1980/82 Rubin & Ashburner CHSL
Scott

2005 Bangalore
2006 Hinxton

Student Seminar 20 min
2 per night 26 Tadashi

9-12:30 Lectures website for downloads

1906 – William Castle, inbreeding on fecundity
1st to domesticate to laboratory, feed banana
short life cycle, lots of eggs by ♀, cheap & easy

Nobel prize Kentucky 1908 – THM, pattern of pigmentation "trident" on thorax, discovered genetic variation, but difficult to study

1910 – white eye male; sex linked inheritence shown in ~2 months (Morgan's greatest discovery)

an – Doncaster had discovered sex linkage earlier in moths abraxis (2-3 yrs)

Sturtevant – ug
Muller – grad
Bridges – bottle washer, found vermillion
1910 – 1936 foundations of ~~genetics~~ genetics
- physical basis of mendelian factors not understood
- E.B. Wilson proposed that chrom carried Mend. factors
  - but not universally accepted, e.g. Bateson
- 1916 Bridges phd thesis: chromosome proof of heredity
- Bridges, dupl., deletions, sex determination, cytogenetic maps, nomenclature, stock lists
- Sturt, genetic maps (1913)
- linkage Bateson & Punnet in chickens 1906
- mapping was novel

Sturt - 1972)
- strength of linkage varied; 5-6 genes, construct into linear map (= chromosomes)
- superb at inferring chrom mechanics from genetic data
- taxonomy

Muller
- ionizing radiation (x rays, gamma rays) 1928, Nobel Prize
- until 1928, phenotypic mutants, spontaneous
- almost as revolutionary as cloning/sequencing
- chemical mut. Lotte Averbach England — mustard gas
  Rappaport (?) Russia > military secret
- Ed. Lewis EMS
- by 1936
  - chrom aberrations, genetic basis of sex determ, genetic correlates of meiosis, begin of dev. genetics
- Don Poulson (Caltech) embryonic phenotype of mutation, phenotype of lethals, Notch. X-linked
- lethals, recessive known by Bridges by 1912, earlier in mice
  - effects on emb. phenotype, hyperplasia of nerv. system.
  - moved to Yale, teaching Ted Wright
- Hadorn, Zurich, lethal factors in Development
- emphasis on mut, not wild type
- 1945 - 1970, Dros not strong, D.O.E. funded,
  - Ed Lewis exception
  - very advanced chrom mechanics complex genetics, but no purpose, intellectual challenge, US
- change by developmentalists, Lawrence, Garcia-Bellido in Europe → US. Madrid, Cambridge, Zurich Basel
- 1966 1st molecular Ritossa & Spiegelman
- rosy Herschel Mitchell, xdh } pre-molecular
  vermillion trp oxidase } links btwn genes & proteins

- Ritossa & Spegelman - bobbed, sex linked
  most proximal to centromere, many alleles,
  variable, revert to wildtype, alleled on Y
  - showed 18 & 28s RNA → bobbed
- Hogness, Stanford pre1980
  - TEs Rubin/Finnegan
  - histones
  - Ubx Bender, positional cloning
  - chrom walking
- 1978 1st seq hsps histones
- 1 postdoc lifetime per gene cloned.
- DNA sequencing, MG very dangerous, hydrazine, rocket fuel
- late 1980s, John Sulston, physical map; C. elegans
  Bob Waterston
- physical maps, sequencing, Ath 1999
- 2000 WGS, started Apr 1999, announced 1998
  Sep 1999 1st assembly

Late 1970s
- transgenesis, transformation in bacteria was effective tool
  yeast - Fink, Cornell
  Begg (?) Edinburgh
- 1982 Spradling & Rubin, P-element transposition
  - hybrid dysgenesis, sterility not complete, fertile flies ↑ sperm ♂
  - Stocks CT announced before published in 1983
  - distributed strains & plasmids
  - Golic, exogenous recombinases

- Success of flies: - utility as experimental organism
  - small chrom #, known by Nettie Stevens 1907
    (nice phase karyotype)
  - polytene chrom 1936 Theophilus Painter
    - map rearrangements/deletions/duplications (Bridges)

- Lindsay polytene mapping ⇒ physical map
- polytene mapping in situ perfected by Pardue
- resource sharing

- counter examples Datura, Blakeslee yeast
- ~~[redacted]~~
- life history
  - synanthropic
  - cosmopolitan, except extreme altitude/latitude
  - generalist
- origin of D. mel, not a question until mid-1950s
- early strains all N. American, current lab strains also N. am, collected in 1920s
- Meigen 1830s described ~~[redacted]~~ european D. mel
- N. Am D. mel not native
- biogeography & molecular variation

discovery
- D. simulans, Sturt, 1919
- D. yakuba, Burla, 1954
- 1970s Tsacas, David, collecting in Africa
  6 more species
- West of rift Om ⇒ Ds

biogeography
- yak savannah
- teis tropical forest
- erecta only in W. Africa, only breed on pandanus
- orena only found 1 time in Cameroon
  lots of heterochromatin
- sec only some islands in seychelles, merula octanoic acid, [same concentration mel will die]
- mau only mauritius
- san only santomea, volcanic island, yakuba at bottom
- closest to mel subgroup are in India/S.E. asia

1. Hist. of Dros Genet
2. Life history & biogeography
3. taxonomy

No.

Date

- Taxonomy & Classification — no good fossil record
- W. Hennig, entymology, phylogenetic analysis method
- cladistics, monophyletic groups
- monophyly vs paraphyly
- ancestral vs derived characters

pleisiomorphy apomorphy

phyl. – Arthropods, inextensible cuticle, dorsal nerve cord, tracheal resp syst.

class. Insecta, 6 legs

- hemimetabolous — larva & adult similar in form
  - hemiptera
  - homoptera
  - orthoptera
  - odonata
- holometabolous — 210 Triassic
  - strepsiptera (sister to diptera)
  - lepidoptera
  - hymenoptera
  - coleoptera
  - siphonoptera (fleas)
  - diptera

orders

subord. diptera – hind wings reduced to halteres

125 K species
clusio fungar clocks

nematocera – mosquito
= long antenna
= sclerotized head capsule

brachycera

↳ no head capsule → prothorax

= aschiza

= schizophora

– cyclorapha, tano cuticle

- acalypterate
- calypterate — wing lobe housefly

→ >70 families

4 acalypterate

- associate w/ higher plants
- diversified (w/plants) in late cretaceous 100-65 mya
- none in N.Z., many in Australia
- larvae associated w/higher plants or their degrading

o Drosophilidae

~4000 species

- center of diversity is SE asia

No. life hist. cat microorganisms

Date

- wing venation, bristles & arista/antenna define group
  - Steganine - no active Adh, e.g. Amiota, amber fossils from eocene
  - Drosophilidae
    - larva adapted to high [ethanol]
    - vegetable-based fermentation
    - by yeast / bacteria
    - e.g. repleta group (hydei exception) are associated w/cactus, e.g. mojavensis
    - eg D. pachea (Bill Heed)

subfamily

- insects (arthropods) cannot synthesize steroids, but needed for cholesterol
  - ecdysone

- permitted by active Adh, single gene
- Adh- 4% eth will kill
- Tapinus
- chymomyza (agression, boxing)
- Drosophila (paraphyletic)
- 40 genera, relationships poorly known

Q: are tephritid & drosophilid Adh's homologs

yeast 2 Adh's

genera

- Steve Benner
- ancestor nonfermentative, 100 my
- origin of fermentative,

- Sturtevant laid down taxonomy of Drosophilinae

subgenus
- Drosophila, 4 spiracles, interrupted tergite, ♂ bow
- Sophophora, 2 spiracles, black ♂ tergites cont.

→ Idiomya (1-2K species)
- Hawaiian Drosophila
- big island Idiomya
- endemic species → sexual selection

species groups
- melanogaster — 110 species
- obscura — holoarctic
- willistoni — subtropical
- saltans

species subgroups
- melanogaster 9 sp
- montium biggest, SE Asia, Africa
- ananassae

- SE asia → Africa ~20 mya prob. Af & Asia seperated by Tethys (now Med & Red sea)

Genetic Analysis

- w
- eye pigment cells contain
  - brown pigments - ommochromes - from tryptophan
  - red " " - pteridines - from guanine
- white gene is transporter for precursors of pigments
- vermillion, defect in brown pigments
  brown, defect in red pigments
- Morgan showed w was sex linked

$+/+ \times w/Y$ P

$w/+ \times +/Y$ F_1

F_2

w/Y	+/Y	w/+	+/+
♂	♂	♀	♀

♀ w/w ← shows that w is sex-linked and not just sex-limited
w/+

♂ w/Y
+/Y

- recessive - lower case | superscripts for alleles
  dominant - uppercase | semicolon for diff chrom
- Bateson, early British geneticists, "presence-absence" hypothesis
- apricot, intermediate phenotype, behaved as allele of white
- $w^1/w^a \times +/Y$

  ↓

  w^1/Y w^a/Y

  - allelic, segregate
  - multiple alleles disproved
  - only see white or apricot ♂
  - never see all 4 classes predicted by 2 locus system!

w^{29e11} 29 May 1911, abbreviated to w^{29e}
(day month year)
- introduced by Bridges
- avoids numerical series clashes in distributed network

nature of μ

- prior to 1928 all μ spontaneous
- low frequency 1/million
- Ed Lewis - Red Book ~1965

ltw/Y ; ltw is allele of scute
su(ltw)/su(ltw) su(ltw) found by Bridges

example of supression, interaction

→ found that su(ltw) could suppress phenotypes of many mutations, all spontaneous, no x-ray/chemical, not all spont.

- majority of spont μ are transposable element
  - ☆ collects all μ & assess % by TE
  - ✱ use inverse PCR to find gypsy in su(ltw)
- stable μ, very, very rare reversants

nondisjunction

w/w ♀ × w⁺/Y

↓

w/Y	expected		
w⁺/w			
exceptional progeny	white ♀ wildtype ♂	] ~1/2000	matroclinous patroclinous

- exceptional progeny at rate < spont μ & occurs very regularly
- non-disjunction, Bridges, failure at meiosis I
- if failure at meiosis I, egg has 2 X
- crosses predict XXY ♀ & XO ♂
  test using cytology
- nondisjunction on 2nd & 3rd leads to lethality, 4th can be observed
  → implications for meiosis (not perfect, error rate higher than μ rate) & sex determination

sex

- Y not male determining
- X:A ratio, balance hypothesis, 1925 Bridges

- Bridges hypothesis was widely accepted, even for humans
- 1950s ~~[illegible]~~ showed Y determining
- no sex hormones in Dros / insects
- butterflies, mab & female in same org
- gynandromorphs, Morgan, rare
- sophophora, sexually dimorphic:
  - ♀ larger than ♂
  - ♂ sex comb
  - ♂ pigmented on abd.

gynandromorphs

- gynandromorphs are normally 50:50 down midline
- ~~[illegible]~~ not possible w/ circ. sex hormones
- cause is nondisjunction in mitosis or chrom loss in mitosis

fate mapping

- Sturt, used ~~gynandromorphs~~ mosaics for fate mapping in D. sm to study development.
- 10 nuclear divisions
- pole cells
- cellularization
- map fate of blastoderm to adult structures
- 1968 Sturt, Ash & Garc. Bellido & John Merriam 1970 J. exp. Zool
- chrom loss only occurs very early in dev
- w^a/Y & w^a/w^a show same phenotype
  w^e/Y darker (?) lighter than w^e/w^e 1932 Muller

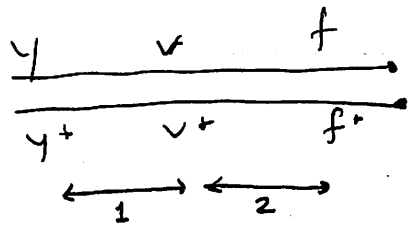
we dosage effect

- Curt Stern ~~[illegible]~~ studied dosage compensation
- dosage comp established after sex determination
- if chrom loss after dosage comp / sex deter then lethal to cell lineage

- Lilly Morgan's wife discovered w/w that 100% non-disjunction

- attached X chromosome
  - XO male is sterile
  - Y has sperm/fertility factors, ~16 genes (Anderson)
  - allowed genetic proof that X-overs occur at 4 strand stage of meiosis, after replication
  - reverse metacentric, eg c(1)RM ← unstable
  - X-ray → breaks, efficient in males to create attached X
  - c(1) double X c(1)DX Muller
    TA (tandem acrocentric)
  - $X^* Y \times X\hat{X} Y$
    ↓
    $X^* Y \quad X^* X\hat{X}$
    cross mutagenized males to attached X ♀

- mapping



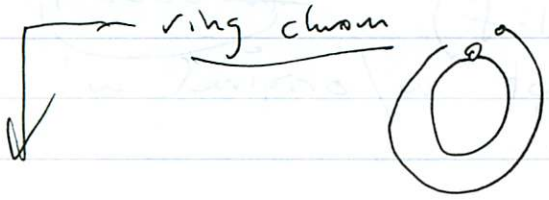
× w.t. ♂

y v+ f+ region 1 recomb
y+ v f
y v f+ region 2 recomb
y+ v+ f
y v+ f double recomb
y+ v f+

- Muller found that double x over is < product of single x-overs ⇒ "interference" 1915
- genetic basis of interference is unknown
- C. elegans, absolute interference, only 1 Xover per chrom
- interference stronger between regions closer on genetic map

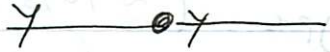
- found chrom where all recomb were double x over but no single x-overs

ring chrom



ring - nd het can only have $ double x-overs

- worked out by genetics then test w/ cytology
- "unstable rings" generate gynandromorphs at high rate, hard to keep stocks, molecular/genetic basis of instability unknown
- tandem metacentric at metaphase makes a spiral



can be used to generate ring chromosomes

- exchange locks chrom together, ideal if homologs x-over, not btwn sisters
- can use ring to see if sister x-over occurs, loss of ring in progeny bc 2 centromer
- only innermost chromatid
- genetic engineering shows gene order
- x-overs 1st seen cytologically
- Bateson & Punnet → linkage
- Strut (?) showed genetic x-over
- 1932 Muller 6th congress genetics (see also dosage comp)
- nomenclature of alleles

$w^a/w^a = w^a/Y$ dosage compensation

$w^a/Df(w) < w^a/w^a$ paler w/ 1 copy

w^-/w^a; $Pp(w^a) > w^a/w^a$ darker than ♀

- Dfs can occur in het, but only ↑ ~1 Mb

- Df 1st made by Bridges (after X-rays ?)
- many ways to Dp
  free duplication Dp (1:f)
- could do same dosage expt w/ original w+ no change in phenotype.
- classes of mutation

  w⁻ amorph loss of fxn / null

  w^a hypomorph partial loss of fxn / leaky

! "never study messy mutation" always know nature of allele, preferably use null.

- assay for allelic type (~90% reliable)

  M/M vs M/Df(M)
  = null
  < hypomorph

- can't just sequence / western, Dros is good at reading thru null

mostly dominant, not rare
- hypermorph → rare
- antimorph
- neomorph

gain of fxn alleles

antimorph reverted by deletion (Sco) sensitive to dose (Dp) opposed wt product

neomorph → new fxn, temporal, spatial (eg Antp) insensitive to dose can be reverted by deletion, mostly chrom ablerations

hypermorph Dp(M) > M/M > M/-

- Minutes (bristles small, not body)
  - Dominant M/+
  - recessive lethal m/m ~~dead~~
  - small bristles
  - develop delayed
  - large # of genes
- haploinsufficient (coined by Lindsley)
- great majority of haploinsufficient are minute
- +/Df ⇒ minute → remainder affect fertility, flightlessness
- proteins required in stoichiometric, not catalytic concentrations
- 1st hypothesis that minutes were tRNAs, general family of genes
- ~60 minute loci
- 88 cytoplasmic ribosomal proteins genes (CRP), 79 proteins
  ~~75~~ mtRP
- 64/65 minute → CRP, not to mtCRP
- 1 locus haplo & triplo lethal TP1
- 2 minute loci, not additive most severe Jack Schultz
- not all dominant m are haploinsuff.

- struggle to find / temp, x-ray effects on m because looking for visible
- easier to find lethals, Muller

  l/+ × l/+

  wt.

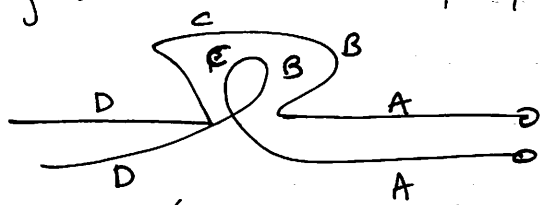
  ~~l/l~~ l/+ +/+

- but hard to keep lethal in stock
- Muller thought to have 2 closely linked lethals

$$\frac{l_1\ +}{+\ l_2} \times \frac{l_1\ +}{+\ l_2}$$

- balancing, balanced lethals
- no meiotic genetic recomb in ♂, flies/higher diptera mosquito do have ♂ recomb

- why? no answer ★
- annanassae, low level ♂ recomb
- known very early that no ♂ recomb
- first found wild chrom that x-over suppressors naturally occuring inversions -1916 ↑ -1936
  took many years to connect x-over suppressors to inversions
- very polymorphic in nature
- took x over suppressors, found spont ♀ & ♂ showed that map was inverted
- 1920 mel - sim maps same order except 3R
  genetic evidence
- 1933 polytene chrom ~~[illegible]~~
  - easy to show
- somatic pairing 1916 Metz in mitotic, also in interphase
  ★ big question ★ no mechanistic understanding
- significance not known, but consequences
- pairing of inversions to form a loop



- 1935 mel/sim hybrid Pctw
- isolate lethal on inversions ⇒ stable balanced system

balancer

- balancer chrom must have:
  inversion
  recessive lethal
  (Dominant marker)
- how to keep an x-linked lethal?
  - instead of recessive lethal, have recessive ♀ sterile

- mutagenesis
  - use ♂, why?
  - more ♂ gametes
  - sperm metabolically inert
  - oocyte, large cell,
- finding mutations

X_m/X_m ⊗ $\xrightarrow{mg}$ X_p/Y

↓

single X_p^*/X_m × X_m/Y

↓

X_p^*/Y	dead
X_m/Y	— 1
X_m/X_m	> 2
X_m/X_p^*	

if x-linked recessive lethal

1 ♂ : 2 ♀

problems: semi-letrality
small samples (no sig diff ♂ v. ♀)
lose mutation

- Bar start 1925.

$ClB/+$ × $\xrightarrow{mg}$ X_p/Y

↓

$X_p^*/ClB \otimes X/Y$

↓

X_p^*/Y if lethal no males

ClB/Y dead

X^*X

XX

strategy used to show that X-rays ↑ μ

radiation implications

non-quantitative assay

- CIB assay must recover μ from het ♀
- Basc
  Bar w^a scute
  invasion but no lethals
  Muller-5, M-5 named by Curt Stern
- repeated - don't use free w.t., use markers not relevant to cross to guard against contamination, non-virginity

Basc/Basc ♀ × *~~/Y ♂♂

↓

Single ♀ X*/Basc ⊗ Basc/Y

↓

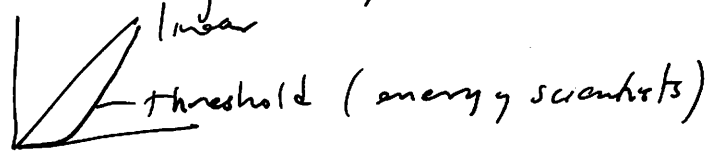
if lethal no wild type males can select ♀ balanced because bigger red eyed

Basc/Y → X*/Y

Basc/Basc

Basc/~~Basc~~ X*

- 1949 Curt Stern Rochester, 1st used Basc assay
- atmospheric testing banned because of Muller's work & evidence for dose response
- 4% per kilorad of X w/ sex linked rec. lethal
- 5krad ~ 20% of X w/ lethal
- human dead w/in 3 days at same level of radiation knocks out gut epithelium, dividing cells
- X ray not gamma
- dose response, linear argued by Muller



- Curt Stern addressed this question
- Basc assay used for chemical testing
- replaced by Ames test & cell culture assays

- xrays good for breaking chrom. 30% of lethals are obvious chrom aberrations
- chemical mutagenesis
  formaldehyde, mustard gas
- early chemicals not well suited
- ethyl methane sulfonate EMS, Fahmy's → Alderson → Lewis, adult feeding strategy
  25 mM EMS, 60% of X have ≥1 lethal
- C|C → AT mutations

Cy0/Sco × +/+ ♂ ♀

↓

single ♂ +*/Cy0 ♂ × Cy0/Sco

↓

Cy0/+* ← +*/Cy0

~~Cy0/Cy0~~
Cy0/Sco
+*/Sco

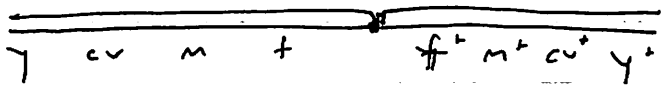
↓

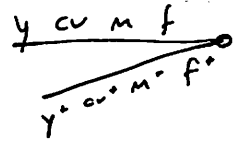
Cy0/+*
+*/+*

- Attached X in ♀ , Anderson
- y cv m f

C(1) RM

y+ cv+ m+ f+

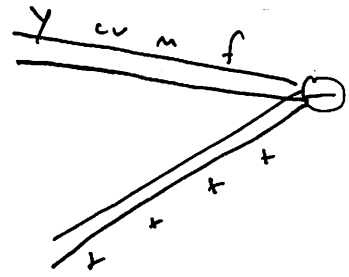




× X/Y

- phen w.t. ♀, produces recomb daughters

y	10%
y cv	10%
y cv m	10%
y cv m f	2%

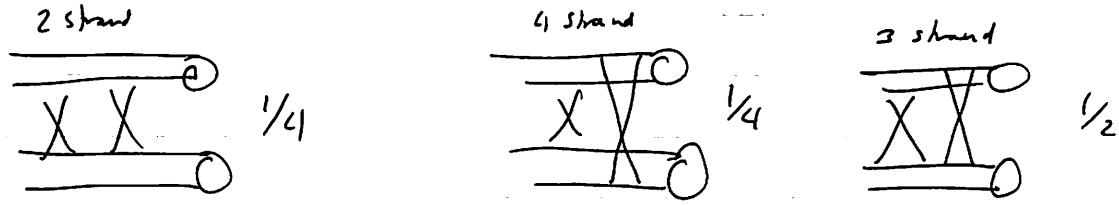


1st mei division homologs
2nd mei div. sisters (like mitosis)

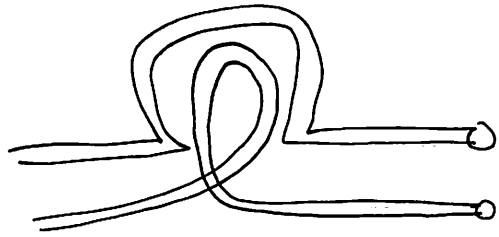
- tells us that X-over occurs at 4-strand stage
- can involve any non-sister chromatids
- attached X allows 2 chromatids to be assayed
- at least 2 errors in Bridges paper, position of centromere wrong (at tip)
- compound chrom also allowed centromere to be mapped

- non compounds, single exchange on X around 60%
- exchange not proportional to physical distance
- double X-over 30%

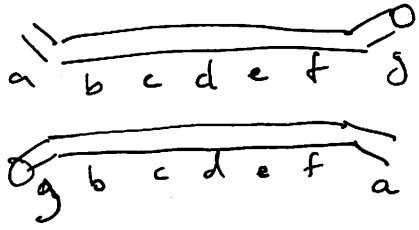
- DCO can involve same or diff sisters
  1/4 use same sisters, 3/4 use other sisters



- no effect of interference on chromatid choice
- genetic interference unrelated to chromatid inteference
- 3% TCO
- 7% No cross over, NCO, segregate using distributive system
- meiosis occurs in ♀ in egg w/no cell division
- inner most nucleus is only product of meiosis that is fertilized
- inversions as x-over suppressors

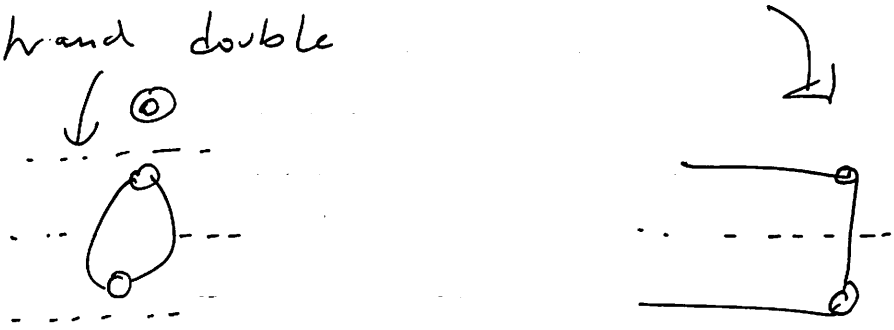


- In(1) sc^{8}
- recover no SCO, but same frequency of DCO



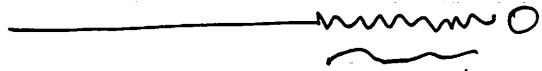
- Start & Beadle, no egg mortality, inv. don't suppress X Over, they suppress recovery of x-overs
  fertility normal
- often get patrilinous sons, but never matrilinous daughters

- SCO causes acentric & 2 outer norms
- 4 strand double



↳ leads to eggs w/no X chrom

- Fig 14, p 107 of Roberts
- predict 3:2 ratio of DCO : patroclinous males
  - Sandler said this was "true science"
- Isabel Thomas noticed 3:2 DCO : NCO
- large para centric suppress exchange by suppression of recovery
- medium size inversion
- In (1) dl-49

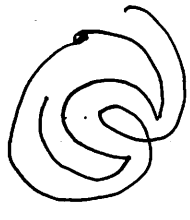


40% of X is het.
no cross overs in het.

- SCO w/in inversion cause dicentric, can't recover
- suppression of X over near breakpoints is not due to elimination
- Ed Novitski (advisor to Ptashne) & Braver 1954
  2nd hardest to read
  1st is Kramer

made reverse metacentric with inversion

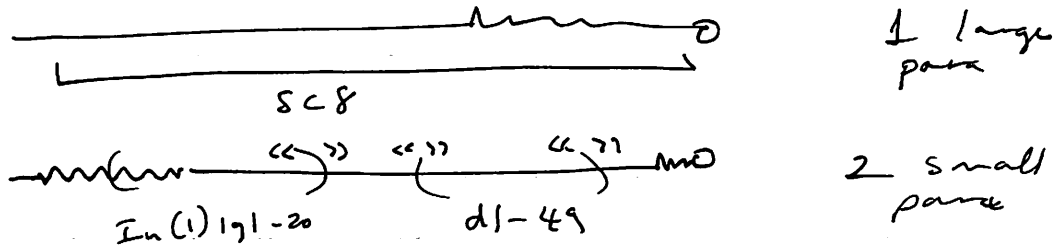
- found suppression on both sides of breakpoint



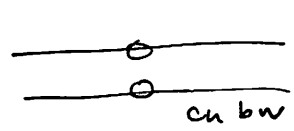
- inversions suppress occurrence of exchange as well as recovery
- Dobzhansky "compet. pairing model" explanation why x overs suppressed, but wrong, Gong showed pairing is fine using FISH.

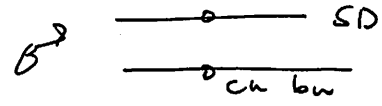
- pericentric inversions
  - reduce fertility
  - cause duplication & deletions
  - all have 1 centromere, so no dicentrics / acentrics

- balancers
  - on X use FM7



  - FM7 x wt 1/5,000 x overs per meiosis & X-overs are not viable
  - Tiling experiments show gene conversion events btwn balancers & w.t.
    - on other chrom, SM & TM, somewhat worse
    - usually have 1-2 pericentric & ~~1~~ paracentric
  - choose balancer w/ breakpoint closest to gene of interest.
    SM6 ok, SM3 has weaknesses
  - Ishiraizumi





cn bw is white eyed

↓ no white eyed progeny

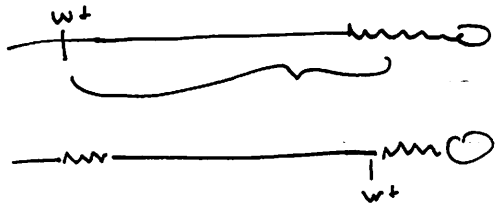
SD

- cross in other direction is fine
- meiotic drive

haplotype preserved by inversions

- ever sporting displacements, Muller

PEV



In(1) w^m4

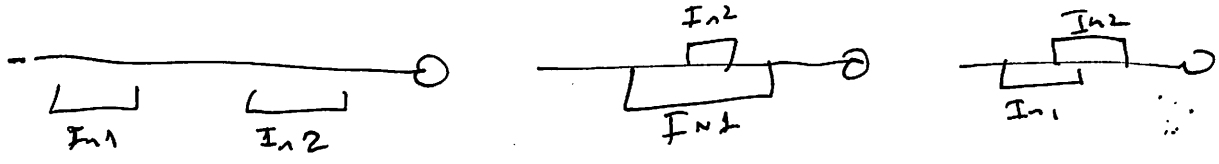
white mottled 4

position effect variegation bc of inversions

- when white displaced, many "mutations"/sports
- chromatin modifier genes discovered by E & SV of Var
- Gunter Rueter, Tom Guilliat
- Hershy Chase expt DNA is heredity material
  - Heaven - go to heaven & given expt novics do expt every day for eternity
- claim: gene order doesn't matter, inversions show
- epigenetic decision made in embryo
- use of inversions in phylogenetics

phylogenetics

- 1935 start natural pop poly for inversions
- D. mel 6-7 common inversions, many others
- other species complex inversions (2-3 rel. to standard)
- arbitrary standard chrom



- Stt liked puzzles (crosswords, ~~inversions~~ anagrams)

- A B C D E F G H •
  A C B D E F G H •
  A C F E D B G H •

  ↕ Shirt idea

- unrooted trees
- Will. Hennig, outgroup
- Dob. showed same btwn species, D. azteca
- 2 heroic studies Wasserman - repleta 70-80 species
  Carson - St Louis - Hawaiian 120 species
- Green, mosquitoes, SE Asia, cladistics, rooted tree

translocations ↓

$\rightsquigarrow + ; + \times \frac{Sco}{Sm1} ; \frac{Sb}{Tm3}$

$\frac{+}{Sco} ; \frac{+}{Sb} \times + ; +$

↓

$\frac{+}{+} ; \frac{+}{+}$ ✝

$\frac{Sco}{+} ; \frac{+}{+}$ expected, only see ★

$\frac{+}{+} ; \frac{Sb}{+}$ "pseudo-linkage"

$\frac{Sco}{+} ; \frac{Sb}{+}$ ✝ - translocation

- only segregation that leads to full complement gametes are opposite (not adjacent)

  T(2;3) 39A, 72c

- adjacent - dup/def - same centr go together
- alternate - full complement - diff cent go together
- breakpoints suppress recomb. Dob. 1934 ← compet. pairing model
- 5-6 numbered units suppressed

corn mice in flies not true in yeast

- FISH shows exchange doesn't suppress pairing
- for inversions in yeast, bc exchange ↑↑ ~320 per meiosis, cp 5-6 for flies
- Haldane's Rule, exchange scaled to have ≥1 exchange per chrom.

2° Nondisjunction

- Bridges 1916

w/w ♂ × +/y

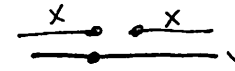
primary non-disjuction: w/w/y × +/y → 1/2000 nondisjunction

~~F1~~ w ♂ wt ♀ regular progeny

secondary non-disjunction: w ♀ wt ♂ exceptional progeny

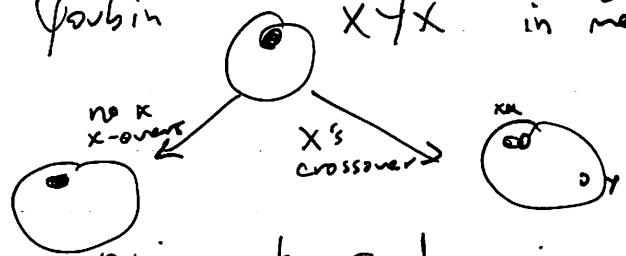
5-8% nondisjunction

- both types of non-disjunction occur at meiosis I
- XX ⟷ Y, not undergone exchange
- Bridges argued that ←XY→ ??
  ←X→ goes at random
- max freq of 2° non-disjunction is 50%
- Stut & Beadle found excess of 50%
- Cooper 1945, miranda, X_1 X_2 Y (don't x-over)
- if X ~~—~~ NCO



trivalent

- predicts the 2°ND & NCO, can see 1 80% 2°ND
- Y with 1 arm has 50% 2°ND
- Yoshin XYX in early meiotic prophase

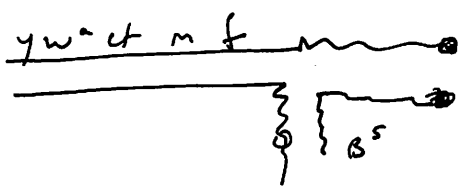


✓✓ - cen pair at random in yeast, after x-over homologs pair

- welcom
- job advert
- RSE/BSS

?? X-overs & segregation

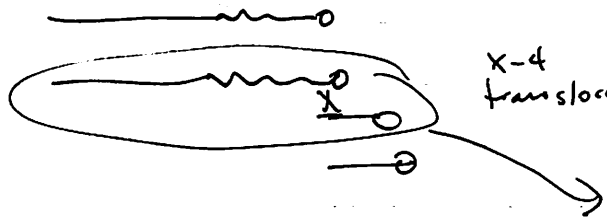
X - Y translocation.



× y w ct m f

• X-cent
○ Y-cent

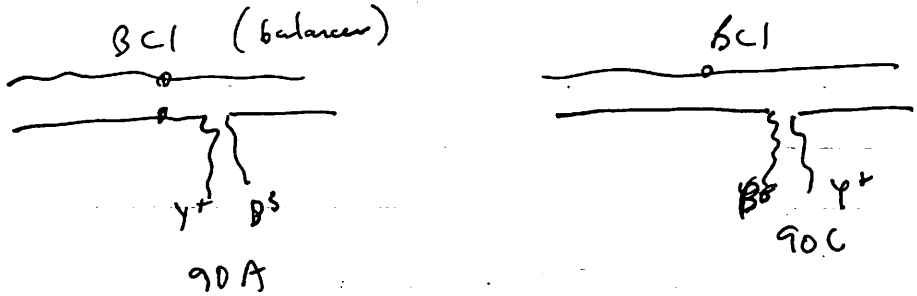
- ½ B^S ♀
- homologous centromeres segregating at random
- X over on arm is preventing homologous centromeres & forcing non-homologous to segregate & ignore homolog
- exchange commits centromeres to segregate (Dean Parker)
- Dean Parker - Half translocation paradox



X-over by X ray will cause 2 chrom to segregate, & thus both can't be obtained

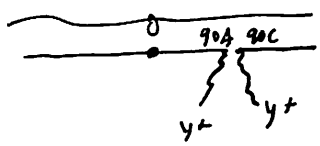
✓✓ - cen 5 / cen 3 switches in yeast
segregate properly, not cen. homology
x-overs ensure proper pairing

deletions using A-Y translocations



?? how did ?? y+ get on Y

- A-Y translocations mapped by polytene
- ♂ wt eyes yellow



△ 90B

- select ♂ y, B^S dup of 90B
- def/dup over balancer
- Sandler, segmental aneuploidy Lindsay, etc Auf
  - found 80 haploinsufficien Merriam X-chrom
  - found triplo lethal
  - problems: Lose B^S, lose y, X-ray breakpoints are "casserole" of seq.
- 1st Done by Patterson & Brown
- y dup on Y chr also includes sc, so hairy wing

Summary

- X-over btwn any 2 chromosomes commits them centromeres ~~regate~~ to segregate from each other
- breakpoints of inversions & translocations surpress X-over in their vicinity
- het. inversion suppress x-over by 3 mechanisms
  1) breakpoint suppression
  2) crossover elimination (pericentric)
  2) production of aneuploid gametes (peri)

SM3 & FM7

Sunday

Duplications

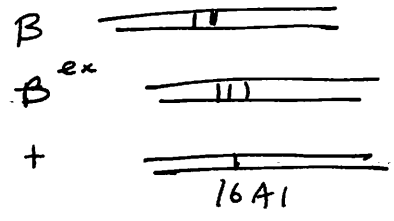
Bar
= B/B reverts to round eye (1/1600 Zeleny)
- only in ♀ see high rate

+B+
+B fu
inv
6.2 map units — 0.25 map units

1925
Stort & Bridges thought X-over may be involved.

Scored 20,000 progeny, 8 revertants of Bar
7/8 revertants fu or +fu, recombinants
2 flies, more extreme form of Bar, both recomb.

- Stort inferred that revert & extreme alleles were complementary products of exchange
- 1936 Bridges & Muller indep. report polytene



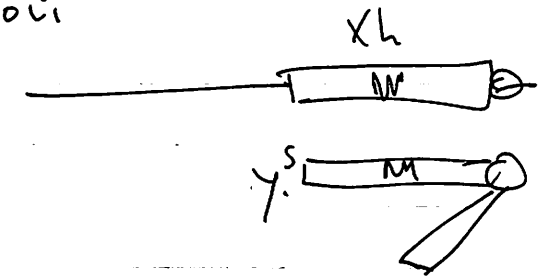
- tandem duplication
- how explain dup w/ exchange?
- unequal X-over
- Lonin & Peterson worried about 1 revertant that was not recombinant

f B odsy
f B +

50,000 progeny
32 B → B+ w/ X-over
3 B → B+ no X-over

- maybe intrachromosomal, show by B → B+ using B/deletion
  2 mech: 1) sisters
  2) same chromatid
  } test using ring chrom
- Mel Green 1968 ring Bar/del
  show get revertants, but no extreme Bar proves that same chromatid exchange can occur, no evidence for/against sister chrom exchange
- BB/+ phen more extreme than B/B
  3 4 2 2
- total # less impt than position, "stable position effect" Ed Lewis
- Bar stone has only 1 copy
- color blindness, opsins on human X
- bobbed
- 18s/28s rDNA, similar phenotype to bristle phen of minute

- 2 loci



- copy # matters
- Bridges postulated repeated genes 1915

bb°/Balancer x bb/Y bb⁻ no rRNA on Y

↓

bb°/bb → expect all bb, but 20% stably
bb/Balancer revert

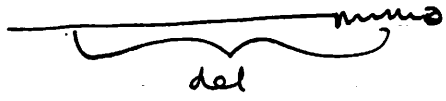
- Tartof post. sister unequal exchange could amplify rDNA array

reciprocal mitotic ?

- use ring chrom to test, no amplification in ring
- can also show reciprocal product ⇒ sister chrom exchange

1) - tandem dup production, use minutes
irradiate ♂ cross to minute/balancer
screen for non-minute dup of minute+

2) - free duplications (big deletions)



- used to define centrome

3) - insertional duplications / translocations, γ-rays

- 1956 Vernon Ingraham (student of Sanger)
hemoglobin dups.

Meiosis

[- indep assortment / rearrangement allele gene order not impt.]

McClintock 1933

- 3 important things:
  1) pair
  2) x-over (physically interlocks, commits cent to seg.)
  3) homologs segregate M_1
- pairing in C. elegans, Abby Dernburg
- telomeres not needed for Dros. pairing
- somatic pairing in G_1 ~~& G2~~ disrupted in S
  so pairing needs to be established in meiotic prophase
- how much exchange & where is it?
- 56-60 map units on X
- exactly 1 xover per chrom ⇒ map length = 50
- why greater?

	NCO	SCO	DCO
E_0	100%	~~0~~%	
E_1	50%	50%	
E_2	25%	50%	25%

Data

1000 progeny
~~495~~ NCO
~~495~~ SCO
~~50~~ DCO

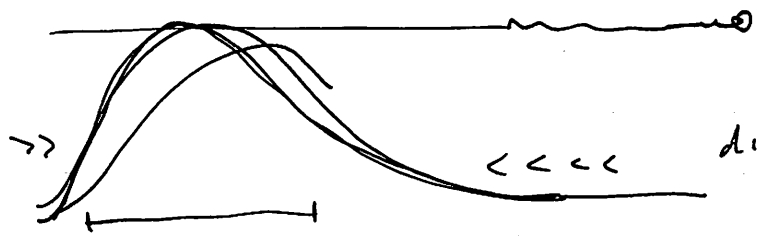
observed data →

$$NCO = E_0 + \tfrac{1}{2} E_1 + \tfrac{1}{4} E_2$$
$$SCO = \cancel{0 \cdot E_0} + E_1 + \tfrac{1}{2} E_2$$
$$DCO = \tfrac{1}{4} E_2$$

- Zwick ML tetrad analysis
- interference in small intervals so strong that DCO not relevant btwn close markers
- real #s

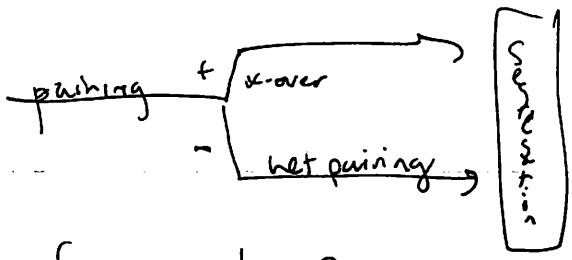
E_0 = 5% (8-10%)
E_1 = 65%
E_2 = 30%

- autosomes E_0 little lower
- much larger E_0 expected under poisson if 1.1 x-over per meiosis
- where is it ?



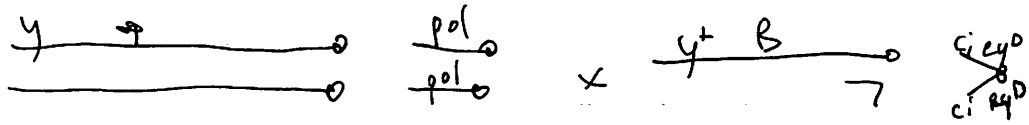
- "centromere effect", prevents exchange
- delete cent het, psh exchange further towards telomere

- exchange not proportional to unit length
- telomere effect
- cis & trans effects in ——
- 1.1 - 1.2 x-over per arm, except 4 (0)
- x-over "pirates" sister chrom ~~exchange~~ cohesion to lock homologs b
- 1/4000 x non-disjunction, but 5% NCO why not 5% ND
- het. pairing, back-up system to ensure seg in NCO
- chiasma & non-distributive system



★ - fusion of 2 arms ⊕ NCO since NCO % is same per arm & 2 arms indep.

- mid 1960's Lindsley & Sandler, 1st screen for a process meiosis, wild type extraction, 1 μ mei-332
- use ND as phenotypic assay
- Baker & Carpenter, EMS screen, 1972
  10 μ 114 drom screened



look for

y, pt ♀
y+ B ♂

mei 9 - protein that resolves Holliday jxn
mei 4 - ATR
mei 352 - makes exchange prop to map length
clip3A, kinesin

mei 218
mei 38
mod - no distributive, 90% exceptional progeny ~~chrom~~
many 4 loss, only 50% max ND with no distr. system
- 2.5% (½ of 5%) NCO × ND

prl screen | c(3)G, ca nd

now ~100 meiotic μ in Drosophila

- types of meiotic mutants

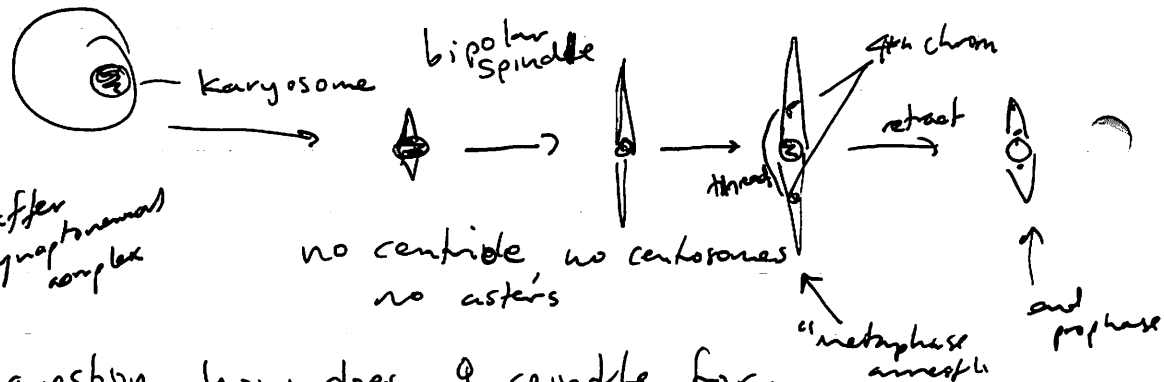
exchange
exchange defective < req to make DSB 68, 22, trem
not meiog mus312

precondition mutants = 288, 332, 219, 352, mcm5, rec replication, sig. in proteins

segregation
seg. defective

- can't find meiosis genes that are also involved in mitosis
- genes in meiosis that are dispensable for viability & fertility
- How to ~~get~~ bypass requirement in viability?
- T.S. M
mosaic analysis
- prior to 1977 nobody tried to visualize meiosis
- Nokkala & Puro, drecin oocyte

- ♀



- big question, how does ♀ spindle form
- textbook meiosis is ♂
- 4th has DNA linkage through threads

- ♂ no synaptonemal complex, no DSB, no recomb

X - Y pairing thru rDNA array
collochore

* higher rel rate of indels on autosomes no DSB in ♂ so less DSB induced indels.

- Kaufman X del bb°, transgene of rDNA
- intragenic spacer
- nucleolus cohesion remainder → pair X Y rDNA
- chrom 2 histone pairing genes
  3 ??
  4 colloc chrom site

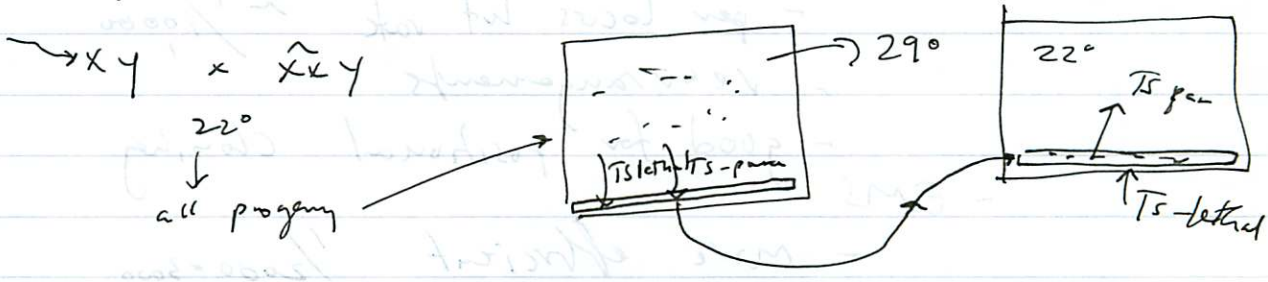
	recomb	centromere	4th recomb
♀	Y	N	N
♂	N	Y	N

Hawley 1992 - het pairing in 4th derivatives necessary
Kaufman 1996 - het (>70 kb) pairing sufficient
Dernburg 1996 - cytological evidence

- recomb in ♀ on 4th at 29°C or triploid

Screens

- TS μ in nervous system Daniel Falk
- paralysis



- screen vs. selection
  - screen → filter manually
  - selection → only get μ of interest

selection not more specific, may miss weak

- Dom. vs. recessive

↳ autosome test in F1
easier to find
small fraction of μ

Dom
1) gene encodes a structural protein, (not enzyme) that is required in specific quantity

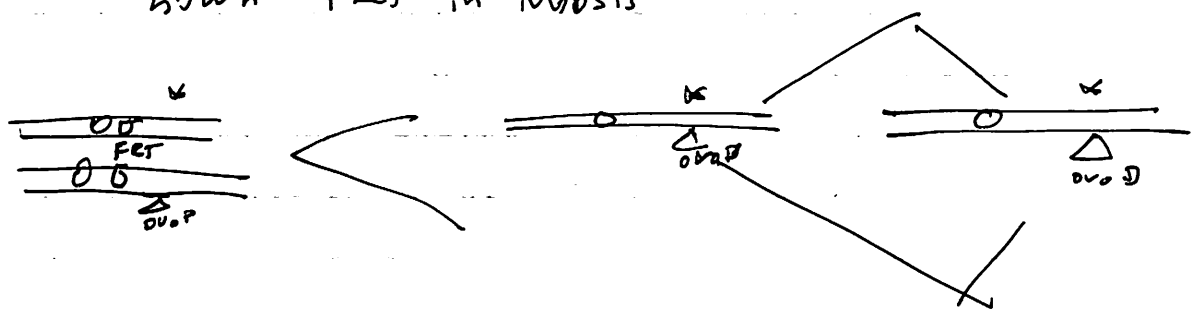
- enzyme efficiency makes substrate limiting, thus most enzymes $\mu \rightarrow$ recessive
2) get "abnormal form that is poisonous "antimorph"
3) misexpression "neomorph"
4) easier, but can't always get

Rec
most genes can μ to recessive, except for redundant loci
1) works for most gets
2) much more work

Mutagen choice

- X-rays
  - safe,
  - per locus but rate ~ 1/10,000
  - rearrangements
  - good for positional cloning
- EMS
  - more efficient, 1/2000-3000
  - dangerous
- transposons
  - easy to clone
  - reversable
  - not very efficient on 1/100
    adh <1/100,000
  - biased

- what are 2° screens
  - ovo° tumors in ovaries, mitotic proliferation
* FLP - fRT hs-FLP, catalyzed recomb btwn FRT in Mitosis



- cross to C(2) EN ♂
  * ♀ germ-line clone × C(2) EN ← Ed Lewis
    if ND
- screen → μ → balance → complementation test → map

- want multiple alleles
- non-complementation screen
* complementation test
  - recessive
  - same phenotype
  - make het a/L → μ? alleles
    a/b → wt diff genes
  - can lie if modular independence
    wt ⇒ intragenic complementation (same gene) rudimentary, enhancers (but inter-diff)
  - can lie if interacting proteins that
    mt don't complement (diff gene but inter same)
    nc33 B-tub-
    ⇒ second site non-complementation M. Fuller
    ⇒ dominant enhancement Bridges

* SSNC screen
  - ~~need 2 to have~~ SSNC μ, need to have same phen as double μ

- SSNC defect cant be mimicked by OE
  control for not being haploinsufficient
- 71 - 81 Hartley

SSNC { type 1 - allele specific μ at both loci
2 - allele specific μ at 1 locus
3 - non specific at both loci
haplo-insuf at both loci

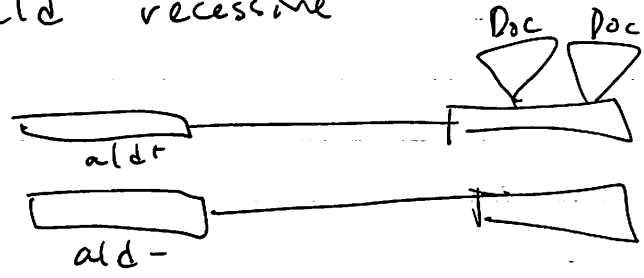
- SSNC, need good sensitized "bait" & good secondary screen

* Dominant enhancement
  Simon .... Rubin
  serenless, RTK, TS

p(sev^{TS}) ; $\frac{+}{+}$; sev^{+} 22.7 → w.t.
24.3 → μ RT missing

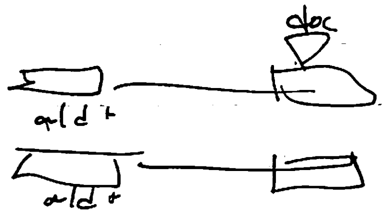
- can we find μ that make serTS dom at permissive temperature
- F1 dom enhancement, look for rough eye.
- secondary screen μ on its own in eye
  use clones to avoid lethality

ald recessive



- ald+ is being shut down by Doc
- Doc μ of germline trx

- suppression only works in cis



w.t.

- if RNAi then would work in trans) should be

- 7 suppressors / 1500 lines μ, 3R
- His2AV

Dideoxy
  fluorescent dyes
  automatic seq → capillary
  cycle seq

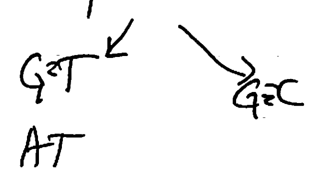
- mult hits in diff. μ ⇒ same gene → good hit
- get 1 hit, reseq other μ in that gene (not whole genome)

- insertional alleles (P) are usually weak hypomorph
- excise imprecisely
- general rule, want > 1 hypomorphic allele
- always find complete loss of fxn before genetic analysis of complex traits
- μgenesis done in marked μ background
  don't μgenized w.t. chrom
- gratuitous markers
- swap backgrnd chrom
- be extremely careful abt background μ
- if ⇈ μ rate, may be polymorphic μ in original stock
- make chrom isogenic before μgenesis

pr/pr × CyO/Sco
↓
100 single ♂ pr/CyO ♂ × CyO/Sco ♀
pr/CyO ♂ & ♀ pr/CyO
↓ pr/pr

select most isogenic

- not entirely isogenic: new μ & recomb w/ balancer
- can save a lot of work downstream
- freeze down some isogenized flies to sequence after EMS
- ~~Isofe~~ inbreeding, 4 chrom 17 gen for 95% isogenic, lethals, inversions
- EMS alkylates $^{e}G \equiv C$



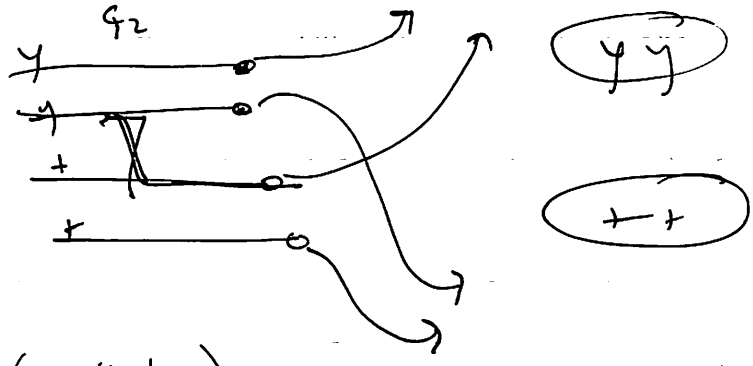
- replication leads to mosacism could be in germline
- can confound screens for lethals
- solve by making μ isogenic
- replicating instability Auerbach, nature unknown
- <u>screens for chrom abberations</u>
- can use pseudo-linkage for Translocation screens
  recomb suppression for In & suppression of minute for dup
- μ of choice is x-rays
- X-ray induced breakpoints are messy
- each division is ~~Mb~~ 1-1.2 Mb 120Mb/100
  lettered subdivision 150-200 Kb
- deletion of <1 division will survive (~1Mb)
- deletion kit 80% of genome, why not 100%
  haploinsufficient, seq limits unknown
- FRT based deletions, seq level resolution
  clean breakpoints

transvection
- Bridges, diff alleles of ubx complement bx^{1}/bx^{34e}
- Lewis complementation of ubx alleles depended on pairing in somatic cells
- somatic pairing ~~mitosis~~ & interphase & metaphase
- mitotic crossing over Stern 1936 Genetics
  y/+ yellow patches on wt background

Clonal analysis — gynandromorphs — fate mapping
= mitotic x-over
- flp-frt

sex dimorphic region

- can't be x-loss, if in post tergites predict blackened patches, not plain. ♂



- somatic (mitotic) x-over, can also occur in pre-meiotic x-over (not just soma)
- 4 divisions in ♂ germline.
- Hans Becker X rays ↑ mitotic x-over
- clonal analysis
- replaced FRT stocks, flp-FRT
- mitotic x-over ♀ = ♂, unlike meiotic
- RNA system required for mitotic but not meiotic pairing

bx^1 / bx^{34e} wt.

$R(bx^1) / bx^{34e}$

R = Inversion or Translocation

- hyp: that rearrangement weakened somatic pairing & predicted that ~~dose~~ transvection dep in somatic pairing
- R need to be near Ubx 89B, not just any rearrangement will do
- Madeleine Gans Zeste z/z yellow eyes

$\frac{w^1 \quad z^1}{wt \quad z^1}$ w.t.

- y, many diff alleles, same trend but diff phenotypic variation
pairs of y alleles w.t.

- eya, y, w, bx, dpp show transvection
- intragenic complementation pairing dependent.
- allelic exclusion in immune cells & olfactory cells

P element & transgenic analysis

- hybrid dysgenesis, white.
- p-element, structure, IR & TP
- exogenous DNA disrupts P-tp
- use helper TP, but helper can also integrate
- rosy, ry^- drosopterin biosynth. defective. Xdh
- only few % xdh ⇒ w.t.
- rosy not cell autonomous, diffusable allow few cells ⇒ w.t.
- Bob Levis helper w/no IR, "wings clipped"
- good hands few % transformation
- nearly all lab strains don't have p-elements
- marker, early ry^+, w^+, mini white
- size ↑ efficiency ↓
- hobo, Minos, mariner, hermes, piggyBac – looper moth
  hydei mav. mus f-ni moth host → baculovirus
- allows reversion assay, precise excision
- Δ2-3:99B, Sb
- imprecise excision → deletions ☆
- remobilization rate varies ??, does this depend on ts ??
- local hopping
- homing
- position effects
- P into heterochromatin silenced do insert
- insulators to buffer ~~pos~~ pos. effects (SupoR P)
- Fischer, Brand GAL4-UAS
- enh trapping lacZ
- combine enh trap w/ GAL4 construct

transposition ↓ remobilization

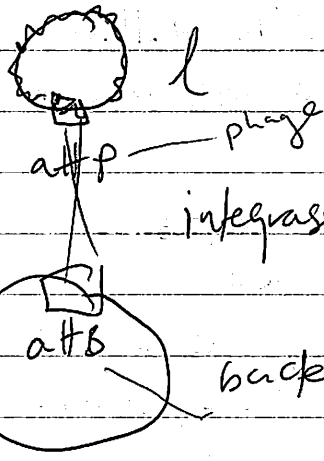
- choice of promoter for galf, hsp
  Actin, da, elav, ubi.
- inducible cell killing, Ricin A
  Diptheria A
  Hid

- FLP FRT

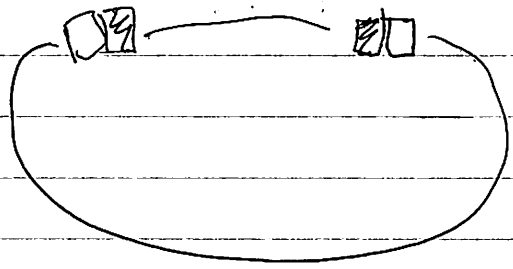
- ΦC31

FRTs are identical

attb ≠ attp diffe



makes



2 hybrid sites
integrase doesn't
recognize hybrid sites

- ΦC31 can go in in both orientations, but same place, select same orientation
- 30% of null → lethal
- ends-out easier, KO, precise
- ends-in

- EP lines

- Dutch Book 1925
- Bridges & Brehme 1942!
- Lindsley & Grell 1968 at Oak Ridge
- Lindsley & Zimm 1992 (press in 1989)

UNIX NROF

- L & G mutations listed
- L & Z genes listed
- illustrated by Edith Wallace, THM artist
- 1988 Lindsley & Ashburner
- 1992 Flybase
- mod, model org. databases
- relational databases
- L & Z, lit. refs, ex. 16 times in diff forms
- 1st commercial, sybase/oracle
- open source, mysql, postgresQL
- schema

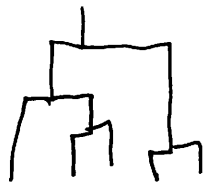
Haripeng

flies Africa 8×10^6
Europe 1×10^6

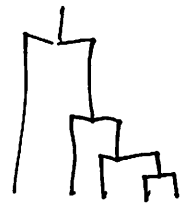
human 10^9

$$p = \frac{2^{n-1-s}}{(n-1)!}$$

n samples
s branchpoints w/ 2 descendents in sample



less likely



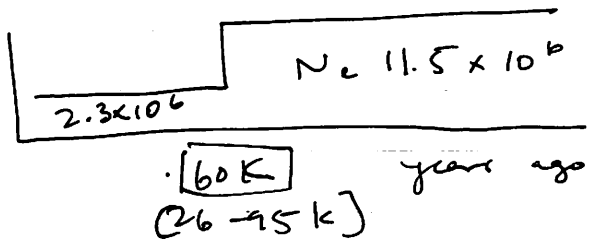
more likely

$$\frac{\theta(1 + k_{max})}{2\mu(1+\theta)}$$

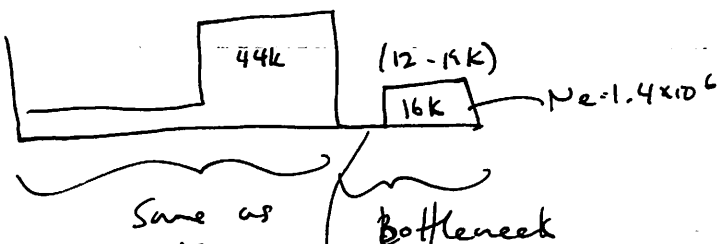
k_{max} = maximum # diff b/tw two seq in your sample

- varying mutation rate, more above mean
- Zimbabwe, skewed toward rare
- European, too few rares
- $\mu = 1.443 \times 10^{-9}$, 10 gen per year

Af



- transition during inter-ice age period
- savannas prevailed during glacial maximum
- expansion before last glacial maximum, transition to domestication.
-



founder 2.9x10^3 [700 - 14,000]
duration 340 [20-1000]

- compact mutation freq spectrum, dilce Lasky's no/21/107
- tendency for sweeps in proximal half of x
- 1/3 of windows Europe
- 1/4 of windows Africa
- 2 overlapping

> candidates for adaptation

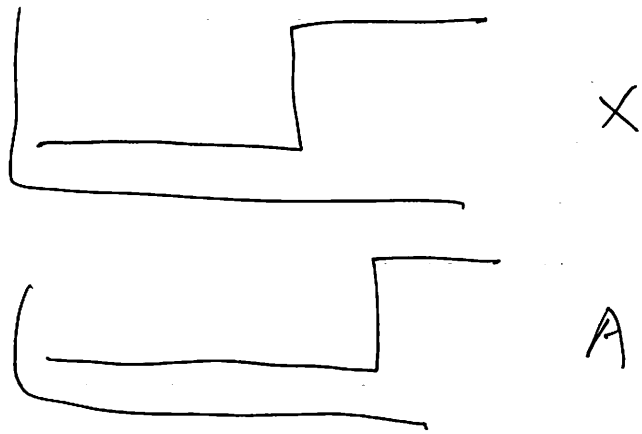
- very few μ w/ $s > 1\%$
- Europe has shift towards higher s, because can't observe low s in low pop site
- bias on detecting dist of s as fxn of N_e

0.061×10^{-9} ~ 150 adaptive μ on X during 60K years, Af

0.088×10^{-9} ~ 58 " " 16K " Eur

per site per gen

- X/A
- sex ratio $N_X/N_A = .56 - 1.125$



units of N generations

- X has higher rate of adaptation
- 3.5 my → modern

400 cc 1,400 cc

Chau-T. Ting Genetics of Speciation

- F1
- Haldane 1922
- genetics of reproductive isolation
- biol species concept, limitations: fossils, asexual
- pre/post zygotic isolation
- how many genes / nucleotides responsible for species differences
- classical, molecular, population genetics of speciation
- Haldanes (1922) rule, heterogametic sex, original observation true in many taxa
- causes of Haldanes Rule, why effect of sex chrom
  - dominance theory
  - fast-male theory
  - fast X theory
  - meiotic drive
- D-M model related to dominance theory ∴ HR easily explained on X if incompatibility is X-linked (∴ therefore "dominant" in ♂)
- F1 ♂ is always "unbalanced"
  ♀ is balanced
- can we make unbalanced ♀? attached-X
- 1985 Jerry Coyne 1st test of dominance theory using attached-X
  sterility → unbalanced ♀ is fertile
  ⇒ Dominance theory cannot explain HR
- Orr 1993 same test for inviability
  also (Stvrt / Ashburner)
  imbalanced ♀ inviable
- introgression, how many factors involved in sterility
- multiple loci w/epistatic interactions (sim-many, sim-sec)
- Sawamura 2000, introgression in rescue background
- True Segal marker introgression
- Presgraves more incompatibility genes on X

- many genes
- sterility in male is most common phenotype
- all regions act recessively
- large X-effect caused by higher density of hybrid incompatibility on X
  - neck effect, strong interactions
  - Dobzhansky

- meiotic drive
- animals often have X-linked drive systems
- sex ratio not 1:1
-

♀♀ ♂♂

$XX \times X^D Y$

↓

all ♀♀ strong drive sex ratio

↓

XX $X^D Y^S$ suppressor on Y could be elsewhere

↓

50% : 50% strong selection

- meiotic drive can participate, but does it?
- recomb inbred lines, Dermitzakis 2000 (differs from progression, just backcross) → F1, F2, then sib mating
- Tao, driver on X (dox) inverted repeat
  - not much young (umy) retrotransposition
- mother of dox, ancestral locus

HMR
JYAlpha
Lhr
Nup96
OdsH

- mel ♀ x sim ♂
  no males
  look for male rescue use natural strains
= also can rescue female fertility
- Hmr, X-linked, ~~fertility~~ deficiency mapping, transformation

Hmr 1 is P-element insertion

- Zim ♀ x balancer ♂
  Chung-I did cross, 1 week later
  no maggots
- 60 pairs Z, 60 pairs M
- Z female choose Z males
- prezygotic, ~20 genes Z-M

- dsaf2 loss of fxn in M strains
  16 bp del in dsaf2 promoter
  Z is wild type

- Z males interfere w/each other to get mates Z Z or ZM competition
- male-male competition in African pop
  but not in Europe

Ting Seminar

- immigrans
- sim clade, ♀ F1 fertile
- rapid evolution (except for transposition Orr)