

*How common should induced or spontaneous mutation be in a population not under selection?*

*Before we address this question in bacterial, let's indulge in thinking about us....*



- Achondroplasia is a *completely penetrant*, autosomal dominant disorder characterized by disproportionate short stature -- the arms and legs are short compared with the head and trunk.
- More than 80% of the people who have achondroplasia are born to parents of *normal* stature and represent a new germline mutation

Michael Dunn is best known for his recurring role as the villain Dr. Miguelito Loveless on *The Wild Wild West* (1965-68).

## *Achondroplasia and the mutagenic male*

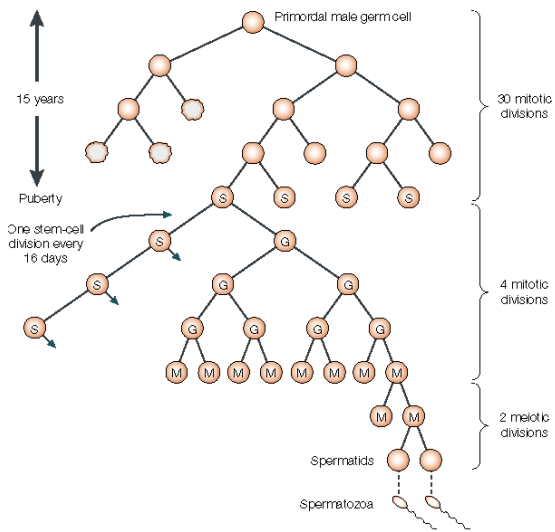
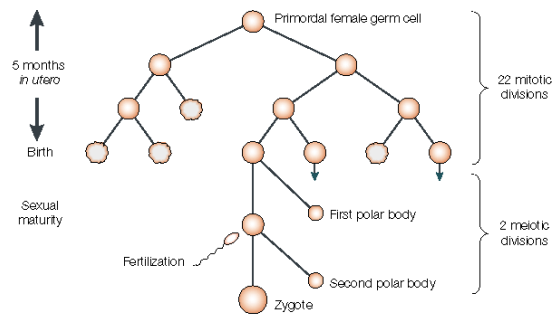
*An estimate of the mutation rate in FGFR is based upon the accumulated data of newborn studies in four cities:*

- In a total of 242,257 births, seven infants had achondroplasia
- From these numbers, the rate of mutation of the normal to the achondroplasia allele is calculated to be
- $1.4 \times 10^{-5}$  mutations in the achondroplasia gene per gamete =
- 1 mutation per 69,216 copies of the gene

- It has been established that fathers are the source of all achondroplasia mutations
- The probability of having an affected offspring increases exponentially with the father's age
- 50% of children with achondroplasia are born to father older than 35 years old.

# Cell divisions during oogenesis and spermatogenesis.

S, stem cells; G, gonial cells; M, meiotic cells.  
 The total number of cell divisions in the life history of an egg is 24.  
 In males this depends on the number of stem-cell divisions, which is greater in older males.



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*Every time a human cell divides it has to replicate  $6 \times 10^9$  base pairs of DNA*

*Every time an *E. coli* divides it has to replicate  $5 \times 10^6$  base pairs of DNA*

- *Every time a base pair is copied, there is a very small but finite probability that an error will be introduced at that site*
- *Furthermore, if the DNA molecule has sustained damage that remains unrepaired, then mistakes will occur during DNA replication of that site by so-called error-prone bypass polymerases*
- *What sort of damage occurs routinely to DNA?*

## Variation in genome-wide mutation rates within and between human families

**J.B.S. Haldane proposed in 1947 that the male germline may be more mutagenic than the female germline<sup>1</sup>. Diverse studies have supported Haldane's contention of a higher average mutation rate in the male germline in a variety of mammals, including humans. Here we present, to our knowledge, the first direct comparative analysis of male and female germline mutation rates from the complete genome sequences of two parent-offspring trios. Through extensive validation, we identified 49 and 35 germline *de novo* mutations (DNMs) in two trio offspring, as well as 1,586 non-germline DNMs arising either somatically or in the cell lines from which the DNA was derived. Most strikingly, in one family, we observed that 92% of germline DNMs were from the paternal germline, whereas, in contrast, in the other family, 64% of DNMs were from the maternal germline. These observations suggest considerable variation in mutation rates within and between families.**

Mutation underlies all heritable genetic variation, and the observation that a mutation has arisen *de novo* can be highly discriminating in identifying causal pathogenic variation in individuals. Attempts to measure mutation rates in humans fall into two broad categories: direct methods that estimate the number of mutations that have occurred in a known number of generations and indirect methods that infer mutation rates from levels of genetic variation within or between species. Previous estimates of germline base substitution rates range from **1.1 to 3 X 10<sup>E-8</sup> per base per generation**. This variation is caused, in part, by uncertainty or assumptions in key parameters, such as divergence times between species, generation times and ancestral population sizes. Furthermore, all previous estimates represent an average across multiple generations and/or an average of male and female mutation rates. Consequently, the previous studies provided no information on how mutation rates vary between individuals of either the same or different sexes or between gametes within an individual. It has been proposed that the mammalian male germline may be more mutagenic than the female germline because of the greater number of cell divisions in the former. Subsequent studies based on whole-genome sequences of human and chimpanzee have indicated a sixfold difference in mutation rate between male and female germlines, averaged across ~5–7 million years of independent evolution of the two lineages.

High-throughput sequencing enables whole-genome analysis of mutation rates in human pedigrees and promises to revolutionize our understanding of how mutation rates vary between sexes, individuals and families

*The power of bacterial genetics lies in the potential  
for detecting rare events*

*We will use E. coli as our model organism to examine  
rates of mutation*

*Why?*



*E. colicultures multiply to vast numbers – HOW VAST?*

*E. coli cultures multiply fast – HOW FAST?*

*Prokaryotic geneticists used the same strategies as eukaryotic geneticist to*

- explore the principles of inheritance patterns
- define the existence of genes especially before the era of molecular genetics

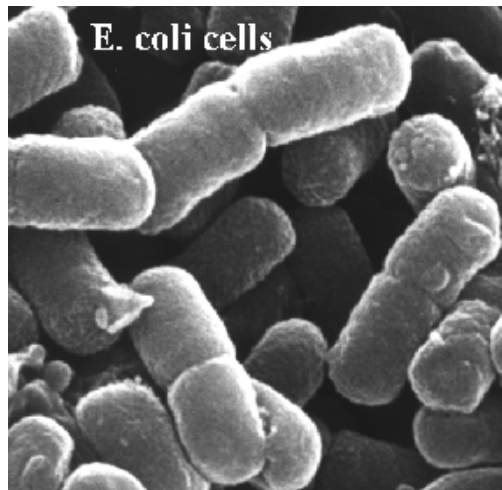
*What are these strategies?*

## *Similar Themes in Eukaryotic and Prokaryotic Genetics:*

- Use model organisms to explore basic questions about heredity
- Use phenotypic variation to explore hereditary processes and to define the existence of specific genes
- Cross organisms with different phenotypes!

**As with eukaryotic genetics, bacterial genetics relies on phenotypic variants of the organism under investigation**

**What sort of phenotypic variation can we observe for single-celled organisms?**



## *Phenotypic variation*

### *4 categories of mutant phenotypes:*

- Chemical building blocks (or other compounds) that the cell needs/does not need. Such as ?
- Energy/carbon sources that the cell can/can't utilize
- Stuff that can/can't poison the cell
- Other (colony color or morphology)

*Nutritional mutant:*

1. inability to grow on medium lacking a specific nutrient
2. inability to metabolize and particular energy source (sugar)

→ typically results from a mutation in a gene that specifies an enzyme in a

(1) biosynthetic or

(2) biodegradative pathway

Composition of media

genotype	MM + glucose	MM + g + arginine
arg <sup>+</sup>		
arg <sup>-</sup>		

MM = minimal medium G= glucose  
 arg = arginine (amino acid)

Composition of media

genotype	MM + glucose	MM + lactose
lac <sup>+</sup>		
lac <sup>-</sup>		

lac = lactose

***Resistance mutant:*** mutant strain that is able to grow in the presence of some specific metabolic inhibitor which wild-type strains are sensitive to



**Selection** for Forward mutation

$rif^s \rightarrow rif^r$  (*rif* = the antibiotic rifamycin)

**Screen** for Forward mutation

$lac^+ \rightarrow lac^-$  (*lac* = lactose)

**Selection** for Reverse mutation

$lac^- \rightarrow lac^+$