Wellcome Twist Advanced course

- Red-book 1st ed, last pre-molecular mutant list
  - shows phenotypes of mutants
- easy short life cycle, high fecundity (200+ offspring/♀)
- safe, not a pest, no allergies, easy to work with
- eukaryotic ✗, higher evk.
= simple genotype, allows rearrangement
- many μ, ½ current, maybe a mutant in every gene
+ application to human biology
- cheap, Falk anecdote (ignorant)

Fly husbandry

♂ × ♀
→ eggs → larva → pupae → ♂ ♀
200 maggots

- 8 hrs until receptive (not 10-12!)
  older male will "rape" 5-6 hr ♂
- critical role of "virgining"
  1) clear & look < 8 hrs
  2) use only young virgins
  3) hold virgins for 1-2 days, look for larval tracks
     ♀ will lay eggs
  4) use μ stocks that show contamination
  5) μ (hid) stocks to virgin, hsp promoter sxl-gfp embryo sorting (↑ all ♂ die)

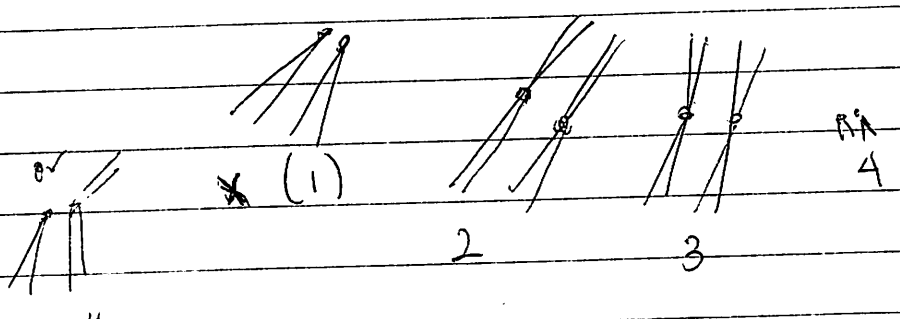
Design

? 18° overnight → Scott says don't twist it?

- ♀ store sperm, requires virgining
- keep 2 copies of stocks, different places
- mites: can alter results of cross
  jump from ♀ → eggs & kill eggs
- <21 days all vials
- 18 days, 24.5°C
- dark vials

- 4 chrom genes

- Ms. Stevens worked out karyotype of Dros.

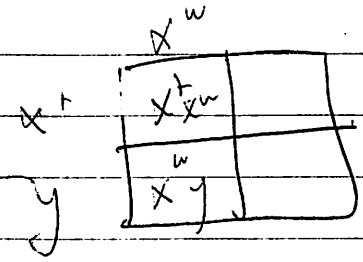


- 13th mvc. division pair → stay paired) ??
- or it pairs in G1 in each cell division
- 3rd instar larva, aceto aerd/orcein stain
- triploids are viable, fertile
- genes & chromosomes
- Mendel, Moravian monk
  1) purity & constancy of gene
  2) law of the gene (2 copies) particulate inheritance
  3) independent assortment
- ebony follows Mendel rules
- early μ, white didn't follow rules
- white → nomenclature
  - W w normal nomenclature
  - w⁺ w flies
  - 1st system - >1 allele, some dom/some recessive
  - case sensitive
- wt doesn't mean wild, it means the stock doesn't carry μ allele

♂♂ w × ♀♀ w⁺ → w⁺ (red) → red, white (all ♂)

♂ w⁺ × ♀ w → w ♂, w⁻ ♀

- one of the first u didn't follow Mendel's rule
- Stevens solved riddle via cytology
- eg. color vision



undergrad project
only score 100 + demonstrate sex linkage

- in order to explain chromosome theory needed to use a violation of Mendel's law.
- how do we prove this hypothesis ↑?
- Bridges, 48 yrs
  - greatest geneticists w/ McClintock
  - McClintock described as Greek God.
- Dean Parker did u work for Muller's nobel prize, irradiated w/ ice block, rotate
- do w/ 2000-3000 progeny
  - 2998 red ♀ / white ♂
  - 1 red ♂
  - 1 white ♀

? would you have recognized this result as important, or contamination of parents?

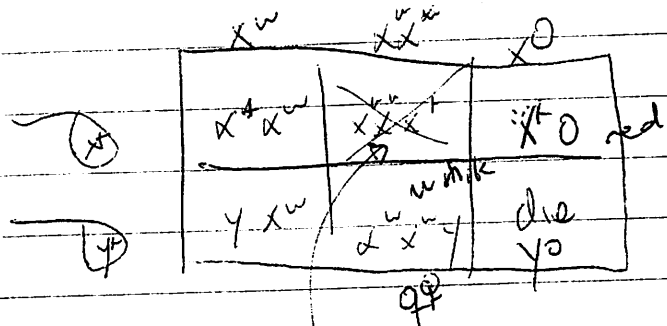
Bridges repeated expt & found repeatable frequencies.

not parents
- red ♂ is sterile, not parent
- white ♀ lead to high freq of white ♀ daughters
- didn't always come on 1st day of cross

- first has to solve XX x sex differentiation
- second, errors in meiosis
- third, XXX diploid autosomes not OK
- exception proves the rule

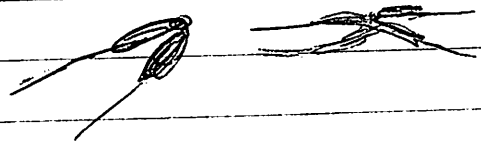
wet seq

- bridges postulated nondisjunction, go to same pole
- homologs pair → recombine → segregate
  match lock move

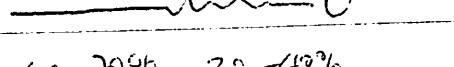


Bridges predicted triploid ♀ dies
but
- recessive/dominant
  - most genes are limited by substrate not by enzyme
  - structural genes can be dominant since amount of product matters
- Nathe Stevens did adult meiotic spreads from oogonia showed XXY ♀ are triploid
- proves chrom theory b/c XXY is exception to Mendel
- begins field of cytogenetics
- identified nondisjunction which is impt for birth defects in humans (trisomy 21)

Cytology — larval brain mitotic



ev - 98-99% of genes
wet - highly repet, simple seq.



60-70% 30-40%
ev wet

~,00 / 8000

3/band

150 / division

polytene rep & seq decoupled

2 → 1028, no cell division

differences in degree of replication

60% of genome ~~polytene~~ not does polytenize

- nomenclature
- ~1000 bands per chrom arm

low on 4th

50 bands / division, 20 divisions / chrom

- deletion mapping link physical (cytological map) & genetics
- 1 gene / 1 band

exceptions < , >

are bands missed

- triploids, chrom 4 will x-over, also heat shock
- ♂ does not x-over in ♀
- ♂ no recombination

LV Morgan ♂ w^+/Y × ♀ w^-/w^-

- lost 1 found fly ↓ w/w ♀ XXY

backcross to w^+/Y ♂ ↑ nondisjunction 4-10%

" secondary nonadisjunction "

- 1916 problem stated
- but w/ LV Morgan's fly

$X^+/Y - XXY$

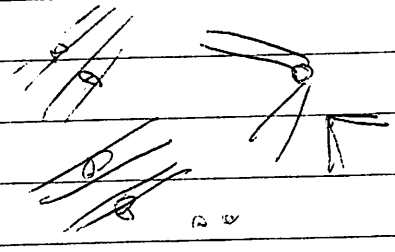
↓

100% nondisjunction

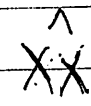
♂ all red

♀ all white

- N. Stevens solves problem thru cytogenetics



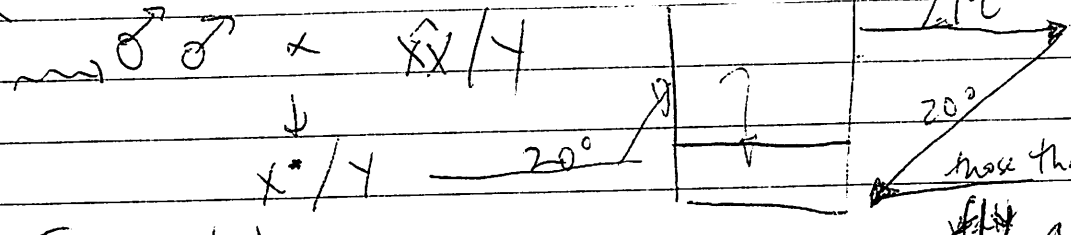
attached X or compound X



C(1)RM

compound 1st reverse meiosis

- chromosome mechanics in 1950's/60's game to create all types of compounds
- Darrell Falk
- EMS (warnings, melanomas) high efficiency
  X-rays - messy
  transposons - weak hypomorphs



- Ts paralytic

KU - blood
opine auxotroph

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

- compound X, pure transmission ♂→♂ useful for μ screens & making stocks

Balancers Muller

wanted to show X-ray cause recessive lethals

X/Y → 3500 Rads → minimal effect on fertility/viability

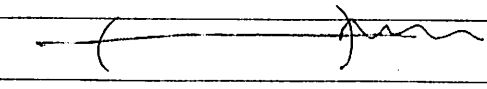
(350 Rads LD 50 human in 3 days Kills immune cells)

$X^* \rightarrow X^*/X$ how to keep μ (test early generation)

- need to keep & keep from recombining w/ homolog

Balancer 1) inversions suppress recovery of recombinants
2) carry a strong dominant μ

2a) carry at least 1 recessive lethal μ
not true for X chrom (recess. female sterile)



basc, (B, ac, sc)
Muller 5 (M-5)

= people use FM7 not M5
- "M-5 test" (can be used like Ames test)

$X^*Y \times M5/M5$ < many no rec lethal

↓

$X^*/M5$ cross intersex
$M5/Y$

look for round eyed ♂

yes → no μ
no → μ on X recessive lethal

- allows lethal to be kept & homozygotes to be made

↳ variant of FM7 with sin (♀ sterile)
- "new" generation of balancers w/ MS-GFP
- use to test genotype of embryonic lethal
- New Zuker Plan
  - 2-3 TL
  - Zuker lines have secondary μ
- autosomal balancers suppress recovery of recombinants, X chrom suppress recomb directly

★ polymorphism can prevent gRNA-based gene disruption
- Monica Justice, cre-lox in mouse

22/6/06 - Mapping

- X-linked lethals can't do complementation testing
- recombination mapping still relevant (although Muller claimed he explained data)

a's Marey map
Y chrom story
SNP mapping

♀ mirror of venus
♂ sword & shield of Mars

$\frac{y \; w \; ct \quad m \; f}{Y}$ × $\frac{+}{FM7}$ ♀♀

↓

♂♂ × $\frac{y \; w \; ct \quad m \; f}{+}$ don't virgin

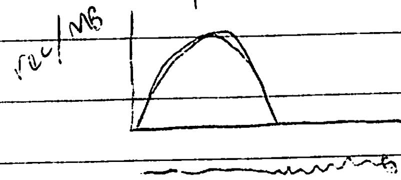
66% 1 x-over
30% 2 x-over
5% 0 x-over
<1% > 1 xover

typical ♀ meiosis

y	w	ct	m	f	200	400	score sons ♂
+	+	+	+	+	200		
y	+	+	+	+	5	10	
+	w	ct	m	f	5		
y	w	+	+	+	45	90	
+	+	ct	m	f	45		
y	w	ct	+	+	125	250	
+	+	+	m	f	125		
y	w	ct	m	+	125	250	
+	+	+	+	f	125		

assume all single xovers

? why is major class non xover if 1, 2 x-over per meiosis & only 5% non xover?
- Haldane's Rule all meiosis have one xover per chrom
- map distance ∝ physical distance is a lie!
→ A! bc xover occurs at 4 strand stage
- 4 strand stage any 2 non sister chromatids (Sturtevant)
- genetic maps measure freq of recombination



map length = recomb fraction
1 map unit = 1% recomb

CyO – Erwin Oster

$\frac{y\ w\ ct\ m\ f}{y}$ x $\frac{lethal}{FM7}$

→ $\frac{l}{y\ w\ ct\ m\ f}$

♂♂ x

Bridges & Painter

- alternative strategy is more work → df mapping
  - but no counting!

200
0 → tells that l is proximal to ~~white~~ yellow

0
5

0
45

60
65 — l is btwn ct & M, & assume ½ btwn

125
0 → l is distal to minature

XDf/y ; CyO/Dp x l/FM7
↓
l/Df ♀♀ CyO

( ) — Cy — Dp

( ) ; CyO — l

- balancers makes this possible
- SNP mapping, induce lethal on know chrom
  - 300 progeny, all non-lethal
  - single fly preps, seq center
  - large # of loci
- complementation test set of new μ before mapping minimal set

mini chrom
Dp centromere

- Robin did F1 dominant en/sup screens

Chromosome Aberrations

Inversions

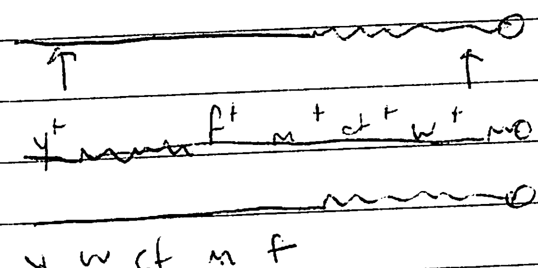
a b c d e f g h
a b g f e d c h

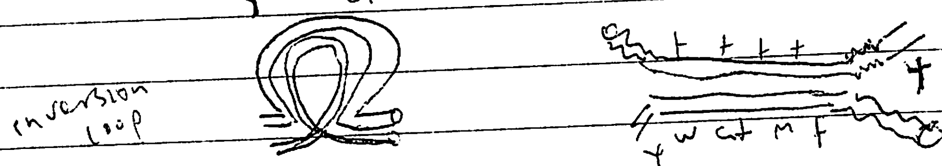
- nothing lost, nothing gained
- pericentric includes centromere
  paracentric centromere not in inversion
- size also impt, large >3/4 arm 15-20 # units
  small <10 units < 1/2 chrom arm

- balancers have combination
  x - 1 large, >2 small para
  auto combine peri + >2 para

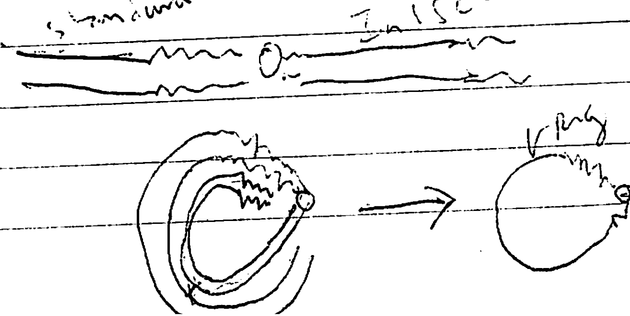
large para

In(1) sc[8]



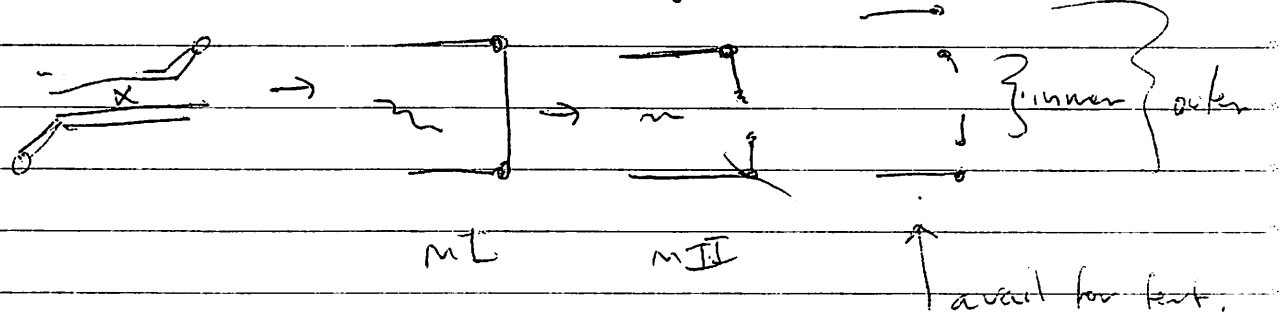


- no single recombinants, why? acentric/dicentric
- same fertility
- maybe just xovers don't occur?
  no, double x-overs can be seen
- Anderson & Beadle

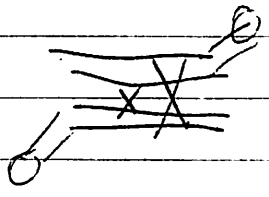


single x-over
- proses that (recomb) does occur in inversion that
- freq of ring recovery says single xovers occur at normal freq 60%
- ♂ meiosis, checkpoints, apoptosis if all chrom are not paired
- ♀ meiosis, doesn't have √points since only a few eggs produce (what abt fish egg ~ sperm)
- Sturtevant & Beadle 1936
- Dros Doyle meiI meiII all 4 products in a row, only innermost nucleus is available for fertilization.
- 500 x more force on cent than required to move it
- 5000 x more force than needed to break phosphodiest. bond

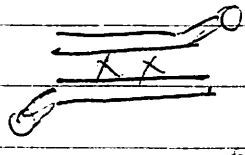
⇒ break dicentric bridge



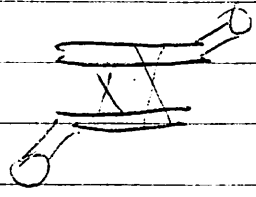
double xover



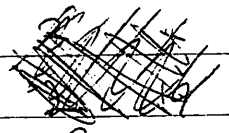
4 strand double 2 nullo x



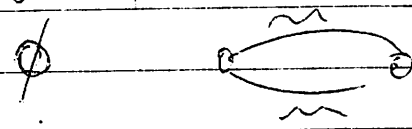
2 Strand 2NCO 2DCD



3 Strand (2 types)



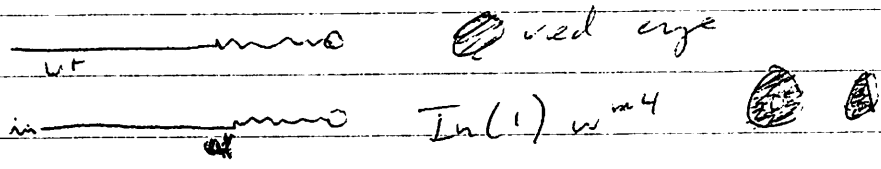
4 strand



3 DCO NCO

2 DCO NCO DCO NCO

- only at hot speciation was double bridge
- predicted ratio of patroclinous ♂ : doubles
  non-disjunction
- Isabel stone (Texas) did X's before St & Red
  didn't know about cell biology of fert.
  showed predicted ratio but didn't explain result.
  (similar to Ms Franklin story)
- large inversion suppress recovery of X over
  do not suppress ~~cover~~ (autosomal balancer)
  except around breakpoints
- Muller, "over-sporting displacements"

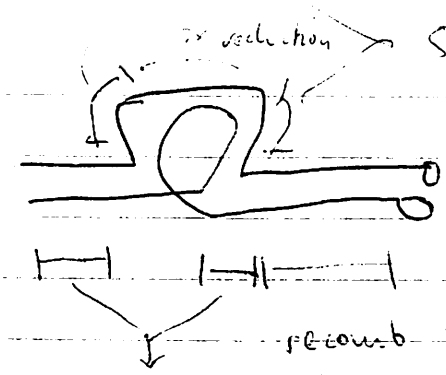


- heterochromatic spreading, reset in germline
- PEV, w^+ is normal, epigenetic effect
- many large inversions variegate for w, y, etc.
- enh/sup heterochromatin / PEV
- inversions are viable ⇒ genome order not impt.
  except for Hox etc.

small pox

In(1)Δ49 4F-12A, in middle of X
- where most of exchange normally occurs

"1/2st transmits dicentric
" centromere strength"

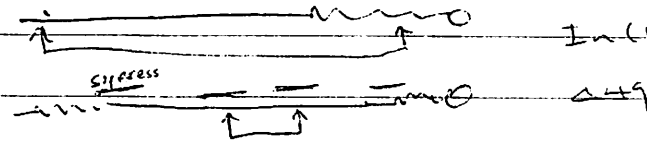


recomb normal rate

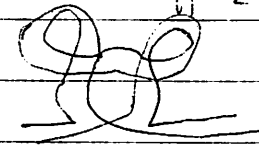
recomb reduced 5-10 fold

- why do recomb frequencies change, when they can't lead to acentric/dicentric
- heterozygosity for breakpoint suppress exchange directly since bivalent cannot synapse in small inv

FM7 used In(1)sc8



each small inversion supp 2-3 #'d units



- FM7 pairs in Muller's mouse fashion
- topology of DNA in region of breakpoint is similar to X-over
- recall interference, possibly inversion breakpoints cause interference → suppress exchange
- correlation of species w/ inversions & interference
- inversion explain balancers & effect of balancers

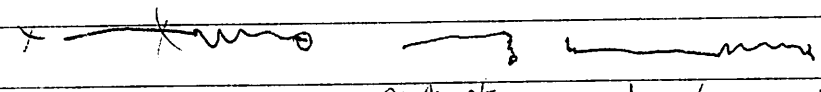
Dicentric inversions

not acen/dicen

- xover inside peri → dup/del but has 1 cen.
- peri in nature have mut that suppress xover
- how much aneuploidy can Pros tolerate?
  del < 1 numbered unit
  60 regions that are haplo insufficient

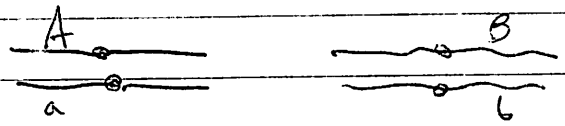
- dup < 2-3 numbered units
- most perihave both breaks in euchrom.
- don't use peri for dup → use PRT

Translocations

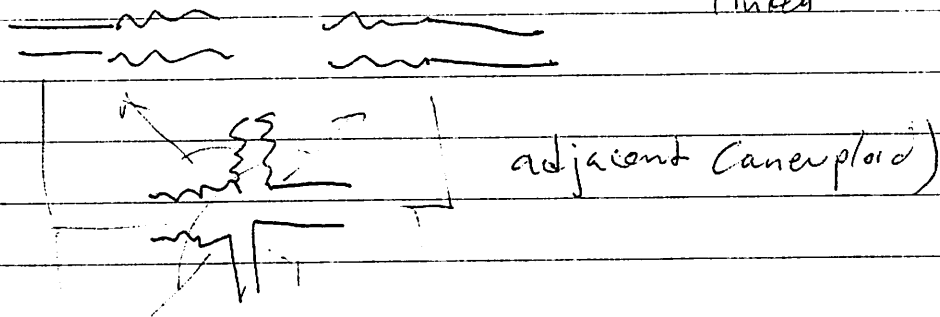


4 ——— part of X carried by 4th centromere

- strong dominant expr. of exchange (like small para)
- ♂, create ψ-linkage (only in ♂) & suppress

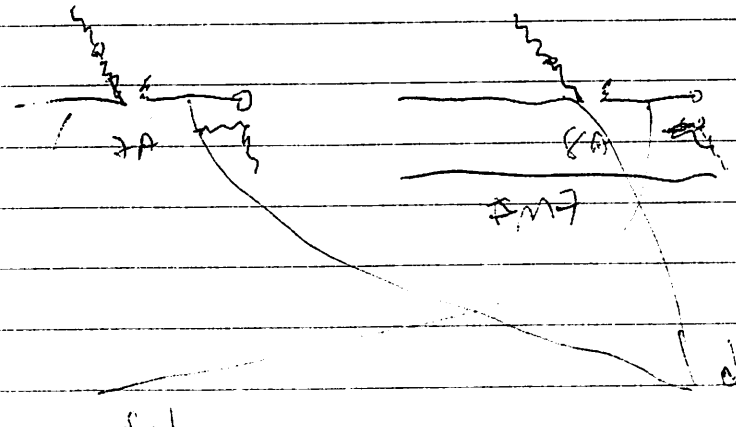


≈ oper to be linked



- 1st observation pale Altenburg (took in Muller student of Morgan)
- Segmental aneuploidy (Lindsley & Sandler 1968)
  - ♀ translocation in nearby regions
  - ♂ meiosis: no synapsis, no xover, no gene conversion

Transposed & not which regions can dup/del



cross 2 trx to each other to see which can permit (lack) dup

reeves → genomes 454
Satellite DNA

Telomeres

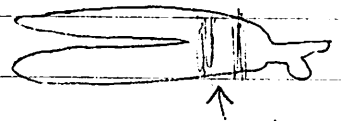
- early obs that deletions always retained last few distal bands
- McGrone Parker & Muller indep. disc. no terminal del
- only way to get rid of bands was to build a ring
- Parker thought they found a term. del, but had 2L term. bands.
- Muller published 1st using his own fly
- telomeres discovered in Dros., but don't have typical telomere repeats.
- are telomeres necessary for fxtion
- M2 flies, can create terminal del chrom will shorten every generation capture tel from another chrom or de nov

Centromere

- primary constrictions
- Bridges cmor - thought cen near yellow
- long runs of satellite
- epigenetic state, histone modification
- only single centromere

- minimal chromosome size? 1/20th of 4th
- maximum size in ♂ but not ♀
- 8 min mitosis, ≠ miss S phase
  mid blast 80-100 minutes (cycle 13-14)

- 11E construction, Hota Muscle genes



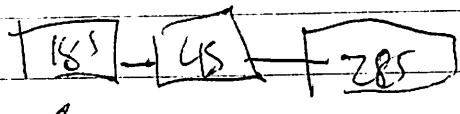
18S rRNA
28S

NO is more lightly staining
nucleolar organizer

- X & short Y have Nucleolus Organizers
- equal to bobbed loci. (bridges) 1938
- 220 rDNA repeats (160 on X, 60 on Y)
- bobbed are partial deletions
- deduced that it was a duplicate
- X^{bobbed} / Y^{bobbed} → W.T.

<120 copies strong bobbed phenotype

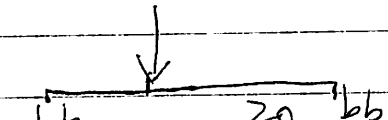
- why bristles, bristle requires a lot of protein



- Ritossa who found that rDNA = bobbed (bb)
- Tartoff bb/Ybb⁻ × Df /Bal

Qualitative shift

- Stubble reversion μ rate 20%



- called magnification
- doesn't happen w/ bb/Ybb+

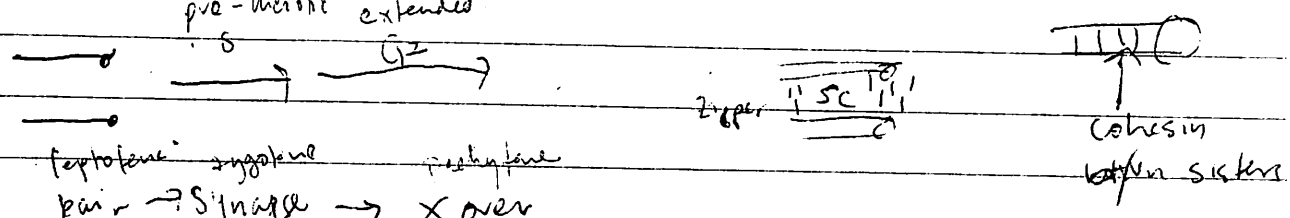
- Ritossa bb/Ybb+ × XX/Ybb⁻

bb/Ybb⁻ × $\hat{X}\hat{X}$/Ybb⁻

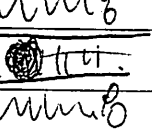
- observes quantitative shift in bobbed phen (less strong)
- in unpaired regions, see unequal pairing as if trying to pair w/ self
  unequal x-overs
- Ritossa proposed alternative model involving episomes

- resolution by Hawley, independently repeat expt.

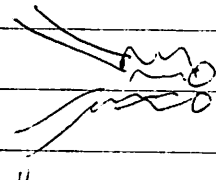
Meiosis

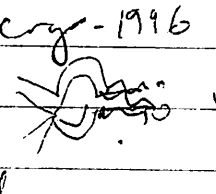


- pair → Synapse → Xover
- (SC) - no SC in Dros ♂
  SC hold chrom in place until Xover
- Down syndrome / Klenfelters (XXY) nondisjunction
  i.e. of failure to xover
- backup system to handle no xover on 4th
- programmed double strand breaks, WGS
- DSB occur before synapsis (synapsis will occur w/o DSB)
  but resolution occurs after synapsis



- recombination nodules
- difficult to find unpaired chromosomes
- Dros may have evolved pairing dependent gene expression mechanisms → transvection
- now cohesive forces → repulsive forces
- diplotene & diakinesis
  SC breaks down & now cohesin & Recom hold
- in Dros ♀, hat holds all chrom together
  but does not repel, only enchrom.
  Tiemberg - 1996


if no xover


if xover

- 4th all synapsed since mostly hat
- Bruce Nicklas explained fxn of xovers

- ♀ nuclear envelope dissolves, a centrosom
- ? how do ♀ build bipolar spindle, up until metaphase I centrosomes present, then disappear
- chrom organize spindle from inside out
- then freeze 1.5 days, pass through oviduct then anaphase I & fertilization
- C3G 1922 1st meiotic pr, no Xovers in C3G
  C3G is transverse filament protein
- claret nondisjunctional, shortening (1st in 1) sim)
  2 genes 350bp apart, deletions at this region are very common
- equational, Jack Schultz, lost
- Sandler & Lindsley, wit. ♂ in biol process
  1968 meiotic mutants, mostly a failure meis 332
- trigger to anaphase → dissolve cohesin ↓ temp can not be lowered
- Charlotte Auerbach, englishi, EMS
- Ed Lewis showed ~~EMS~~ EMS as ♂ in Dros DTS
- Baker & Carpenter, 200 X chrom, 9 mei mutants, EMS
- Hawley P-screen, 10 yrs ago
  FRT 3R, 2L

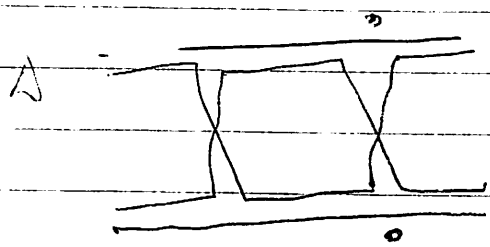
Recomb defective meiotic mutants

- E_0 = 5 % Weinstein Harvard 1988
  E_1 = 60 %
  E_2 = 33 % 4th chrom E_0 = 100%
  E_3 = 2 % X balancers E_0 = 100%

X nondis 1/1000 ⇒ other mechanism

- null recomb
  C3G, mei W68 (spo11), mei P22, mei-F9

- intrachromosomal(effect but balanced on 2 ↑ xover
- recomb defective
  - 2 classes:
mei-9, mus312 + 1) lower freq (10%), but otherwise normal
+ 2) preconditioned mutants.
affect final resolution steps in recombination.



do one or both junction

Szostak model

Terry orr-Weaver did work

- chromatids are intertwined so how do we separate chromosomes.
- 2 single strand nicks on X both = DSB
  ∄ nicks at ●

⇒ recombination

2) defined as if residual exchange is normal
no interf, don't respond to mutness of exch
(largest class (3/4)
no idea how the work
- clay

segregation defective ψ next in seminar

Hawley Seminar:

- central dogma of meiosis
  pair → exchange → disjunction
- largest killer of humans is failures in meiosis
  ~~X~~ embryo has Turners (1X). 40% spont abort
- % nondisjunction ≪ freq of non-exchange
- most nondisj. are non x-over
- no exchange on 4 bt segregates
- pair → exchange → disjunction "revised view"
  → non-exchange → achiasmatic

"distributive system"

- why study achiasmatic ~~[illegible]~~, omit exchange
  just study pairing & segregation
- ~~Hawley 1992 het. required for segregation~~
  Karpen 1996 sufficient " 350 K
  Dernburg 1996 showed that proposed achiasmatic
  pairing actually exists
- achias. X's are delayed in getting to plate
- ald, Mps1, inhibits activation of separase
- ald⁻ not metaphase arrest (anaphase like)
- spindle assembly checkpoint is required to
  delay the onset of anaphase long enough
  to allow co-orientation
- het regions held together not by protein
- achiasmatic het pairing held together by DNA threads
- threads are heterochromatin
- concerted evolution
- fold repl. in repeats → repair from homolog not
  from sister
- topoisomerase involved
- model doesn't differ from gene conversion
  - no SC
  - no DSB
- Xover commits centromeres to each homolog
- NOD ~ homolog to motor kinesin
  not a motor
  ? maybe bind to ⊕ end to stimulate
  polymerization

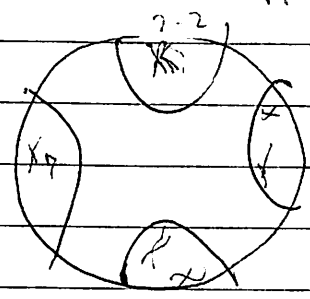
(margin: what seqs are involved in het)

Male Meiosis

- X-Y held together by rDNA collochore
- no SC, no recomb

maps have 1 less and add telomeres

-? what happens w/ DSB & SC in ♂



"go to your room" chrom not locked together

- mutants that specifically affect certain parts not observed for ♀ meiosis
- most μ only affect ♂ or ♀, but not both (except dub)
- Rulb: ♂ ♀ meiosis differs
- ?. does ↑ affect TE life history?

Dipt, Hymenoptera vs bee, Siphonaptera (flea)

- Diptera, 1 of 5 major holometabolous
- insects/arth, grow by molting, discontinuous
- cockroach - hemi
- metamorphosis in holometabolous morph & ecol. diff btwn larva & adult

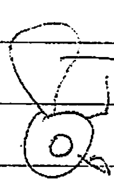
1.) egg/embryo, - no growth only gas exchange vitelline mem. chorion
2) larva, 3-4 instars, growth
feeding stages in last instar (minor peak ecdysone)
ecdysone, major peaks → molting Wigglesworth founded insect physiology
ecdysone receptor + USP
3) pupariation, crawl out of food contract "tan" skin
form pre-pupa, last molt into pupa inside
puparium,
juvenille hormone → insulin, insulin + ecdysone → molting
↓ ecd ⇒ metamorphosis
molec. bsis of JH is poorly understood
?. hormone receptor?

prothoracic gland

- [illegible] JH synth in ring gland, around esoph
- inside resp. environment steroi
- P450s involved in steroid biosynth.
  - µT have no whole (es. Shadow [illegible])
- JH in corpus allatum
- brain hormones control synth of ecdysone
  - light + metab → trigger
  - eclosion hormone
  - bursicon, tanning of adult cuticle
- Bridges 1920s introduced yeast as a medium
- filter feeders, eat yeast & bacteria as larvae
- * grow in micro-org rich environments, fermenting substrates
  - sugar → alcohol ↑ 12-13% alcohol in a yeast ferment. env.
  - ancestral Adh → very efficient alcohol detox enzyme
  - made by fat body, highly abundant
  - not orthologous to mam. Adh
  - some Dros. have secondarily lost Adh, specialized to non fermenting substrates
- flower or fungal feeders, leaf miners, aquatic carnivorous (Hawaii), gills of land crabs
  - predation
- adapt by using ferment. substrate
- repleta (hydei, mojavensis) rotting cacti
  - specific relationship fly-cacti, not pockets
- 3800 Drosophilid species (could be 2x)
- restricted geo. distr., exceptions like mel
- 800–1,000 living in Hawaii endemic
  - pesticides in sugar plantations
  - predatory wasps
) risks
- endemic to specific islands of Hawaii
- morphological innovations

- little work in evo-devo exploiting these mnou.
- lek behavior, (first described in grouse) Rombias
  → strong sex sel.
* most species display & mate on breeding site
- cosmopolitan flies, (D. mel), synanthropic (free living)
- 6-7 other cosmo. or pan-tropical
- ecologically versatile, opportunistic feeders
- newly cosmopolitan
- buzzatii, pan-tropical, opuntia (prickly pear)

* Diptera
- sister to mercoptera (stone-flies) or siphonoptera (fleas)
- first fossils, triassic, 245 mya
- nematocera (mosquitos) primitive — no puparium, larva have head, multi jointed antenna
- brachycera
  - non-cyclorraphan (muscomorpha)
  - cyclorraphan (pupate w/in harder larval skin)
    rotate 360, male genitalia, (myosin 31B ~~opposite rotation~~)
    - Aschiza
    - Schizophora (ptilinum)
      - acalypterates (Dros) ~~tephritids 70 fam~~ 10 superfam
      - calypterates (Musca) tse-tse



lower cretaceous 140 mya
1st dros → amber, eocene, Baltic 45 my
(cretaceous, canada, lebanon, S. Africa
dros → miocene, 15 mya, D. Republic

Grimaldi & Evolution of Insects

> most acaly exploit flowering plants, coevolve w/angiosperm
- radiation of angiosperm, cretaceous
- 75-80 mya origin of family (late cretaceous)
- Hennig dipterist, invented cladistics
- no acalypterates in N.Z. endemic so evolved since split of NZ from gondwanaland 80 my. (unless loss on NZ)

- fleshy fruits arose in paleocene after K-T
  + fossils & morphology of birds (as habitat)
- yeast have 2 classes of Adh
  1) fermentation
  2) metabolism
* - Steve Benner? which is ancestral? (2)
  fermentation is a derived characteristic
  needs sugary substrate
  - est date 75 mya
- >40, <100 age of family
- Drosophilidae (sister unclear) one of Ephydroidea (superfamily)
  - Steganines, [illegible], nonfermenting, [illegible]
  - Drosophilinae, fermenting, detoxify EtOH

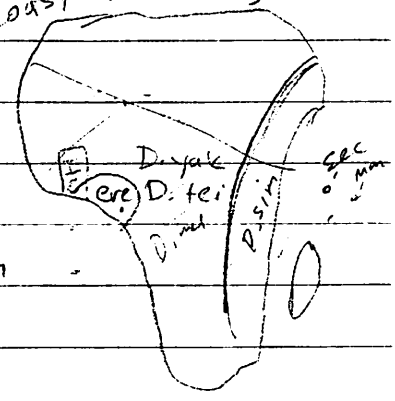
- [illegible] a bee, Cacoxenus, european, artificial nest
  parasitic

Drosophilinae

Zaprionus — white stripes, Africa, pan-trop

- Drosophila
- Chymomyza — 1st desc by Sturt, boxing behavior
- Drosophila — 4 filament, discontinuous abdominal band
- Sophophora — 2 chorionic filament, abdominal pigmented band
  - obscura — holoarctic
  - saltans, willistoni — neotropical, origin of P element
  - melanogaster — oriental, Africa, 180 species, 12 subgroups (African origin)
    - montium, largest, 90, oriental + Africa
    - mel, 9, African
    - anan, tak, suzukii — oriental

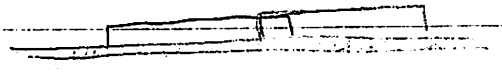
- Meigen 1834 melanogaster, 1906 Bussy Inst Castle 1908 Morgan
- Quackenbush sent S/Ard ♀ mel" → D simulans 1919
  - distinguished by shape of genital arch
  - not as strongly tolerant to EtOH
  - hybrids in wild Lipari Islands
- Burla 1954 D. yakuba, Ivory Coast (savanna)
- Tsacas, David, Lachaise
  - teissieri (forest)
  - erecta, pandanus fruit
  - orena, top of mountain in Cameroon
  - sechellia } island endemics (morinda shrub fruit)
  - mauritiana
  - saotomea, yellow, look like y⁻



- morinda not native to seychelles, indonesian 6-7 AD mariners, smells like roquefort, octanoic/hetanoic acids, lethal to D mel
  ? morinda mel. ecology
- highest species diversity in W. Africa
- rift valley, continues to Red Sea, very dry
  E of rift S >> M W of rift M >> S
- rift is 2 mya
- mel left W. Af >2 times
  - through now-disturbified Saharan region
    .5 mya
  - recent long haul
- 7 strains differentiated
- 20 mya Af → Eurasia, previous tethys
  differentiation in birds/mammals
- Dms can migrate long distance
- Hawaii
  midway → Aleutian islands
  NW
  - Drosophila
- Guppy - origin of fish name studied long dist dispersal

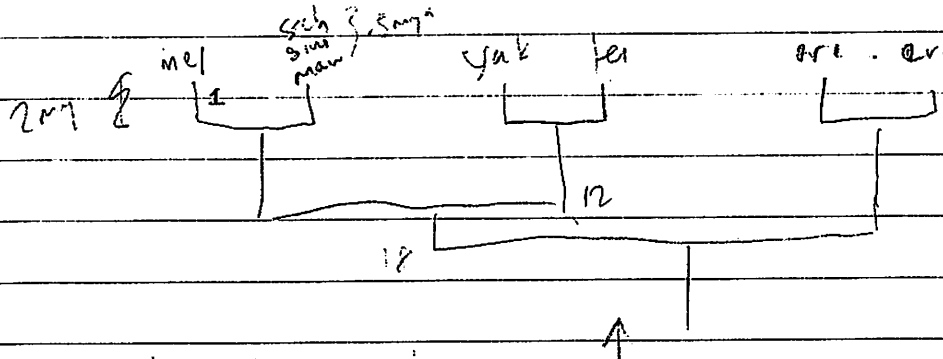
Painter

- 1933, Painter discovered polytenes
  Levitsky
- 1935, banding pattern was colinear w/ genetic map
- Sturt collecting pseudo, going for inversions
- loops form bc of somatic pairing



Sturt had intuition that overlapping inversions could be used to make cytogenetic

- replicate Wasserman
  hawaiin picture using Carson
  melanogaster Ash & Lemmon



- use tak to polarize this tree
- Sturt w/ sim collected visibles, mel-sim hyb
  - interspecific complementation test
  - genetic maps are same, except 3R
    Plunkett & Sturt 1926 all mapping, no poly
  - 1st evidence of inv. diff btwn species
    Patau - aneuploidy in humans trisomy 13
    Horton
    mel ♀ x sim ♂ mel ♂ x [illegible]
    behavior easy cross [illegible]
    ♀ sterile ♂ [illegible]
    ♂ fertile ♀ [illegible]

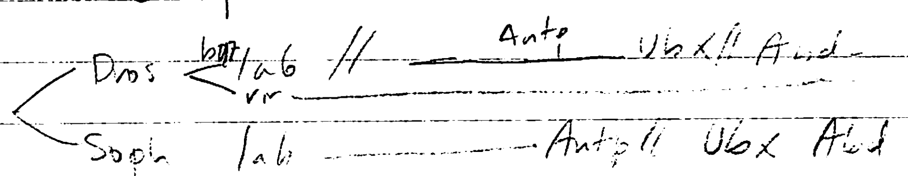
=> need sim X, can show by alternate X

- agametic sterile

- ? why sterile ♂ in hybrids ? mechanism
- temperature sensitive
- Watanabe 1970s, Lhr, dominant
  - rescues ♂ lethality (but still sterile)
    - formal genetic proof that lethality is under genetic control,
- Hutter, tried to find Lhr in D. mel
  - ukraine strain
    - Hmr, hybrid ♂ rescue
- Dp Hmr lethal in hybrids
- Hmr is a Myb, 20% divergence

Barbara Negre

- Ranz 2001 23-599 Kb ($\bar{x}$ = 188)
  6.3 ± 1 breakpoints/Mb
- breaks at random
- what abt gene complexes?
- HOX, in mel broken into ANT & Ubx
- Anopheles complex intact

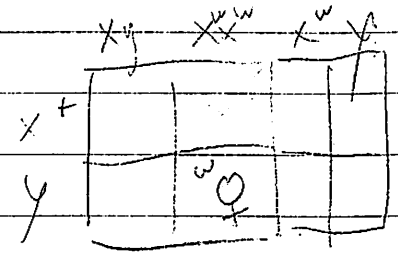


- whole protein genes are still near pb in bz
- pb break at 3' end, no 3' enhancers
- pb change in expr pattern but not related to split.
- each Hox in
- A. gam [illegible] in complex but no [illegible] in complex
- estimate rate of expected rate from whole chrom
  - compare to observed
- all genes near a break or [illegible]
  → no evidence of constraint in loop

~across metazoa: splits in rapidly developing species

Scott Hawley ← learn, teach, write

$X^+/Y \times X^w/X^w$

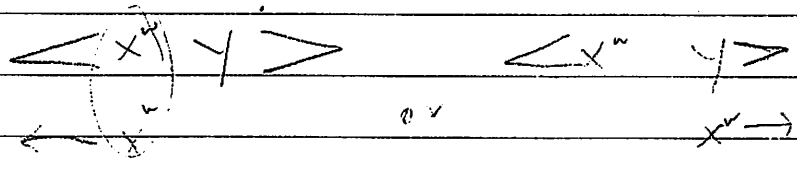


↓ 1/2000 ultra ♀

$X^{nd}X^{nd}Y \times X^tY$

↓

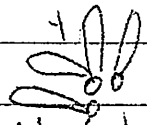
- 50-100 fold ↑ in rate of disjunction "secondary nondisjunction" (doesn't mean at meiosis II) — 4-8% nondisjunction
- Bridges couldn't explain, but proposed a competetive model
  $XXY \rightarrow XY$ or YY (presence of $2X$, $1Y$)
- ~450 cases 2° nondisj. involved non-exchange chrom. 95+% used marked chromosomes
- can't pair, can't X-over, or vice versa
- Eo,



predicts 50% nondisjunction if no exchange

- alternative model — trivalent — Cooper

late pairing



mirandа-like only after no X-over

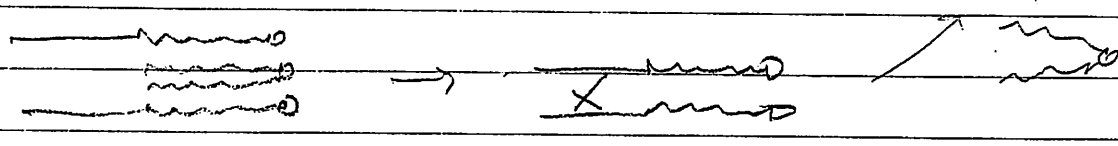
- Grell — availability
  — site shape, 1 meta. will orient 2 acrocentrics

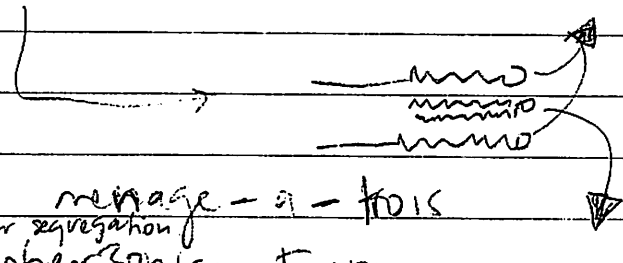
no association with NEB

- XXY associated in germarium (early prophase)
- all models are wrong

begin paired
if X over, XY association breaks down
if no X-over, maintain [illegible] till late

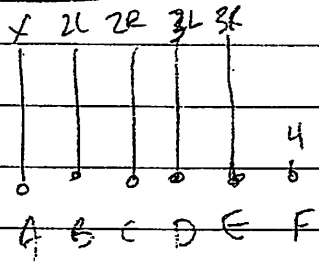
- 2° nondis is for of trivalents
- X-over, lock hom. cent together
- homeolog — divergent homolog from another species Dean





- cytogenetic manage - a - this
- explains Robertsonian Fusion
proper segregation

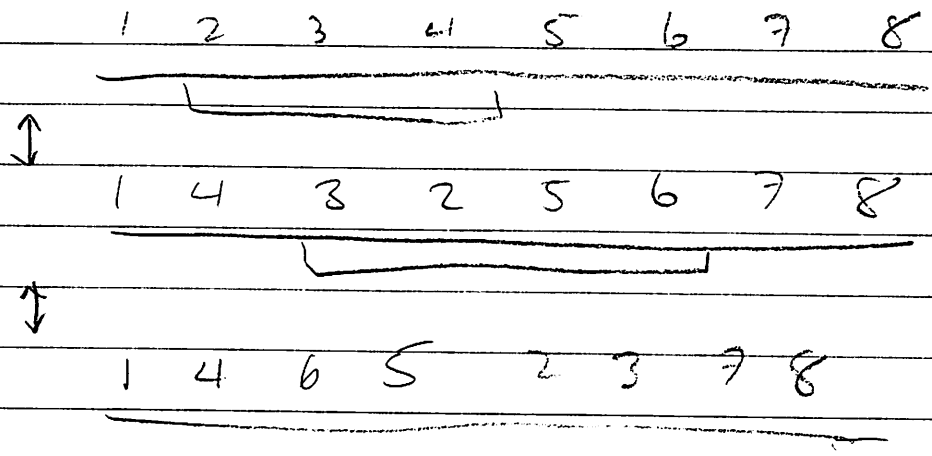
M. Ashburner Chromosomal Evolution



- no interference in mauritiana (Scott)
- D. pse 3x map of D. mel

- 1932, Ithaca, Muller proposed that fusion/fission
- Robertson, first described in grasshoppers (attached X's)
- tobacco mouse
- Novitski (thesis) & Sturtevant conservation of genetic mutations (1942 ish)
- paracentric inversions
- Sturt found natural X-over suppressors "C chromosomes"
proposed on genetic grounds "inversion"
- Sturt & Beadle 1936 mechanism
- by then, polytenes available
- para => peri (rare) robusta
- mel 5 common cosmopolitan, autosomal, para
- freq high ~ 20%, all simple

- nearly all are simple in mel
- in pse, complex overlapping inversions
- overlapping inversions → order



- C element, chrom 3, all on one arm
- not atypical for one element to be more affected than others (but see obscura)
- closely related species differ by fixed inversions
  " " level of poly inversion
- poly sampling in pse by Dob.
- phylogenetics in obs & willi by Dok
- Wasserman & Carson culmination
- assumptions: single event in a lineage
  - never been rigorously tested
- no effect of X over ~~is~~ consequence of recomb outer ?
- pericentric cytosynaptic derivatives, derived from single X over in peri
- Charles Metz, milnbc chrom evolution
- Michael White's review was peak of this trend
  UK → Texas → Australia (communist, McCarthy)
- 106/183 species ~58% seq poly
- 22K - 56K inversions fixed in genus
- dogma, ectopic exchange
  but, 2j poly inversion

- Q-P - melsim
- invert dup (detectable)

- evidence from the lab that TEs
- mel/sim (conserved) 3R long, 0 short in sim
- junction regions have inverted duplications
- comes in 2L P
- 24 transpositions, 21 replicative / retrotransposition
- (yakuba) retrogenes are rare
- 55 breaks / 29 inversions
  breakpoint sharing
0.015 ~.02 breaks/Mb/My
- w/ inverted dups 20/29 del
- no dup - close breaks
  - break at Δ end
  - lost secondary events
- TE effects not strong
- 28:1 on yakuba lineage

Databases & Resources - MA

Dutch Book 1925
- Morgan, Sturt, Bridges
- 3 yrs before X-ray induction
- all spontaneous
- visibles, some lethals

- N was first lethal to be studied in development
- 1928 Columbia → Caltech
- 1932 DIS Bridges

Bridges & Brehme 1942
- published after Bridges death — listed by mutant allele

Lindsley & Grell, E 1968
- Oak ridge
- "Red Book"

Lindsley & Zimm G 1992 — listed by gene
- last data ~ 1989

? dist of synonyms for gene!
volume "performance ..."

- 1992 Fly meeting idea → launch FlyBase
Gelbart, Merriam (UCLA), Ash...
- 1992 first grant Merriam, Gelbart, Kaufman
- 1996-2006 formal collab w/ BDGP
- original plan was L&Z in a database
→ electronic files
→ Muller late 1930s → 1942 bibliography
- ... (student of Muller) took over biblio, at Indiana
~ 100,000 journal papers
- maintain by "curation" 4-6 curators
- triage, 15 top journals
100 others scanned
- 60,000 ..., 20,000 pdfs
- rare Russian papers translated under CIA/Israel program
- Oct 1993 first web browser
- Kauffman web presence & images
Matthews - stock lists
- names of genes, priority rule (follow zoological ...)
- dpp, Notch, ...
- Dl, ...
- ... 13,000 10% matched to known genes
- IDs ... no semantic meaning
- Protein coding genes

- μ alleles & sequence N, dpp
- sequence CG
- μ alleles curved
- status uncertain lost stocks
- sybase back end
- chado, postgreSQL, postgenomic
- structured controlled vocabularies
- GMOD

B. Charlesworth

- cardinal is a natural polymorphism
= inversion poly.
- relatively few poly in morph traits
- typically quantitative variation
- C.V. of most metric traits 5-10%
- heritability is 20% - 80%
- "concealed variability" recessives shown by inbreeding
  - reduced performance (viability)
  - increased variability
- closing of chrom by inversion
- $\frac{Cy}{P_m} \times \frac{+}{+}$ ♂ ♀ P_m is allele of brown

$\frac{Cy}{P_m} \times \frac{Cy}{+}$ (no crossing over)

$\frac{Cy}{+} \times \frac{Cy}{+}$

↓

expect 2 Cy : 1 +

- 30% homozygous lethal
- 2nd + 3rd 1-2 lethals per genome

- non-lethal reduction in viability is ~20%
- freq of steriles is lower, non-sterile are reduced in fert.
- lethals are typically non-allelic (2% allelic)
  ⇒ rare in any given gene

? what % of fly genes ↓ to lethal?
25-30% in Adh region (vitzl)
⇒ affects estimated # of genes very roughly
- Sved, completion, showed isogenic is ~15% flies
- end of 50's classical - Muller, rate del vs with balanced - Dob, study of inversions

- Wright & Dob "wrote" a paper on neutral [illegible] but not ever published
- Dob saw fluctuations in freq. each year
- Dob's insistence in het. adv. caused breakdown in relations w/ Wright
- molecular genetics
  - unbiased
- Lewontin (Dob student) & Hubby (Chicago school)
- prot. slightly neg., Starin (Harris) neg. (L&H)
- molec. phenotypes, Mendelian
- Dob was right abt 10% of poly loci
  - ↑ 40% of loci w/ >10% alleles
  - 13% of loci are het. (many unt.↑)

heterozygosity ⇒ gene diversity

- refuted the classical view of low poly
- biased, only 1/3 of aa can be detected
  - too insoluble mem or rare proteins TF
  - no silent changes

RFLP
- mostly snps 25% indels, some large TEs
- Kreitman, high variation 10X allozyme
  - many silent/synonymous
  - SNP density is metric
  - expected 3% AA poly, see 1.
  - → most aa changes are del.
  - silent/noncoding may be governed by drift.
- T & θ same when n=3, necessary mathematically
- E coli genome 0.05
  D. mel (Af) 0.02
  H. sap 0.001
- genome studies are as unbiased as possible.
- Forms of selection: purifying, directional, balancing
- divergence, multiple hits JC is NM

- saturation, D ~ .75 random sequences
- $K = 2\mu T$, neutral evolution
- Kimura 1968, Wright derived but no data
  → est μ from obs K & poor T
  $K < 2\mu T$, purifying selection
- N_e 10^6, don't need strong selection to prevent aa from fixing,
  ⇒ explains why biochemist can μ many aa w/ no effect but seqs stay similar over time
- similar conclusion for directional selection.
- $\bar{U} = 0.80$, deleterious
- 4×10^{-9} point sub μ rate, same as electrophoretic
  - 2-3× times molecular clock
- no real evidence for $\sigma^2 \neq 0$ μ rates
- most sel is purifying
- genetic death per generation e^{-U}
- Malik & Henikoff (2001) $K_a/K_s < 1$ but MK tst
- selective sweeps, distance ~ r/s
- Kim & Stephan simulated sweeps, recent (.25 N_e)
- $10^6 \times .25 \times 10$ gen/yr = 25,000 yrs ago
- frequency distributions, assumes derived state known
- bottleneck, Af → Eu., D > 0 if partial
  D < 0 if complete
  diff parts of genome will show +/- D, and ↑ variance in Taj D
- sel sweep ~ bottleneck at one locus
- avoid species w/ bottlenecks, D. miranda
  - neo-X sweep at Eve!
- Bordeaux mixture, metabolomics
- Ometto, bottleneck model based approach.

Selfish DNA

- 1st observ. X-drive, Gershenson
- why are they here? TEs 3 Seq distinction
- most families seq sim, but w/defective
- adaptive vs. selfish DNA
- some cases of adaptation, but cannot explain prevalence
- most visibles are TE insertions
- n ↑ transp & excision
- few solo LTRs (ectopic recom in Dros is rare)
- rates of movement, by in situ hyb.
- 21 lines, 27 new insertions, 1 excision
- trans >> excision of Pf 500 10^{-9}
  1.2×10^{-4} 4.0×10^{-6}
- 1 new mutant in DHPLC screen
- based in situ towards
- n per family, additive quant trait
- $\bar{n}(\mu - v) \Longrightarrow$ all sites that can be occupied, should be but most sites are at low freq
- occupancy distribution < 0.5
  RFLP unique
- 23000 gen ~ 2,000 yrs (10X increase in abundance
- Forces controlling element abundance
  - neutral model rejected $N_e s \gg 1$ (12.5), equilibrium

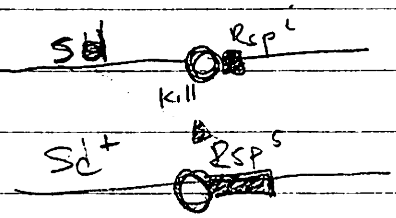
a - self-regulation P-element
b - sel. against insertions, few in genes, but μ in lab
so nothing stopping insertion into genes
c - ectopic exchange
self regulating, works on intergenic regions
d - direct effect on fitness

a - no $v > V$
b - indeed ~ site exhaustion, $S \sim V$

c - Langley / Charlesworth ↑ in low x-over
- B, w dup little work
- ectopic exchange - could be pre meiotic
can occur btwn sisters
- X chrom cf - mn 7 - 10
distal most 2-5^ths

SD - B. Charlesworth

- D - drive allele violates Mendels law
- no genes required for sperm (all but 4th, 4th not essential)
- all SD in ♂, overproduction of sperm
- some fertility loss, but if D gains it will drive
- Hiraizumi wild flies to cn bw/+ -
small fraction produced all wt
1-2% of w.t. chrom are SD in wild
2^nd chr
- SD homozygotes are sterile ♀
- Hartl, PhD SD is 2 genes Sd & Rsp
Sd⁻/+ and
- SD, RanGap dup, truncated protein Ganetzky
- Rsp, CI W., 2R, repeat 120 bp, ↑ repeat, ↑ sens



SD chrom are Sd Rsp^i
Sd Rsp^s is suicide

50% Sd^+ Rsp^i
50% Sd^+ Rsp^s in wild

- SD chroms have peri & paracentric inversions

S.H. - add recessive lethal to prevent homozygosity of SD
B.C. not always true, (true for t-allele)

- Sex ratio distortions (SR) all in ♂ no mechanisms
  - ♀ in D. obscura
  - Start of Dob in D pse involves an X chrom Xr, such that sperm w/ Y die
  - Xr, inversions
  - XR (3L) ST pse is same arrangement SR pseudo CINs
  - recens driving X is non recomb
  - Dyer, one haplotype, complex of inversions not paired in polytene chrom
  - balancer FM7
  - cryptic distortion in sim, X linked Y ♂ have suppressors
- distortion causes distruction
- double m to get SD, requires close linkage
- primary disadvantage is sterility
- recens X is "balancer"
- approach to eq: is
- multiple genes, adapt, suppress X-over
- Fisher & Dob (inversions), SD is good example

Evol of Y

- Muller 1914 proposed proto-X / proto-Y model
- some human X-Y gene pairs in evt mam is autosomal in marsupial
- Darwin proposed model for sex evol in plants
  - hermaphrodite ↔ ♀ knockout male fem / ♂ " " female
  - need to be closely linked otherwise neuter
  - linkage constraint
- C. elegans, accordingly hermaphrodite males are produced by nondisjunction
  - Otgroups are dioecious

- no hormonal sex determination in flies, not entirely true

- if ♂ rare, het sex (♀ in lepidoptera)
- Degeneration of Y
  - Muller
  - μ on Y can however become homozygous
  - NE
  - 3x : 1y pop size for Y
  - lack of recomb.
- Hill-Robertson
  - 1st proposed by Muller & Fisher

Greg Gibson

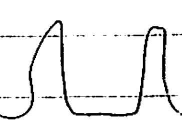
- background mut genet. Antp/Scr
- what abt genetic background?
- Hogness & Waddington work
- use Dros to study complex traits
- disease, animal/plant breeding
- 2 flies more diff than human-primate
- classical: many genes of small effect (R. A. Fisher)
- QTL: few genes of large effect (10-12)
- QTL → QTN (goal)
- 5 million SNPs in fly genome
- many questions can be studied at higher level than QTN, reduction not always necessary
- "emergent properties"
- oligo genic discrete → continuous
- environment blurs distinction b/w classes
- all under additive contribution
- "discrete" complex trait disease/no disease
  - threshhold
  - mendelic
- penetrance - % of genotypes w/ phenotype
  expressivity - severity given penetrant

- if Mendelian traits were a part of human experience then ancient Greeks would have discovered it
- heritability is not taught, maybe political bad name
- what are most "heritable" traits
  skin, hair, height
  opera, religion ← not genetic

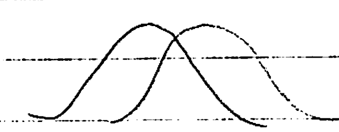
$$h^2 = \frac{V_G}{V_G + V_E}$$

- heritability says nothing abt diff btwn populations since it is a w/in populations
- Af. Am IQ fallacy assumes V_E is same
- h^2 is not a property of individual
- can't infer from diff btw pop & know of genet ⇒ genet diff.
- h^2 valid for single pop, single "point" in time
- vertical (genetics), oblique (teacher→stud) (parent-rep.) & horizontal (stu→stud) (child-child)
- ⓪ height n=13, need large sample
- 25-30% of genes expressed in egg, maternal effect
- variance among lines
- isofemale line (1-3 ♂ sperm) → single 15+ gen sib-pair mating
- isogenic by balancers, double x-overs, gene env
- ANOVA

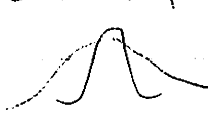
90	vs	100
89		99
91		101



	90		100
same means	110	vs	120
	70		80



- eval diff means by measuring dispersion around mean
- $R/S = h^2$
- estimate # genes, F2 analysis

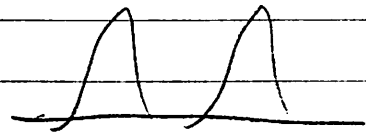


narrow - more genes
wide - fewer genes

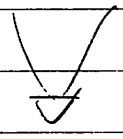
100,000
20,000

5000 generations human

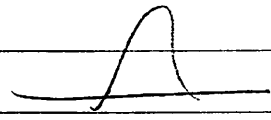
- wide variation in est # of genes
- modifier changes expression of highly penetrant
- transgressive segregation
  grandchildren more diff than grandparents



P if pure breeding



if same env.



F1 should have same variance

- canalization
- 17-18 whiskers on mouse/leg
  tabby ↓ whiskers ~6-20, $\bar{n} = 12$
- perturbation: change mean & variance → decanalization
- additive vs multiplicative model
- epistasis, interaction in QG terms
  diff usage of word from mol. genetics
  → btwn & w/in loci
- dominance, under & over
- "molecular existentialism" - epistatic effects
- eg. eyeless, humidity dependent
  miniature, temperature
- if N.S. powerful, why not identical?
  diff environments
- maybe mutational effects ("quantum force")
- 20 yrs at M.A. ~ standing variation
  ~100 generations (not same at mol level) ⇒ pleiotropy
- $\frac{V_M}{V_E}$ is 1/1000 V_E $V_G \sim .5$
- Morrison dystrophy from new alleles

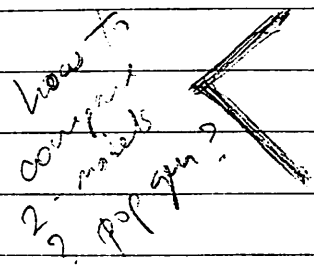
~~next~~ complementation rescue

270 cM size genome

- pleiotropy - pumilio embryogenesis 3 memory ...
  - may explain upper limits
- P screen for bristle
- introgression, backcross to males
- QTL, lower limit 5% effect on trait
  - similar to linkage mapping but use correlation not perfect association
- mapping multiple genes simultaneously
- QTL limits 5 cM ~ 100+ genes
  eg wing shape 10-15, 3 expts, each diff
  add all up ~ 250 cM
limit of resolution - statistical problem since they not fully penetrant
- not # markers or sample size
- RIL allows more accurate QTLs since you can make mult measurements, reduce V_E
- dCAPs - few $ per SNP
- all markers can be used to map Mendelians

- LD mapping - a la human genetics
  use recomb history
  linkage mapping in F1000 not F2
- hap map 100,000 blocks 200K
- flies 50 bp hap blocks, need to type all snps to get QTN
- association studies infer causality from correlation
- variation rec. rates in flies (selectable)
- Schlotterer paper diversity / pop struct in Asia
- can't use assoc. study in flies (too much recomb)
  → use candidate gene approach
  but can get to QTN
- egfr specifies ... development

- w.r.t. shape is less variable than size "pure-string"
- ∴ likely that "bowing up" shape so veins are impr
- ras ityn
- multiple testing
- even # of sites associated w/ trait even though few (∅) sites are associated
- common alleles, large effect "Beavis effect" genetic effect + sampling effect
- human genet only believe after several studies
- hitchhiking mapping 10^{-5}
association mapping (diff scale)
- Butthead effect false negative effect



one major QTN

vs

multiple small QTN

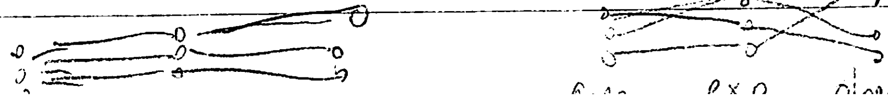
- same problem as QTL at diff. scale
- w.r.t. modularity of GRF
- Second site vs. out crossing to find modifiers
↑ hard to map
- decline in ins. signaling as age heart failure problems
- reversion, mapping, single or limited only certain class of μ, don't target all genes

---

- canalization vs. homeostasis (buffering) (evolved)
- redundancy is a mech fo. homeo, but evolved for

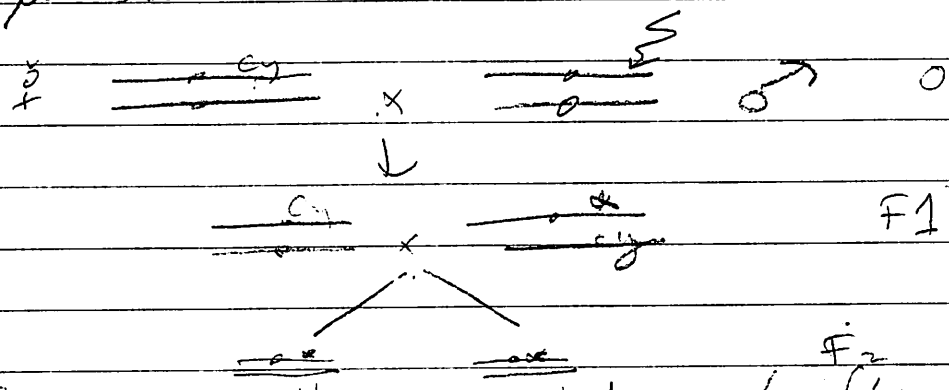
1st systems biologist ?
- "creod" Waddington
- Andreas Wagner "Robustness & Evolvability in Biological Systems"
- Laszlo Barabasi "Linked"
- "Freakonomics"
- Schmalhausen
- open up genotype → phenotype "black box"
- is h^2 contribution of h^2 of expression
- mixed model analysis of expression
- genotype x sex x age ?, what is biggest contribution
  age ≤0%
  sex ≤0%
- eQTLs & QTT (quant. trait transcripts)
"genetical genomics" eQTL
- Illumina : exp & genotype

Schadt
- 15% of 23,000 genes w/ ~25% variance (unusually high for weight etc. traits)
- stronger effects tend to be in "cis"
- eQTLs clustered
- most genes controlled by ≥1 QTL
- structure of eQTLs is unknown
- much non-additivity in eQTLs (Russel for late Dec.)
- nicotine sensitivity
- ornathine amino transferase
- transcriptional cliques, groups of genes w/ similar bimodal profiles
- TFC, transcript frequency classes
- plasticity ~ global responses by class of individuals (novice bees etc)
- more diver. in MA than WT ⇒ stab sel
- Paabo ~ many genes div b/c ~ neutral


w/ canal.

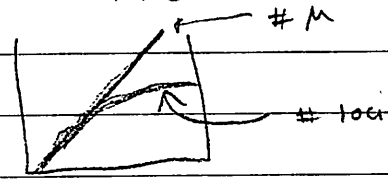
D. St. Johnston

- Lewis screened adult phenotypes
- Heidelberg screens 1980
  - saturation
  - embryonic phenotypes
  - whole genome
- F3 μ screen



F2 → F3 plan

- DTS T1 kills non balancer/μ flies
  - don't need to sort flies at F2
  - → similar to HS-HID
- saturation
  - mis-wormer, 0 ♯ 1 class frequent



- 80% sat, 5000 clones.

N-V, Roux Archive

- not all genes are equally mutable (assumption of Poiss)
  - clumpy, 1/20 EMS μ
  - synthetic phenotypes
- rapid devel ⇒ if can be provided maternally, it will
- Heid. screen was successful since many essential genes were not found, just spatially restricted zyg
- Weyer's is a horse barbitrate
- exoskelaton → cuticle → plan (key to success)
- what did they not find (new technology)?
- zygotic trans at mid-blas to derm, so upstream can't be zygotic → look for maternal μ

- maternal effect screen F4 same as zygotic but take viable F3 & look at progeny
- trans both sides of special rubber band
- zygotic - mostly unique phen
maternal - large groups w/ same phenotype
⇒ 4 classes of genes 4 signals
- what did nat not find?
- μ for internal tissues (only cuticle)
- Tear & Goodman Heidi screen on fixed embryos
- Gao et al 1999 dendritic phenotypes
- wg pathway components (not spatially restricted)
only looked for AP (pattern) defects
- Only find 1st essential fxn but if supplied maternally it can be missed
- Weischaus attached X & X-Y translocation
↳ Dev, 1988 essential fxn & location on X
- deficiency kit → missed in the 1st screen
- 7 cellular blastoderm } Zygotic
(gastrulate
- Simon et al (1991) Cell 67: 710
- enh of sevenless (RTK, R7)
- sensitized background, μ rescue transgene
- tissue specific
- assay is pseudo pupil
- F1 screen, need to recover μ in fly assayed
- RTK, src exciting, pathway & substrates not known
- biochemists wrong RTK doesn't have targets besides itself, recruits RAS
- each ommatidia is "replicate" expt
- eye is dispensible
- Banerjee Dom suppressors (opposite) rasGAP
Hafen

- ... a few degrees ...

- enh/sup screens w/ sev. didn't find genes
  - ... (lethal if μ
  - ... use eye-specific sev enh → drive
    - EMS, only in eye, post mitotic cells
    - X-rays used since EMS hits one of 2 strands → mosaics (see in eye but not in germline)
      * 1 more generation to get rid of mosaicism
      * cytologically visible, breakpoint in Southern
  - EMS older or more mature, efficient, but mult hits, TS 1/100 alleles some % small Δ
    diff types of allele (allelic series)
    sperm storage ↑ Δ from EMS
  - most enh/sup in eye
    - non essential
    - easy read out
    - multiple indep events in each ommatidia
    - can use molecules not normally expressed in eye
  - adv: ~1, save time 100x more flies
    screen whole genome, not just chrom
    only visible ones in eye, not fxn
    drivers sev < GMR < eyeless
    (report mitotic control for cell div phen
  - dv: suppress expr of construct, not product
    eg. mod of GAL4, don't use GAL-UAS
    (make proper construct, secondary screen necessary)
    not all genes are dosage sensitive, eg. rdl
    some μ affect unrelated process (noisy)
    many μ give no phen in wildtype background
    (redundancy?)
    don't know where μ is ∴ only are expressed in sensitized background.

- get around 1st essential fxn?
  - Dom en / sup screens
  - clonal screens

Garcia Bellido — FLP / FRT Golic Science 1991
  - exchange in G2
  - somatic pairing → makes system efficient
  - Xu & Rubin, adult
  - F1 screen, since only a clone, recover from mutant directly
  - wing bubbles, integrin mediated adhesion (orig loss GFP)
  - germ line clones, look for embryonic phenotypes
  - can't screen for ♂ specific phen on X.
  - Brand & Perrimon, GAL4 - UAS
    GAL80 inhibits GAL4, enh traps are expressed in mult cell types, need to switch off
    combinatoric GAL4 / GAL80 → more specific
    ubiquitous GAL80 TS

Luo Stanford? — MARCM, Reuter et al Dev 130:1203
  - only mark μ clones, single cell
  - eg neuronal pathfinding μ
  - living flies
  - F2 screen, lines guaranteed to recover μ (avoid mosaicism)

mapping FRT know arm, but each arm separately
DFS - FLP (dominant female sterile) Chou & Perrimon 1996
  - stage 2-6 kill egg chambers
  - clone ovo^D μ
  - select for germline clones rather than mark clone
  - 100% of progeny from mitotic clones
  - cross is from ♂ can't recombine onto DFS-FLP
  - Perrimon et al (1996) 50% of P's have maternal effect, 4 new seg pol μ

Mosaics ? ... FRT Chrom.

- many mod of sig signalling found
- Ovo^D + GFP select & mark F2
- inducing clones via Hs-FLP, ↑ random, in space but controllable in time.
- cloner requires mitosis, in adults many tissues not dividing

- driver - FLP, specific tissue production
- mark chrom w/ mini white on non-μ chrom
- bkll transpose w/ recessive lethals
- slow growth with mutate μ in non-recomb cells, get out-competed.

- more reproducible, less unintended pheno consequences, less work than Hs
- more specific than MARCM
  - get all cells of a type, rather than random
- "eye as an independent organism" to find μ
- pinhead screen Stocker (2003) Neuf. Cel. Biol. S:559

FLP/FRT screens Summary

adv: find μ, regardless of other process
F1 since find recessives

disadv: 5 sep screens, 1/arm
1/th of cells proximal to FRT sites
all cell-autonomous effects

Mutagens

EMS 1/1,000 efficiency 7 per chrom
mosaicism in F1
Makes genes harder to clone

x-ray's 1/10,000 ragged ends
no mosaicism
rearrangements

P-element 1/50,000 - 1/100,000
trys genes, TPCR
P-cold spot → ...

Practical Tips
- Isogenize chrom you are going to μ
  - need independence of μ
  - need to know SNP haplotype
- do a first screen 1st
- balancer, map, complementation test are more work than screen
- specific & laborious are better than non-specific easy
  Dickson (seizure known mouse) vs Tipsy (behavior)

- SNP mapping
  - visible & SNPs on same mapping chrom
  - cleans up chromosome in mapping process
  - 2 stage visibles → SNPs
  ~ 50 kb

Forward Genetics w/ RNAi screens
- S2 cells good for basic cellular processes
- VAS RNAi
  - guarantee "saturation" post-mitotic
  - know the relevant gene
  - less perdurance (to RNA not DNA) problem in clones
  - don't require cell division
  - 20,000 lines hard to cross into complex backgrounds
  - not loss of fxn (↓ expr only)
  - some cells refractory to RNAi (generative & CNS?)
  - still need a μ
  - can't make rescue constructs to map fxl domains
    ↓ natural RNA not working
  - behavior
  - targetted screens
  - [illegible]

D.S.T

? is bcd RNA modified?
in vitro shows same

localized RNA
- B-actin polarized cell behavior
- mother/daughter Ash1
- macho-1 D-V

bcd / osk - A/P

grk - D/V

- ectopic bcd is sufficient to organize Anterior
- bcd localization stage 8 oogen.
- Macdonald no x-link shift expts
  - many proteins found, general RNA bind prot.
  - but unlikely to be impt.
  - propose complex of many, low specificity
- exu necessary to make bcd competent to localize
- Swa, Dgrip75, γ-tub 37C, Msps - indirect role in bcd local, anterior microtubule function
- staufen needed to stabilize bcd localization
- bcd gets localized to microtub spindles

} all that is known

- contrast to osk where 20-30 genes are known
- mech of localization changes over time
- Swa required for efficient trl of bcd
- multistage process covers components of process in indirect screen for head defect
- larson (lsn) Vps 22 part of complex
  ESCRT - sorting pathway multivesicular body in late endosomes
  μ in Vps cause tumors bc receptors are kept on since not packaged into multives body
- other members of ESCRT complex not involved in bcd localization
- yeast 3-hybrid RNA acts as bridge
- UV x-linking, competition assays

Vps22 ... complex but doesn't bind

1 45°
2 ...
3 ...
4 ...

- bcd recruits Vps36 to Ant
- staufen not required to get bcd - Vps36
- can't X-link DS RNA to protein
- ESCRT has 2 fxns
- Vps36 has GLUE dom. to Vps22

- osk localization requires 1st intron splicing and uses Exon Jxn complex
- several mechanisms
  1) + end transport
  2) flow
  3) movement away from cortex

Kent Golic

P-element (2.9 Kb)
- 31 bp IR, 150 bp terminal required
- 2-3 intron only spliced in germline
- engineered elements are non-autonomous

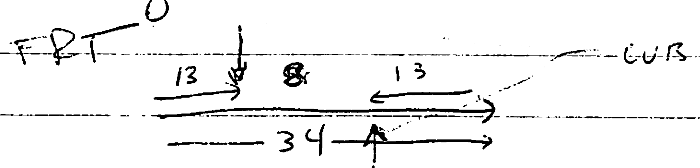
>1 TE allows mobilization of 1 w/o moving others
- helper, eg wings clipped
- prior to 90 min, prior to cell membrane formation
- kill old embryos on slide by blowing up.
- 8 Bp PD ~ bend 3 Rio 1997
- imprecise excision ~ exonuclease after excision
- 5' preference hypo-morphs
- piggyback, no bias, but no imprecise excision
- revert 17 bp left + 8 bp

~~8|7 17/8~~

in cis — or — inverted single strand (6); compl.

⇒ precise excision

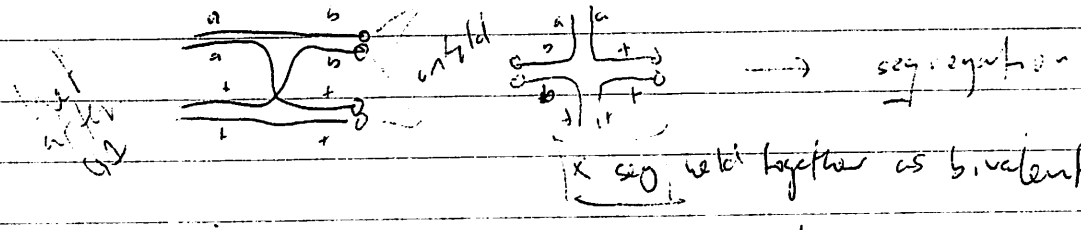
= P goes in lots of places - good (diversity of) & bad (variability)
- revertants often leave a few bp behind even if phen is reverted (?? what are implications ??)
- repair off sister → restore, often incomplete
- repair off homology → gene conversion → precise
- repair off non homologous position to introduce seq into a position by gene conversion (need a marker)
- site specific recombination
  - control for PEV
- FLP - FRT yeast 2μ plasmid
  CRE - lox phage P1 bact (consti. expr of CRE causes lethality φlox sites ?)
  integrase - attP/B φC31
- all target sites have orientation, not symmet



- excision ⇄ integration, equilibrium away from integration
  limits recombinases for transformation system
- can change FRT & lox sites still work but can't work w/ wt or each other
- close inverted FRTs will creates acentric/dicentrics
  direct repeats → duplications
  ▷ - use 2 diff FRT sites to make transformants
RMCE - recomb mediated cassette exchange
  FLP - Handler
  Cre-lox - Small → integrates whole plasmid
  integrase - Calos (2004) / Jing Wu → cassette exchange leaves vector behind
Bateman et al 2006
- as efficient as P-element transformation
- < 10% have integrated elements, so efficient that loss of white is sufficient, so no marker is needed in construct
- orientation of construct not specified since same att? sites used at both ends

- exchange on one end puts whole plasmid in bac, get out w/one more round of recomb.
- BAC ? may not be phy possible because of shearing during injection process
  - need ATT markers
- Mosaics - 2 genotypes
  - chimeras, transplantation
  - genetic
    1) lineage analysis
    2) time & place of gene action
    3) cell autonomy
    4) Cellular effect of lethals

Baker et al 1978 events that culminate events to form n clones
- twin spots are evidence for mitotic exchange
- C. Stern 1936 X vs Z segregation
  - considerations more impt when X rays used
  - 1000 rads ½ cell death, embryos fine
- PRT recomb can occur btwn homologs
→ eye color 2 pathways
  - ommochromes (brown) x sc
  - pteridines (red) x bw
- use induced recombination to investigate models of segregation (X v Z) in mitotic exchange
⇒ Z seg essentially never happens



- exceptions when exchange near bp
  - sister cohesion converted to homolog cohesion via X over

- exchange in g1 or exchange in g2 w/ x seg
- similar to meiosis
- single exchange leads to 3 genotypes (parent & twin)
- MARCM requires FRT to control exchange
- gal4 & μ & FRT on same chrom arm; other components elsewhere in genome
  - Minute - dominant slow growing, recessive lethal
    Newsome et al' 2000
    Very useful if μ has growth disadvantage
- Maternal effects & germline clones
  DFS dominant female sterile, ovo^D, early defect
  useful to study early lethal genes
  - 3 genotypes, 2 DFS, 1 viable — has μ homozygous
  - >1 ovo^D required for full ♀ sterility
  - difficult chrom to build get ovo^D onto FRT, recomb in ♂
  - how to distinguish twins w/out molecular marker?
  - size & shape of clones are dependent of time in development and cell type
  - very little cell mixing so most clones are from different events
  - 60h wng clones large, 96hr small
  - use of "incident" markers to mark clones
    Q. influence process? control by reversing markers

- cloned gene analysis to produce conditional dominant lethal, etc.
- FRTs inverted in intron & 3' inverted, reversible
- excise or invert, pair of all of gene & separate marker
- FLP-out

1/5 2.0 video not covered

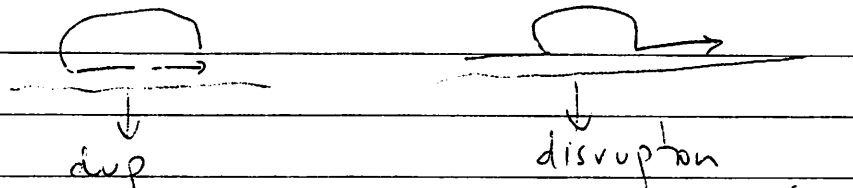
- allows island of activated marker in background of no marker activity.
- later in dev ↑ # clones ↓ size of clones in eye
- small w⁻ clones can look dark w/ direct light

Turning beg into μ

- ~~random~~ vs. targetted (RNA. 3 ho. mol. recomb)
- yeast 1978
  - Hinnen, Hicks Fink
  - transfo. mation leads to homol. recomb.
- mouse 1987
  - Capecchi
  - ES cells
  - positive & negative selection, random insert mostly
- Pros - African Rivers 2000
  - bypass cultured cell requirement
  - rare cutting endo. nucleases
- ~~target~~ targeting precedent, ? gap repair by gene conv.
  - DSB is recombinogenic
- need 3 components ISCE-1, flped out cycle & 2 element in construct to produce ↑
- targeting is predicted to make tandem dup
- not always observed
  - 2 copies integrate
  - repair by double
  - deletions
- need to do Southerns to verify structure of integrated region (PCR can be foled)
- ♀ >> ♂ efficiency, 1/500 ♀ gametes
  - ♂ random insertion > targetted as well as ↓ rate
- no real explanation for differences, practically, ♀
- ? extent of homology > 5 kb is OK

? both homologs ?

- other questions?
  Is this generally useful?
  can we produce μ?
- ends in vs ends out



- ko IIS? to, also complicated events, partial deletions
- different donor p's have diff efficiency on targeting (pick >1 homolorment)
- what adt effects on neighbors — yes, need to use WT transgene to complement Δ
- add μ to donor → distance dependent >800 bp both recipient copies
- ends out is quickest way to ↑ μ (dirty)
- ends-in gives specific μ (pt sub etc)
  2 step allelic substitution (target & reduction)
- I-CreI used to generate second DSB
  " " has endogenous recog sites in rDNA
  cross onto fresh rDNA to avoid changes in rDNA
- use water bath for IIS-FLP step
- rapid dangething 2 rounds of IIS-FLP gets rid of ftres where donor didn't move

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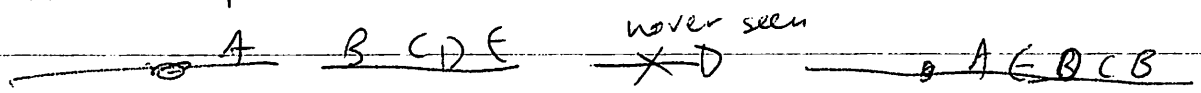
Kent Gelis — ends of DNA

- telomere: prevent end-to-end fusions
  " rearrangements
  " check-point response
  solve end-replication

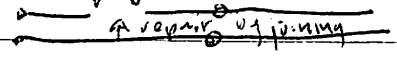
Muller & telomeres

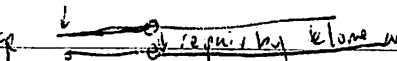


two terminal inversions → A

" " deficiencies

- telomere is a functional definition
- telomeres are polymorphic
- since telomere repeats are not essential
  ⇒ telomere fxn is not seq specific (same as centromere)
- telomere loss → soma - cell cycle arrest
  germline - telome addition
- DC chroms are engineered to permit dicentric bridge formation w/ no phenotypic effects
- fates in embryo can be breakage or not but nuclei are fated to go to S/M
- still see phenotypes when dicentric bridge occurs in non-essential chrom
- same phenotypes observed in hypomorphic ATM, etc mutants
- see apoptosis in cells that are being instructed to differentiate, when go thru cell cycle again
- apoptosis occurs after cell cycle arrest that can last for days
- telomeres from broken ends are capped w/ hope, but don't have het-A
- resulting broken chroms are short or long short are unable, long are semi-fertile
- why diff from muller?

X-ray  2 broken ends



dicentric bridge, one broken end

- some cells can escape cell cycle arrest & differentiate

RT receptor is good example of gene expression not strictly tx to gene txn

Bruce Baker

- EMS, mitotic x-over, fate maps in early 70's → dev. genet.
- XAA ♂
  XXAA ♀ } Bridges balance theory
  XXAAA Metafemale
- tra, etc were available
- tra an example of Bridges male factor: no tra → ♂
- from classical perspective, define the null phen → wt txn
- is tra cell autonomous? what is null phen?
  what is epistatic relation of diff genes? when/where txn?

1. Null? cross to deficiency Muller's definition of a null
   ? a/a to a/Δ, equal null, ok for recessive
   - only Minutes are het dom., usually dom are g.o.f.
   ? what abt dom, revert dsx[D] to null by x-rays
   & cross to recessive
2. autonomous? use mitotic clones
   - if cell has ♂ in ♀, then autonomous
3. when txn?
   - make clones at diff times
   - temp. shift w/ TS alleles — usually thermolabile in protein txn
   (tra is active ~ puparium formation)
   (# sex combs bristles, indep from morphology)
4. how μ relative to each other?
   cp single & double μ for genes w/ diff phen

- Sxl, dies as embryos, how do you by pass early defect
  ⇒ clones
- dsx, unlike other genes in pathway, had txn in ♂ & ♀, precedent w/ mating type in yeast
- cline, schedl, Baker, Belote, Nothiger
- result, all genes in sex det hierarchy
  use alternative splicing
- do alt splices act directly or indirectly
- constitutive activ. fxn construct

Sxl ↓ ○ Sxl ↓ → make tra constit.

- constitutive expression up⁻, down⁺⁺ = down⁺
Up works thru down only
Up⁻ down⁺⁺ = up⁻, branch in pathway or order of pathway, no real conclusion
- constitutive mutant test is powerful way to order μ, but not a symmetrical test
- Sxl only in flies
dsx (mab-3 in worms, Dmrt 1 in mouse)
ix conserved, interspecific gal4 UAS rescue
?, what abt parthenogenetic species?

Dosage Compensation

- problem of gene dose on sex chromosomes
- Muller ♀ w/ 2 leaky recessives = ♂ w/ 1 leaky
- show both X active?
het cell autonomous marker (eg w^{a}) → mosaic = inactivation
no mosaic, both expressed in all cells
- what is phenotype of dosage comp μ? lethal, sex sp.
Lucchesi - Belote F3 sex specific lethal screen
- screens: intellectual goal - ideal) incongruent get
practical goal - lab) μ that aren't
⇒ need second screen!!
eg. sex determination μ all ♀ → ♂ looks like dos. comp. μ
- msl's male specific lethals (all autosomal)
- how do we know these are involved dos. comp.
- could be Y, male sp. physiol fxn, one X region
XX tra ♂, msl⁻, ok, not male physiology
XO ♂, msl⁻, die, not Y
1 replication 2. gene expression: X^{dup} ♂ msl⁻, die, not 2 X
- polytene, X same diam as ♀ ...
thinner in msl⁻
- Sxl Lucchesi
- hyper transcription of ♂ X

- MLE (cloned in Baker lab)
- all MSLs bind to some regions, complex all present
- not simply DNA condensation dependent
- compensasome, msl 1 & 2 form core
  - modify chromatin of X
- Sxl represses msl 2
- nox transgene → autosome, induce compensasome formation @ secondary sites
- spreading model: 30-40 initial sites → complete compensasome → spreading
- direct evidence for spreading on X
- X→A dup, always see X binding pattern with no entry sites
  A→X no spreading into A
- entry site model replaced by affinity model
- rox transgene may have been misleading since rox RNA is where compens. assemble.
- neo-X attract msl by changes in cis
- evolution is piecemeal, gene by gene

open questions
- What is seq on X?
- What is biochemistry of histones?
- How do you build a band?

? lack of
- pairing → not likely
- transgenes are difficult to recapitulate binding

Targets of Sex determination

- Yp made in fat body, hemolymph → egg

divide
- need sex hier. to activate & maintain Yp genes
  dsx^M → ♂ Yp dsx^F → Yp

tissue level
- sex hier. need to form acces. gland, transcripts w/in acc. gland are regulated w/in the organ
- genital imaginal disk

control of Sxl + tra germline not affected

? pathway connecting Eject. Duct ?

tra - should switch sexes to see if sex regulated

- dsx is instructive in ♂/♀ genital disc primordia
- clones of ♀ in ♂ proliferate, thus not cell autonomous
- sex of A-P organizer determines fate of genital disc
- sex ∃ Segmentation genes involved in differential
- target of dsx: candidates, enh traps, microarray
- bab, post pigmentation: $dsx^F \rightarrow$ bab
  Abd-A ⊣
- branchless (FGF) ∃ breathless (FGFR) male disc rotary
- expression of FGFR allows migration by recruitment
- sex hierarchy controls FGF expression
  dsxF ⊣ branchless
- sex is fixing at same level as HOX genes
  (⊣ modulation doesn't incur genomic cost since same binding sites)

Q: what are direct targets of dsx?

- microarray, somatic differences
  fewer genes diff in some than germline.
- all internal gametalia gene expression is tissue specific but directly by sex
- 12 microarray targets, none direct
- gal4-UAS screen, see disc in live animals

Sex ∃ Behavior

- Sxl ∃ tra control sex ∃ behavior
- dsx, controls sex but not behavior

Green gus 1995

- pulled out fru by probe to 13bp x6 repeat which tra recognizes
- null for P1 transcript no courtship behavior

behavior → cell autonomous?

- leaky & defective in recognition
- fru is BTB-ZF TF
- P1 trx has 100 aa ∫ unique to all
- 2000 cells in CNS
- fru expressed at highest level during larval → adult remodeling
- fru necessary from earliest stage → innervation of sex organs
- insert GAL4 by transl recomb into fru^M P1 ⇒ express in every cell where fru is transient
- UAS-GFP persists in cells where fru is transient
- ♂ & ♀ same pattern of fru expression is fru sufficient for courtship behavior
- UAS-trx drives trx in fru cells & make ♀ ✓/♂ behavior

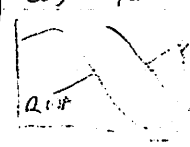
⇒ Fru is sufficient to make male

- visual, olfactory, auditory, & tactile
- antibodies don't penetrate cuticle
- multiple fru+ cells in a row ⇒ circuit
- male-male habituation, fru required
- courtship conditioning, ♂ → ♀ ↓ receptivity → ♂ less likely to court for ~3 hrs
- do fru+ cells have other fxn.
  drive TS shibire
- only recognize male when sex circuitry is fxn
- use UAS-RNAi to 100 AA fru^M
- knock in Φ by gene, put in others (GAL4, LexA) by Φ

- fru trx how to set up a circuit

b. Kyriacou

- genetics of behavior, 1960's started, selection + strain comparisons, didn't yield many results
- Students of Tinbergen & von Frisch
- early 1930's, Benzer, EMS single pt m in genes which have behav. phenotype
- Benzer was interested in neuro system not behavior
- "time, love, memory", J Weiner (1971) 3 major phen. of interest
- Konopka, screened for flies w/ clock μ
- flies sleep, 12 hrs
  - ▷ attached X, first 60 lines found per
    - per^L 29
    - per^S 19
    - per^0 - arrhythmic
  - per is not a vital locus, del is viable
  - Hall & Kyriacou picked up per work
  - 160-200 wing beats/sec in flight
  - hum & pulse
  - inter pulse intervals (IPI) vary in cyclical pattern, shows neurogenic effect (not myogenic)
  - song cycles & IPI, sympatric character displacement
    - ∃ female recognition (but no independence on phylogeny)
    - ▷ two components are independent & impt.
  - recip. mel-sim hybrids rhythm maps to X, IPI maps to A
  - rhythm of 1 min & 24 hrs, are they same?
  - yes, per^L long IPI per^S short IPI
  - cloning of per, Hall/Rosbash & Young - 1984
  - Per rescue in circadian & song domain
  - RNA cycles in fly head, & protein cycle



neg auto-regulation

- 2 features: need delay for ⊖ feedback
  need short half-life of protein
- photoreceptors have per, 10,000 more per

per expression
- 3 grps of lateral neurons, 3 grps of dorsal
  rest in photoreceptors
- LNd - evening > gal4/gal80 expts
  LNv - morning
- ZTO, per nuclear localized
  12, cytoplasmic
- LN determing behavioral clocks not peripheral
- per-luciferase, cycles apparent in isolated leg, etc
- no master clock, only for behavior
- per protein, thr/gly repeats
  founding member of PAS domain, dimerization dom
  Sim is another, has bHLH
- PAS / LOV, respond to light, oxygen & voltage
- T/G repeat → remove → sim like song rhythm
- mel/sim differences map to T/G region of per
  interspecific transgenics mel 20, sim 24
- single gene of large effect, adaptive divergence.
- T/G ↑, ↑ song rhythm length
- ring X, tra all male w/ clones of per+ & -
- brain+ thor- 24 hrs, song messed up > decoupled
  thor+ brain- song ok, 19 hrs
- how many genes cycle, microarray, 2 groups
  50% concordance, diff expt. design
- per, other genes don't cycle
  650 genes, non cycled, trx +/-
- bouton size varies day/night, cellular change
- tim overgrowth at bouton, no effect on 24 hr clock
  song is rhythmic but long
? is neuronal branching related to song rhythm?
- is song only major gene effect
- D. pse transgene per shows dom. effect
  & maps to 3' end of gene

per, tim ID using a population assay

- per transformants show associative memory
- dom, single locus, affect both ♂ & ♀
- nonA, no-ou = transient exp potential
- dissonance maps to nonA, song gene maps to eye physiology defect
- every song µ is visual µ, but not vice versa
- vir nonA trans rescue mel nonA
- OP locomotor response is fine in transformant so not a "partial rescue", but real
- major genes common? morphology? bristles
- timeless, per mRNA affected & vice versa (mRNA · protein) same delay, cycles as per
- tim & per bind via PAS domain, mutually translocate to nucleus
- clock & cycle are bHLH-PAS, E boxes CACGTG in per/tim promoters
- clock cycles in a loop, clock is gene that links clock loop & per/tim loop
- native cycling enhanced by cryptochrome induced per degradation
- PER genes in mammal 3 + 1 ψ, poorly conserved
- human per gene has same phos site µ

conclusion
- clocks historically initiated neurogenetics
- impt mech in gene regulation
- impt for evol, speciation
- mechanism for circadian rhythm is not used in courtship song (molecular)

Fly learning — Benzer student
- 1974 Quinn's olfactory learning paradigm
- classical conditioning, smell & shock, pop assay
- 1976 dance isolated

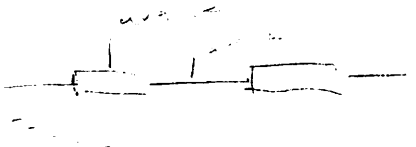
Pavlov's Flies 1985

- 1980 Tim Tully, enhanced Quinn assay 90% Flies condition
- RoboTrainer
- dunce - cAMP phosodiastenase
  amnesiac - neuropeptide
  rutabaga - adenylyl cyclase
- learning ↓ affect acoustic systems, central pathway
- ♀ are more receptive if hear song, memory index
- dCREB - cAMP pathway components implicated in learning & memory
- mushroom body ... memory genes
- over-express GA (rutabaga), learning index ↑ in mushroom body but not other parts of brain like central complex
- amnesiac expressed in DPM neuron, modulates memory in mush body
- memory trace  0 hrs composed of short, medium, anesthesiac, long memory components
- Tully spaced training increases memory
- LTM depends on protein synthesis for spaced training but not for cramming
- dCREB 2, 2 isoforms, hyp-act form ⇒ photographic memory (1/1 in cell mgt, controversial)
- ☆ stop codon in transgene ↑ Perazzona 2004
- Davis
- rutabaga affects LTM but not LTM
- LTM localized to α-lobes of mush body
- Hebbian process - memory is built up by connections of
- Shibire^{TS} synapses, disrupt during learning or retrieval
- ↑ gene expression → synaptic growth → circuitry
- ↑ no strong evidence for modified growth

Steve Russell "probe vs. target" reversed

- functional genomics of gene expression.
- high throughput changes the way we deal w/ data
- reverse northern → multiplexed → manage
- ordered set of frags
- single channel - computation difference
  dual channel - competitive differences
- PCR - full length cDNA, hetero probes, cross-hyb
  - 3' frag from genomic DNA, good if no cDNAs, requires genome sequence/annotation
- oligo - 70 - operon
  50 - compugen
  30 - amersham
  25 - affy
  } rely on gen. seq.
- 40 K element on 1 slide,
- inkjet oligo synthesis, in situ, programmable, not in house
- phosphoramidite vs photolithography
- maskless micromirror oligosynthesis - Nimblegen
  400 000 elements
- efficiency determines quality, off-line you can purify in situ, take what you get >99%
  ⇒ problems w/ background & sensitivity
- intern length probe is most specific
- sensitivity ↑ w/length
- noise/bias ↓ w/length
- 50-100 bp probes are good, trade-off btwn spec. & sens.
- optimize equivalent binding energy across oligos w/ Tm
- 18 labs print INDAC
- sacrifice sensitivity for specificity, increase sample
- 150 £ INDAC, Affy 300-600 £
- Affy ~25 bp, lack spec/sens, multi probe per gene
  - match/mismatch design

mRNA arrays



- Most people ignore mismatch data
- what abt polymorphism?
- subtract mismatch from match & avg over probes per gene
- must amplify to detect mRNAs
- label - 1st strand synthesis (Arbeitman)
  - 3' end of RNA
- Eberwine at T7 pol in 1st strand synth
  - ~1000 fold amplification Klebs 2002 "overamplified"
  - ↑ amplification ⇒ ↑ bias
- PCR amplification RT + 2 primers
  - SMART cDNA synth.
- end labelling 1 mRNA → 1 label
  - use of branched oligo deindrimers
- Eshuis (2006) PLoS Genetics, gfp+ cell sorting
  - recombing gal4/gfp onto μ chrom

- Experimental design (Yang & Speed Nat Rev Genet 2002)
- time course T_1 v T_2 v T_3 vs. $T_1+T_2+T_3$
- biological replication is most impt, pool to save $
  - 3 smooth biol replication
- wt vs μ 4 biol 3 dye swap ~8 slides
- spot finding, normalisation
- idea behind norm is that majority of genes are unchanged
- M^{diff} vs A^{ave} $\log_2$ scale
- Affy norm MAS 5, Speed GCRMA is best
  - affy estimates are poor for genes at low expression levels
- good correlation btwn Affy & Spotted results
- Splc, N. bicaps ~40% of non exon detect expression, genome-wide
- Affy genome tile array, 25 bp every 35 bp (10b NO)

- J. Munak
  - 60% known exon
  - 9% EST
  - 23% intron
  - 7% intergenic
  - significance
  - unannot exons
  - in mammals

IgB ~ integrated genome viewer
- Affymetrix & Jaenisch chip
- chromatin immunoprecipitations
- chip array reproducibility higher than expt. array
- good correspondence btwn 1 kb tiling vs. high res genome tiling array
- couple w/ cell sorting
- depends on quality of antibodies
- DamID, leaky promoters or overexpression leads to no resolution, try to cripple processivity
  ⇒ can't drive in tissue specific manner
- Similar results DAMID vs Chip
- Andrea Brand N. enable gen data

Proteomics
- yeast 2 hybrid, 9% in common in 2 expts
- very noisy data, why? diverse, foreign conditions
- Mass spec:
  - Peptide Mass fingerprints
  - need genome & good annotation
  - post translational mod change mass
- Maldi-TOF → peptides
- tandem Mass spec → get AA seq from daddy fragmentation
- 2D gels charge & mass
- DiGE Gong (2004) Development 2D gel w/ Cy5 & Cy3

2.1 PD 97.6 11.6

- membrane proteins don't go into gels
- [illegible] of ~1000 genes
- ICAT, Rudi Aebersold, Moseley 2001 Trends Biotech
  2 labels, heavy water
- ITRAQ more labels
- Peptide Atlas www.peptideatlas.org Tandem MS
  ~5% of spectra can be interpreted

RNAi knockdown

- R. Paro's amplicons had T7 & T3 incorp-n!
- Perrimon Lab
- reproducibility is good
- Second generation Michael Boutros's
- UAS constructs
- Echeverri & Perrimon NRG 7:373
- cell arrays, print dsRNA