Meiosis:

Lecture 4
A. Wedel

46 chromosomes
[2 copies each of 23]

\[\Rightarrow\] each sperm or egg cell has a reassorted set of 23 chromosomes to contribute to the next generation.
Chromosomal reassortment

2a

8a

2a

8b

2b

8a

2b

8b
Recombination:
Willem Stemmer's Approach to Evolving Super Bacteria:

- Start with plasmid bearing an ampicillin-resistance gene.
  ⇒ want to evolve a better gene (a gene which codes for a more effective product)

1) A large number of identical plasmids are cut into small pieces (~50 basepairs) with DNase.
2) These pieces are reassembled into concatenated plasmids by PCR, by virtue of the fact that pieces from different plasmids will overlap.
3) The result is linear, concatenated plasmids

amp resistance  amp resistance  amp resistance

4) Cleavage with a restriction enzyme results in linear, full length plasmids, which can be ligated to re-form circles.

amp resistance

amp resistance  plasmid

5) The plasmids can be re-introduced into bacteria, which then replicate, the plasmid to high numbers.
6) Because the process of reassembly by PCR after DNase cleavage results in mutations, each reassembled plasmid carries mutations, some in the ampicillin-resistance gene.

\[\text{ampicillin resistance} \]

7) Improved variants of the ampicillin resistance gene can be selected by growing the bacteria in a near-toxic concentration of ampicillin.
8) Plasmids from these bacteria which survive elevated concentrations of ampicillin are purified and put through the whole process again, crucially as a pool.

⇒ Repeated chopping up and reconstruction of plasmids continually introduces new mutations, but most importantly, allows recombination between different versions of the ampicillin-resistance gene, such that beneficial mutations can be brought together without concomitant deleterious mutations.

⇒ Within 5-6 generations, Stemmer had improved the activity of the resistance enzyme by several orders of magnitude.

Control experiments with an equal rate of mutation, but no recombination, produced enzymes only a few fold more active.
Strategies for the evolution of cooperativity:

1) Physical linkage

Example:
- Tethering identical molecules together via rolling-circle transcription

$\Rightarrow$ Production of an RNA strand from a circular template results in concatemers.

![Diagram](DNA template connected to RNA polymerase)
2) Isolation in space

Example:
- confinement of molecules in liposomes

Liposomes are small cell-like bubbles made of lipid molecules. Molecules can be confined to the interior of the bubbles.

If a desired reaction causes a change in light absorbance or fluorescence, liposomes containing active molecules can be selected.

liposome containing RNA molecules

Device for selecting liposomes of different absorbance/fluorescence