N	F	94
e	N	f	3
E	n	F	5

RF=(45+40+89+94+3+5+3+5)/1448=0.196

The E and N genes are separated by 19.6 m.u.

5. The map (ta-daaa!)