


## Evolution at Two Levels in Humans and Chimpanzees

Mary-Claire King and A.C. Wilson



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### What did we know prior to 1975?

- 1700's: Linnaeus and others of that time considered "Great Apes" to be the closest relatives of humans.
- 1859: Charles Darwin published *On the Origin of Species*, proposing the concept of evolution.
- 1925: Raymond Dart suggested that *Australopithecus africanus* was the midpoint between apes and humans.



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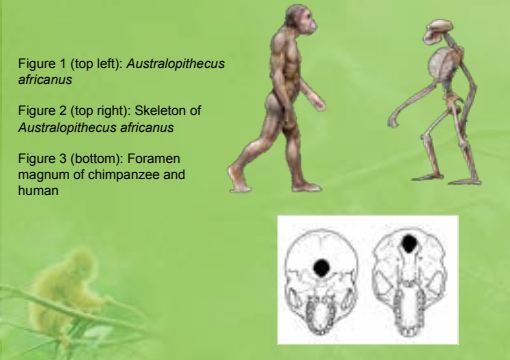
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Figure 1 (top left): *Australopithecus africanus*

Figure 2 (top right): Skeleton of *Australopithecus africanus*

Figure 3 (bottom): Foramen magnum of chimpanzee and human



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- 1967: Sarich & Wilson: ape/human divergence 4-5 MYA
- Conventional wisdom: ~23 MYA
- 1974: "Lucy" (*Australopithecus afarensis*): 3.2 MYA



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So think about this!?

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



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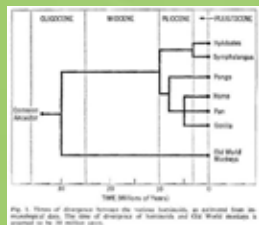
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- Before the 1950's: No common way to measure genetic distance between species.
- 1950's: Comparing proteins and nucleic acids of species would allow for estimation of genetic distance.



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**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



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**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 know for the molecular clock and mitochondrial eve.



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**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



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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
- Microcomplement fixation of larger proteins results in approximate measure of the difference between the amino acid sequences




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Table 1. Differences in amino acid sequences of human and chimpanzee polypeptides. Lysozyme, carbonic anhydrase, albumin, and transferrin have been compared immunologically by the microcomplement fixation technique. Amino acid sequences have been determined for the other proteins. Numbers in parentheses indicate references for each protein.

| Protein                      | Amino acid differences | Amino acid sites |
|------------------------------|------------------------|------------------|
| Fibrinopeptides A and B (2)  | 0                      | 30               |
| Cytochrome c (4)             | 0                      | 104              |
| Lysozyme (13)                | ~0                     | 130              |
| Hemoglobin $\alpha$ (4)      | 0                      | 141              |
| Hemoglobin $\beta$ (4)       | 0                      | 146              |
| Hemoglobin $\delta$ (5, 6)   | 0                      | 146              |
| Hemoglobin $\gamma$ (5, 6)   | 0                      | 146              |
| Hemoglobin $\epsilon$ (5, 6) | 1                      | 146              |
| Myoglobin (7)                | 1                      | 153              |
| Carbonic anhydrase (4, 12)   | ~3                     | 264              |
| Serum albumin (10)           | ~6                     | 580              |
| Transferrin (11)             | ~8                     | 647              |
| Total                        | ~19                    | 2633             |

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- 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




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### 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

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Table 2. Electrophoretic comparison of chimpanzee and human proteins. In the first column, Enzyme Commission numbers are given in parentheses. N = the number of chimpanzees analyzed, both in this study and by other investigators; abbreviations: MW, molecular weight; aa, amino acids; ub, ultracentrifuged ultracentrifuge; EDPA, ethylenediamine-pyrocatechol; Secreted proteins differ most frequently for the two species than intracellular proteins (197).

| Enzyme (E.C. number)                                  | Amino frequency                      |                              | Probability of identity† (S <sub>i</sub> ) | Comments and references   |
|---|--------------------------------------|------------------------------|--|---|
|   | Human <sup>a</sup> (N <sub>H</sub> ) | Chimpanzee (N <sub>C</sub> ) |  |   |
| Intracellular proteins                                |                                      |                              |  |   |
| Aspartate phosphatase (3.1.3.2); N = 95               | 0.79                                 | 0                            | 0.69                                       | Rat cells; 15,000 MW; 100 aa; cytoplasmic; pH 5.8; starch electrophoresis (24, 73)  |
| ADP <sup>b</sup>                                      | 0.96                                 | 1.00                         |  |   |
| ADP <sup>c</sup>                                      | 0.95                                 | 0                            |  |   |
| Cytochrome c  | 1.00                                 | 1.00                         | 1.00                                       | Mitochondria; 22,000 MW; 269 aa; cytosolic; identical based on amino acid analysis (7); possible heterogeneity in man (74)          |
| Glucose 6-phosphate dehydrogenase (1.1.1.43); N = 111 | 1.00                                 | 0                            | 0  | Rat cells; well buffer in alkaline buffer, pH 8.1; not buffer in EDTPA buffer and isozymes, pH 7.1, starch electrophoresis (24, 73) |

$$S_i = \frac{\sum_j y_{ij}}{N_i}$$

$$\sum_j = 1 \uparrow A \downarrow 1 \equiv x_{ij}$$

X = humans  
 Y = chimpanzees  
 i = locus  
 j = allele  
 S<sub>i</sub> = probability of identity

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$$1/S = 1/L (S_1 + S_2 + \dots S_L) = 0.52$$

• This represents the probability that the alleles at one locus will be the same. This happens 52% of the time.

Fig. 2. Electrophoretic comparison of 41 proteins from humans and chimpanzees. The probability of identity (S<sub>i</sub>) expresses the likelihood that at locus i, human and chimpanzee alleles will appear electrophoretically identical.

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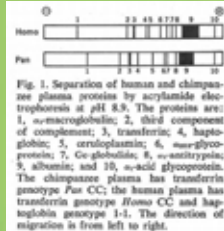
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Results of the Electrophoretic Comparisons



Note: 7, 8, and 10 are identical in both species!

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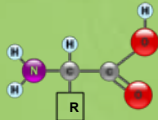
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Agreement Between Electrophoresis and Protein Sequencing

- In order to find the average amino acid sequence difference, you need to estimate the proportion of amino acid substitutions detectable by electrophoresis.
- Amino acid substitutions can only be detected if a change in net charge of the protein occurs.
- For this study:
  - $C=0.27$ , which represents the proportion of detectable point mutations




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Poisson Distribution

- The Poisson distribution is required to correct for two things:
  - The underestimation of the number of amino acids not completely identical
  - The number of amino acid changes that may have occurred more than once




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

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




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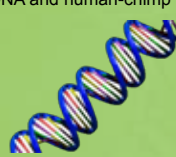
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### Comparison of Nucleic Acids

- Hybridization of DNA
- Debate over  $\Delta T$ 
  - 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.
- $\Delta T$  = the difference in dissociation temperature of re-annealing human DNA and human-chimp hybrid DNA




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- % difference of N.A. sequences =  $k \times \Delta T$ 
  - k = the calibration factor estimated between 0.45 and 1.5  $^\circ\text{C}$
- 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 =  $0.011 \times 3000 = 33$  bp

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




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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

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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

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

$$D_H = -\log_e (1/L \sum_{i=1}^L \sum_{j=1}^L P_{ij}^2) = -\log_e (1/L \sum_{i=1}^L \sum_{j=1}^L x_{ij}^2)$$

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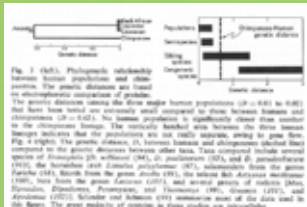
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- According to Table 2 data:
  - $D_{HC} = 0.65$
  - $D_H = 0.05$  ∴  $D = 0.62$
  - $D_C = 0.02$
- An average of 0.62 electrophoretically detectable codon differences per locus between homologous human and chimp proteins

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



Also: The  $\Delta T$  between re-annealed human DNA and human-chimp hybrid DNA is less than sibling and congeneric species

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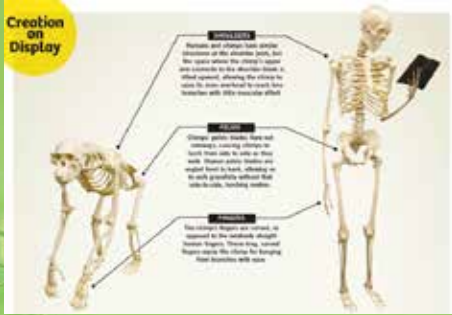
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Although molecular similarity between chimps and humans is extraordinary, anatomy is far more different than sibling species




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- 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




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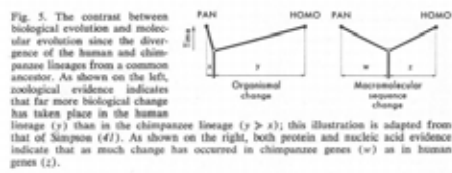
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Chimps have evolved slowly compared to the human lineage in terms of anatomy and adaptive strategy




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### 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

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- 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?!?!?

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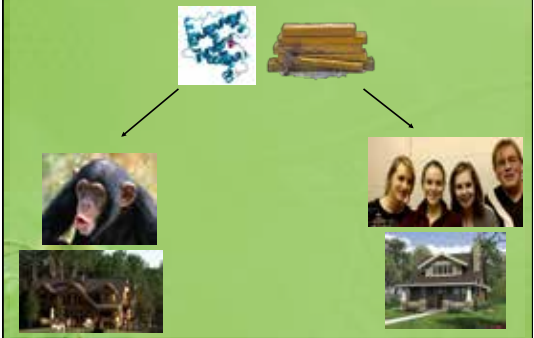
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If they are supposedly so closely related, what explains the variety of differences between the two species???



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### 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

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### 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



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