Computational Biology

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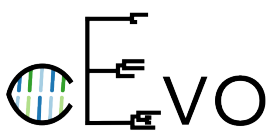
Computational Evolution
Department of Biosystems Science and Engineering

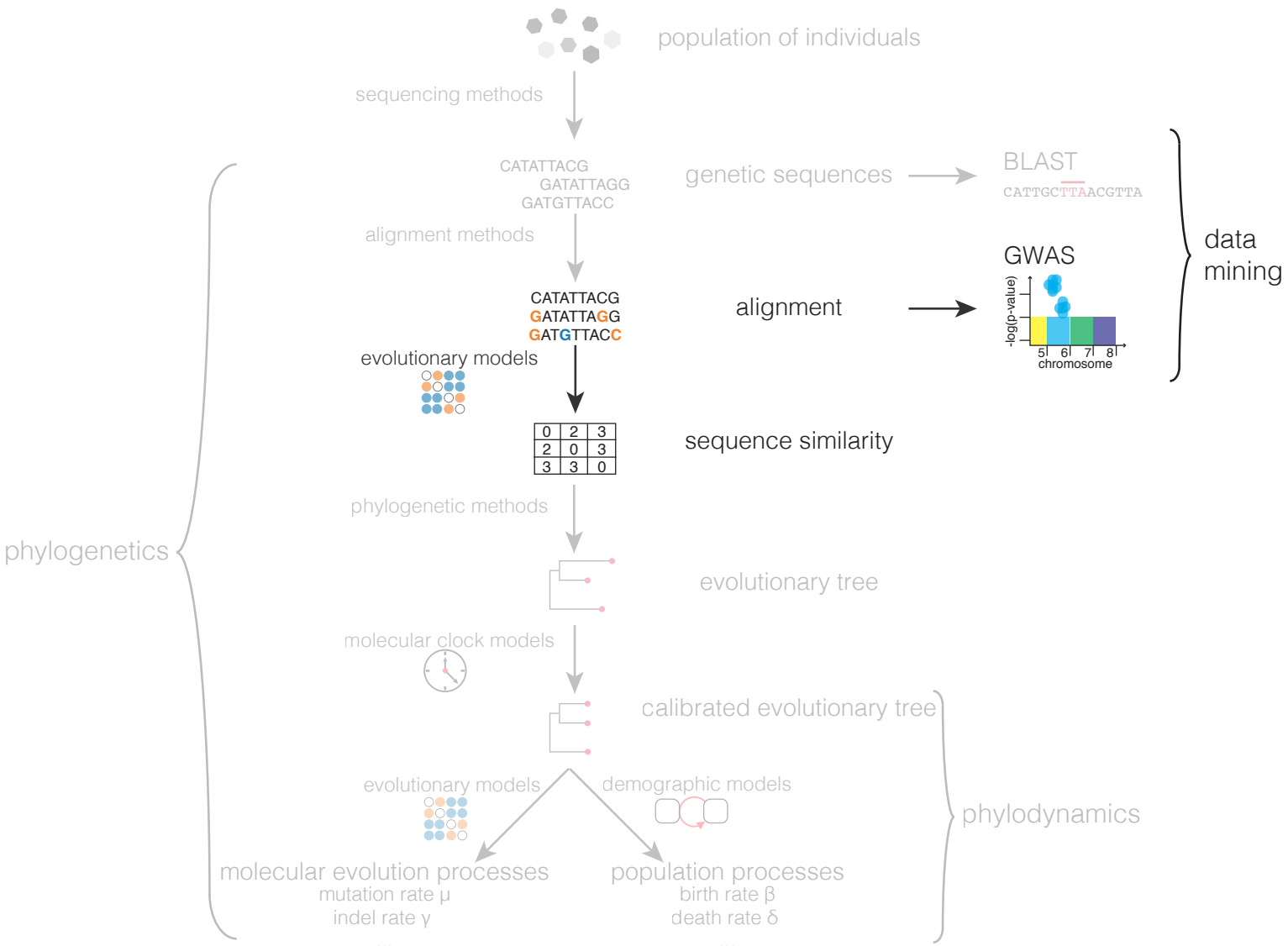
HS 2023



Alignment, BLAST Questions

- What are the weaknesses and strengths of the different alignment methods (dot-matrix method, Smith-Waterman, Needleman-Wunsch, BLAST)?
- With which alignment methods do you get an optimal alignment?
- Do you obtain the same alignments when using different scoring schemes in the Smith-Waterman and Needleman-Wunsch algorithms?

Overview and outline of lecture 03



Genome Wide Association Studies.

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Research question: Can we identify genetic risk factors for common diseases?

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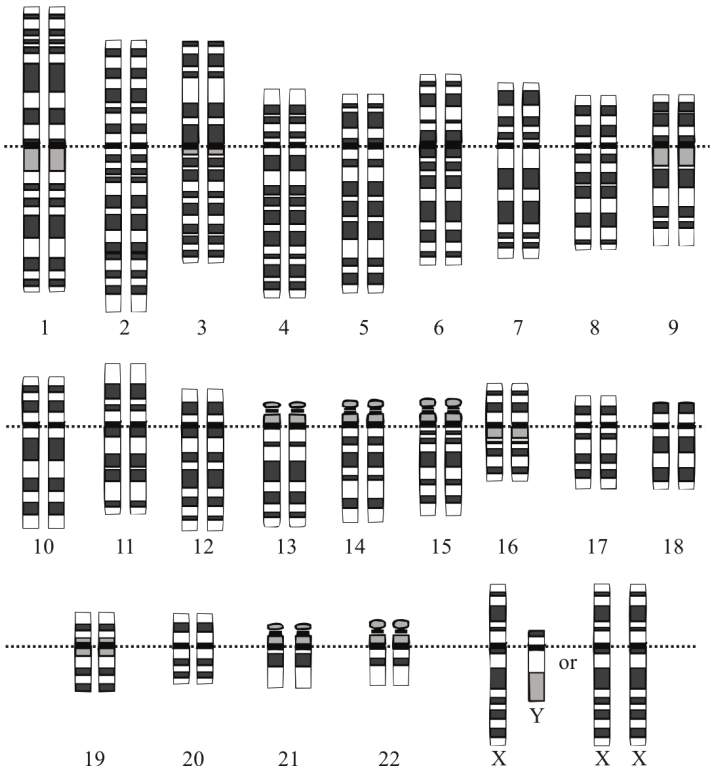

The Human Genome

en.wikipedia.org/wiki/Human_genome

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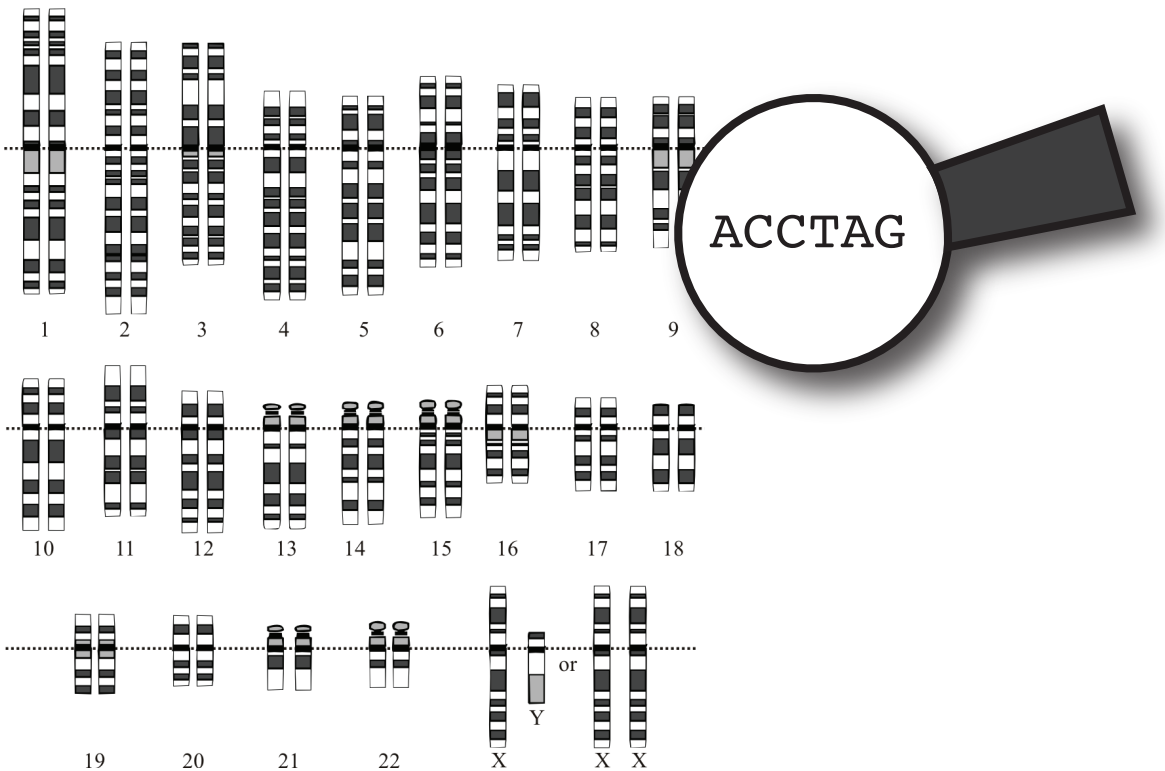

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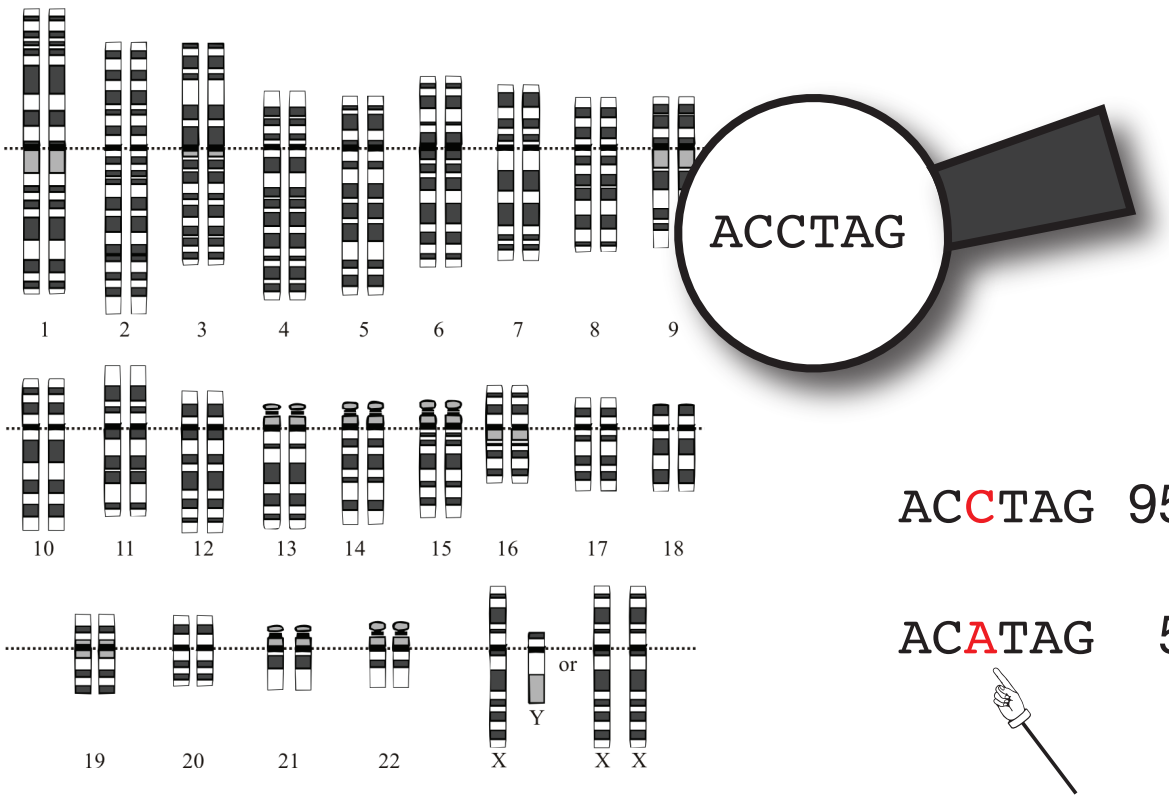

ACCTAG 95%

ACATAG 5%

of humans

The Human Genome

Single nucleotide polymorphism(SNP)

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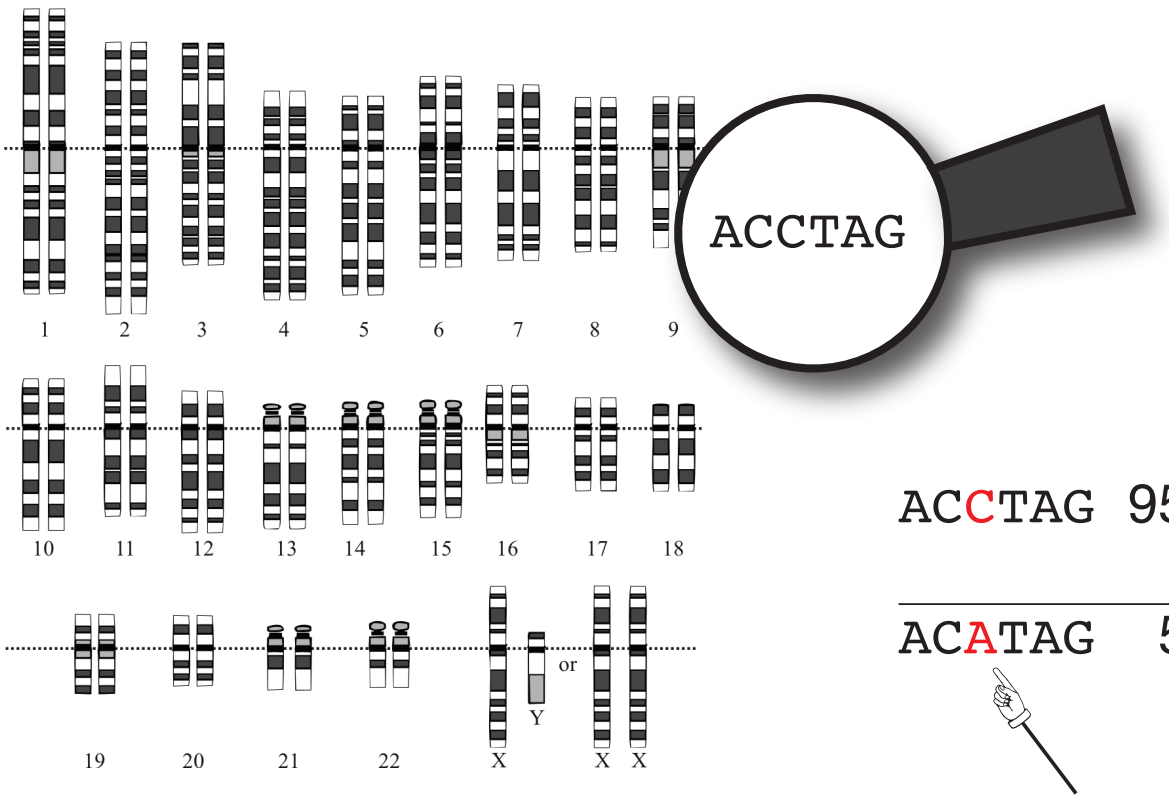

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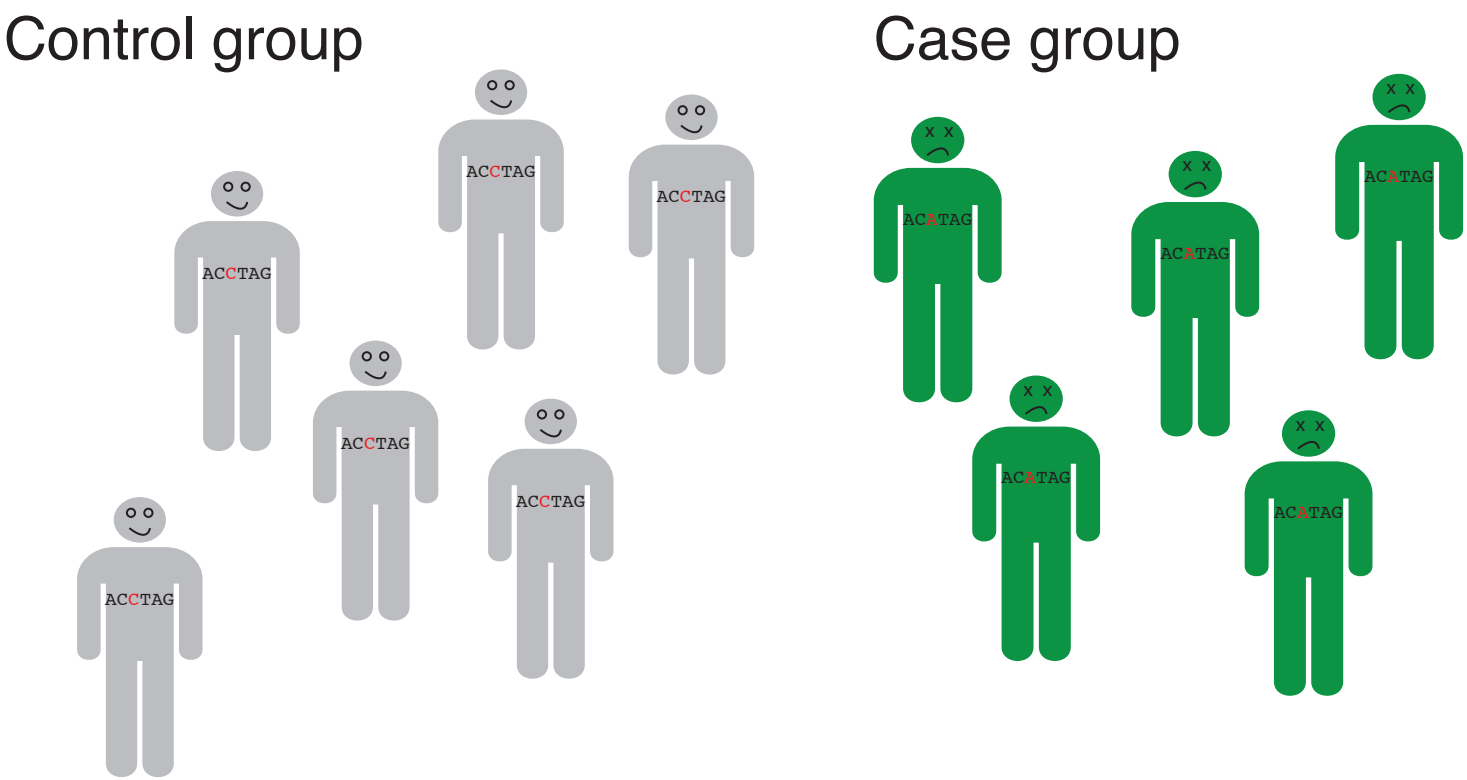
Genome wide association studies

Case-control setup – The most commonly used strategy:

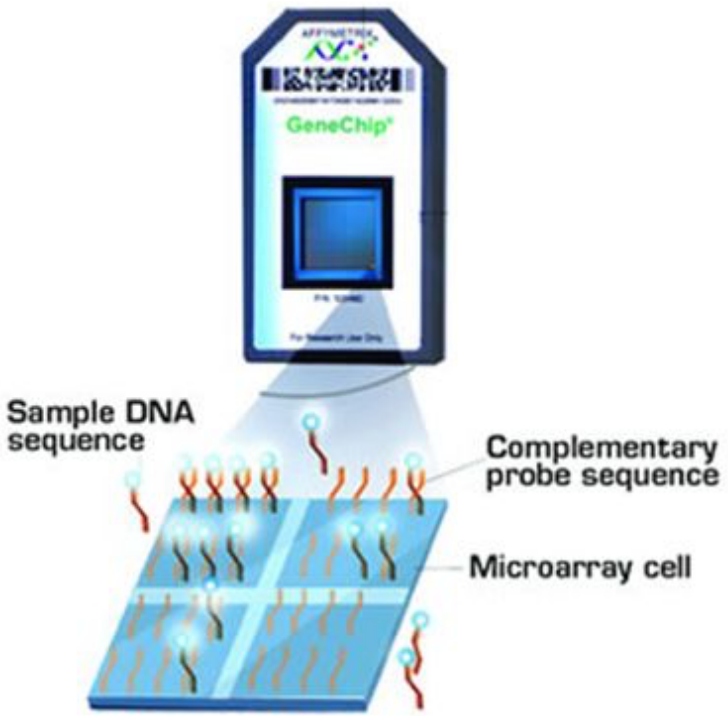
- two large groups of individuals: one healthy control group, and one group with a certain disease (e.g. diabetes)



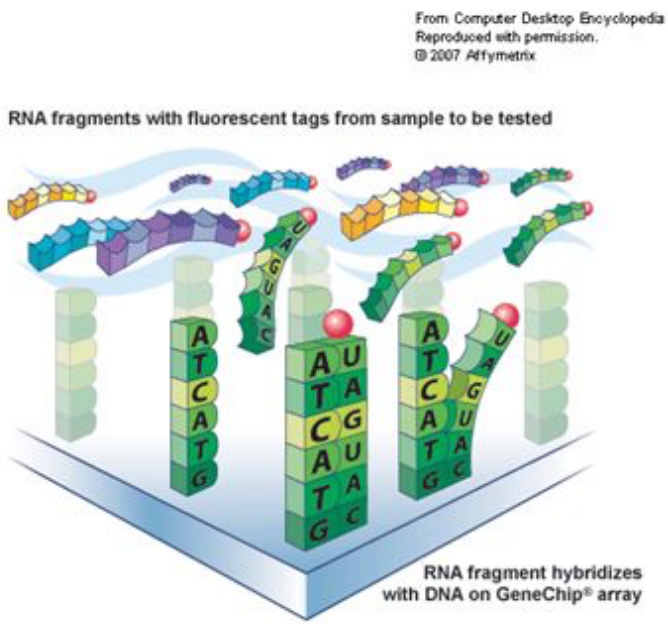
Genome wide association studies

Case-control setup – The most commonly used strategy:

- two large groups of individuals: one healthy control group, and one group with a certain disease (e.g. diabetes)
- all individuals are genotyped for the majority of known SNP locations (Illumina, Affymetrix)



(Liu 2007)



(Affymetrix)

adapted from `http://slideplayer.com/slide/8465760/`

Genome wide association studies

Statistical analysis

- case control setup: two groups (i) patients with disease, (ii) healthy patients
- for each SNP, count the number of healthy individuals without this specific mutation, $\mathrm{H_N}$, and with the mutation, $\mathrm{H_S}$, as well as the number of diseased individuals without the mutation, $\mathrm{D_N}$, and with the mutation, $\mathrm{D_S}$

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$$\mathrm{OR} = \frac{\text{odds of having the disease amongst individuals with minor variant on SNP position}}{\text{odds of having the disease amongst individuals with major variant on SNP position}}$$

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$$\mathrm{OR} = \frac{\text{odds of having the disease amongst individuals with minor variant on SNP position}}{\text{odds of having the disease amongst individuals with major variant on SNP position}} = \frac{\mathrm{D_S}/\mathrm{H_S}}{\mathrm{D_N}/\mathrm{H_N}}$$

Genome wide association studies: Example

Observed

	case	control	total
minor	2104	2676	4780
major	1896	3324	5220
total	4000	6000	10 000

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Definition: p-value

Given a random variable X and a realisation (observation) x. Let us assume a null hypothesis $\mathcal{H}_0$, which is a statement on the distribution of X. The p-value is the probability of observing x or a more extreme realisation under the assumption the null hypothesis was true.

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Definition: Significance level

The significance level, α, is a value that one defines before performing a statistical test. If the p-value lies below this significance level, the observed result of the random experiment cannot support the null hypothesis which leads to rejection of the null hypothesis.

Toolbox: Fisher's exact test

Fisher's exact test: Statistical test to examine the significance of the association between two kinds of classifications (here: being diseased, having minor variant).

Contingency table:

A \ B	B_1	B_2	
A_1	a	b	a+b
A_2	c	d	c+d
	a+c	b+d	n= a+b+c+d

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Thus our null hypothesis is:
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Toolbox: Hypergeometric distribution

To be able to phrase the null hypothesis in terms of a probability distribution, we need to consider the hypergeometric distribution, which is best explained in terms of an urn experiment. Assume an urn with r red and s black balls.

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$$P(R_k = i) = \frac{\binom{r}{i}\binom{s}{k-i}}{\binom{r+s}{k}}$$

Toolbox: Fisher's exact test II

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Toolbox: Fisher's exact test II

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☞ p-value$= \sum_{i=a}^{a+b} \frac{\binom{a+b}{i}\binom{c+d}{a+c-i}}{\binom{n}{a+c}}$

Toolbox: Pearson's χ^2-test

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  One calculates the deviance between observed and expected numbers based on a hypergeometric distribution:
  e.g. # expected cases with minor variant $= 10\,000 \frac{4000}{10\,000} \frac{4780}{10\,000}$

Observed $= (O_{ij})_{i,j\in\{1,2\}}$

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1912	2868	4780
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To test whether this result is significant, we calculate the following test statistic:

$\chi = \sum_{i,j=1}^{2} \frac{(O_{ij}-E_{ij})^2}{E_{i,j}}$

Pearson proved that this statistic is χ^2-distributed.

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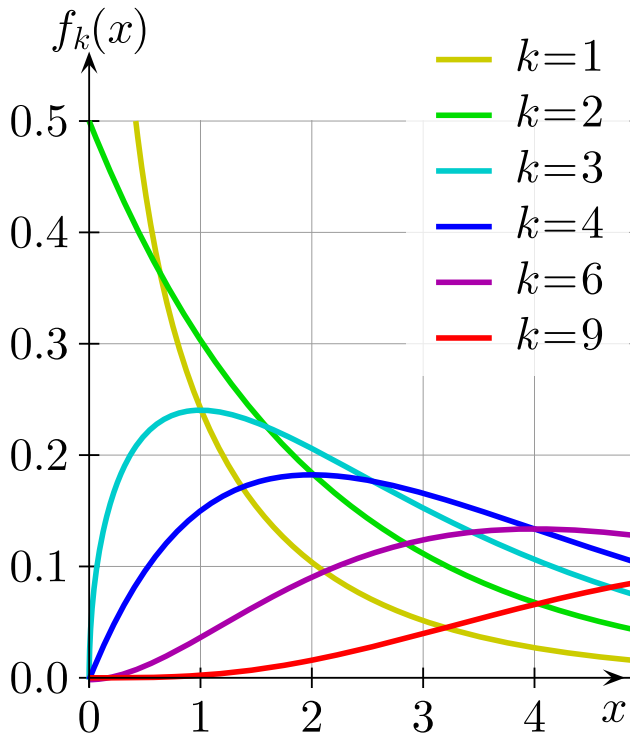
The p-value is then the probability of a χ^2-distributed random variable being $\geqslant \chi$:
In our case $\chi = 61$ and thus p-value $= 5.055 \times 10^{-15}$

Toolbox: The χ^2 distribution

Assume n i.i.d random variables $Y_i \sim \mathcal{N}(\mu, \sigma^2)$. The quantities $X^2 := \sum_{i=1}^{n} \frac{(Y_i - \mu)^2}{\sigma^2}$ are then χ^2- distributed with n degrees of freedom.



- It can be proven that many statistics are approximately χ^2_n-distributed.
- This simplifies hypothesis testing or estimation of confidence intervals.
- $P(\chi^2_n = x)$ is a short (and sloppy) notation which means: the probability that a test statistics S which is χ^2_n-distributed, is $\geqslant x$, i.e. $P(\chi^2_n = x) = P(S \geqslant x)$

further reading: [Sokal and Rohlf, 2012]

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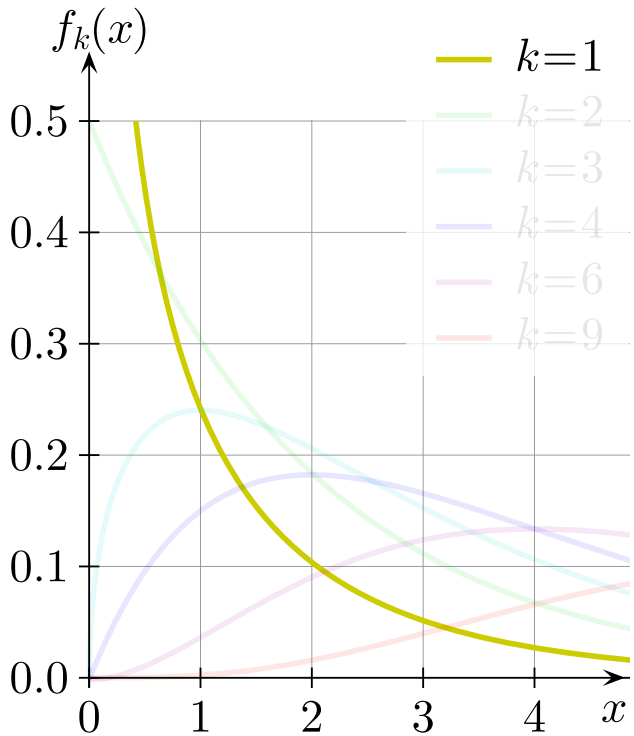

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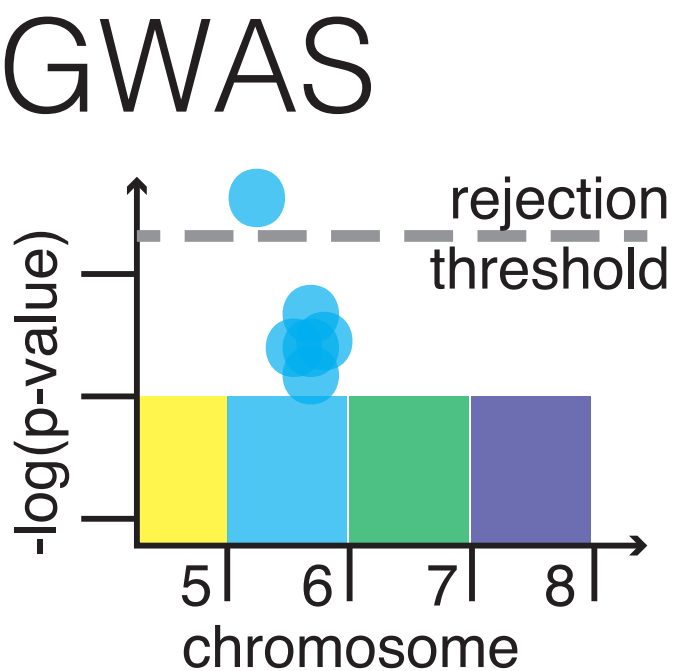
Genome wide association studies

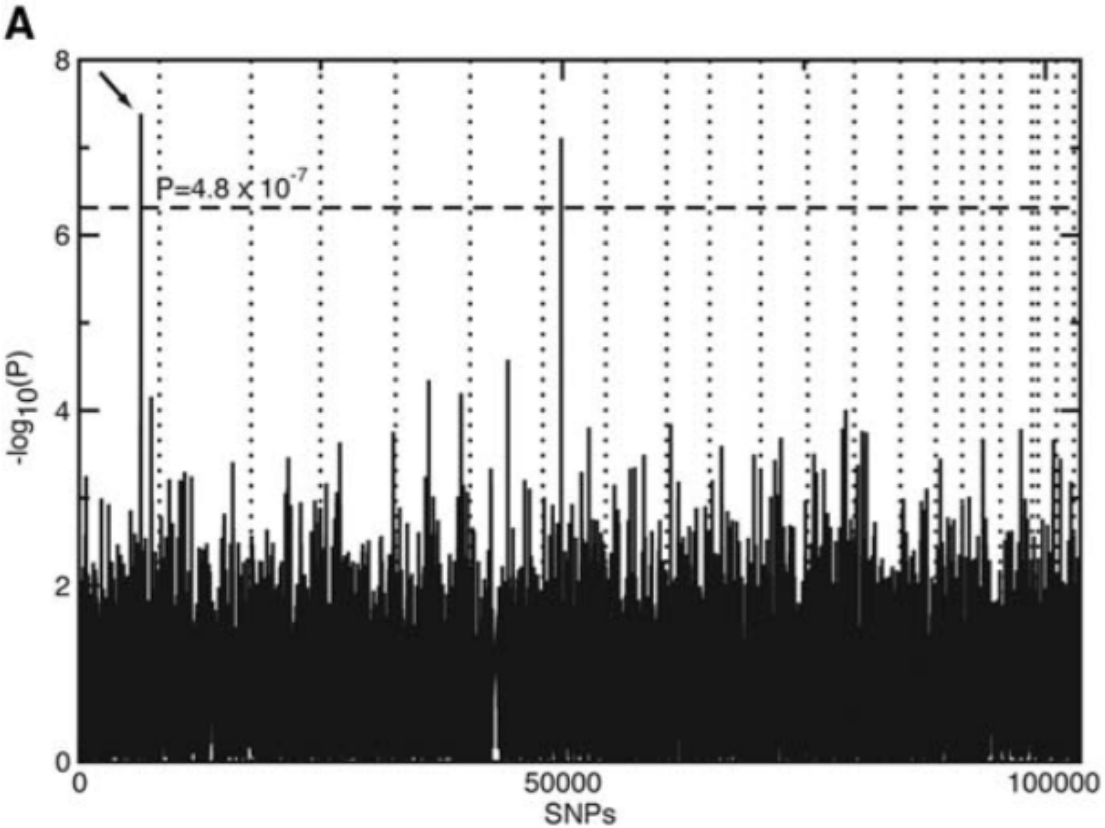
Typical results

- graph with the SNP positions (sorted according to chromosome) on the x-axis and -log(p-value) on the y-axis
- SNPs (as well as chromosomes) with extremely low p-values could be associated with the specific disease



The first GWAS

- first GWAS used to identify SNPs in complement factor H gene associated with age related macular degeneration
- 96 affected individuals and 50 healthy controls [Klein et al., 2005]



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- However, as of 2011, 1 200 GWAS have been conducted; over 200 diseases and traits, and almost 4,000 SNP associations have been found. GWAS approaches have been extended to address the above listed criticism.

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Further reading:

- [Pearson and Manolio, 2008]
- [Bush and Moore, 2012]
- `https://www.ebi.ac.uk/gwas/`
- `https://easygwas.ethz.ch/`

Quantifying variation between sequences.

Motivation

We want to infer phylogenetic trees. Phylogenies depict the genetic relatedness of sequences:

```
A C T A G C T G  human

A G T T G C T G  chimpanzee

A C T T G A T G  gorilla
```

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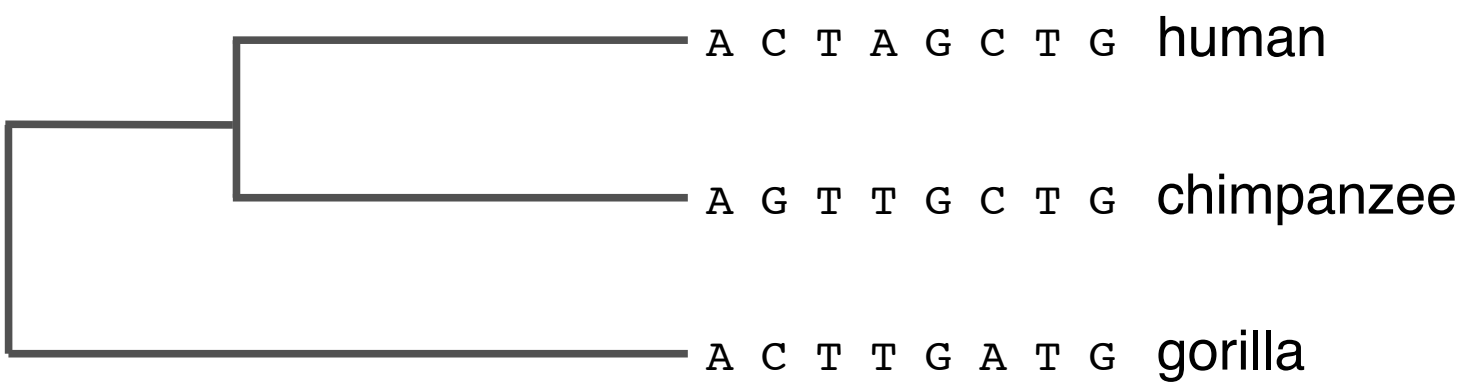

How to measure the sequences differences

Example:

```
ATTACGAC
TCTACGAC
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- Hamming distance:
  - number of segregating sites
  - count the sites that vary
  - in our example 2
- p-distance:
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Example from last lecture: *triosephosphate isomerase*:

```
NGTTDQVDKIVKILNEGQIASTDVVEVVVSPPYVFLPVVKSQLRPEIQVAAQNCW
||....!..!.|!|..|.!.:.  .||||. | .!|.:.!|||...! ||||||!
NGDKASIADLCKVLTTGPLNAD__TEVVVGCPAPYLTLARSQLPDSVCVAAQNCY
```

Hamming-distance 35

$\mathrm{p} = 0.636$

Molecular evolution models.

The fundamental problem

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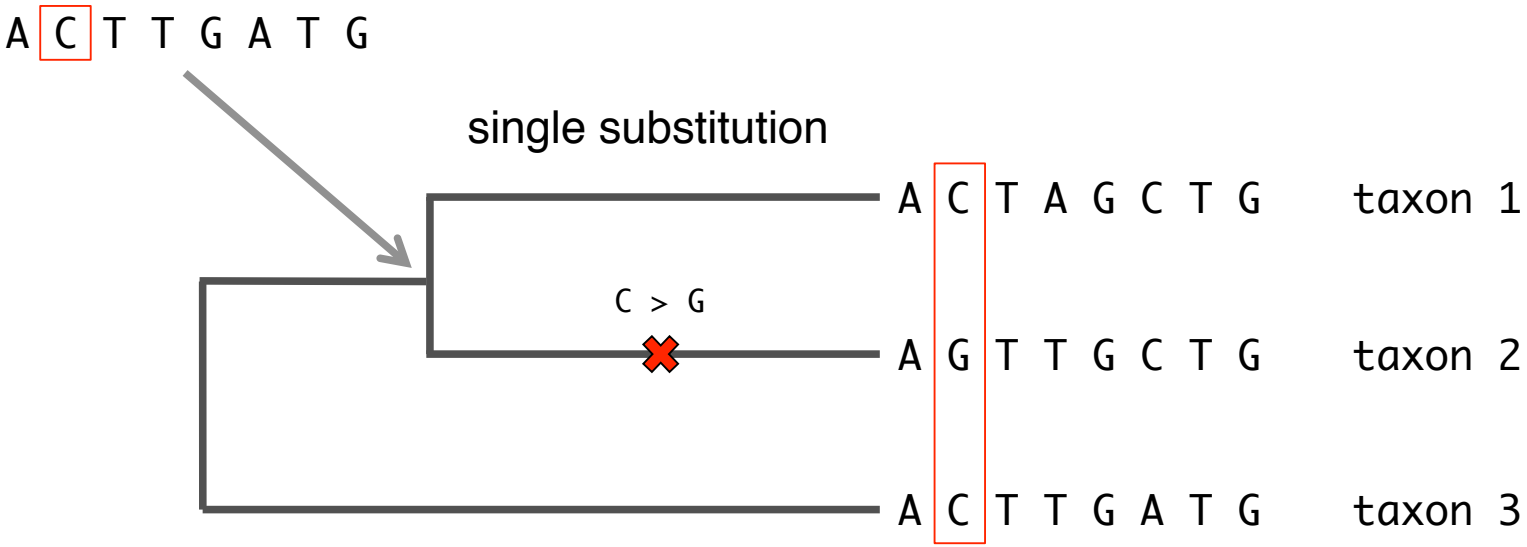

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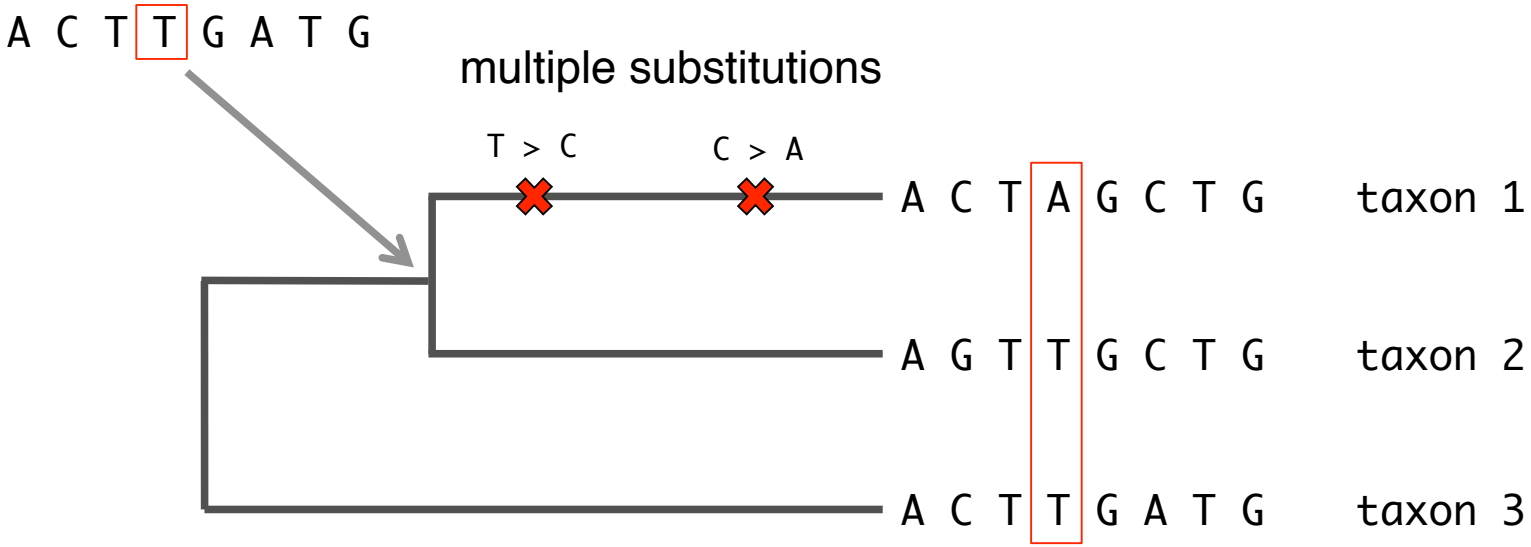

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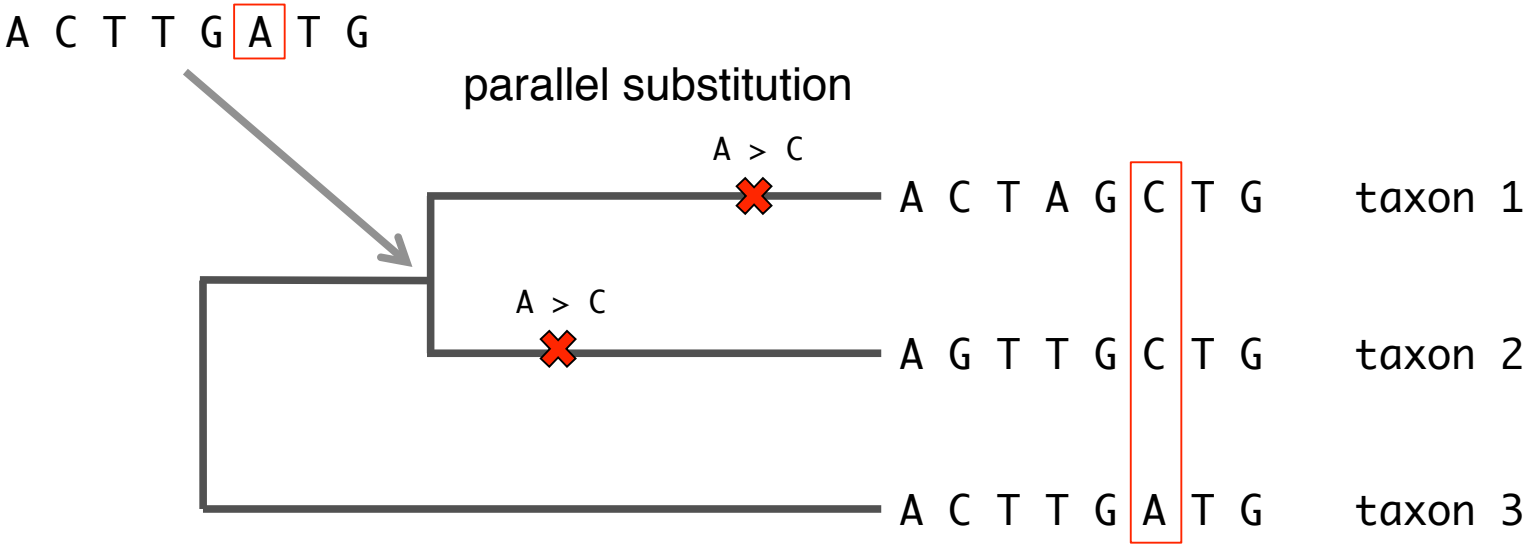

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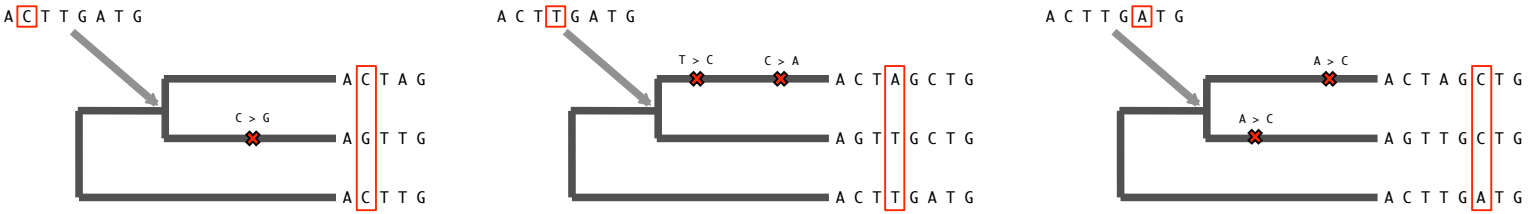


Problem of phylogenetics:
We observe sequences but not their evolutionary history. Thus we have to take **all** possible evolutionary trajectories into account.

How can we account for the evolutionary steps in between?

Sequence distance

Definition: Distance between two sequences
The distance between two sequences is the expected number of nucleotide substitutions per site [Yang, 2014].

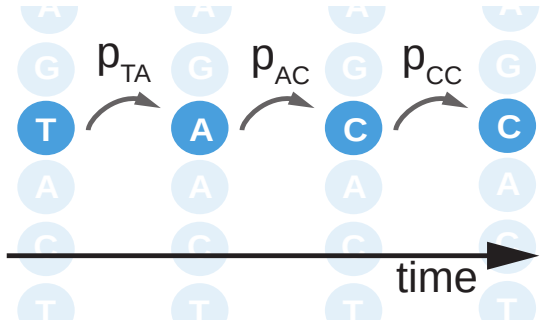
- This definition includes all (non-visible) evolutionary steps in between two sequences.
- We need to employ a mathematical model for estimating the distance, as we do not know the evolutionary steps. This model must include
  - (stochastic) process modelling the substitution through time
  - substitution rates

Nucleotide substitutions as a Markov chain

Definition of a Markov chain (see also [Ross, 1996])

stochastic process, i.e. a series of random experiments through time

Nucleotide substitutions as MC



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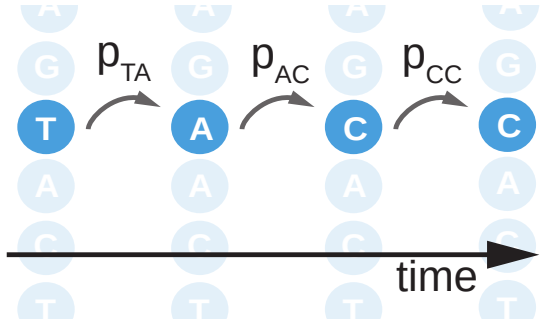

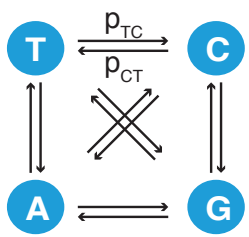

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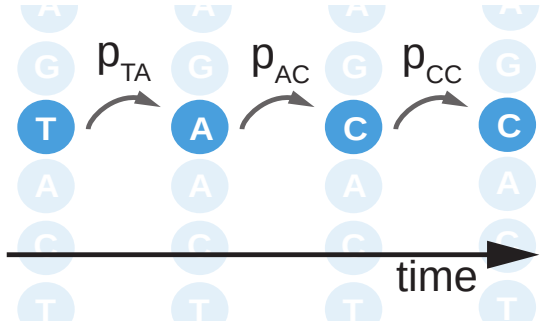
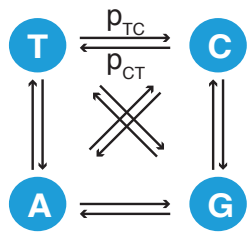
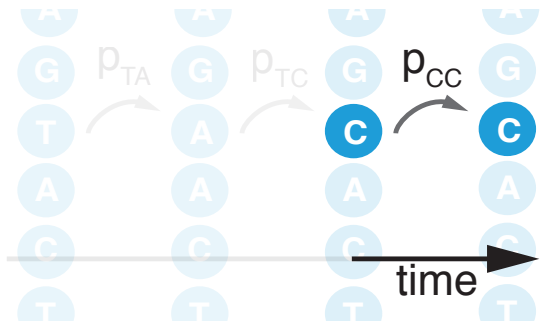






Mathematical toolbox: Markov chains

Definition of a Markov chain (consult [Ross, 1996] for further information on MC):

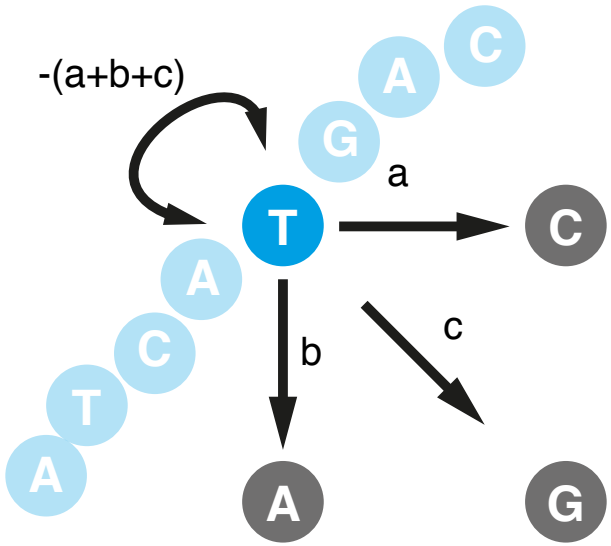
1. Stochastic process, i.e. a series of random experiments through time
   - a series of random variables $(X_t)_{t \in \mathcal{T}}$. If $\mathcal{T}$ is discrete, the Markov chain is called **discrete**, otherwise **continuous**.
2. lives on a state space and jumps to the different states
   - state space $\mathcal{S}$
3. **memorylessness:** the probability of jumping to a state only depends on the current state
   - $P(X_{t_{n+1}} = x_{t_{n+1}} | X_{t_n} = x_{t_n}, X_{t_{n-1}} = x_{t_{n-1}}, \ldots) = P(X_{t_{n+1}} = x_{t_{n+1}} | X_{t_n} = x_{t_n})$

If the transition probabilities on the state space do not change over time, the Markov chain is called **time homogenous.**

Nucleotide substitution as a Markov chain

State space of each nucleotide position: $\mathcal{S} = \{\mathrm{T}, \mathrm{C}, \mathrm{A}, \mathrm{G}\}$

Example: Assume the process is at state T



Substitution rate matrix:

$$Q = \begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \text{-(a+b+c)} & \mathrm{a} & \mathrm{b} & \mathrm{c} \\ \mathrm{d} & \text{-(d+e+f)} & \mathrm{e} & \mathrm{f} \\ \mathrm{g} & \mathrm{h} & \text{-(g+h+i)} & \mathrm{i} \\ \mathrm{j} & \mathrm{k} & \mathrm{l} & \text{-(j+k+l)} \end{pmatrix} \end{array}$$

Rates versus probabilities

Rate: measures events per time unit

- deterministic, fixed quantity
- examples: birth rate (offspring per year), substitution rate (nucleotide changes per unit time)
- describes averages

Probability: measure of chance that a random event occurs

- stochastic
- examples: obtaining 6 when throwing a die, time to substitution (when seen as a random process)
- describes an exact event

From rates to probabilities

Let α be the **rate** of an event E happening per unit of time. The **probability** that this event happens in a very small (infinitesimally small) time step $\Delta \mathrm{t}$ is defined as $\alpha \Delta \mathrm{t}$. We denote the time until the event happens with the random variable X. The probability that the event does not happen in $\Delta \mathrm{t}$ is:

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$$P(X > \tau) = (1 - \alpha\Delta t)^k = (1 - \alpha\Delta t)^{\tau/\Delta t} \xrightarrow[\Delta t \to 0]{} e^{-\alpha\tau}$$

The limit at the end of the latter equation holds true, because of the definition of the exponential function $e^x = \lim_{n\to\infty}(1 + \frac{x}{n})^n$.

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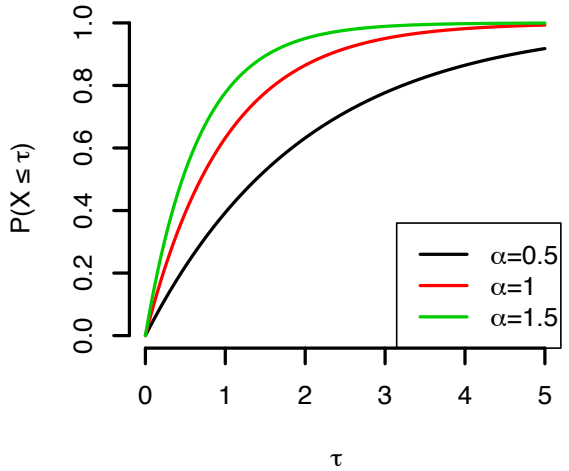
$$\implies P(0 \leqslant X \leqslant \tau) = 1 - e^{-\alpha\tau}$$

From rates to probabilities

$P(0 \leqslant X \leqslant \tau) = 1 - e^{-\alpha\tau}$ is the **cumulative density function**



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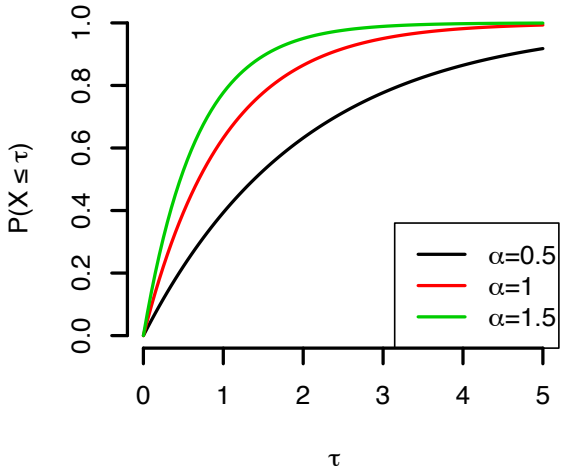
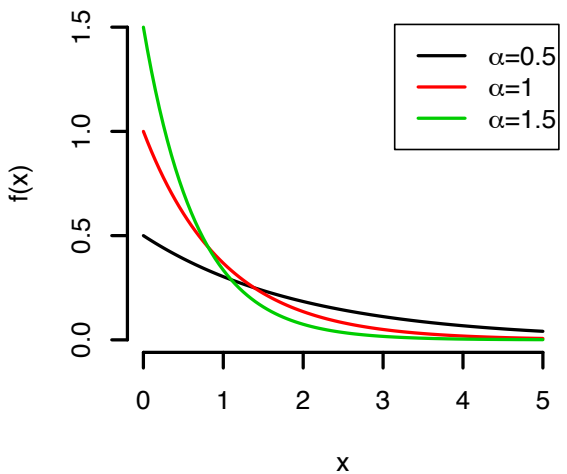


$f(x) = \frac{dP}{dt}(x) = \alpha e^{-\alpha x}$ is the **probability density function** of an **exponential distribution**



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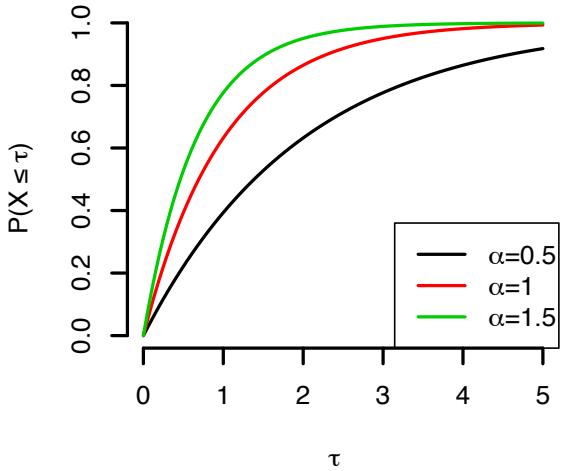

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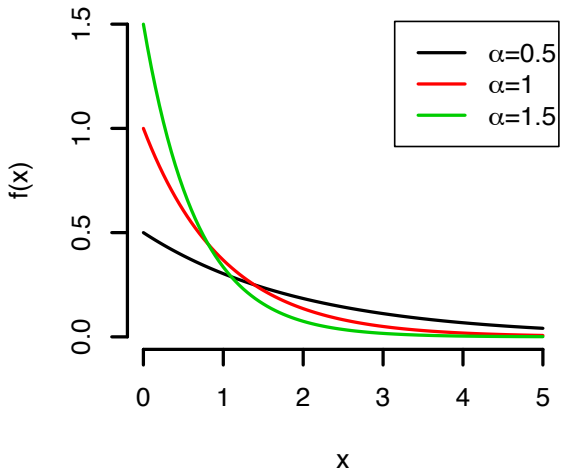

An event occurring with rate α means that it occurs after an exponentially distributed waiting time with parameter α.

From rate matrix to transition probabilities

Let $\mathrm{P}(\mathrm{t}) = (\mathrm{p_{ij}}(\mathrm{t}))_{\mathrm{i,j} \in \mathcal{S}}$ be the *transition probability matrix* with all probabilities that given the Markov chain is in state i at time 0, the Markov chain will be in state j at time t. When we look at an infinitesimally small time step $\Delta\mathrm{t}$, in which only one event can happen, we can calculate the transition probability at time $\mathrm{t} + \Delta\mathrm{t}$ with the same trick as used for the transformation from rate to probabilities:

$$\mathrm{P}(\mathrm{t} + \Delta\mathrm{t}) = \mathrm{P}(\mathrm{t}) + \mathrm{P}(\mathrm{t})\mathrm{Q}\Delta\mathrm{t}$$

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$$P(t + \Delta t) = P(t) + P(t)Q\Delta t \qquad \Leftrightarrow \qquad \frac{P(t+\Delta t) - P(t)}{\Delta t} = P(t)Q$$

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Now we let $\Delta t \to 0$, thus it follows:

$$\lim_{\Delta t \to 0} \frac{P(t+\Delta t) - P(t)}{\Delta t} = \frac{dP}{dt}(t) = P(t)Q$$

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This is a differential equation with the solution:

$$P(t) = e^{Qt}$$

!!The substitution rate matrix defines the transition probabilities!!

Meaning of $\mathbf{P(t) = e^{Qt}}$

As just derived:

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Meaning of $P(t) = e^{Qt}$

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$$P(t) = e^{Qt} = \sum_{i=0}^{\infty} \frac{(Qt)^i}{i!}$$

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$$P(t) = e^{Qt} = \sum_{i=0}^{\infty} \frac{(Qt)^i}{i!}$$

Every possible series of states is visited in time t!

☞ One can obtain the same result using the theorem of Chapman-Kolmogorov

Why Markov chains are a great model for nucleotide substitutions

- memorylessness: a nucleotide substitution happens independently from the substitution history at this site
- substitution rate matrix defines the transition probabilities
- the transition probabilities take into account every possible substitution path

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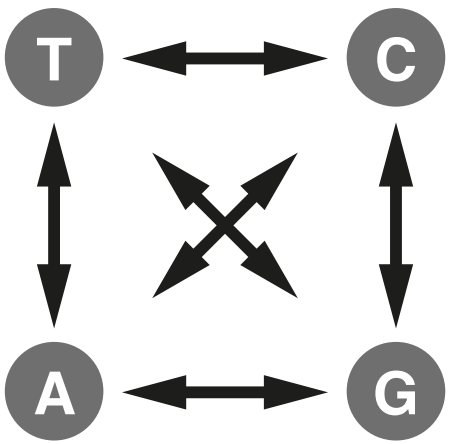
- applying theories of linear algebra we can calculate the transition probability matrix according to:
  $P(t) = e^{Qt} = U\,\mathrm{diag}(e^{\epsilon_1 t}, e^{\epsilon_2 t}, e^{\epsilon_3 t}, e^{\epsilon_4 t})U^{-1}$
  - cookbook recipe provided on moodle
  - for further information on how to derive this formula please consult a textbook on linear algebra

Substitution rate matrices and their properties.

The easiest substitution model: JC69

JC69:

- named after TH Jukes, CR Cantor: Evolution of protein molecules. 1969 [Jukes and Cantor, 1969].
- all substitution have the same rate, λ



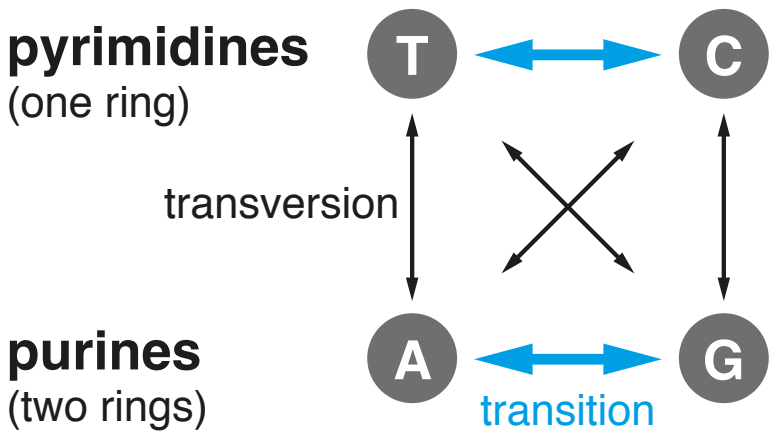
Substitution rates:

$$\begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \cdot & \lambda & \lambda & \lambda \\ \lambda & \cdot & \lambda & \lambda \\ \lambda & \lambda & \cdot & \lambda \\ \lambda & \lambda & \lambda & \cdot \end{pmatrix} \end{array}$$

Accounting for transition/transversion: K80

K80:

- named after M Kimura: A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. 1980. [Kimura, 1980]
- transitions happen at rate α, transversions at rate β



Substitution rates:

$$\begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \cdot & \alpha & \beta & \beta \\ \alpha & \cdot & \beta & \beta \\ \beta & \beta & \cdot & \alpha \\ \beta & \beta & \alpha & \cdot \end{pmatrix} \end{array}$$

Including equilibrium frequencies: TN93 and HKY

TN93:

- named after [Tamura and Nei, 1993]
- transitions between $\mathrm{T} \leftrightarrow \mathrm{C}$ happen at rate $\alpha_1 \times$(nucleotide equilibrium frequency)
- transitions from $\mathrm{A} \leftrightarrow \mathrm{G}$ happen at rate $\alpha_2 \times$(nucleotide equilibrium frequency)
- transversions happen at rate $\beta \times$(nucleotide equilibrium frequency)

Substitution rates:

$$\begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \cdot & \alpha_1\pi_\mathrm{C} & \beta\pi_\mathrm{A} & \beta\pi_\mathrm{G} \\ \alpha_1\pi_\mathrm{T} & \cdot & \beta\pi_\mathrm{A} & \beta\pi_\mathrm{G} \\ \beta\pi_\mathrm{T} & \beta\pi_\mathrm{C} & \cdot & \alpha_2\pi_\mathrm{G} \\ \beta\pi_\mathrm{T} & \beta\pi_\mathrm{C} & \alpha_2\pi_\mathrm{A} & \cdot \end{pmatrix} \end{array}$$

Including equilibrium frequencies: TN93 and HKY

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- transitions between T $\leftrightarrow$ C happen at rate $\alpha_1 \times$(nucleotide equilibrium frequency)
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- Note that the special case of $\alpha_1 = \alpha_2$ was described earlier [Hasegawa et al., 1984] and is named HKY

A more general substitution model: GTR

GTR (REV):

- generalised time-reversible model
- based on three papers:
  [Tavaré, 1986, Yang, 1994, Zharkikh, 1994]

Substitution rates:

$$\begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \cdot & a\pi_C & b\pi_A & c\pi_G \\ a\pi_T & \cdot & d\pi_A & e\pi_G \\ b\pi_T & d\pi_C & \cdot & f\pi_G \\ c\pi_T & e\pi_C & f\pi_A & \cdot \end{pmatrix} \end{array}$$

\+ quite flexible

\+ time-reversible

\- not completely general

Time reversibility

- mathematical condition, making calculations and simulations easier

$$\begin{pmatrix} \cdot & a\pi_C & b\pi_A & c\pi_G \\ a\pi_T & \cdot & d\pi_A & e\pi_G \\ b\pi_T & d\pi_C & \cdot & f\pi_G \\ c\pi_T & e\pi_C & f\pi_A & \cdot \end{pmatrix}$$

$$= \begin{pmatrix} \cdot & a & b & c \\ a & \cdot & d & e \\ b & d & \cdot & f \\ c & e & f & \cdot \end{pmatrix} \cdot \begin{pmatrix} \pi_T & 0 & 0 & 0 \\ 0 & \pi_C & 0 & 0 \\ 0 & 0 & \pi_A & 0 \\ 0 & 0 & 0 & \pi_G \end{pmatrix}$$

The most general substitution model

UNREST:

- unrestricted model first described in [Yang, 1994]
- each substitution has a (different) rate

Substitution rates:

$$\begin{array}{c} \\ \mathrm{T} \\ \mathrm{C} \\ \mathrm{A} \\ \mathrm{G} \end{array} \begin{array}{c} \begin{array}{cccc} \mathrm{T} & \mathrm{C} & \mathrm{A} & \mathrm{G} \end{array} \\ \begin{pmatrix} \cdot & a & b & c \\ d & \cdot & e & f \\ g & h & \cdot & i \\ j & k & l & \cdot \end{pmatrix} \end{array}$$

\+ most general case

\+ all other models are special cases of UNREST

\- mathematically very complicated and not handy to use

\- not time-reversible

- Further models described in [Yang, 1994]

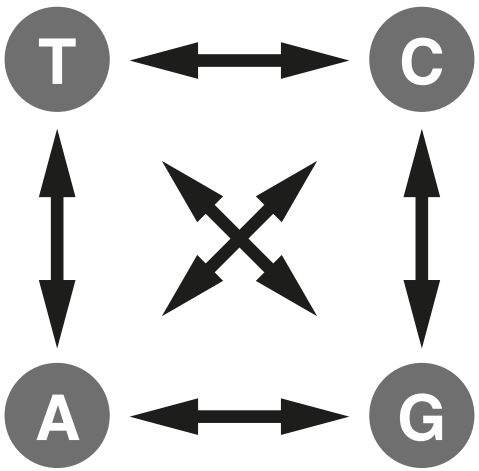
Substitution models

model	parameters	description
JC69	1	all substitutions have the same rate
K80	2	accounts for transition and transversions
HKY	2+3*	distinction between transition and transversions, including equilibrium frequencies
TN93	3+3*	different rates for transitions
GTR	6+3*	general, but still time-reversible
UNREST	12	most general, not time-reversible

* equilibrium frequencies of nucleotides

Calculating transition probabilities and sequence distance.

JC69



Substitution rates:

$$Q = \begin{pmatrix} -3\lambda & \lambda & \lambda & \lambda \\ \lambda & -3\lambda & \lambda & \lambda \\ \lambda & \lambda & -3\lambda & \lambda \\ \lambda & \lambda & \lambda & -3\lambda \end{pmatrix}$$

JC69: Approximation for small time, t

If t is very small in comparison to λ, we can use the first two terms of the Taylor expansion $P(t) = e^{Qt} = I + \sum_{i=1}^{\infty} \frac{(Qt)^i}{i!}$ to get

$\rightarrow p_{i,j}(t) \approx \lambda t$ and $p_{i,i}(t) \approx 1 - 3\lambda t$

Example: Assume a region of the human genome evolves according to JC69 at the rate $\lambda = 2.2/3 \times 10^{-9} \frac{\text{substitutions per site}}{\text{year}}$. The probability that starting with a T, after $t = 10^6$ years, we observe a C is

$p_{TC} = \lambda t = 7.3 \times 10^{-4}$

and that it still is a T is

$p_{TT} = 1 - 3\lambda t = 0.9978.$

What we learnt today

- case control setup in a GWAS
- sequence distance included non-visible substitution steps in between
- we model sequence evolution with a Markov chain framework
  - memoryless
  - toolbox: from rates to probabilities
  - sequence evolution model is defined by its substitution rate matrix
- rate assumptions in different substitution models:
  - JC69: all substitutions have the same rate
  - K80: transition and transversions have different rates
  - other (more general) models: [Yang, 2014]
- approximation of distance for JC69

GWAS, molecular evolution: Questions

- In a GWAS, why can you not reject your null hypothesis if the p-value is $< \alpha$?
- Why is the Markov Chain model a good model for sequence evolution?
- Why is it not advisable to reconstruct a phylogeny based on the Hamming distance?

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