

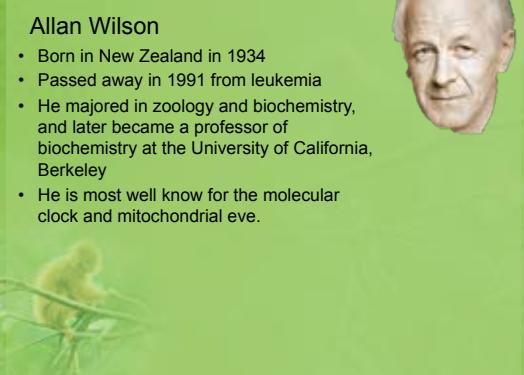
Mary-Claire King

- Born in 1946
- American Human Geneticist
- Professor at University of Washington
- 3 major accomplishments: identifying breast cancer genes, using genomic sequencing to identify victims of human rights abuse, and discovering that chimps and humans are 99% identical



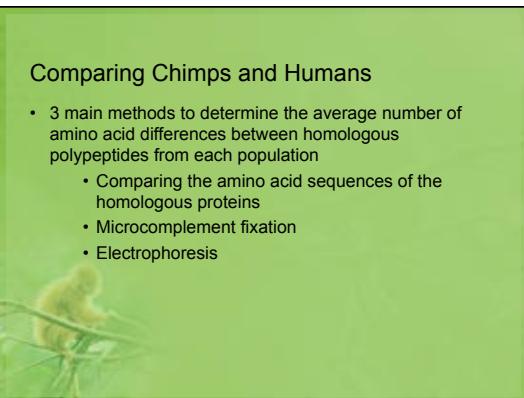
Allan Wilson

- Born in New Zealand in 1934
- Passed away in 1991 from leukemia
- He majored in zoology and biochemistry, and later became a professor of biochemistry at the University of California, Berkeley
- He is most well known for the molecular clock and mitochondrial eve.



Comparing Chimps and Humans

- 3 main methods to determine the average number of amino acid differences between homologous polypeptides from each population
 - Comparing the amino acid sequences of the homologous proteins
 - Microcomplement fixation
 - Electrophoresis



Sequence and immunological comparisons of proteins

- Amino acid sequences have been studied on several human and chimp proteins
 - Limitations did not allow for conventional sequencing of larger proteins
 - Microcomplementation fixation of larger proteins results in approximate measure of the difference between the amino acid sequences



Protein	Amino acid differences	Amino acid sites
Fibrinopeptides A and B (3)	0	30
Cytochrome c (4)	0	104
Lysozyme (13)	≈0	130
Hemoglobin α (4)	0	141
Hemoglobin β (4)	0	146
Hemoglobin γ_1 (3, 6)	0	146
Hemoglobin γ_2 (3, 6)	0	146
Hemoglobin δ (3, 6)	0	146
Myoglobin (7)	1	153
Carbonic anhydrase (4, 12)	≈3	264
Serum albumin (10)	≈6	580
Transferrin (11)	≈8	647
Total	-19	263

- Average degree of difference between human and chimpanzee proteins is:

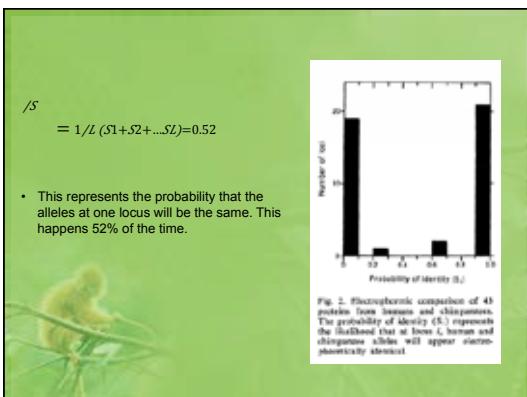
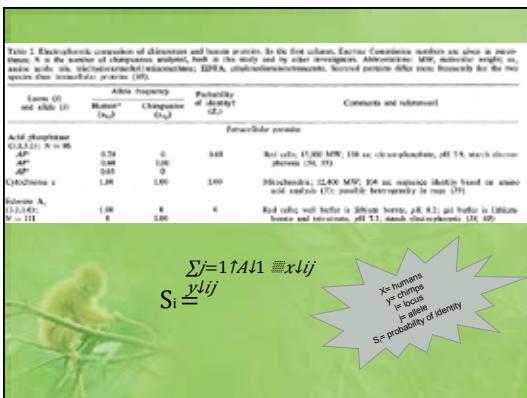
$$19 \times 1000 / 2633 = 7.2$$

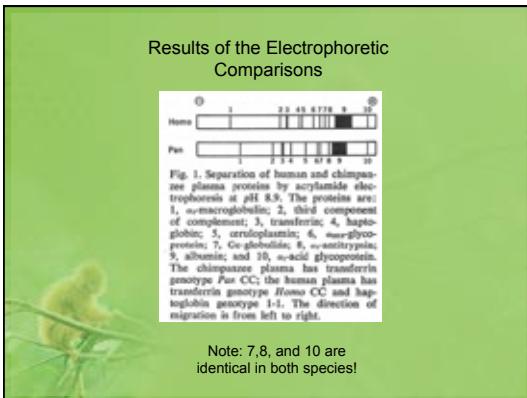
- This represents the number of amino acid substitutions per 1000 sites
 - The sequences of human and chimp polypeptides are on average more than 99% similar

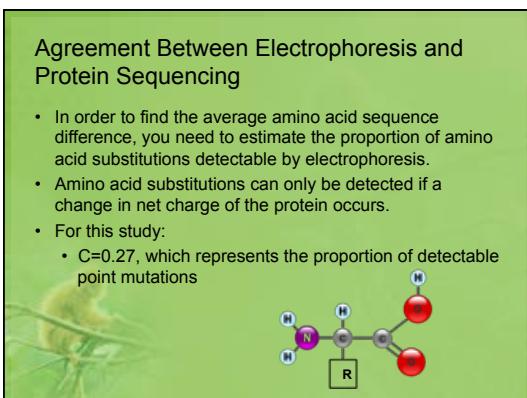


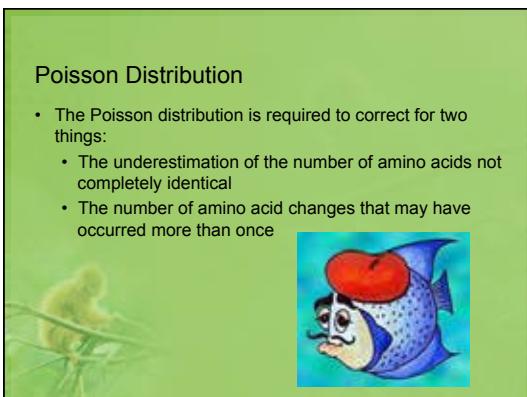
Electrophoretic comparison of proteins

- Electrophoresis was used to estimate the average amino acid sequence difference between humans and chimpanzees
- To do this they:
 - Used starch gel electrophoresis for analyzing the intracellular proteins
 - Used acrylamide gel electrophoresis for the secreted proteins
 - Set the electrode chamber at pH 8.9
 - Used amido black stain









Keep in mind

r – # of amino acid substitutions
 c – proportion of substitutions detectable
 m – expected # of substitutions (mean of poisson distribution)

- $P(r) = (mc)^r e^{-mc} / r!$
- $P(0) = 0.52 = e^{-mc}$
- $mc = 0.65$
- $m = 0.65 / 0.27 = 2.41$
- $2.41 \times 1000 / 293 = 8.2$



Comparison of Nucleic Acids

- Hybridization of DNA
- Debate over ΔT
 - Kohne said $\Delta T = 1.5^\circ\text{C}$ below human annealing temp.
 - Hoyer said $\Delta T = 0.7^\circ\text{C}$ below human annealing temp.
- ΔT = the difference in dissociation temperature of re-annealing human DNA and human-chimp hybrid DNA



- % difference of N.A. sequences = $k \times \Delta T$
- k = the calibration factor estimated between 0.45 and 1.5°C
- Example Calculation:
 - % difference = $(1.0)(1.1^\circ\text{C}) = 1.1\% = 0.01$
- If the length of the DNA is 3000 bp, then:
 - Difference of N.A. sequences = $0.01 \times 3000 = 33 \text{ bp}$

Note: For every amino acid difference, there are 4 nucleic differences; more changes in the DNA than protein



Reasons why this happened?

- When the third position changes there is no effect on the amino acid
- If the substitutions were equal between all nucleotides in a codon, there would be 1.4 to 1.7 nucleotide changes for each amino acid substitution
- This shows that this must be happening in the third position or non-coding regions
- All analyzed proteins with important functions were conserved, so this occurred in proteins that did not have a vital role

Genetic Distance

- Genetic distance is estimated using electrophoretic data by using standard estimate of net codon differences per locus

$$D = D_{HC} - (D_H + D_C) / 2$$

D_{HC} = estimate of variability between human and chimp populations
 D_H = variability within human population
 D_C = variability within chimp population
*= also a measure of the heterozygosity in human and chimp populations

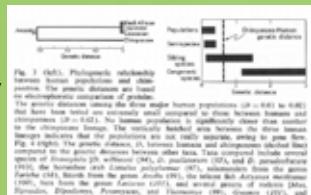
$$D_{HC} = -\log_e S$$

$$D_{HC} = -\log_e \left(\frac{1}{L} \sum_{i=1}^L \sum_{j=1}^{T_1} \sum_{k=1}^{T_2} \delta_{ijk} \right) = -\log_e \left(\frac{1}{L} \sum_{i=1}^L \sum_{j=1}^{T_1} \sum_{k=1}^{T_2} \frac{\delta_{ijk}}{T_2} \right)$$

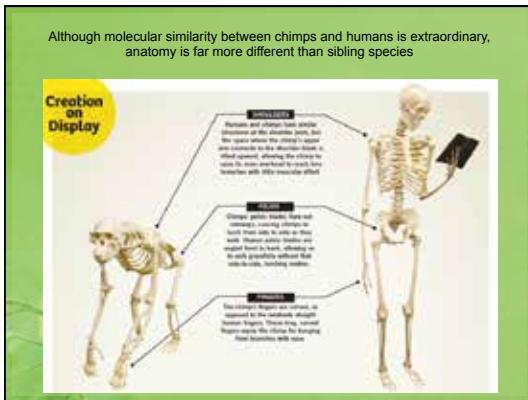
- According to Table 2 data:
- $D_{HC} = 0.65$
- $D_H = 0.05 \therefore D = 0.62$
- $D_C = 0.02$

- An average of 0.62 electrophoretically detectable codon differences per locus between homologous human and chimp proteins

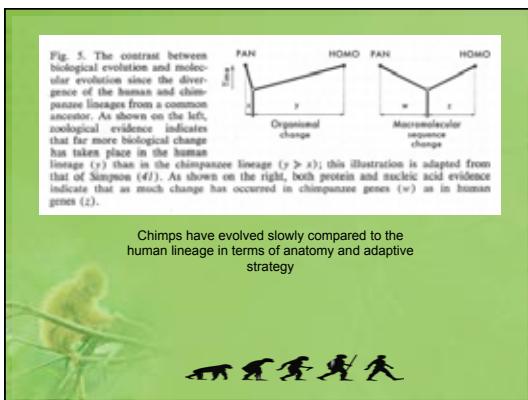
Note: The genetic distance between humans and chimps is very small, corresponding more closely to the genetic distance of sibling species



Also: The ΔT between re-annealed human DNA and human-chimp hybrid DNA is less than sibling and congeneric species



- Chimps and humans evolved at the same rate at the molecular level
 - Each side undergoes different changes, but at the same rate
 - Example, hemoglobin in humans and myoglobin in chimps
 - Even though the rates of change were equal, there was a great difference in their morphological evolution
 - The adaptive shift in humans was not accompanied by fast protein or DNA evolution



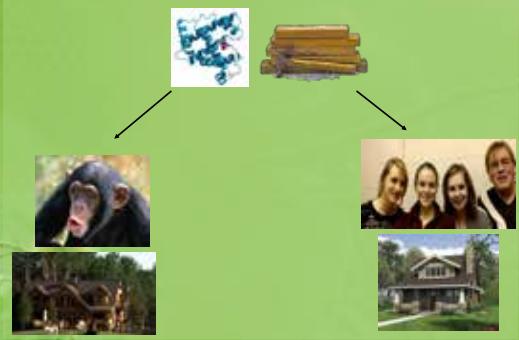
Molecular Basis of Evolution

- Ohno suggested that major anatomical changes usually results from mutations affecting the expression of genes
- He hypothesized that:
 - The time of activation or level of activity of a single gene could greatly influence embryonic development
 - So the differences in chimps and humans could result from genetic changes in a few regulatory systems, while amino acid substitutions would rarely have an effect
 - Gene rearrangements may be more important than point mutations as sources for evolutionary changes in gene regulation

- Humans have 46 chromosomes and chimps have 48, but the arrangement of genes on the chromosomes are different
- Banding studies indicate that at least 10 large inversions and translocations and 1 chromosome fusion have occurred since the two lineages diverged

So.....have you thought about
our question?!?!

If they are supposedly so closely related, what explains the variety of differences between the two species???



Summary and Conclusion

- All methods indicate that the average human polypeptide is more than 99% identical to its chimpanzee counterpart
- Non-repeated DNA sequences differ more than amino acid sequences
- The genetic distance between human and chimpanzees is very small; macromolecules and anatomical/behavioural features of organisms can evolve at independent rates
- A small number of genetic changes in systems controlling the expression of genes may account for the major organismal differences

How does this help us now and in the future?

- Developmental biology
- Understanding of the evolution of whole organisms
- Timing of gene expression during development