Cytogenetics: (after Jackson): "the study of the correlated behavior of chromosomes and their attendant genes or other markers in meiosis and mitosis through nuclear, cell, and recombinant generations." as well as their evolution, structure, and biology.

- We take genes and chromosomes for granted, but at one time, they were remarkable discoveries
- History gives insight into the thoughts and processes that led to this realization
  - In many ways, is almost a comedy of errors

How did they determine that genes existed and were on chromosomes?

The Cell Theory

- 1590: Development of the compound microscope
  - Original use: parlor game
- 1665: Robert **Hooke**, curator of the Royal Society, discovers cells in cork and pith of alder trees. Publishes *Micrographia*
- The next 100 years were without any major studies
  - Poor quality of microscopes limited possible observations

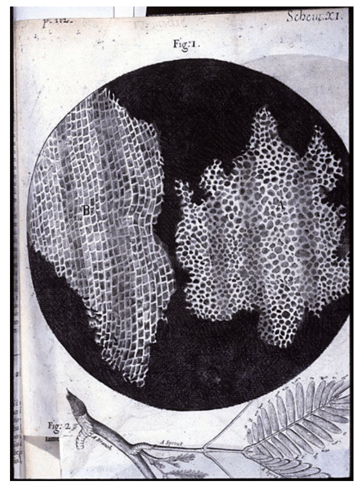


Figure 1. Hooke, Robert, 1635-1703. Micrographia: or some physiological descriptions of minute bodies made by magnifying glasses with observations and inquiries thereupon. London: Printed for John Martyn, 1667.

Most people believed in preformation (as opposed to epigenesis)

- If everything was preformed, there was little reason to study it
- Only argument was whether preformation was in the cell or in the egg
- Most people favored the sperm, even though contradicted by cases of parthenogenesis

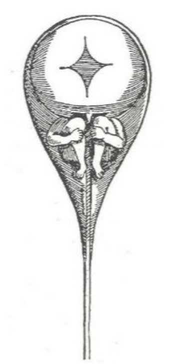


Figure 2. Nicolaus Hartsoeker, 1694, Essai de diotropique

Figure 3. https://en.wikipedia.org/wiki/Caspar_Friedrich_Wolff
https://en.wikipedia.org/wiki/Karl_Ernst_von_Baer
https://en.wikipedia.org/wiki/Marcello_Malpighi

1790 - 1820: Chicken embryology

Wolff and von Beer: Differentiation from undifferentiated egg

Malpighi: Preformation in chicken eggs

- Actually looked at eggs that had been incubated in the summer heat. Nevertheless, his preformation view prevailed

https://www.physik.uni-augsburg.de/theo1/hanggi/History/Robert_Brown_Vortrag.pdf
https://www.sutori.com/item/theodor-schwann-839f
'www.schleiden.uni-jena.de

- 1825-1830: lenses corrected for chromatic aberrations and spherical aberrations become commercially available

- 1833: Robert **Brown** discovers the cell nucleus as a component of all cells, and establishes a nucleated cell as the basic structural unit of living plants

- 1838: Matthias Jacob **Schleiden** (botanist): first to insist nucleus was of central importance, but thought that nuclei formed from protoplasmic granules

- 1839: Theodor **Schwann** (zoologist) found that animals are also made of cells

__Cell theory__: the cell is the fundamental unit of organization, structure, and function
Based on **Schleiden and Schwann:**

- Problem: Where did cells come from?

Where do cells come from?

- Schleiden and Schwann favored free formation from protoplasm (the fluid contents of a cell, as defined by J.E. **Purkinje** in 1840), with the nucleus forming first.

https://en.wikipedia.org/wiki/Robert_Remak
https://en.wikipedia.org/wiki/Carl_N%C3%A4geli
https://en.wikipedia.org/wiki/Rudolf_Virchow

1841: Robert **Remak**: embryonic blood cells are derived from division of previous cell

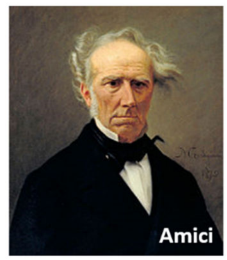
1846: Karl von **Nägeli**: plant cells are derived from division of a previous cell. Thought nucleus still formed from protoplasm.

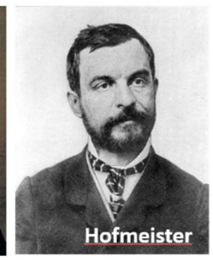
1855: Rudolf **Virchow**: "omnis cellula e cellula" --Every cell comes from a cell

Origin of new individuals

- Gametes not recognized as cells for some time
- Purpose and function of fertilization was not understood
- Observation was made that pollen tubes grew towards the ovule
  - Thought that ovules were incubating places for the pollen tubes
  - Schleiden and Schwann were working with cucurbits, in which the pollen tube enlarges and persists

1849: **Amici** and **Hoffmeister**: In higher plants, embryo comes from the egg, not the pollen tube





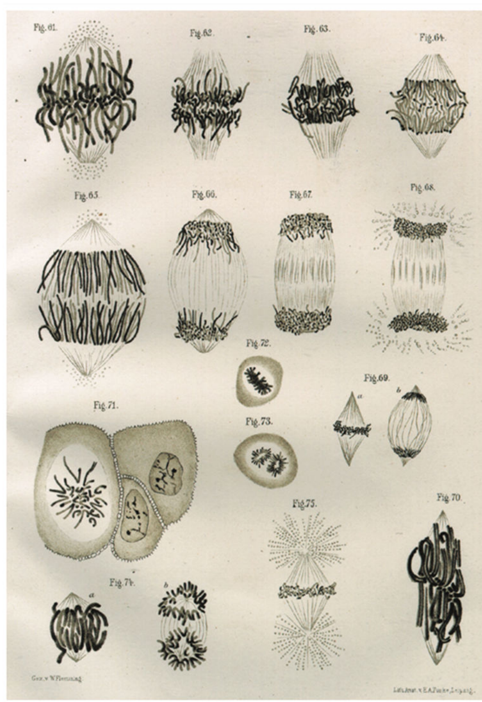
https://prabook.com/web/wilhelm.hofmeister/3723302
https://en.wikipedia.org/wiki/Giovanni_Battista_Amici

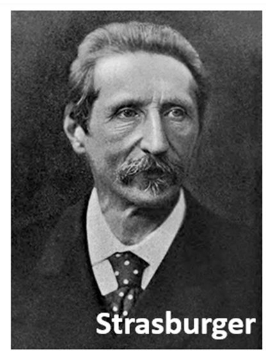
The chromosome theory of heredity

1870: Eduard **Strasburger &** Walther **Flemming**: established physical continuity of nuclear contents between cell generations-- nuclei were no longer formed anew each time– ie, observed nuclear division

1874-1900: The Classic or Golden era of cytogenetics

- Discovery of chromosomes, fertilization, mitosis, meiosis, and apomixis
- Better fixatives and stains (the coal tar dyes -- all carcinogenic)

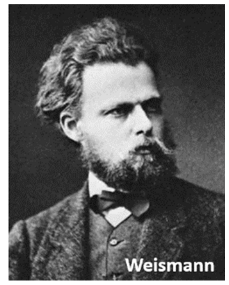


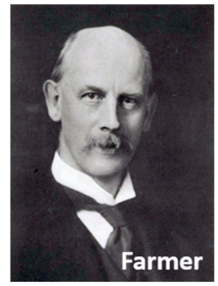


https://www.deutsche-botanische-gesellschaft.de/en/about-us/promoting-young-scientists/eduard-strasburger-prize/vita-eduard-strasburger/
https://www.uni-kiel.de/grosse-forscher/index.php?nid=flemming&lang=e

- Microtome and improved microscope
- Condensers with numerical apertures similar to that of objectives

Cytogenetics is currently experiencing a revival driven by genomics





https://alchetron.com/Oscar-Hertwig
https://en.wikipedia.org/wiki/Carl_Rabl#/media/File:Plate_17_Carl_Rabl,_Photograph_album_of_German_and_Austrian_scientists_(cropped).png
http://www.vliz.be/wetenschatten/beeldbank.php?pic=41628

https://kids.britannica.com/kids/assembly/view/32294

https://www.jstor.org/stable/769107?seq=1#metadata_info_tab_contents

1875: Oskar **Hertwig** discovered fertilization in sea urchins: Fertilized egg has 2 nuclei, one from the egg itself, and one from the sperm

1877: Eduard **Strasburger** discovers fertilization in Spirogyra

1879: **Strasburger** discovers the chromosome

1882-1884: **Flemming, Carl Rabl**, and **Edouard van Beneden** describe the behavior of chromosomes during mitosis (a term coined by **Flemming**) in Ascaris, 2n = 4: Chromosome number in somatic cells was constant

1883: **van Beneden**: chromosome & and % contributions (egg and sperm) were equal

This made it obvious that a reduction division must occur in order to prevent chromosome numbers from doubling each time fertilization occurs

1887/8: August **Weismann**: Concept that reduction of chromosome numbers in gametes offsets the increase during fertilization. First to unite cytology with genetics.

- However, the thought was that ½ the chromosomes simply degenerated
    - Realized there must be two types of cellular divisions:

Mitosis:		→		(longitudinal)
?:		→		(transverse)

Oskar Hertwig and T. Boveri, working with *Ascaris*, found longitudinal divisions during the reduction of chromosome number, but disregarded it as was not transverse as Weismann thought it should be.

-Later, Strasburger and E. Overton found the same in plants.

Details of chromosome reduction were by J.B. **Farmer** and J.E. **Moore**.

- Moore coined the term **maiosis** to describe the process, from the Greek "meioun", which means to diminish.

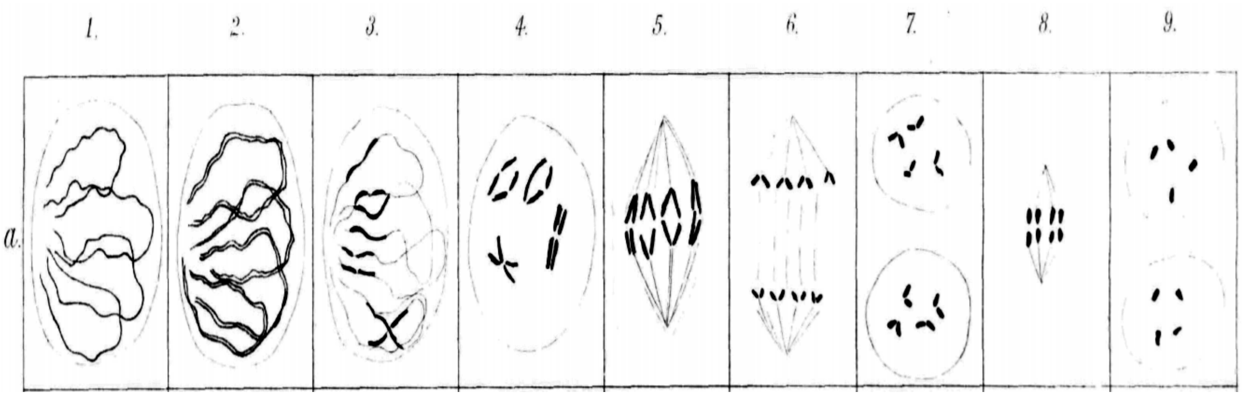
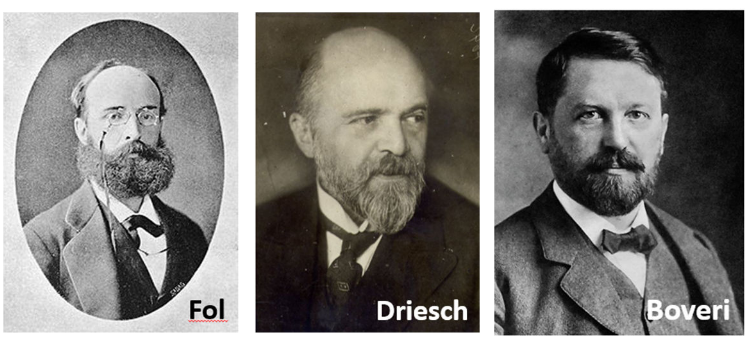


Figure 4 Figure 4 Farmer JB & JES Moore. 1905. On the maiotic phase (reduction divisions) in animals and plants. Quarterly Journal of Microscopical Science 48: 489–557

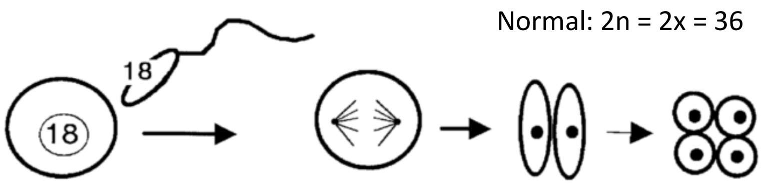
1888 Discovery that all chromosomes are not alike



https://en.wikipedia.org/wiki/Hermann_Fol
https://alchetron.com/Hans-Driesch
https://fineartamerica.com/featured/theodor-boveri-american-philosophical-society.html

Knew ahead of time that:

Normal: 2n = 2x = 36



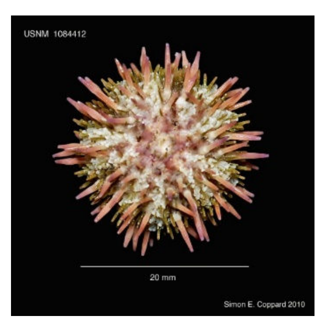
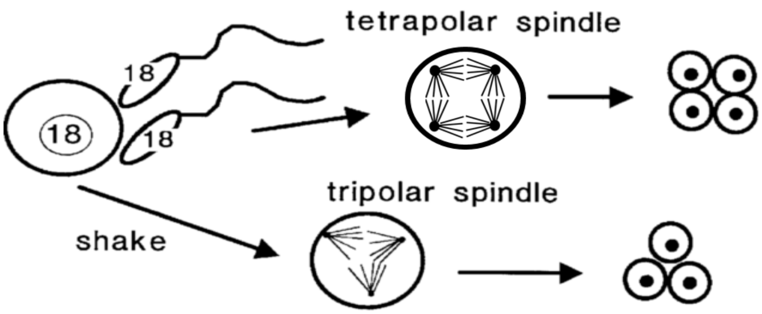


Figure 5
https://eol.org/pages/600396



Dispermic eggs are triploid– that is, they have 3 sets of chromosomes that are split into 4 nuclei in tetrapolar spindles. With more nuclei than genomes, it is not possible to get complete sets into any one nucleus.

- Previously knew:

- Herman **Fol**: dispermic eggs were abnormal after the blastula stage for some unknown reason
- Hans **Driesch**: looked at 80 dispermics → all were abnormal
    - Fused 2 zygotes → got normal development

- ∴ the disturbance is not due to chromosome number alone

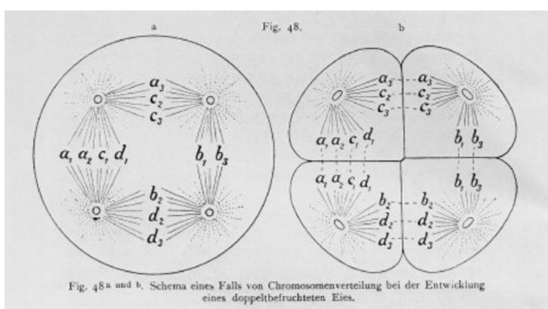


Fig. 48a und b. Schema eines Falls von Chromosomenverteilung bei der Entwicklung eines doppeltbefruchteten Eies.

Dispermic zygotes have 3 copies of each chromosome. The role of diploid mitosis is to ensure non-random distribution of chromosomes. If that is disrupted, distribution of chromosomes gets a random component. Diagram by Boveri, 1904.

-Boveri: concluded that abnormalities due to unequal chromosome distribution:

- Observed dispermic zygotes and found nuclei of differing sizes

- Ruled out importance of total chromosome number as:

- normally have 36 chromosomes
- parthenogenesis → normal urchins with only 18 chromosomes
- tetraploids → normal urchins with 72 chromosomes

- Ruled out de-arrangements of cytoplasm, as the cytoplasmic distribution of dispermics was similar to that of normal

- ∴ <u>normal development happens only if each cell gets the correct set of chromosomes.</u>

- I.e., individual chromosomes must have different qualities → concept that chromosomes come in sets

If chromosome distribution is at random, would expect more normals from tri than from tetrapolar spindles

- Looked at: 1500 tetrapolars → 1? normal = 0.1%
  719 tripolars → 58 normals = 8%

- **Driesch:** 4-cell stage in Ca-free water → cells fall apart, and each develops into small but normal individual
- Theodor **Boveri:** used Driesch's technique to separate cells from dispermics
  - Chance of getting the correct chromosomes in 1 cell > in 3 or 4 cells
  - Tripolar separations should also give a greater number of normal separations than tetrapolar separations

- 23 tetrapolar → separated into 92 cells → 17 normal = 18%
- 34 tripolar → separated into 102 cells → 44 normal = 43%

Percent of normal gastrulae from one zygote		
	Tripolar (n = 34)	Tetrapolar (n = 23)
4		0.0
3	14.4	4.5
2	22.8	4.5
1	40.0	54.5
0	22.8	36.5

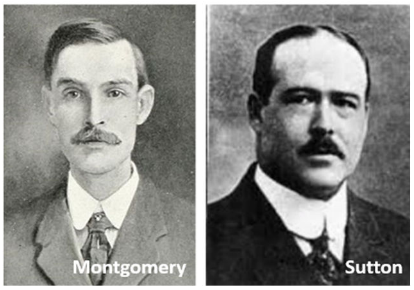
Conclusions:

1) Normal development depends on normal combination of chromosomes established at fertilization and maintained by mitosis
2) Chromosome distribution is upset at mitosis in dispermic zygotes
3) Normal combinations can occur:
   - Tripolars: Cells and intact embryos
   - Tetrapolars: Cells only (sample size)
4) Different chromosomes have different roles in development

1900: **Mendel**'s work is rediscovered

- Raises the question whether chromosome movement follows the laws of heredity

The chromosome theory of heredity



https://en.wikipedia.org/wiki/Thomas_Harrison_Montgomery_Jr.
https://en.wikipedia.org/wiki/Walter_Sutton

Figure 6 On the morphology of the chromosome group in Brachystola magna, 1902

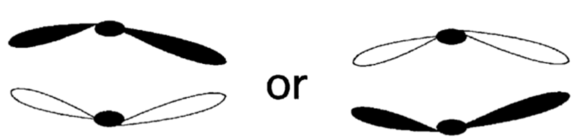
1901: Thomas Harrison **Montgomery** describes meiosis in insects

- Did the cytological observations that explained Mendel (but did not do so himself)
- Noticed constant size differences which appeared in symmetrical pairs
  - These paired at meiosis
  - Were homologues, one from each parent, and separate at meiosis

1902: Walter Stanborough **Sutton** "The Chromosomes in Heredity"

The chromosome theory of heredity

1) Somatic chromosomes are in 2 groups: 1 from& and 1 from % parent
   - Based on van Beneden, Boveri, and Montgomery
2) Individual chromosomes are identifiable and continuous throughout the life cycle
   - After van Beneden, Boveri, and Rall
3) Each chromosome is genetically different
   - After Boveri
4) Pairing of homologues occurs at meiosis
   - After Montgomery and himself
5) Homologues separate at meiosis
   - After Montgomery and himself
   - *First to relate this to Mendel's law of segregation*
6) Speculated that orientation of chromosomes at metaphase I was at random, and was the basis for Mendel's independent assortment



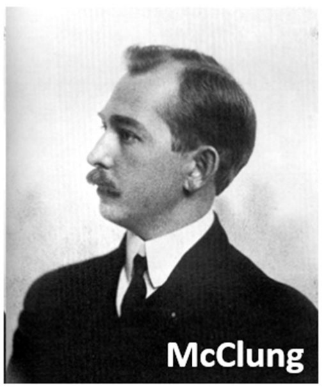
Conclusion: Sutton applied Mendel's laws to cytogenetics

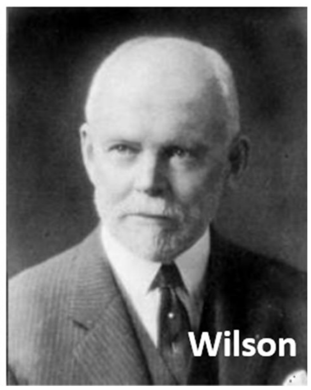
Sutton and Boveri

Chromosome theory of heredity– gave simple definition for genes on chromosomes and explains the patterns of heredity

- Beginning of cytogenetics

The sex chromosome





http://www.esp.org/people/
https://www.britannica.com/biography/Edmund-Beecher-Wilson
BRYN MAWR COLLEGE LIBRARY, SPECIAL COLLECTIONS

1902: Clarence Erwin **McClung** discovers the accessory chromosome

Suspected was the male sex determinant
Previously **Henking** studied meiosis in Orthopteran insects

- Unequal distribution of nuclear component at meiosis
- Resulted in 2 types of sperm, 1 with and one without the X factor

- Is a chromosome in nature
- Present in all cells up to meiosis
- Present in half the sperm
- Responsible for or involved in sex determination
- First time anyone associated a particular trait with a particular chromosome
- However, thought that X sperm were the %

1905: E.B. (Edmund Beecher) **Wilson** - 50:50 segregation in sperm
1905: Nettie **Stevens** - Males come from sperm with large/small chromosomes
1909: E.B. **Wilson** & = XX, % = X_

Independent assortment

1913 – Estrella Eleanor **Carothers**

https://en.wikipedia.org/wiki/Eleanor_Carothers

The cytological basis for independent assortment

- Independent assortment of 2 pairs of alleles paralled by independent assortment of chromosomes
- Needed 2 pairs of heteromorphic chromosomes, as in % *Brachystola* grasshoppers:

Figure 7. X = the accessory chromosome; c denotes the heteromorphic large/small

Used Sutton's drawings, plus more of her own
- 1st was the X/O pair
- 2nd was a large/small heteromorphic pair

- Looked at 300 Anaphase I divisions:

Left: X/large at top; O/small at bottom: $^{154}/_{300}$ = 51.3%
Right X/small at top; O/large at bottom: $^{146}/_{300}$ = 48.7%

- There were two possible combinations, and each occurred half the time.

1935: Carothers found that *Trimerotropis* % grasshoppers had from 1 to 8 heteromorphic pairs per individual
- Differences in size, position of centromere, or spindle attachment
- Selected one with four heteromorphic pairs (X/O and 3 large/small)
- Looked at 100 meiocytes at anaphase I (gives 200 cells after 1st division)

Recovery of:	N	Probability	Expected	Observed
A given large chromosome	× 200	½	100	100
2 given large chromosomes	× 200	½ × ½	50	46/47
3 given large chromosomes	× 200	½ × ½ × ½	25	22/21
All large chromosomes	× 200	½ × ½ × ½ × ½	12.5	8

<u>The first use of stats in genetics</u>

Calculating probabilities

Next, looked at 100 meiocytes. If the probability of recovery of

a small homologue = a = ½

a large homologue = b = ½,

Calculate probabilities by expanding the binomial $(a+b)^4$: Begin by incremental exponents

$a^4b^0 + a^3b^1 + a^2b^2 + a^1b^3 + a^0b^4$, then use Pascal's triangle to get the coefficients:

```
0                     1

1                 1   +   1
                  ↓
2             1       2   +   1
                      ↓
3         1       3       3   +   1
                          ↓
4     1       4       6   +   4   +   1
```

and there are *4* chromosome pairs, then $(a+b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4ab^3 + b^4$, where:

Recovery of:	Probability	Expected	Observed
Any 1 large chromosome	$4a^3b$	50	48
Any 2 large chromosomes	$6a^2b^2$	75	84
Any 3 large chromosomes	$4ab^3$	50	48
All 4 large chromosomes	b^4	12	8

- <u>Convincing evidence for independent assortment of chromosomes</u>
- <u>Further evidence that genes are on chromosomes</u>

Non-disjunction

<u>Final proof that genes were on chromosomes</u>

1913 – Calvin Blackman **Bridges**

- Non-disjunction as proof of the chromosome theory of heredity
- PhD thesis: *Genetics* 1:1-53; 107-163
    - Journal started by George H. Shull

Figure 7. mblhistoryproject.tumblr.com/post/130907349268/people-of-the-lab-calvin-bridges

- Was at Columbia, with Morgan, Sturtevant, and Muller

- Used *vermilion*, a sex-linked eye-color trait in Drosophila:

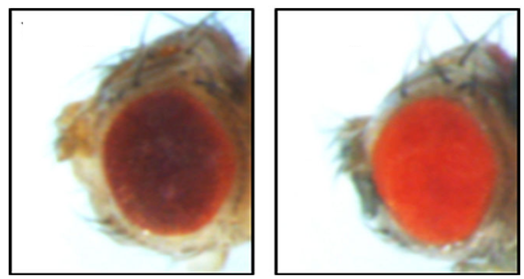


Figure 8. Red eye color in Drosophila. https://bio-protocol.org/e3147

	vermilion ♀ (*v*/*v*)		×	red ♂ (+/Y)		or	red ♀ (+/+)		×	vermilion ♂ (*v*/Y)	
			↓						↓		
F_1	red ♀ (+/*v*)		+	vermilion ♂ (*v*/Y)			red ♀ (+/*v*)		+	red ♂ (+/Y)	
			↓						↓		
F_2	¼ red ♀ (*v*/+)	¼ verm. ♀ (*v*/*v*)	+	¼ red ♂ (+/Y)	¼ verm. ♂ (*v*/Y)		¼ red ♀ (+/+)	¼ red ♀ (+/*v*)	+	¼ red ♂ (+/Y)	¼ verm. ♂ (*v*/Y)

However, the cross of *v*/*v* × +/Y (ie, the F_1) gave exceptional progeny every once in a while:

1) vermilion & (sex-linked trait from their mother) = $^1/_{5000}$
2) red % (x-linked trait from their father) = $^1/_{1200}$

- Could explain these results if both X chromosomes in & went to one pole during meiosis (<u>non-disjunction</u>)

Primary non-disjunction

v/*v* × +/Y
↓

v + + → red ♀
v + Y → vermilion ♂

normal

$^1/_{5000}$ (*v*/*v*)	+	(+)	→	*v*/*v*/+	→	usually dies
$^1/_{5000}$ (*v*/*v*)	+	(Y)	→	*v*/*v*/Y	→	exceptional vermilion &
$^1/_{1200}$ (--)	+	(+)	→	+	→	exceptional red sterile %
$^1/_{1200}$ (--)	+	(Y)	→	Y	→	lethal

-Bridges confirmed above results cytologically

Secondary non-disjunction

vermilion ♀ (X/X/Y) × red ♂ (X/Y)
↓

♀:	96% red	4% vermilion	exceptions
♂:	96% vermilion	4% red	

	Eggs - 4 types:			
♂\♀	X^vY	X^v	X^vX^v	Y
X^+	X^vX^+Y red ♀	X^+X^v red ♀	XXX die	X^+Y exc. red ♂
Y	X^vYY verm ♂	X^vY verm ♂	X^vX^vY exc. verm ♀	YY die

Predictions: (all verified cytologically)

1) ½ red & should have extra Y
2) ½ vermilion % should have extra Y
3) exceptional red % should have Y and be fertile

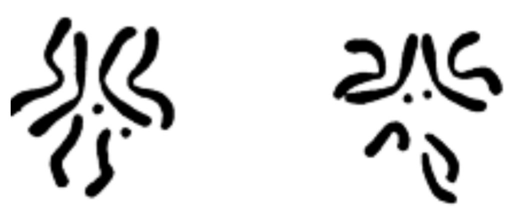


Figure 9. A normal XY (L) and an XXY daughter (R) from an exceptional mother.

<u>Significance:</u> showed that genes are on chromosomes

In summary at this point:

- Primary non-disjunction: XX → XX
- Secondary non-disjunction: XXY → X **+** XY, or → XX + Y

1922 – Lilian Vaughn **Morgan**

Attached X

- found 100% non-disjunction in Drosophila
- caused by attached X chromosomes

Figure 11. A ♀ with an attached X. Such XXXY females survive, while regular XXX condition is lethal.

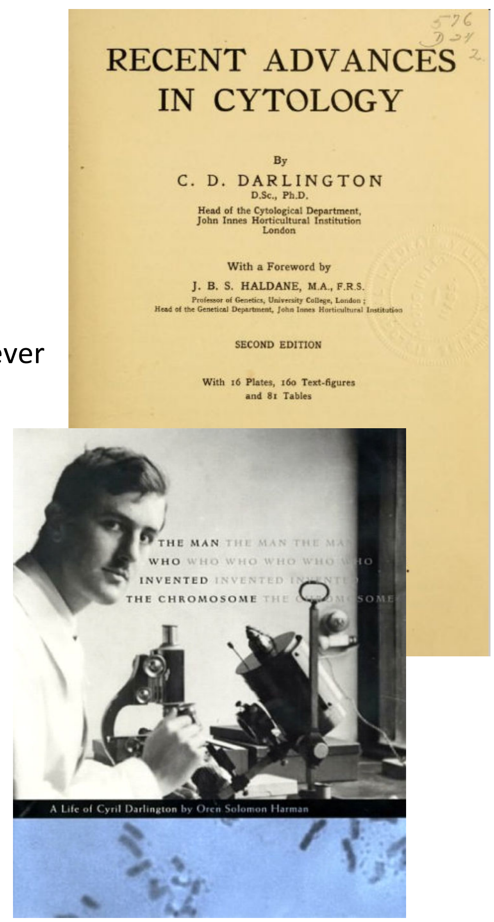
Figure 10. Wikipedia commons

The Big Picture

1932 – Recent Advances in Cytology, by
Cyril Dean **Darlington**

- Seminal book on chromosome behavior and evolution

- Speculative extrapolations
- Provided explanations for CO, sporogenesis, etc, but never tried to prove them or show they were correct
    - Very controversial
    - Most has been shown to be correct
        - Taken a whole century



Darlington: Hypothesis → data (like Mendel)
Prevailing view: Data → explanatory hypothesis

Like many of his contemporaries, lost credibility by embracing eugenics