MA 21/02 Intro to Dros

- 1906 1st Dros paper Castle, 1st experimental inbreeding fecundity
- D mel, 1839 described by Meigen
- culture, bananas
- 1908 TH Morgan sought org to study evol in lab, Columbia U.
- 1st variants pigment on thorax
- 1910, single male white-eyed fly
(- 1869 Mendel, 1900/1901 rediscovered) Morgan not convinced
- Morgan noticed new pattern of inheritance
- EB Wilson - 1895 → 1925 last ed of cyt. book
- 1907 Natalie Stevens n = 8

♀ ≫ II ≪ ♂ ≫ IV ≪

by convention, ♀ = x ♂ = y

w♂ × wt♀ → all wt.

wt♂ × w♀ → ♂ w, ♀ wt

- 1st evidence that Mendelian factors were physically carried by chromosomes
- Science, 1910, start of Dros. genetics (1st great achiev. of Morgan)
- 2nd g't achiev. of THM, group at Columbia
- Calvin Bridges, bottle washer 1939 college job
  - found vermillion, THM hired him
  - maps, found many μ
  - 1st cataloguer
- HJ Muller (Joe)
  - 1st aberrations, translocations
  - what is nature of gene, μ
  - 1928, Science X-rays/ionizing rad → μ

Natural History — Morgan 1942 dies

- Stadler 1928 in maize showed similar result
- Muller → Texas → Sov. Union → 1936 → Spain → England → Indiana
  - political active against atmospheric nuc. test.
- Alfred Sturtevant (died 1971)
  - supervised Levit
  - greatest classical geneticist in Pros
- 1912-1936 Morgan School esti class genet as a discipline, mechanisms
- Dobzhansky first started work on pop. w/ Stvt
- Stvt started comparative work

- Drosophilidae ~4000 species, 28 in india
- most geographically restricted, ex Hawaiian
- late 1950s, Texas group
- D. mel cosmopolitan, commensal, ecologically versatile, breeding conditions variable, temp tolerant
- 12°-30° C late tolerance
- most temperature sensitive to humidity
- Origin in Africa, W. Africa, Zimbabwe
- egg → egg 10 days at 25°C
- ♀ are very fecund, couple hundred eggs a day for many days
- Bridges introduced agar media & incubators 25°C

♀ —24 hrs (chorion)→ L1 —24 hrs→ L2 —24 hrs→ L3 —48 hrs→ "tan pupae" —metamorphosis→ prepupae

- Saprophagous, eat bacteria & yeast
- ecdysone, steroid hormone
- eclosion hormone preferences
- burrow adult cuticle hardens
- ♂ mate w/in few hrs 2 hrs
- ♀ can't mate w/in 1st 8 hrs, lag in sexual maturity

line of weakness operculum

- Zimbabwe
- ACPs
- ~~Drosophila~~ hawaiian dros, molecular clock

Chap 6 - life cycle

- w/out lag, Dros genetic would be much harder
- ♀ will remate, store sperm
- sperm precedence

Taxonomy

- Arthropods
  - crustacea
  - insecta
  - arachnida

  - 3 tagma (head, thorax, abd)
    - thorax: prothorax, mesothorax, metathorax
    - abdomen segmented
- head
- thorax segments may fuse
- 6 legs; 1 pair on each part of thorax
- hemimetabolous, homometabolous
  - cockroach
  - metamorphasis

orders: beetles, moths, bees, flies (major grp.)

sub orders: nematocera (mosquitos) thin head in larvae; brachycera

brachycera: orthorhaphous; cyclorrhaphous (have operculum)

cyclorrhaphous: calyptrate (higher flies) tsetse, mites, bottfly; acalyptrate

Herwig noted that present in Australia but not in N.Z. split 180 mill.

- ptilinum - hydrostatic sac breaks operculum
- ~3,70 diff families, mostly vegetarian, origin 80 MYA
  - coevolved w/ higher angiosperms
- eldest insects Devonian 600
  - diptera Triassic 200 mya
- acalyptera arose after KT extinction
- fossil Dros Amber European ~50 specimens, 40 MYA
  - Domin. Rep oligocene 18-20
- Family Drosophilidae

subfamilies: Steganinae (eg Amiota) primative, attracted to eggs; Drosophilinae

- larvae of Dros. exploit fermenting substrate
  sugar fermentation by yeast
- Repleta group grow in fermenting cactus

- many genera, Drosophila is biggest, Zaprionus
- Hawaiian originally in separate genus Idiomyia

Drosophila — Sophophora

Drosophila	Sophophora
Hawaii dimorphic	sex dimorph
~4 dors app.	2 dors app.
split abd. pigments	continuous

→ cosmo — cosmo, brewesier

→ hydei, virilis
  ↳ associated w/ willow ancestral

Start introduced species group

obscura (New world, palearctic) — willistoni S. Am — (180) melanogaster

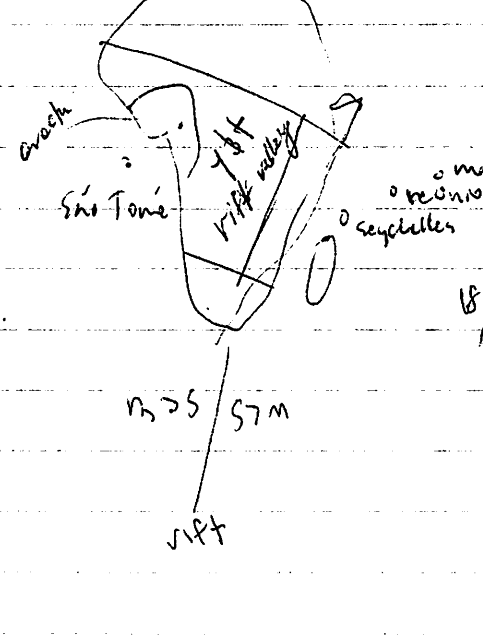
→ greatest diversity of mel (whole family) in SE Asia

11 species subgroups

melanogaster (African) — 9 species

↳ mostare oriental (biogeographically) — montium is both

Biogeography



cosmo
- mel 1839
- sim 1919 from Quackenbush
- (2 Mya) mau
- sec – morinda oxonoic acid [] kill mel
- teissieri – forest fruit
- yakuba – savannah
- santomea
- (18-20 mill) erecta – pandanus fruit
- orena – found 1, Cameroon

→ outgroup is oriental, not montium.

D. mel in Cuba 1860s, W Africa → N. World
- 27, carbon, unsaturated hydrocarbons, pheromones

Polytenes
- Sturtevant, Taxonomist, characterized extent of polymorphism
- 1933 T. Painter, 3 μ in diameter chromocenter, haploid # of chrom
- Metz, 1913, metaphase pairing is relict of interphase pairing in soma
- polytene hypothesis ("many strands")
  $2^8 \times C$ (C = haploid content)
- 2 levels of synapsis, w/in & btwn homologs
- density of ♀ in ♂ is 1/2, classic picture is ♀
- Balbiani 1881 chironomus
- 50:1 packing ratio in bands : interbands
- physical map & genetic map were colinear used translocations

1920 genetic maps of D. sim & D. mel. using visibles was same, save for 1 exception
3R exception, all done genetically!
confirmed cytologically
1936 Sturt & Dobzhansky
highly polymorphic inversions in D. pse
- Dobz. showed polymorph inversions were nonrandom & changed over time.
- overlapping inversions
- mel group - L & A > application
- Thompson Carson

Adh
ethanol → aldehyde
- adaptation to saprophagy
- multiple specialization to tubular flowers (like ipomea)
- adaptation fleshy fungi basidomycetes
- amanita, α-amanatin phalloidin, actin inhibitor
- leaf miners, other diptera
- aquatic
- Stegaminae ← predators
- breed in gills of land crabs (X-mas island)
- insects cannot produce steroids, but need it for molting & cell membrane
-

dispersal - Stratosphere, mats
dating species - fossils, biogeography

Speciation Genetics

Split Simmel 1919
first genetics, then morphology

M♀ x ♂S forced mating ♂ >> ♀

↓

♂ die L3
♀ live, sterile, gonad no germ cell

S♀ x M♂

♀ die as embryos
♂ live, sterile, gametes

- mel parent wins

- Watanabe 1970s, 1975
  found sim strain, allowed ♂ to live, sterile
  sim strain, μ, Lhr, 2♀
- 1st evidence that intersp. viability by a single gene effect.
- 1980 Hilfer, Ukraine strain had μ in Hmr that rescued ♂
  Hmr+m/Hmr+m → ♀♀ die, rescued by Lhr
  Lhr & Mhr interact

downregulatory allele 20% AA id.

♀ × ♂ C164.1 rescues ♀ fertility
use Hmr to rescue viability

Resources 22/02

- The Dutch Book 1925
- DIS 1929/1930 Demerec
- 1942 Bridges/Brewer
- 1968 Lindsley/Grell Revised Red Book
  1992 Lindsley/Zimm Revised (current "?")
  - genes & μ, rather than μ alleles
- 199- = ybase grant
- Bloomington origin was Bridges (Caltech) & Muller (Bloomington)
- Genetics services incorporated

23/02

Nondisjunction as proof of chromosome theory of heredity

w/w ♀ × +/+ ♂ — +/+ ♀ × w/+

↓ ↓

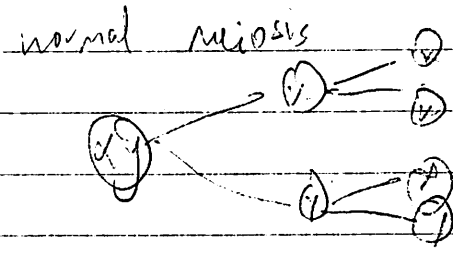
w/+ ♂ — +/Y ♂

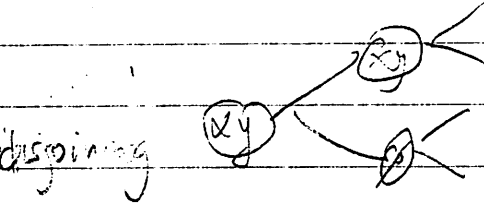
w/+ ♀ — +/w ♀

if large # of progeny, exceptions will be seen w/ ♂ (1/2000)

- patriclinous males
- higher rate than freq of spont. μ
- 10^{-6} for spont. mut.
- Bridges was first to realize/be convinced of patriclinous ♂

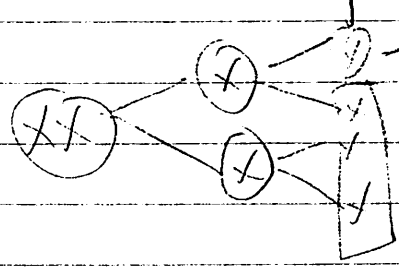
exceptions for this cross w/w ♀, w ♂

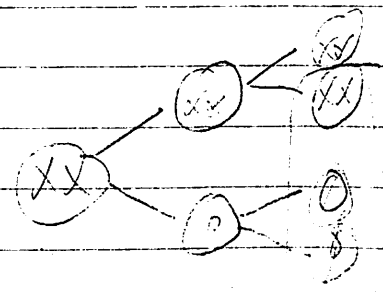




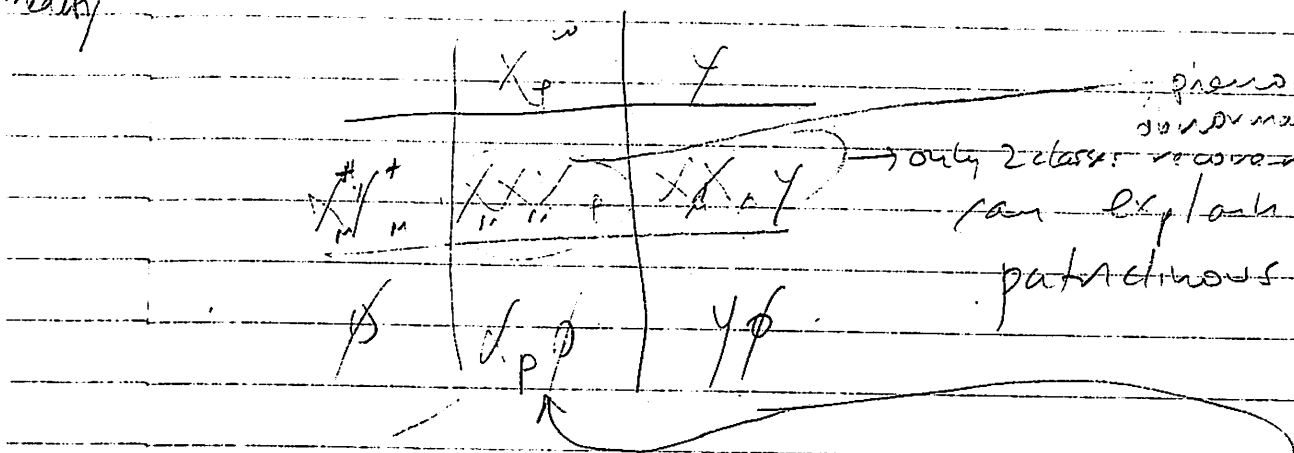
	X♀Y	∅
X_m	X_mY	X_m∅
X_m	"	"

cannot explain patriclinous





diplo-X or nullo-X



be - if assume fly w/ 1 X ⇒ ♂
- fly w/ no Y is lethal, not shown by Bridges

- Ms Nelly Stevens referred to in paper (Dr. Nelly) finding did cytogenetics → find XXY ♀ : ♂ agonia
- equivalent of Klinefelter Turners
in flies ♂ 1/5000 at birth
- by product of expts showed that Y doesn't determine sex - Dros
- this became dogma for all org
- 1959: Jerome showed by cytogenetics in humans that Y has role in sex det

* X.O males are sterile, Y required for fertility

XX | XY, X

	X	Y
X	XXY	XX
Y	XYY	XY

XY ♀ indistinguishable

- non dis 10^{-5} in nei ♀
- Morgan saved collection,
- Bridges showed exception proves rule
- Sutton's chromosome theory

+ Bridges paper also showed that genes
Control more than phenotypic Environmental
process i.e. sex determination

- Bridges proved balance hypothesis for sex det.
ratio of X:A

♀ XX AA 1.0
♂ XY AA 0.5

- one of many phenom shown first in flies then others
- non disjunction in meiosis/ovaries die as embryos
- can have triplo-4 3 triplo-4 (minute M)
- viability of nondisjunction crosses determined by amt of genes

compound X — L.V. Morgan (TH's wife)

y/y x +/Y
↓
y/+ +
y/Y ♂

found sex cross with of

y +
+ ♂

- rumor abt escape of ♀ y ♀
proposed that except could be explained by attached X chromosomes XX
example of a compound chromosome

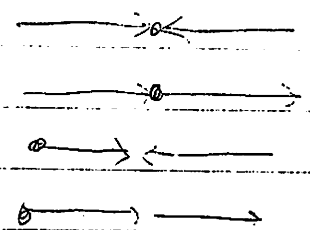
(1) RM
nondisjunction
reverse

	X_p	Y
$\hat{X}X$	~~$\hat{X}X X_p$~~	$\hat{X}XY$
O	X_pO	~~YO~~

	X_p (sct)	Y
$\hat{X}X$	$\hat{X}X X_p$	$\hat{X}XY$
Y	X_pY	~~YY~~

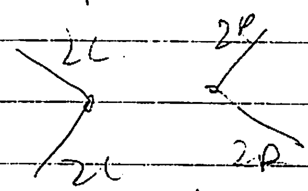
- 4 strand recomb (not 2) Anderson, stud. of Morgan
  used RM

C(1) RM
C(1) TM
C(1) RA
C(1) TA



→ can be used to make ring chrom.

- Novitski Sandler, Lindsley 1950s, 1960s
  fashionable to do chromosome engineering
- made compound autosomes

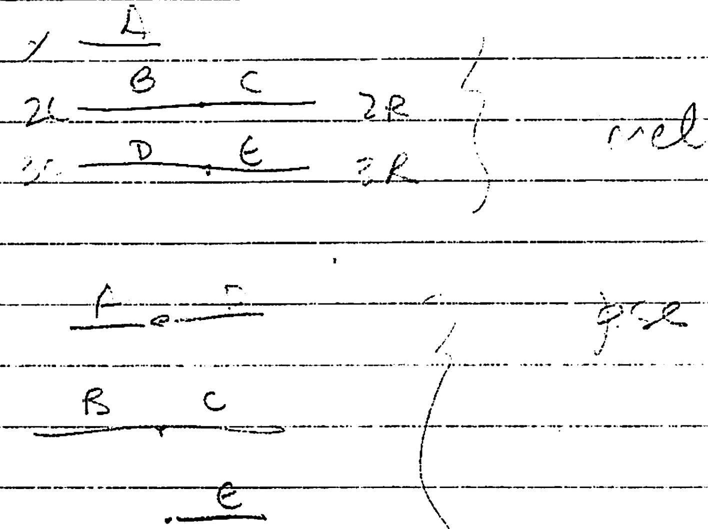


♂	♀ 2L-2R 2L2R
2L-2L	
2R-2R	
2L 2R	progeny
2L 2R	gross'g
0	aneuploid
	all die

- [illegible] meiosis at random
- 2L compound cross [illegible]
  [illegible] compensating
  genotypes [illegible] viable
- called new species
  [illegible] stable
- once you have a compound you can generate
  more compounds
- genotypes w/ aneuploid gametes can be viable

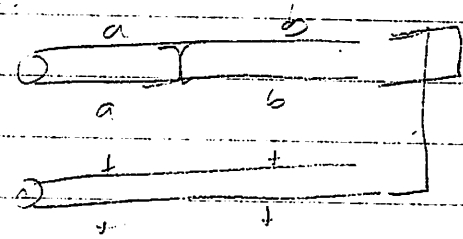
→ If you can create compensating [illegible]

- shows that gamete [illegible] is indep. of genotype
- also shows that gene order is not impt.
- occurs in nature



- Mendel's 2nd law: diff factors segregate indep.
- Bateson & Punnett chickens, comb found that pairs of factors are inherited together "coupling" [illegible] indep. [illegible]
- Morgan found X-linked [illegible] were coupled renamed linkage
- linkage varied, strong → weak
- Sturtevant analyzed results of series of crosses and realized that strength of linkage could be used to map factors in a linear order
- strength of linkage ~ proximity
indep. cytology plants & amphibia Johannsen
meiotic prophase structures - cross - [illegible] btwn non-sister chromatids.

= chiasma were pointed to be physical manifestation of recomb. event



sister chromatids attached to same centromere

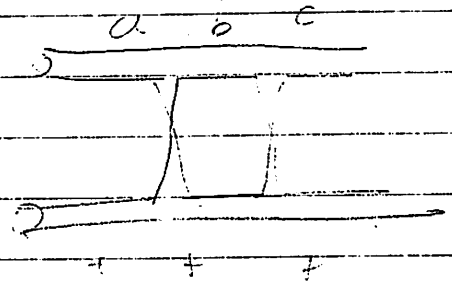
non-sister

- exchange btwn sisters not seen.
- rec I = homologs split
  rec II = sisters split
- recomb rate = freq of exchange / total # offspring
- 50% max
- higher dip. meiotic recomb not present in males
- Haldane when freq recomb differ, always lower in heterogametic sex
  - no recomb in ♀ moths (proves rule)
- over short distances, freq additive
- 3 or more → 8 genotypes
- 1913 1st genetic map by Sturtevant, published before Bridges' cytological paper

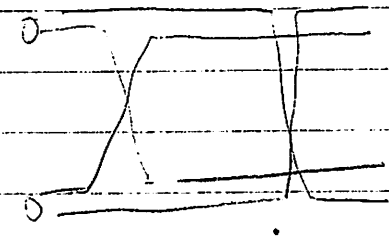
- Newstein, another Morgan protege, 1933 → 1936 (13 yrs after Bridges' expt.)
  calculated prob of observing recomb if sister chrom exchange
  - 3 markers used for 2 exchanges
  - 4 types of double exchange
    - 1 2 strand
    - 2 3 strand } double
    - 1 4 strand

assume no chromatid interference

2 strand double = double x classes equally freq,



0.25



no double crossovers

sum over all double crossover types

Non	single	double
.25	.5	.25

- so, non crossover gametes can come from double exchange!
- 2 H, no sister, sister/no sister equally freq.
- estimate freq of tetrads

E_0, E_1, E_2 ... # of tetrads w/ 0, 1, 2 ...

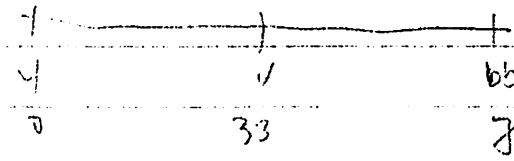
- if equal freq exchange btwn sis & non sis
  - ⇒ tetrads have neg frequency

E_0 5-10

E_1 65 %

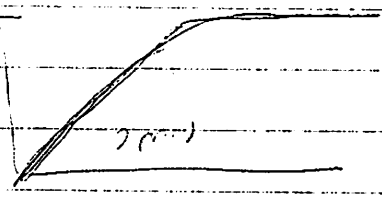
E_2 25-30

- Map units, 1% recomb.



>50 since additive

50%



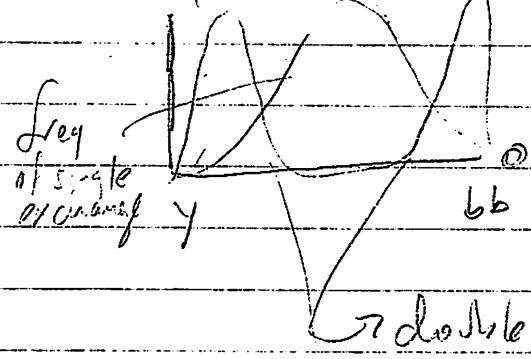
- plateau is because of double cross overs

Map distance

- recomb distance varies by strain, temp, crowding

- under constant, distance varies

- Charles 1938



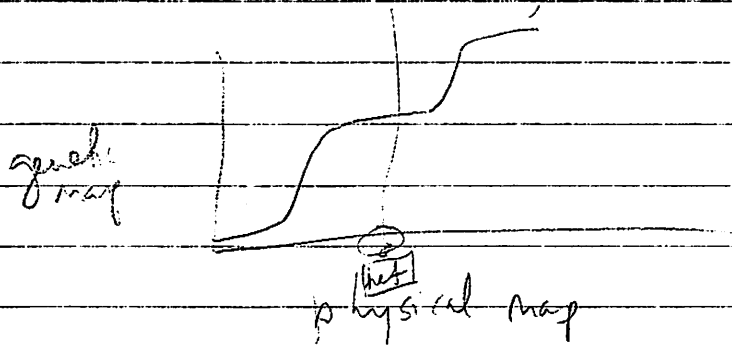
?? does double exchange compensate

→ double exchange

Muller 1915

- prob of double = $p_1 p_2$, Interference not indep events.
- strength of interference 0.05 / (exp = $p_1 p_2$)
- interference decrease w/ physical distance
- col inter is complete, in drosophila absent
- total genetic length 240 (4th)
- 1.2 / chrom arm

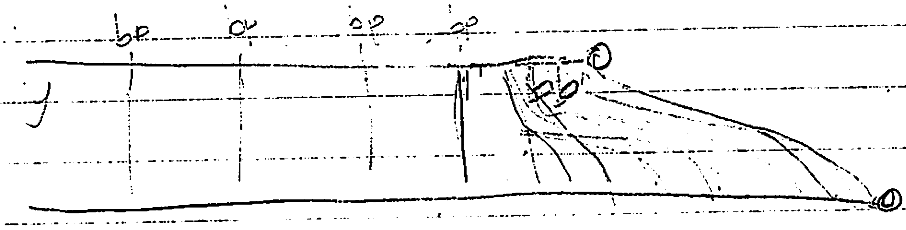
- 4th no recomb too short?



no recomb around centromere

- heterochromatin E. Heitz
- Muller & Painter, Heredity, 192

- Muller made translocations & mapped crossing point & Painter mapped translocation cytologically
compare map & ... showed that on distal 2/3 had ...



Mutation

- 1st visible until late 30s
- "markers", or not interested in origin of μ, what it meant for concept of gene, or what it meant for understanding wt function
- 1928 Muller ionizing radiation
- assay for recessive visible markers, unsuccessful
- Muller's assay, lethality

⤳ +/Y ♂ × +/+ ♀

↓

+*/Y ↓ sex ratio depressed

+/+

low power, lose μ since dead

↓

+*/+ ♀ × +/Y

- carrying chrom through ♀
- good power to find reduction in sex ratio

↓

+*/Y 0.5 ↓

+*/+ 1

- Auerbach, Scotland,
- Fahmy?, few, alkalating agents were mutagenic, predicted when never supersed by x-rays

EMS, ENU

- nature of μ → nature of gene Muller
- 1932 $w^a/Y = w^a/w^a$ same phenotype
$w^{eosin}/Y < w^{eosin}/w^e$ exception
- should be a mechanism to adjust 2:1 ratio
- called this dosage compensation.
solved by Bearman & Mukherjee 1958 Nature
role of trx. f is 2x times ♀
hyperactivation of male x.
- mammals 1964 Mary Lyon random inactivation of 1 ♀ X

$w^a/Y; Dp w^a$ darker than single copy

$w^a/Df w^-$ lighter than 2x

$w^a/Y = w^a/w^a$

$\wedge \qquad \vee$

$w^a/Y\ Dp w^a > w^a/Df w^a$

with w^1 allele all have same phenotype

2 phenomena: dosage compensation
dose effect

w^1 complete lack of fcn "amorphic"] recessive
w^a partial " " "hypomorphic"

- classical [illegible] for alleles [illegible] dose effect

- easiest test m/m vs m/− deletion
- hypomorph can lead to misleading results

- Muller also classified hypermorphic] m/m > m/−
very rare (new fxn (expr.) —— neomorphic
opposed wt allele fxn —— antimorphic] → dominant
not many spontaneous

① Antp/+ dom vis
recess. lethal
② Antp/− lethal

③ Dp Antp ; Antp/+ same as ①, not hypermorph

- deletion of neomorph/antimorph should restore wt phenotype
- duplication of antimorph should increase phen.
- reversion of " " should make recessive loss of fxn w/same phen as Dom. antimorph
eg Sco scutoid
- antimorphs should be suppressed by wildtype allele

m/m = m/+ - recessive]
M/+ - dominance] relative concepts

- env & genetic factors affect dom.

Lindsley 1972

g^+/g^+ —deletion→ $g^+/-$ haploinsuf.
dominant

- $Antp^+/-$ wt, not hap... [illegible]
- most common minutes, n=60, many ribosomal
- genes needed in stoichiometric amts.

<u>Sex Determ (somatic)</u> — genes re sex determ were poorly understood

- hypoth by Bridges in 1916
- Sturt's last expt. paper → tra 1925

♂ X/Y ; tra/tra wt

♀ X/X ; tra/tra

↓

phenotypically ♂ in soma

- 1950s Muller, found X-chr "female lethal"

Fl/+ ♀ die

Fl/+ ♂ live

~~if loss of fxn~~

tra^+ on in ♀, tra^+ can be off in ♂

Fl^+ " " , Fl^+ most "

- da/da ♀♀ x da/da ♂♂

↓

sons OK

daughters die

- found gene on X Ml/+ males die

by reversion showed that Fl & S

were same gene

Sxl < gain of fxn ♂ die; loss of fxn ♀ die

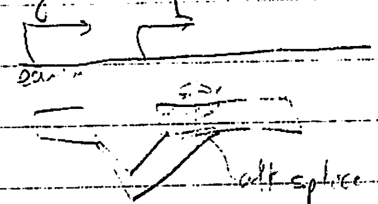
recall Bridges hypotheses: X/A 1.0 ♀
X/A 0.5 ♂

- Screened for μ in num or denom elements
- sis A, sis B, sis C, runt numerators

bHLH bHLH JF JF

- only 1 denominator deadpan
- Schedl / Cline cloned Sxl, showed expressed in ♀ ♂
  Sxl protein only expr in ♀

- expression of X/A genes needs to occur b4
  dosage compensation
-



activated in ♀ =
sets up auto-reg loop

- Sxl splices tra into ♀ active form
- tra splices Dsx into ♀ "
- Sxl also binds to MSL 2 3'UTR &
  inactivates in ♀

Tra involved in Sex Det in Bees

- Whiting proposed ♂ is homo, hemi X, /X, X,
- hetero = ♀ X^1, X^3

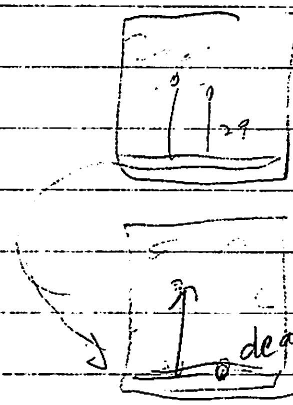
- Morgan showed that single fly can
  be both ♂ & ♀ gynandromorphs
- demonstrated that sex phenotype is
  cell autonomous

Mutations & Mutant screens ST: 2, 02

EMS → X/Y X^X/Y & aim of screen is isolate X-chrom μ, determine in flight

↓

X*/Y



22° → 29
remove tray

22   TS lethal s/by
dead TS -
paralytic

2 types of μ: recessive on X, dom on Auto
- mate X*/Y × X^X/Y   attached X stock

↓ $

X*/Y   X^X/Y

* 4 questions to ask b4 starting a mutant hunt
Q1: Are you looking for:
- μ that affect a broad biological process
(flight, meiosis, olfaction)
- μ that affect a well-defined process
(specific pathway)
- μ in a specific gene
Q2: What kind of μ do you want
- loss of function
- Dom/gain of Fxn
- Ts/conditional
- poisonous, antimorphs

- analysis { dominants can be tricky, rare &
not all genes μ to Dom alleles

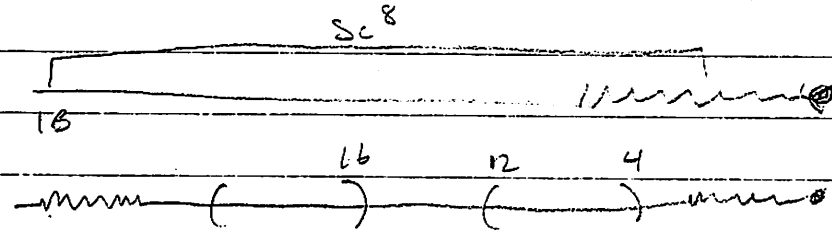
- 1/10 - 1/15 EMS μ and TS
- Q3: How many μ do you need to screen?
  - do you need to achieve saturation?
  - what are your resources
  - how rare are μ you are seeking
  - how hard are you willing to work
  - how will you know when to stop
- Q4: what are you going to do when you get your μ?
  - phenotypic investigation
  - cloning genes (~3 yrs/μ)
  - pathway analysis (only will) (GOT) (Strong athletes)
  - structure-function studies
  - interacting partners
- Now determine the phenotypes we are going to look for
  - diff btwn a selection and a screen
  - char of a good "screen-o-type"
    - simple phenotype (red v white)
    - quant phen are harder
    - easy to assay
    - doesn't pre-exist in stock abt to μ
    - isogenic line
    - pilot screen
- Assays
- choose your poison (mutagen, carcinogen)
  - dangerous! EMS C,C→AC
  - hood, double glove ~~wt brm~~ (reversant proof)
  - P-elements — weak hypomorphs
  - ionizing radiation (1/500 hits/dose)
    - X rays (safer)
    - chrom aberrations
  - wild-type μ (phylogenic/ unknown cause)

- Dan Lindsley & Larry Sandler screened wild type populations in Italy
- ~~one~~ existing EMS-treated chrom (the Tokyo collection)
- T.ling-PCR based screening of gene X in Tokyo collection

Simple Screen for X-linked lethals

- lethal in XY ♂ (not concerned w/ semilethal)
- balancer
  - prevent recomb or prevent recovery of recombinants.
  - set of overlapping inversions
    - paracentric - acentric/dicentric
    - pericentric - deficiency/dup
      - include centromere
  - small inversion do suppress x-over
    - 2 strand doubles inside inversion are ok

FM7



- autosomal balancers aren't as good because to double X-overs
- if you suppress X-over in one part of genome ↑ elsewhere
- attempting to balance with chrom 2 or 3 dangerous
- Must have Dom marker (note Bar reverts)
- presence of recessive lethal to prevent balancer homozygotes
- X dom. are viable or lethal
- Cy & TS, expressed in pupae by fluorescence

– EMS ♂ × Ba... ♀

Bal/Y ♂* × X*/Bal   Muller 5

(single vials)
(don't need to be virgins) ← already mated to brothers

↓

Muller 5 test

X^x/Y Bal/Y   Bal/X^x Bal/Bal

? are there any males w/ bright red eyes?
yes – rid
no – count Bal/Y

↓ $

– test for mutagenicity, estimate dose rate
– if lethal-X, can't make ♂, so cannot do complementation tests

autosomal lethals

EMS → cn bw/cn bw ♂ × CyO/Sco ♀

cn bw^x/CyO × CyO/Sco ♀

could have Sco ♂, no recombs

(single vials)

↓

cn bw^x/CyO × CyO/cn bw^x ♀

(in single vials)

? check for absence of Non-cy progeny ?

yes – rid
no – automatically new stock
can do complementation

- virgining is harder part of work

Y - hs - hid   hs of hid kills males
29° → 34°C 3-4 hrs

DTS - dom temp sens

♀ DTS / Bal x cubw / cubw ♂

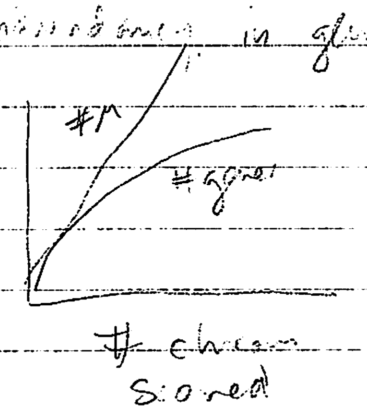
↓

♀ DTS / Bal x cubw* / Bal ♂

↓   all others die at restrictive temp.

29°   cubw* / Bal

? - how do you know when to stop?
- Poisson distribution assumption, estimate ∅ class of Poisson
  - wrong assumption
  - all genes not equally mutable
  - can't get good estimate since ∅ class is not included in average
- when mutations in genes ID, saturation



[illegible]/SNP mapping

?, what do you do next?
P, plasmyback, inverse PCR
if not - need to map

- make recombination using P

Marker P μ marker

- only works w/ certain Ps
- SNP mapping
  - do μ on SNP marked chrom
  - can be expensive, but labor saving

Reverse Genetic Approach

- in silico db
- annotation < GO homology
- P-screen
- assay phenotype
- P-element excision to "rescue" gene function & make deletion

P-element transformation rescue is definitive proof that you have gene.

mus309, Ku - not gene Rm, Blm - real gene Rm
- rescue by Ku, rescues mutation -- Blm
- μ not sequenced in Ku by rio
- shows that transf. is not definitive
- Seq + P-reversion + P-transformation

Analysis of Genetic Pathways

A) Defining of a genetic pathway
double μ w/ nulls determines whether 2 genes belong to same parallel or partially redundant pathways

B) Ordering gene function — epistasis analysis

1) Biosynthetic pathway
synthesis of A depends on presence of B
All μ fail to produce common end product

2) Regulatory hierarchy
indep synth of A & B
μ affects a phen, μ causing

3) molecular epistasis

Botstein & Jarvic ⓒ p 22
conditional μ
isolated "reversions"
showed to be second site suppressors
allele specific suppression
showed that genetic interaction was btwn proteins that physically interacted

two wrongs make a right

M. Fuller
b_2 tubulin screen for new μ in b_2
complementation test → find kin α tubulin
allele specific second site noncomplementation

Suppression Screen

① screen in a te insertion as starting μ
(apt regulation of sporing, etc)

(2) don't use null alleles
(3) use a missense
(4) clean & dominant phenotype
(5) make sure suppression can't be made by temp
(6) show that new μ affects same phenotype
← secondary screen

2nd Site Noncomplementation F1 - screen

Type I physical interaction of 2 μ proteins to produce a poisonous product.
- don't prove that they interact
- allele specificity at both loci
- cannot use a deletion at either locus to get same effect

$m_1/+ ; +/+$ wt
$+/+ ; m_2/+$ wt
$m_1/m_1 ; m_2/+$ mutant
$Df/+ ; m_2/+$ wt
$m_1/+ ; Df/+$ wt

Type II sequestration of the remaining wildtype protein
- dose effect ½ A in A/+
¼ A in A/+ B/b
→ good reason to think proteins interact
- allele specific at 1 of 2 loci
- deficiency will produce same effect
- doesn't prove they physically interact

Type III combined haplo-insufficiency
- reduce ½ A ok
- reduce ½ A, ½ b not ok
- not allele specific

- SSNC
  - can be thought of a dominant enhancement of the 1st pt.

- intragenic complementation

PEV

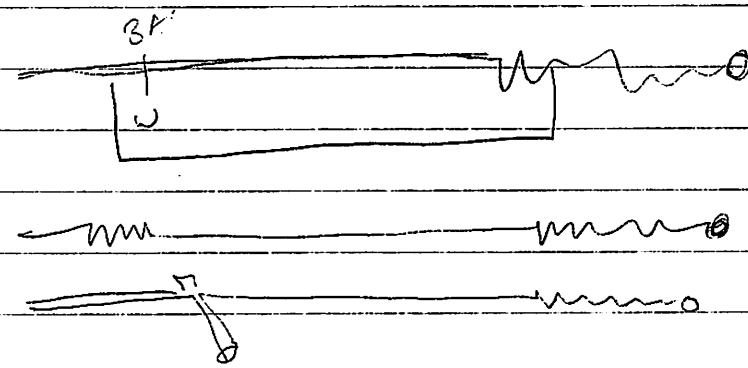
- "ever-sporting displacements" - Muller
- eyes white & red, each eye, but differ

$X^{v}/Y \times X^{\hat{v}}/Y$

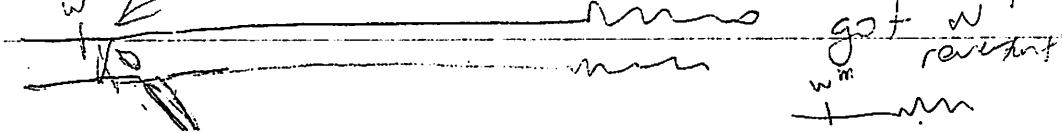
X^{v}/Y also sporting, but in different way

- always associated w/ genetic class of rearrangements

$\frac{In(1)\ w^{m4}}{T(1;4)\ w^{m5}}$ white mottled 4



- renamed PEV — proximity to heterochromatin
- but why is this a gene effect
- Schultz '30's — selected for reversion btwn w, BT



Su(var)205 / HP1

- Tartoff

Fn(1) mite ~x-ray

↓

wt revertants

- see variegation in any cell autonomous gene
- many variegating μ, all rearrangement into het
- brown, het → brown region, brown variegates
  & brown homolog variegates
- mosaicism is w on or off, size of clone
  depends when switch
- adding more het, temp affect variegation
  ↳ suppresses μ, enhanced by removal of het
- large e(var) & su(var)
- b^D
- het associates in somatic chrom, localizes
  in one area of nucleus
- some region of nucleus doesn't have factors for

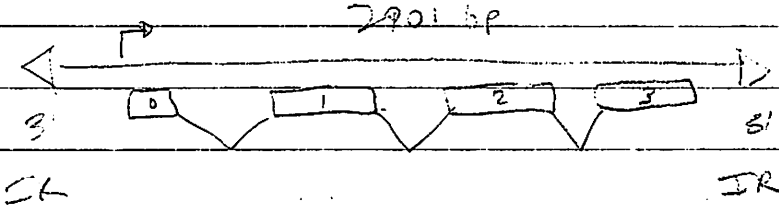
- Dubinen effect het genes → eu variegate
  So & E have opp. effects
- 2 major screens for Dom S(var) &
- su(var)205 → HP1
  dominant because they are needed in stoichiometric

- tandem dup of white, will become
- mitotically but not meiotically stable

25/02

Transgenesis

- Cavalli 80's - desire to transform Dros.
- Ledbetter 70's E.col / ϕ → transduction
- Griffiths - pneumococcus transformation
- Avery & McCarty 40's - DNA responsible for transformation
- Gerry Fink, Cornell 1970; transform yeast
- Ivan 70; early 80 different transposons
- Hybrid dysgenesis → F1 sterility, F2 ↑ μ
- MJ Green - suppressed dys = TE
- Mary Lidwell -
- rediscovered in E.coli - Shapiro
- cloned P-element in W, truncated copy
- M - no P elements, P - many, Fs diff size
- complete ⇒



87 Kd transposase, catalyzes excision & insertion

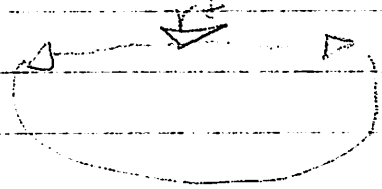
- dysgenic phenotype is germline
- in soma 2-3 intron not spliced

66 Kd not a transposase, repressor of transpos. thru

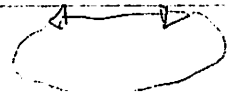
- remove intron → somatic activity → lethality
- each show indep mol mech for somatic repression
- lack repeats, cannot transpose
- Spradling & Rubin, P-element in plasmid

→ non-cell-auton marker

- very little ry needed for w.t. phen (Chovnick)

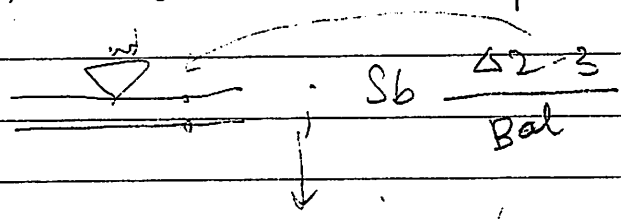


co-inject w/ helper plasmid



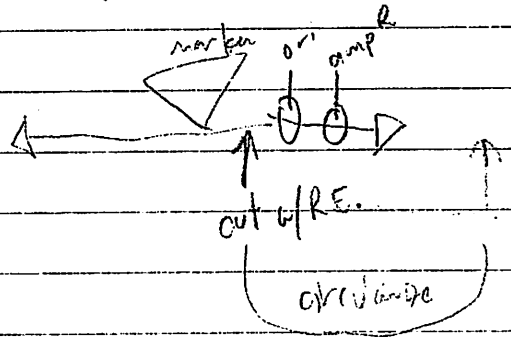
- fly was ry⁻, P-free (lab stocks)
= stocks of RbS announced result, brought vials & distributed plasmid.
- impt. transgene is precisely inserted, but so is helper
- made helper w/ 3' ⇒ removed, "wings-clipped"

2 uses: vectors for transf. & use as mutagens
↑ size ↓ trans rate
w⁺ marker is white
= provide external source of transposase, helper or
- P(Δ2-3) 99B
lacks .2-3 intron
lacks one of its ends
- replicative transposition, DSB repair
- start w/ w⁺ insert or X, look for aneusomic inheritance
- new insert is Dominant so no balancing cross can remove type source

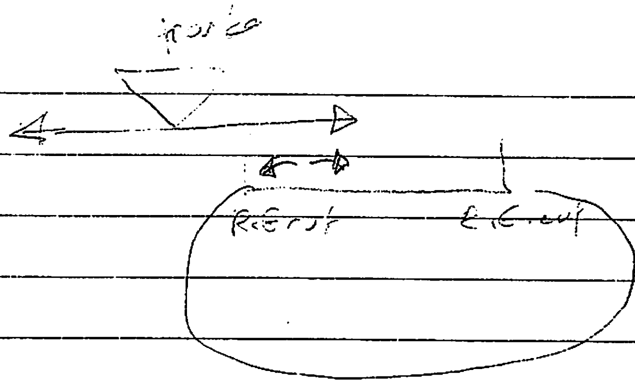


take new insert / Bal

- local transposition is quite common "local hopping"
- hotspots for P insertion Adh cold, esg hot
- map P by genetics, in situ, plasmid rescue



transform E. coli amp
& select amp^R



inverse PCR

85% efficiency

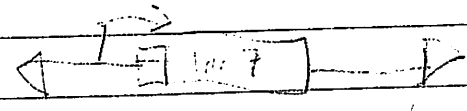
- trans rate is impt for targeted P
- K2, Szeged 3 - multiple insertions !!
- if μ by P - first do reversion by Δ2-3
  select for reversion of phen
  or for w^-
- reversion can be precise or imprecise
- 10% rev rate, 10% prog carry new mu,
  depends on donor site

- first big P element screen
  - Kiss chrom 2 → lethal phen.
  - Szeged chrom 3
  - in situ by Laverty
  - stocks that remain in Bloomington → IPCR
- BDGP Spradling, Rubin, Bellen
  - no phen, all IPCR
  - unique deposited in Bloomington
- NIG, Kyoto
- Exelixis, Spyros-Artavanis, Harvard
- Cambridge - 3000
  70% know/predicted genes have P
  w/in or w/in 1.5 kb upstream

VariantB -

O'Kane B. Gehring enhancer trap in
→ Gehring lab, EMBO, MA interviewed said
wouldn't care   Peter Lawrence

- enhancer trapping

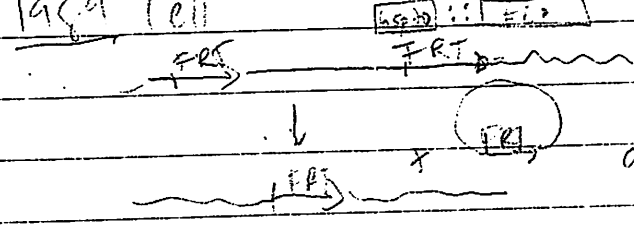


- P. Rorth instead of lacZ, use GAL4
  enhancer trap expresses GAL4
  GAL4 shown to activate in Dros (Struhl, Perrimon bound)
  binds to UAS in 2nd
  2000 3000 EP screens
- P-promoter has been replaced by hsp70 promoter
  Sev, β_2 tub promoters

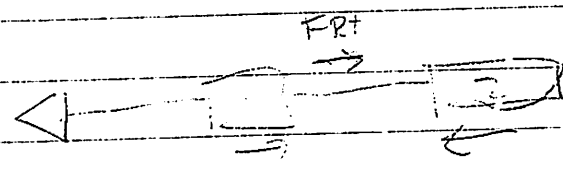
GAL4 :: ER feed estradiol, so not enh trap

- Kent Golic (Sandler, Lindquist)
  - site specific recombinases in bact/yeast
  - FLP 34 bp target FRT
    2 μ plasmid replication by recombination
    double ring resolved

lacZ cell



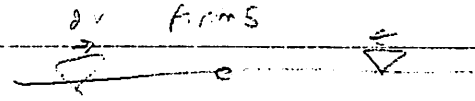
- put FRTs in miniwhite, HS, mosaic eyes variable genotype sperm
- HSp70 not spatial, but temporal control to turn off gene



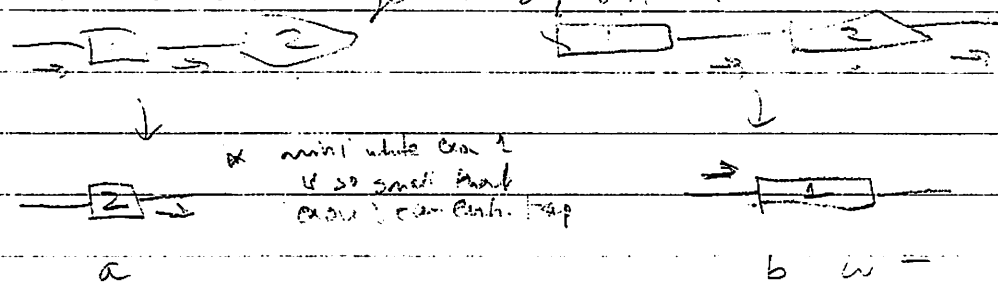
inverted repeats will turn gene on under HS / or will control

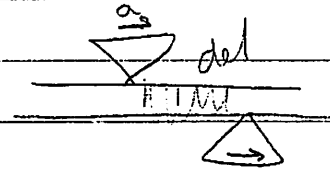
P1 phage Cre recombinase
lox site 34 bp
- Dan Hartl showed Cre-lox works

- FLP can recombine in trans among homologs



pericentric inversions translocations
- Golic 2 white genes w/ diff FRTs





select for red eyes
- delete region btw 2 partial P's
- reciprocal is a duplicate, phenotypically white
- have power in mixing deletions & duplications
- Let Δ in dmos up to Mb in size
- Dmsdel ← EV, acronym

- P-dominant position effect
  - chlamydomonas I-CE-1 18 bp target
  - site specific endonuclease
  - create DSB

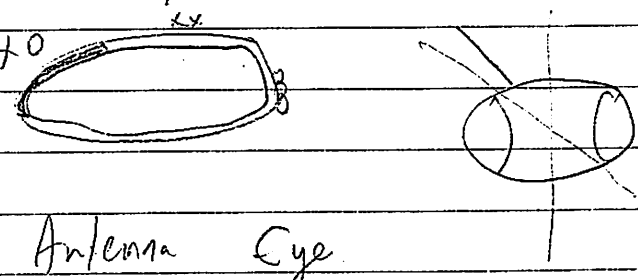
Mosaic Analysis (d.f. cells have diff genotypes)

- Morgan's gynandromorph
  - cell autonomy for sex determ
- not all gyn are bilateral
- used to determine cell autonomy of gene fxn

R(1) ring X first discovered by LV Morgan

R(1) w^{vc} Ring of catchoside

- single X-over betwn ring-rod not obs but doubles almost normal
- ring lost with high frequency in early embryo
  - after dosage comp estab. then loss of X_{ring} is cell lethal
- Strt. sim $\frac{ca}{ca}$ claret chrom loss for X, ↓ (not 2, 3 → lethal)
  - ca^{nd} later Ed Lewis found sim allele in mel
  - deletion of claret & neighbor ncd
- Strt. realized you could use ca^{nd} in novel way

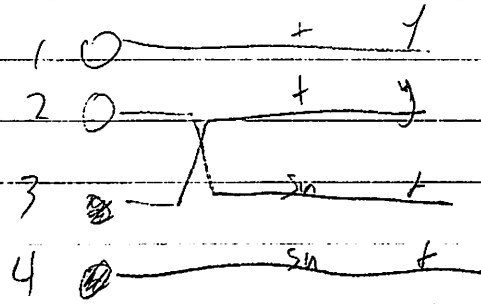


- could be used to map dev. fate on blast, embr.
- if high freq. of separation by clone boundaries than distant on blast map.
- 1977 A.G-B & Morman used Strt 1930 data

- Sayrao: Benzer realized that behav. could also be mapped w/ mosaic analysis

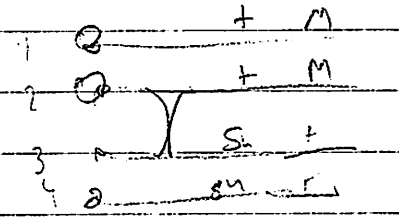
Mitot. Recomb

- Morgan noticed small patches of yellow in y/+ → proposed chrom loss
- Stern was able to reason that in region of sex dimorph, sex is wrong. proposed mitotic recombination



y y	y +	rule is if mitotic recomb then they go to opp poles (segregate)
1 3	1 4	
2 4	2 3	
+ +	+ y	

- produce a clone (patch) of y- cells
- only get y when recomb bw marker & cent.
- twin spots nailed hyp (by Stern)
- freq of spot wt. recomb is low
- x-ray ↑ mit rec.
- induce in spermatogonia show that not rearrangements since sperm are OK.
- b/c x-ray clones are small
- M/+, AG-B



2, 4 Sn +

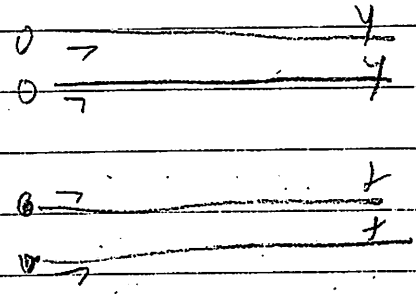
1, 3 + M

made larger clones since mitotic in parent

Dtob 67C

- technique led to compartments in wing

- Golic



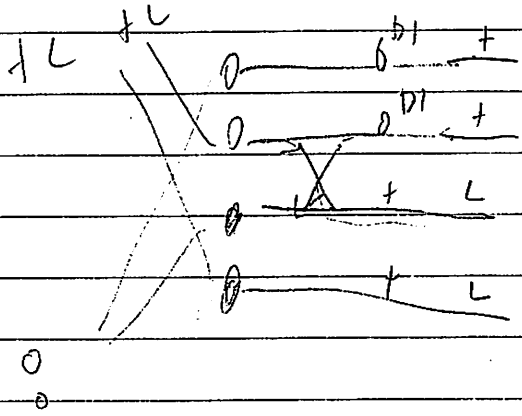
ITS, FLP

now frequency not limited by irradiation.

- GFP, UAS:GFP, GAL80 MARCM

Revision

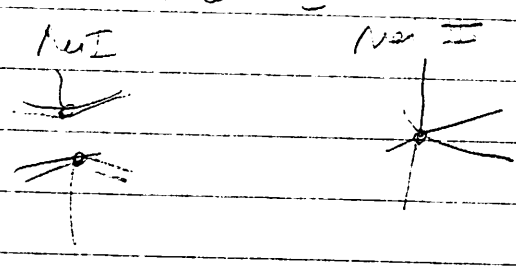
- OVO^{D1} dom. antimorphic allele X-chr, sterile



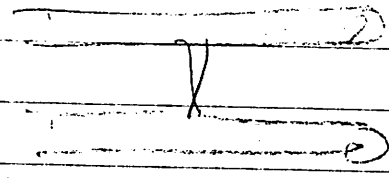
can evaluate distal pt for fxn in oogen. by recovery of progeny

- cloned OVO^{D1} is transformed to all chrom in combination w/ FRT, make germline clones very easily.

26/02 Meiosis



- how do homologs segregate
- pairing → exchange → disjunction
- crossing over occurs at 4 strand stage, after rep



- sister chromatid held together by cohesin complex
- break → retuse, now all chromatid are linked → chromatid or sister ≠ diff homolog
- 1st mei div, cohesion lost along arms
  2nd " " " at centromere
- cross over ± loss of arm cohesion switches chromatid → homolog adhesion
- crossing over locks homologs together
- if X over at tip (not enough cohesion)
  or at base (doesn't disrupt cohesion at mei I)
  then X-over won't lock chrom together
- distance not proportional to physical distance (chiasmata assumed = mapping)
- Haldane said bivalents need slightly more than 1 exchange
- Lindsley/Sandler 1968 screen for mei mps
  mei-S332, mei-S282, mei-S6
- Baker & Carpenter 1972 screen of 350 EMS chrom (mei-9, mei-11, mei-352, mei-416, mod)
- Baker Hall 1974 ? # chrom EMS (ord, mei-W68)
- O'Tousa, Stanfer & Wieschaus Image EMS (ald)
  lost

- Things hit bottom 1986 - no interest stocks lost
- Sekelsky-McKim 1996 P-screen (mei-P37, mei-P26, mei-P21, mei-40)
- McKim screens the 2nd chrom further collection C(2)M
- Peng F-LP-FRT screen in 2004-2005 (mei-A12, mei-A15, mei-A17, mei-C3, mei-B12)

Pairing

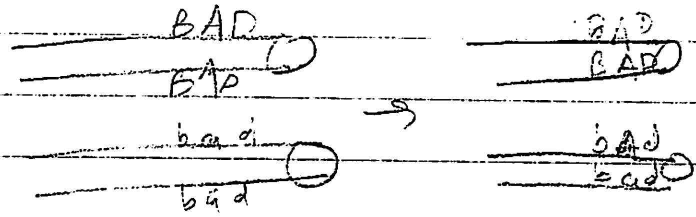
- cycle 5 pairing initiated, 1st cell cycle w/true G1
- visualize by FISH interphase, lacI-GFP
- visualize in mitosis
- maintained into meiosis (no leptotene zygotene)
- polytene I arms both homologs together
- pairing in somatic cells → transvection
- has pairing defective genes in Dros
  - screening wrong tissue
  - prob lethal (wouldn't have seen yet)
- pairing leads to synaptonemal complex (S.C.)
- very diff to study S.C. using EM
- C(3)G doesn't build S.C., recomb defective
- C(3)G can visualize S.C.
- SC may tell centromeres that x-over occurred

Recomb

- many recomb defective μ
- + class I - can't initiate mei-W68 (spo11)
  - mei-W68 - makes DSB
- in human, no DSB no SC
- in flies, no DSB but SC, difference is that flies are paired
- in others DSB → search for homology → pairing

- C. elegans also DSBs indep. but has pairing center which makes hom search
- Why does non-dis screen → recom def μ ? recomb required for seg.
- most mei μ are recomb def
+ class II — required for recomb resolution
  - eg mei-9

Gene conversion — violates Mendel's 1st law



- most DSB are resolved as gene conversion
- only possible in Dros at rosy
+ class II — pre-condition, control distribution of exchange Carpenter Sandler
  [mei-352] exchange ∝ phys. distance
  Klp3A, chrom condensation

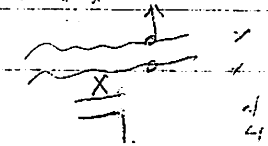
Segregation

- genes for spindle assembly (ncd, Msps, ...) — focus spindles
- genes for chiasmate segregation (ncd, ald?)
- " " achiasmate " " (nod, mtrm, Axs)
- " " sis chrom cohesion

- 1/2,000 spontaneous matroclinous nondisjunction
- 126 recovered, 90% did not cross over
- 1/2 million scored
- 10% had x-over, but distal/proximal
- " 1/2 Francis ch... paradox "

Dean Parker

X-4 translocation



- if exchange → segregate, only exception when spindle fails
- exchange is sufficient but not necessary
- chrom 4, no recomb, still segregate
- chrom X 6-10% no recomb, but non-dis 1/3000
- balance X, freq of spont nondisjunction doesn't ↑
- achiasmic homologous segregation

2nd system

"non-distributive system" — not good term since explains 2nd & 3rd systems

- het tightly associated
- 1976 RB Nicklas Genetics

XX ? 1/2000 Bridges primary nondis

XXY ? 5-10% secondary

almost always involve non-exchange X

defaults to heterochromatic pairing

Bridges 3's a crowd

X ⇔ Y

predicts max freq 50%

⇐X⇒

revised

$X_1 Y_2$ $X_1 Y$ $X_2 Y$

$\leftarrow Y \rightarrow$ $\leftarrow X_2 \rightarrow$ $\leftarrow X_1 \rightarrow$

- Kenneth Cooper, shows higher than 50%

1945 nondisjunction, when recomb ↓

$X_1 Y$ (trivalent)

X_2

new data shows Xs are associated

3/1 system

- heterologous segregation
- non-achiasmatic homol. segregation

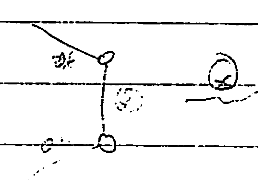
$X_1 ; 44$ X $X_2 ; 44$

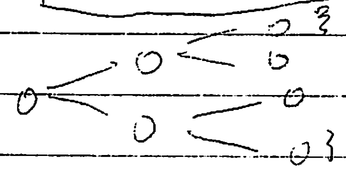
haplo 4 minute

- no pairing of non-homol chrom, but still segregate
- only µ that affect het. seg are those that affect spindle
- "eclipse model"
- Rhoda Grell explain rules for seg
  1) availability, non exchange non hom these will segregate
  2) if 3, then physical characteristics
     - big seg from smaller 2
     - more sim in size
- no centrioles in ♀ meiosis

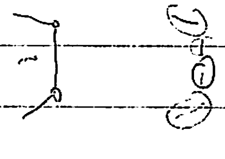
- why mei I b4 mei II? doesn't have to
exceptions in some insects, sex chrom do mei II when autosomes do mei I
- mei II resolves het distal to x-over
- silk moth no x-over in ♀, SC built

Chrom Rearrangements
- 2 types of inversions
- Sturtevant & Beadle 1936
- Sc^8 inversion, het, recovery double x-over but not single x-overs
- single => dicentric bridge
  => dead eggs? - none
  - hatch rate is wild-type



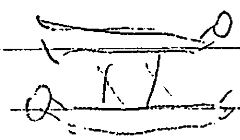
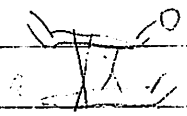
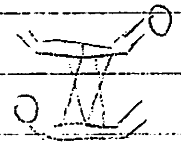


- no cell div, only [illegible] or outer nucleus avail for fertilization



- assume [illegible] of dicentric

breakpoint suppression

	NCO	DCO	nullo X
2 strand 	1	1	0
4 strand 	—	—	2
3 strand 	1+1	1+1	

prediction DCO : nulloX 3:2

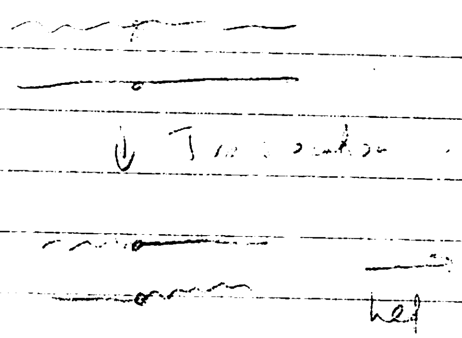
- Thomas & Stone 1934 - empirical obs that DCO : nullox 3:2
- Shown later that 4 products of mei are arranged in linear array

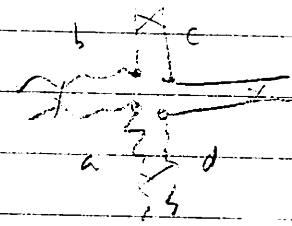
- breakpoints suppress exchange

Pericentric inversions
- SCO w/in inversion → dup / def
- het → zygotic lethality
- Course Congo pop w/ pericentric inversion
  → suppress. X-over
  - relyies on distributive system
  - nod seg at random
- X chrom balancers FM7 overlapping inversions
- autosomes peri & para, doubles do occur
  - choose a balancer with breakpoint near m
- autosynaptic → create dup & def
  ↳ overlapping pericentric inversions

Translocations

T2;3 Altenberg & Muller   Pole Translocation





alternate segregation   b ⟺ d   a ⟺ c   ok balanced gametes
1 adjacent 1   b ⟺ c   a ⟺ d   unbalanced
rare adjacent 2

- dominant region specific sup of exchange
- boundaries to exchange suppression X
- pairing is fine
- X-A translocations are sterile in ♂
  except if breakpoints are very distal proximal
  Y-A fertile except w/ prox def on X

- insertional translocations

Deletions
- 1 Mb ~ 1 numbered division can be deleted
- triplo lethal, others
- 3-4 #'d units for a duplication

Sturt 1925 - Bar, Zeleny
reversion of B to + at high frequency
B/Y ...   B B
B 2   B/y   B/
reversion ...   +/

fv B +
÷ B f

← 4% →

- reverts were also recombinants

1bA1-1bA7 = postulated duplication

2 B ——— unequal exchange

1 wt ———

- then shown by polytene to be true
  eg Bridges & Muller
- α globin peptide sequencing showed hybrid globin, postulated duplicates & unequal x-over

Bobbed

- 18S, 28S, x-het, bb, 240x rDNA array
  similar array on Y
  ~100 copies for wild type
- Y/bb⁻

  Xbb/Yb⁻ x bal/Df(bb)

  → bb*/Df(bb)

  10-40% revert ⟹ wt

1974 Tartoff bb^l ← bb → bb^+
move & less extreme

- all in males w/ mi problems
- doesn't occur if X is a ring (double crossover)
- mitotic chromosome
- Hawley & Gelbic expts

Male meiosis

- X-Y - held together by nucleolus
- no synaptonemal complex
- no DSB, no exchange
- univalents segregate to restricted loc. in nucleus
- then sisters & homol relax
- Vasquez, Belmont & Sedat Curr. Biol. compartment form 3 compartmentalization guide proper segregation
- why no recomb in ♂?
hyp: reduced X-over btwn X & Y
= μ for achiasmatic segregation in effect in ♂ meiosis

MA - Transvection

1953 - Ed Lewis, persuaded Bendle, Air force
- flies irradiated by H-bomb
- paid by AEC
- canister was hot
- test for rearrangement

weak UC ← Ubx / bx 34a show mosaic compl.

8(Ubx) / bx 34e or Ubx / ∈(bx 3, 4)

⇒ het. shows extreme μ phen

- 89E, Ubx

89E 100

breakpoint of ∈ in this region.

Am Nat

- proposed that some synapses of som. homologs
- 1st ev. that som pairing may have some funct consequence
- Madeleine Gans, discovered zeste[1]

yellow eye, X-linked

sexually dimorphic

z^1/Y ♂ red

z^1/z^1 ♀ yellow

red z^1w^+/z^1w^- ♀ white deletion

yellow $z^1w^+, Dp(w^+)/Y$ ♂

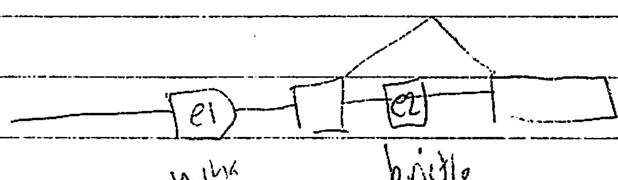
Suppressor of white when 2x white

- Burt Judd & Ashburner showed model is correct
- 2nd pairing dep phenomenon
- many other pairing dep phenomena, serendipitously discovered reflect som pairing
- transvection at yellow is best studied case

y^1 y body y bristles — 2 classes mutants

y^2 y body + bristles

y^1/y^2 complement by transvection



- dpp, eya, other genes
- no mu in somatic pairing

→ need to add c to lecture

Genome facts

Carvalho
Lynch

~175 Mb cytophotometric, diploid, propidium iodide
using a standard (C. elegans)
used to be bird erythrocytes
Let 45 Mb, complex (TE) simple & complex satellites
~~688~~, 360, mono- or dinucleosome
359 in dros 1.688, neutral Cesium
satellites - Roy Britten, discovered ~1970
pericentromeric & Y (has genes)
- 18 & 28S single transcript cleaved
- required for spermiogenesis
- Stern, fertility factors, defined by deletions
Brosseau Iowa ~5 K(short) K(long)
- Y genes dynein motors Hydei >3Mb
- transcr. 1.1%/minute Thummel at 25°C pol II
- form large lampbrush loops
no Y assembly
- 1/4 [done], TEs hard to assemble
40 Mb, 16 genes Carvalho
- Y in pseudo & mel not homologous
- since 16 genes are there more than
6 fertility factors
- Ai deletions are large, could be >1 gene

- ~~in~~ genes Dros 158 → 779
maritiana nasutoides
- 40% GC, 60% CDS 40% noncoding
- 14,000 CDS, early estimates were wrong because
assumption of 0% lethal was wrong discovered 1999
- RT, few gene models inspected by hand
- Mt DNA 16 KB
- 4 introns, 5 exons, allow alt splice

orientation
→ org of promoters
- multiple promoters

dicistronic
IRES

>100,000

?? 30,000?? proteins, further diversity by post-transl.

2500 ncRNA

286 tRNA

2 clusters of rRNA — X 18/28 200 x; Y 18/28 240 x; 2R 5S 120 copies] bobbed

tRNAs are found in clusters
40

31 snRNA - spliceosome

U11, GT.... AG

U12 non-canonical, rare class, pros

25 snoRNA

make base changes to rRNA

- intronic, protein coding genes fxn in protein synthesis

- Ugh1 & 2

150 miRNAs

21-23

nos1, nos2 → msl

>20,000,000
14000

- methylation ↗T 5me → deam →

no CpG underrepresentation, no methylation

perhaps low level of CpT methylation

- RNA editing

A → Inosine deamination (read e.g. as G)

also gives diversity

17 transcripts, all channels, para

U → C

- random gene orientation

- biggest para 229 k, smallest 144 bp Rdh

- nested genes, often antiparallel

- LCP

- nested genes

- Kni, Knrl intronless Knrl can rescue kni;

- overlapping / antisense

- divergent promoters

5'/intron/3'

att / CG 4241 overlapping
vap-33 - retained introns
mod(mdg4), lola = trans-splicing