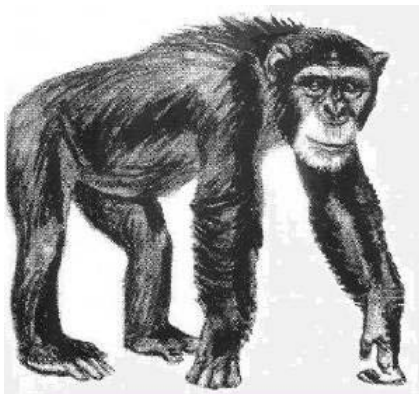


# King & Wilson 1975

## Evolution at Two Levels in Humans and Chimpanzees

"Apes and Humans are 99% similar."



[Home](#)

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[Luria-Delbruck 1943](#)

[Hershey-Chase 1952](#)

[Meselson-Stahl 1958](#)

[Garapin et al. 1978](#)

[McClintock 1953](#)

[King-Wilson 1975](#)

[Sanger et al. 1977](#)

[Jeffreys et al. 1985](#)

[Rothberg et al. 2011](#)

[Hamer et al. 1993](#)

### Background

Mary-Claire King (born 1946) and Allan C Wilson (1934-1991) contributed to contemporary understanding of human genetics and molecular evolution, and are responsible for what we know today about the evolutionary distance between us and our closest cousin *Pan troglodytes* - the common Chimpanzee.

Proteins and their homologs were starting to be analyzed by the scientific world (gel electrophoresis, nucleic acid hybridization, determination of amino acid sequences).

Point Mutations in protein-encoding DNA was thought to be responsible for organismal differences (more on this in the next section).

François Jacob (born June 17, 1920 in Nancy) is a French biologist who, together with Jacques Monod developed the idea that control of enzyme levels in all cells occurs through feedback on transcription. This would come to be known as the operon model of molecular biology.

Emile Zuckerkandl proposed the idea of a molecular clock to measure evolutionary distance between species.

Allan Wilson was the first to apply the theory to an actual scenario (humans and chimps)

Susumu Ohno began to consider the role of "controller genes" and gene regulation to account for these differences upon the introduction of the operon model of gene regulation.

### More Details on Human-Chimp Evolution and Associated Assumptions (c.a. 1970's)

Between 1936 -1947 modern evolutionary synthesis constructed. Two key ideas from this synthesis to keep in mind:

- 1) Natural selection is the primary mechanism for evolution, natural selection can account for both small and large phenotypic differences in organisms

2) Evolution is gradual (gradualism): small genetic changes (i.e. changes in allele frequencies) accumulate over long time periods at a few or many loci and eventually lead to changes in the phenotype

Prior to 1950's: Evolutionary history of organisms constructed based on "organismal," or biological characters (anatomy, behaviour, ecology, physiology).

In the 1950's molecular methods were applied to the study of evolutionary genetics (i.e. gel electrophoresis of proteins) to measure the degree of genetic variation within and between species. Later on, as more molecular markers became available, there was also interest in the study of how genetic variation was distributed throughout the genome.

Assumed that large phenotypic differences that produce the emergence of new species occur due to the accumulation of many small genetic changes over long periods of time (gradualism)

Zoological evidence indicated that humans were far more evolved than chimps from the common ancestor of chimps and humans (See Figure 5 from King and Wilson, 1975).

Therefore, since humans showed much more organismal change (anatomy, behaviour, physiology, ecology) than chimps, it was expected that humans and chimp DNA would show large differences at the molecular level.

Idea that proteins could evolve at rates independent of morphological evolution was extremely novel at the time.

Molecular evolution, and in particular, the idea that the primary force driving the evolution of biological molecules was not natural selection but mutation-drift equilibrium was very controversial. The molecular clock concept was therefore highly debated.

## MOLECULAR EVOLUTION

Biological molecules evolve over time just as the species of which they are a part. Thus, there is a common ancestor molecule of human and chimpanzee cytochrome c that was present in the common ancestor leading to the human and chimpanzee lineages. As each lineage evolved, so did their biological molecules. The cytochrome c proteins in chimps and humans are homologous proteins.

### Purpose

King and Wilson wondered whether or not the molecular differences between chimps and humans (nucleic acid/amino acid substitutions) were substantial enough to account for the phenotypic variance observed between the two species. This was assuming that genetic distance is an accurate reflection of evolutionary separation (the molecular clock theory). Are the genes of humans and chimps similar?

### Questions:

Are the degrees genetic distance obtained from different methods between humans and chimps similar?

Does the degree of genetic distance (measured using four different methods) reflect morphological distances between chimps and humans?

**Main Objective:** Obtain and compare four measures of the genetic distance between humans and chimps based on four methods:

1. Nucleic Acid Hybridization
2. Protein sequencing
3. Protein electrophoresis
4. Immunological Comparison of Proteins via the microcomplement fixation

## Methods / Results

King and Wilson used four methods to determine the "genetic distance" between the chimpanzee and humans:

### (1) Nucleic Acid Hybridization

Estimated Differences in DNA (nucleic acid sequences) can be estimated based on hybridization using the equation:

$$N = \Delta T * k$$

N = % difference in nucleic acid sequence between humans and chimps

$\Delta T$  = difference in dissociation temperature between human-human DNA and human-chimp DNA ( $^{\circ}\text{C}$ )

k = calibration constant ( # nucleic acid substitutions per 100 bases per  $1^{\circ}\text{C}$  difference in dissociation temperature)

\*\* different values of k have been estimated; King and Wilson used 1.0 for human/chimp DNA

**Conclusion:** King and Wilson found that human and chimp DNA is about 1.1% different (11 different bases per 1000)

This indicates there is more variation on the genetic level than the protein level

- may be attributed to redundancy of the genetic code
- the differences in nucleic acids may be found in noncoding sequences, allowing these sequences to not be conserved during evolution

Also indicates that humans and chimps have a similar genetic distance to other sets of **sibling species**

### (2) Protein Sequencing

**Source Data:** Results of protein sequencing were based on homologous proteins in chimps and humans for which sequences had already been published prior to 1974.

**Direct Count Data:** A total of 19 amino acid differences were found among a total of 2633 amino acids among 13 proteins (Table 1)

**Calculations:** But how did King and Wilson turn this into >99%?

**Step one:** express this number (19 of 2633) as the number of amino acid substitutions per 1000 sites:

$$(19 / 2633) * 1000 \text{ sites} = 7.2 \text{ amino acid differences per 1000 residues}$$

but 7.2 is a measure of how different chimp and human proteins are

**Step two:** to convert 7.2 amino acid differences into a number expressing the number of identical amino acid residues per 1000 sites:

$$1000 \text{ sites} - 7.2 \text{ different amino acids} = 992.8$$

**Step three:** convert this number into a percentage  $992.8 / 1000 = 99.2 \%$

**Conclusion:** Based on PROTEIN SEQUENCING, chimp and human DNA is **99.2% similar**

### (3) Protein Electrophoresis

**Raw Data:** King and Wilson determined the number of alleles and frequency of each allele at each of 44 protein loci in homologous chimp and human proteins (see Table 2 from King and Wilson).

#### Calculations:

**Similarity of Electrophoretically Detectable Alleles** based on Protein Electrophoresis as calculated by a Probability of Identity Statistic

i) Probability of Identity for a particular protein locus,  $S_{sub i}$

Equation and example calculation here.

Probability of identity at each protein locus given in table 2 (King and Wilson, 1975). Notice that alleles at some loci are different ( $S_i=1$ ), the same ( $S_i=0$ ), or similar (when  $S_i$  is  $> 0$  and  $< 1$ ). See Figure 2 from King and Wilson.

ii) Probability of Identity for an "average protein locus"  $\bar{S}_{sub i}$

Equation here.

**Semi Conclusion:** The probability that human and chimp alleles will be electrophoretically identical at a particular (average) locus is 0.52 (about one half).

**BUT,** How do allelic differences translate into amino acid differences? Need to calculate the expected number of amino acid substitutions per 1000 sites to compare with direct protein sequence data.

Involves several steps:

(1) Calculate proportion of amino acid substitutions detectable by electrophoresis. Based on the buffer systems used this proportion was determined to be 0.27.

(2) Calculate the proportion of the expected number of amino acid substitutions per protein.

Method: Use Poisson distribution where:

i) the zero class ( $P_{sub zero}$ ) is the number of amino acid residues that are the same in chimps and humans,  $c$

ii)  $m$  is the mean of the Poisson variate, in this case the expected number of amino acid substitutions per polypeptide

iii)  $P_{sub r}$  is the Poisson variate, or the number of amino acid substitutions expected in a given polypeptide, where  $r$  is equal to or greater than zero

**Two assumptions:** amino acid substitutions occur independently, and at random with respect to species since humans and chimp lineages diverged from a common ancestor

See calculation using Poisson distribution to determine the expected number of amino acid substitutions per protein.

**2.41** = expected number of amino acid substitutions per polypeptide ( $m$ )

(3) calculate the number of amino acid substitutions per 1000 residues

2.41 amino acid differences per polypeptide

King and Wilson determined that the average number of residues in all the proteins analyzed electrophoretically was 293 plus or minus 27

Therefore 2.41 divided by 293 \* 1000 sites

= 8.2 amino acid substitutions per 1000 sites

(4) calculate the percentage of sites that are similar to compare with sequencing data (99% figure).

1000 sites subtract 8.2 amino acid differences gives 991.8 similar amino acids per 1000 sites

Divide by 10 to get a percentage = 99.1%

Therefore, based on electrophoresis of proteins chimp and human DNA is 99.1% similar. Compare with 99.2% based on amino acid direct sequencing and immunological methods. Nucleic Acid Hybridization gives a 98.9% similarity between chimp and human DNA.

#### Finally, Calculation of Genetic Distance between Chimps and Humans from Electrophoretic Data

Use Nei and Roychoudhury's Standard Genetic Distance

Remember must account for within species variability (so subtract intraspecies heterozygosity from total variation within and between species)

Calculation [here](#).

Calculated D, genetic distance between chimps and humans is 0.62.

This is displayed visually [here](#) (Figure 3 from King and Wilson 1975).

#### (4) Immunological Comparison of Proteins by the Micro-complement Fixation Technique

## Conclusion

The four experimental methods (above) all indicate almost 99% similarity between the human and the chimpanzee

This makes it clear that molecular differences in the organisms at the macromolecular level could not account for organismal differences

King and Wilson propose that the organismal differences are derived from mutations affecting gene expression.

## Ohno's Hypothesis

=> small differences in the time of activation/level of activity in a gene considerably influences systems controlling embryonic development

=> King and Wilson agree

Suggest point mutations in genes encoding ex promoters, repressors, and receptors are likely

responsible

Also suggest the order of genes on the chromosomes contributes to inversion, translocation, and addition/deletion of genes => **regulatory properties (gene expression)** (likely more important than point mutations)

=> while human and chimp DNA is ~99% similar, humans have 46 chromosomes while chimps have 48

=> only a small number of banding patterns coincide between the two species on their chromosomes

=> banding studies indicate that at least 10 large inversions/translocations and at least one chromosomal fusion have occurred between humans and chimps

1.) The four methods (above) all revealed ~99% resemblance

2.) Nonrepeated DNA sequences differed more than amino acid sequence, due to redundancies in the genetic code and mutations in noncoding sequences

3.) The comparisons all indicate little genetic distance between humans and chimps.

- Classically, humans and chimps have been put in different families

- King and Wilson suggest this is misleading and should be rectified

4.) A small number of mutations affecting gene expression are likely responsible for the huge organismal differences observed

- likely those involving gene arrangement on chromosomes