Spring 2025

BIOS 477/877

Bioinformatics and Molecular Evolution

Lecture 5

1

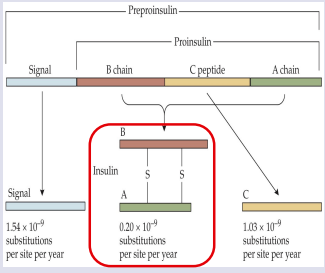
TODAY'S TOPICS

➢ Molecular Evolution - part 3

2

Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$d_N(AB) = u_T(AB) * f_0(AB)$

$d_N(C) = u_T(C) * f_0(C)$

Both mutation rates (u_T) and selective constraints (f_0) affect nonsynonymous rates (d_N)

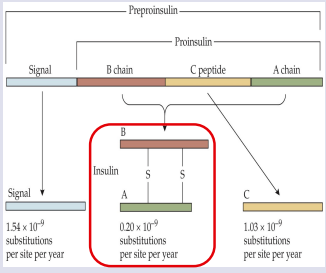
Note: In literatures, K_a and K_S are also used instead of d_N and d_S

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

3

Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$d_N(AB) = u_T(AB) * f_0(AB)$

$d_N(C) = u_T(C) * f_0(C)$

Does it mean:

$u_T(AB) < u_T(C)$?

$f_0(AB) < f_0(C)$?

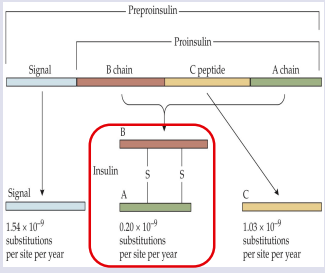
Both mutation rate (u_T) and selective constraints (f_0) can affect $d_N(AB) < d_N(C)$.

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

4

Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$u_T(AB)*f_0(AB) < u_T(C)*f_0(C)$

If mutation rates are constant within a gene,

➜ $u_T(AB) = u_T(C)$

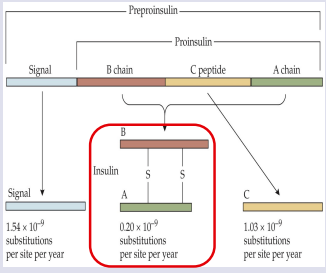
➜ $d_N(AB) < d_N(C)$ can be explained by $f_0(AB) < f_0(C)$

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

5

Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$u_T(AB)*f_0(AB) < u_T(C)*f_0(C)$

If mutation rates are different

➜ $u_T(AB) \neq u_T(C)$

➜ $d_N(AB) < d_N(C)$ cannot be simply explained by $f_0(AB) < f_0(C)$

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

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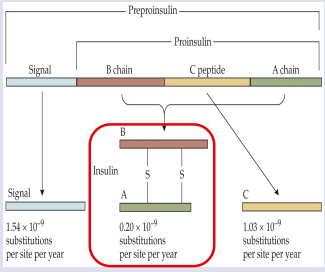
Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$d_N(AB) = u_T(AB) * f_0(AB)$

$d_N(C) = u_T(C) * f_0(C)$

Synonymous substitutions are assumed to be neutral or near-neutral.

$d_S = u_T f_0 = u_T$

where $f_0 \approx 1$ (neutral)

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

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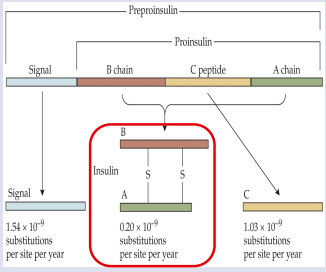
Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)



$d_N(AB) < d_N(C)$

$d_N(AB) = u_T(AB) * f_0(AB)$

$d_N(C) = u_T(C) * f_0(C)$

Synonymous substitutions are assumed to be neutral or near-neutral.

$d_S(AB) = u_T(AB)$

$d_S(C) = u_T(C)$

(Based on nonsynonymous substitution rates estimated from human-rat comparison)

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Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)

$d_N(AB)$ *vs.* $d_N(C)$

$d_N(AB) = u_T(AB) * f_0(AB)$ $\quad$ $d_N(C) = u_T(C) * f_0(C)$

$d_S(AB) = u_T(AB)$ $\quad$ $d_S(C) = u_T(C)$

$\frac{d_N(AB)}{d_S(AB)} = \frac{u_T(AB) * f_0(AB)}{u_T(AB)}$ $\quad$ $\frac{d_N(C)}{d_S(C)} = \frac{u_T(C) * f_0(C)}{u_T(C)}$

$\frac{d_N(AB)}{d_S(AB)} = f_0(AB)$ $\quad$ $\frac{d_N(C)}{d_S(C)} = f_0(C)$

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Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)

$\frac{d_N(AB)}{d_S(AB)} = f_0(AB)$ $\quad$ $\frac{d_N(C)}{d_S(C)} = f_0(C)$

d_N/d_S can be used to identify selection

- Shows only the level of selective constrains (f_0)
- We don't need to worry about mutation rates (u_T)
  - → Even if $u_T(AB) \neq u_T(C)$, d_N/d_S can be compared

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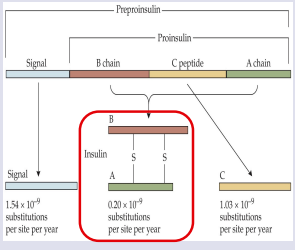
Identifying Selection

- Number of synonymous substitutions per site: d_S (or K_S)
- Number of nonsynonymous substitutions per site: d_N (or K_N)
  - → d_N/d_S can be used to identify selection



~~$d_N(AB) < d_N(C)$~~

$d_N(AB)/d_S(AB)$? $d_N(C)/d_S(C)$

This is the better comparison

Regardless of the mutation rates, we can compare:

$f_0(AB)$ vs. $f_0(C)$

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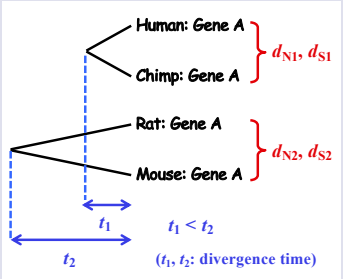
Identifying Selection



$d_{N1} < d_{N2}$, $d_{S1} < d_{S2}$

This may be caused simply by the difference in divergence time ($t_1 < t_2$)

$d_{N1} = u_{T1} * f_1 * 2t_1$, $d_{S1} = u_{T1} * 2t_1$

$d_{N2} = u_{T2} * f_2 * 2t_2$, $d_{S2} = u_{T2} * 2t_2$

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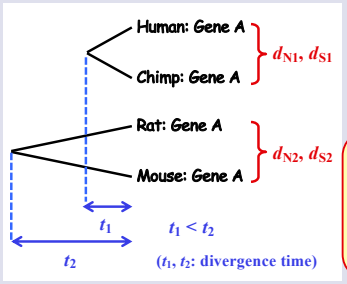
Identifying Selection



$d_{N1} < d_{N2}$, $d_{S1} < d_{S2}$

This may be caused simply by the difference in divergence time ($t_1 < t_2$)

d_{N1}/d_{S1} *vs.* d_{N2}/d_{S2}

By comparing d_N/d_S, the time effect can be cancelled out.

We can compare the selective constraints between genes and between lineages.

$d_{N1} = u_{T1} * f_1 * 2t_1$, $d_{S1} = u_{T1} * 2t_1$ ➜ $d_{N1}/d_{S1} = u_{T1} * f_{01} * 2t_1 / (u_{T1} * 2t_1) = f_{01}$

$d_{N2} = u_{T2} * f_2 * 2t_2$, $d_{S2} = u_{T2} * 2t_2$ ➜ $d_{N2}/d_{S2} = u_{T2} * f_{02} * 2t_2 / (u_{T2} * 2t_2) = f_{02}$

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Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N

➜ d_N/d_S can be used to identify selection

If nonsynonymous (replacement) substitutions are neutral:

$d_N > d_S$ or $d_N \approx d_S$ or $d_N < d_S$?

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Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N

➜ d_N/d_S can be used to identify selection

If nonsynonymous (replacement) substitutions are neutral:

$d_N > d_S$ or $d_N \approx d_S$ or $d_N < d_S$?

$d_N = u_T * f_0$

$d_S = u_T$

If nonsynonymous substitutions are neutral,

➜ $f_0 = 1$,

➜ $d_N = u_T * f_0 = u_T = d_S$

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Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N

➜ d_N/d_S can be used to identify selection

When nonsynonymous (replacement) substitutions are neutral,

$d_N \approx d_S$ ➜ $d_N/d_S \approx 1$

➜ d_S is used as a control
(substitution rate at neutral or near-neutral)

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Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N

➜ d_N/d_S can be used to identify selection

d_N/d_S

$d_N/d_S > 1.0$

$d_N/d_S \approx 1.0$ Neutral (*e.g.*, non-coding regions, pseudogenes)

$d_N/d_S < 1.0$

0

➜ d_S is used as a control
(substitution rate at neutral or near-neutral)

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Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N

➜ d_N/d_S can be used to identify selection

d_N/d_S

$d_N/d_S > 1.0$

1.0

$d_N/d_S < 1.0$

0

Which indicates negative selection?

➜ d_S is used as a control
(substitution rate at neutral or near-neutral)

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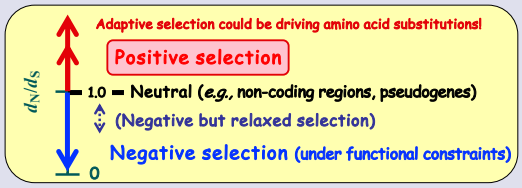
Identifying Selection

- Number of synonymous substitutions per site: d_S
- Number of nonsynonymous substitutions per site: d_N
  - ➜ d_N/d_S can be used to identify selection



- ➜ d_S is used as a control
  (substitution rate at neutral or near-neutral)

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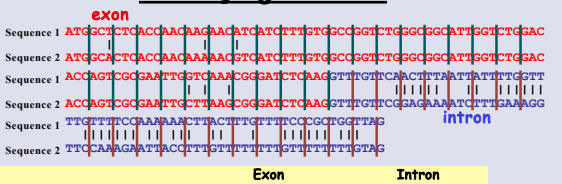
Identifying Selection



	Exon			Intron		
[Codon position]	1st	2nd	3rd	1st	2nd	3rd
# nucleotide sites	31	31	31	23	22	22
# nucleotide substitutions	2	0	4	12	12	14
Nucleotide substitutions/site	0.06	0	0.13	0.52	0.55	0.64

Exon(2nd) < Exon(3rd) | Intron(2nd) ≲ or ≈ Intron(3rd)

Exon(2nd) / Exon(3rd) 0 << Intron(2nd) / Intron(3rd) 0.86 (≈1)

(Similar to d_N/d_S analysis)

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Identifying Selection

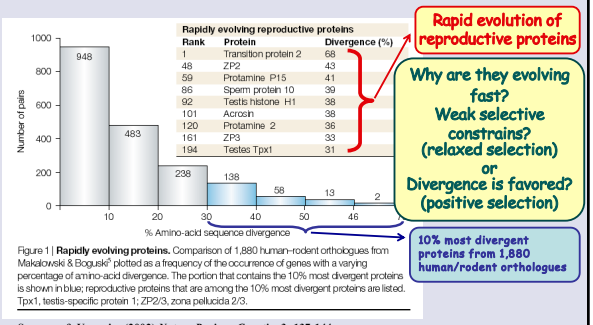

Figure 1 | **Rapidly evolving proteins.** Comparison of 1,880 human-rodent orthologues from Makalowski & Boguski[5] plotted as a frequency of the occurrence of genes with a varying percentage of amino-acid divergence. The portion that contains the 10% most divergent proteins is shown in blue; reproductive proteins that are among the 10% most divergent proteins are listed. Tpx1, testis-specific protein 1; ZP2/3, zona pellucida 2/3.

Rapid evolution of reproductive proteins

Why are they evolving fast?
Weak selective constrains? (relaxed selection)
or
Divergence is favored? (positive selection)

10% most divergent proteins from 1,880 human/rodent orthologues

Swanson & Vacquier (2002) *Nature Reviews Genetics* **3: 137-144**

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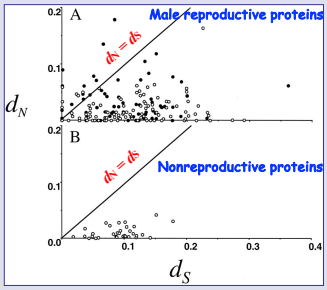
Selection and functions

- Elevated d_N/d_S ratios are often found in reproductive genes



(*e.g.*, genes involved in mating behavior, fertilization, spermatogenesis, ejaculation, or sex determination)

Swanson *et al.* (2001) Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *PNAS* 98: 7375-7379.

See also

Findlay *et al.* (2010) Proteomics enhances evolutionary and functional analysis of reproductive proteins. *BioEssays* 32: 26-36.

Wilburn and Swanson (2016) From molecules to mating: Rapid evolution and biochemical studies of reproductive proteins. *J. Proteomics* 135: 12-25.

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Selection and functions

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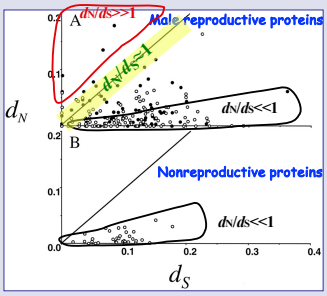

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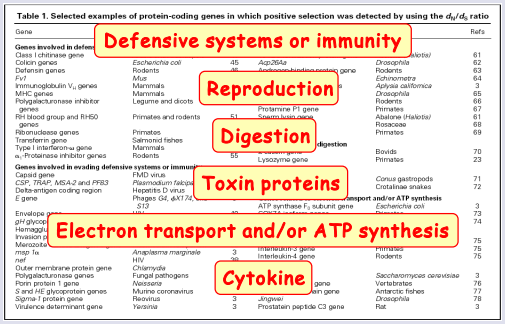
Selection and functions



Yang & Bielawski (2000) Statistical methods for detecting molecular adaptation. *TREE* 15: 496-503.

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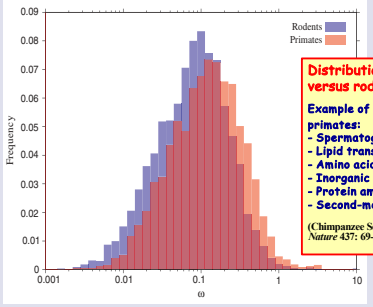
Identifying Selection



Distribution of ω (d_N/d_S) in primates versus rodents.

Example of genes whose ω is larger in primates:
- Spermatogenesis
- Lipid transport
- Amino acid transport
- Inorganic anion transport
- Protein amino acid glycosylation
- Second-messenger-mediated signaling

(Chimpanzee Sequencing and Analysis Consortium, 2005 *Nature* 437: 69-87)

Gibbs *et al.* (2007) Evolutionary and biomedical insights from the rhesus macaque genome. *Science* 316: 222-34.

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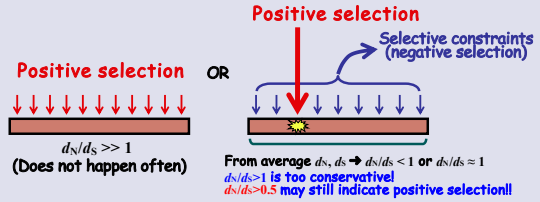
Identifying Selection

- Positive selection usually works within a limited region (*e.g.*, a few particular amino acid sites: binding sites)



$d_N/d_S >> 1$ (Does not happen often)

From average d_S, $d_S \rightarrow d_N/d_S < 1$ or $d_N/d_S \approx 1$

$d_N/d_S>1$ is too conservative!

$d_N/d_S>0.5$ may still indicate positive selection!!

- If we can identify sites under positive selection, we can predict amino acid positions that are important in adaptive evolution

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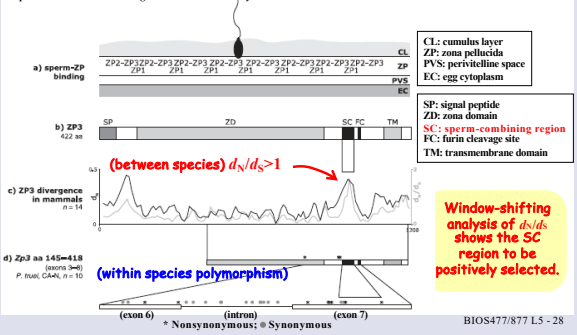
Identifying Selection

Turner and Hoekstra (2008) Reproductive protein evolution within and between species: maintenance of divergent ZP3 alleles in *Peromyscus*. *Mol. Ecol.* 17: 2616-2628.



CL: cumulus layer
ZP: zona pellucida
PVS: perivitelline space
EC: egg cytoplasm

SP: signal peptide
ZD: zona domain
SC: sperm-combining region
FC: furin cleavage site
TM: transmembrane domain

Window-shifting analysis of d_N/d_S shows the SC region to be positively selected.

* Nonsynonymous; ⊕ Synonymous

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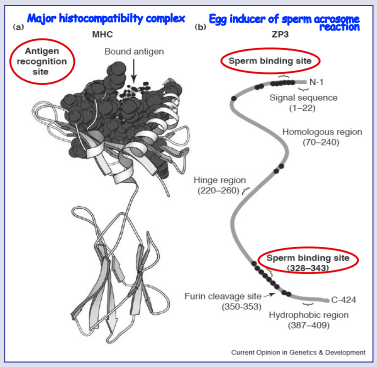
Identifying Selection



Swanson (2003) Adaptive evolution of genes and gene families. *Curr Opin Genet Dev* 13:617–622.

[Originally from: Swanson *et al.* (2001) Darwinian selection drives the evolution of several female reproductive proteins in mammals. *PNAS* 98: 2509-2514.]

● sites:
Predicted to be subjected to positive selection

by using
a maximum likelihood model that allows d_N/d_S to vary among amino acid sites

PAML:
http://abacus.gene.ucl.ac.uk/software/paml.html
(reviewed in Yang, 2007)

See also HyPhy and Datamonkey:
http://hyphy.org
http://www.datamonkey.org
(characterize various selective pressures)

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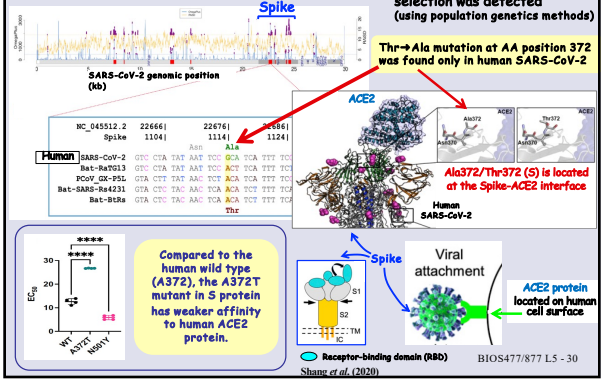
A selective sweep in the *Spike* gene has driven SARS-CoV-2 human adaptation

Kang *et al.*, (2021) *Cell* 184, 4392–4400

Lin Kang,[1,2] Guijuan He,[1] Amanda K. Sharp,[1] Xiaofeng Wang,[1] Anne M. Brown,[4,5,6] Pawel Michalak,[1,7,8,*] and James Weger-Lucarelli[1,9,*]



: Regions where positive selection was detected (using population genetics methods)

Thr→Ala mutation at AA position 372 was found only in human SARS-CoV-2

Shang *et al.* (2020)

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Identifying Selection - Data Quality

The quality of the sequence, the degree of misannotation, and ambiguities in the multiple sequence alignment, all affect identification of positive selection!

Table 1
Inferred Percentage of PSGs as a Function of Sequencing Coverage (PSG: positively selected genes)

Many are based on errors

	Higher quality→ Coverage ≥3×			Coverage <3× ←Lower quality			
	Total	PSG	% PSG	Total	PSG	% PSG	$P(\chi^2)$
Human	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Chimp	1,144	9	0.8	1,836	74	4.0	9.6×10^{-8}
Macaque	896	32	3.6	2,084	488	23.4	8.1×10^{-46}
Mouse	2,493	77	3.1	487	37	7.6	1.3×10^{-6}
Rat	1,841	93	5.1	1,139	217	19.1	3.2×10^{-38}
Dog	1,568	212	13.5	1,412	481	34.1	1.7×10^{-49}
Cow	1,086	54	5.0	1,894	249	13.1	4.8×10^{-14}
Total	9,028	477	5.3	8,852	1,546	17.5	2.3×10^{-166}

Schneider *et al.* (2009) Estimates of positive Darwinian selection are inflated by errors in sequencing, annotation, and alignment. *Genome Biol Evol* 1: 114-8.

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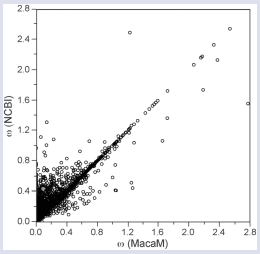
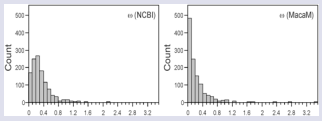
Identifying Selection - Data Quality



Gradnigo *et al.* (2016) Advantages of an improved rhesus macaque genome for evolutionary analyses. *PLOS ONE* 11: e0167376.

Low quality annotation of the rhesus macaque genome inflated estimated ω (*dN*/*dS*).



- Human vs. rhesus genes
- NCBI annotation: draft quality rhesus genome
- MacaM annotation: new high quality rhesus genome (same assembly but different gene annotation)

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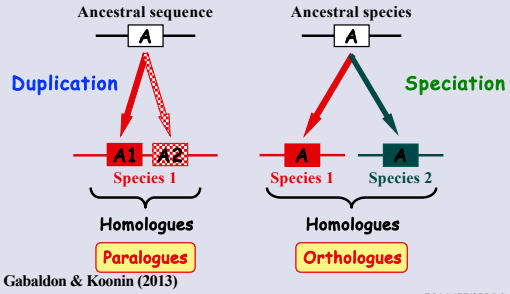
Homologue, Orthologue, Paralogue

➢ Homologues: sequences that share a common ancestor



Read Gabaldon & Koonin (2013)
Mini-reviews: Koonin (2001)/Jensen (2001)/Theißen (2002)

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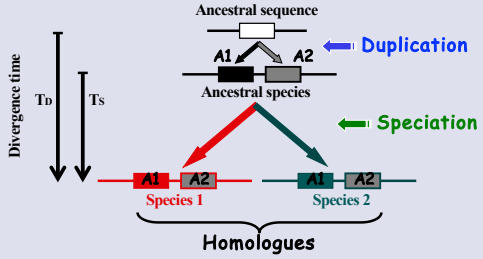
Homologue, Orthologue, Paralogue

➢ Homologues: sequences that share a common ancestor



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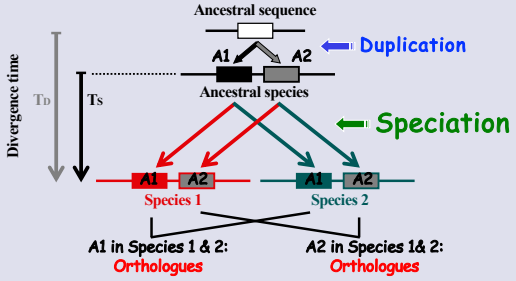
Homologue, Orthologue, Paralogue

➢ Orthologues: derived from a speciation event



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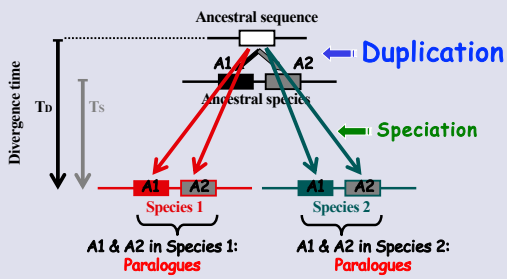
Homologue, Orthologue, Paralogue

➢ Paralogues: derived from a duplication event



A1:Species 1 & A2:Species 2, A2:Speices 1 & A1:Species 2 are also paralogues (paralogues can be in different genomes)

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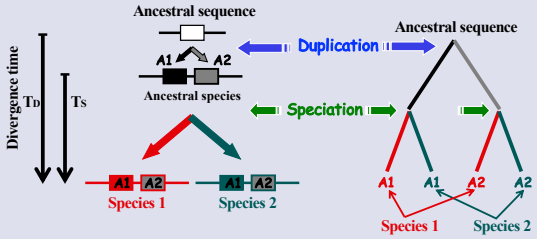
Homologue, Orthologue, Paralogue

➢ Phylogenetic relationship of gene families



A diagram to clarify duplication vs. speciation (a visual help, informal)

Duplication: joined by a horizontal line
Speciation: joined by an inverted Y
See Jensen (2001)

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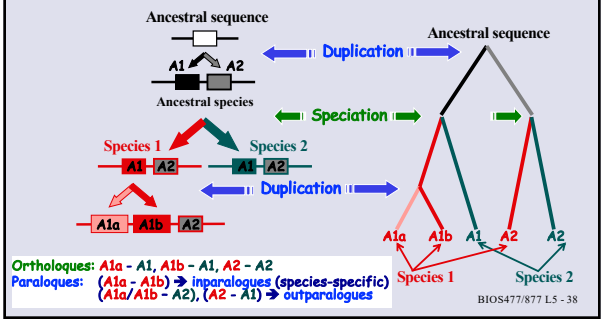
Homologue, Orthologue, Paralogue

➢ Duplications can happen anytime



Orthologues: A1a - A1, A1b - A1, A2 - A2
Paraloques: (A1a - A1b) ➔ inparalogues (species-specific)
(A1a/A1b - A2), (A2 - A1) ➔ outparalogues

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Gene Duplication

➢ Gene duplication creates large gene families

➢ Important means of providing a substrate on which evolution can work

- 15% or more of human genes are duplicates!

1) Duplicated copies: Redundancy
   - ➔ Weaker functional constraint (neutral or near neutral)
   - ➔ One copy can be degenerated to become a pseudogene
   - ➔ Non-functionalization … likely to be lost
2) One copy can acquire a novel gene function
   - ➔ Neo-functionalization, rare but important
3) Sub-functionalization?
   - ➔ Functional complementation between duplicated genes

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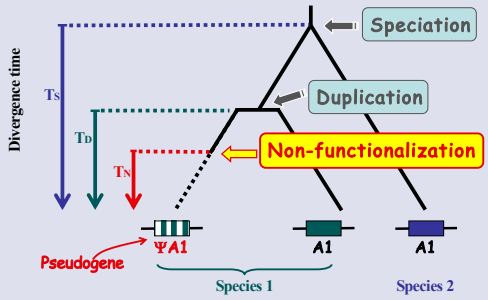
Pseudogene



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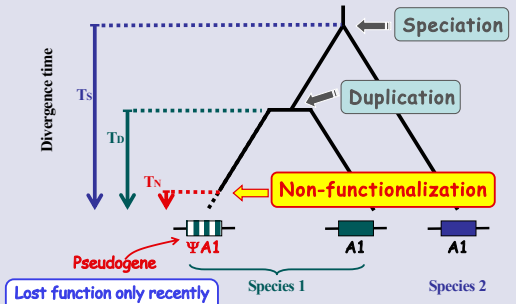
Pseudogene



Lost function only recently ($d_N/d_S \ll 1$)

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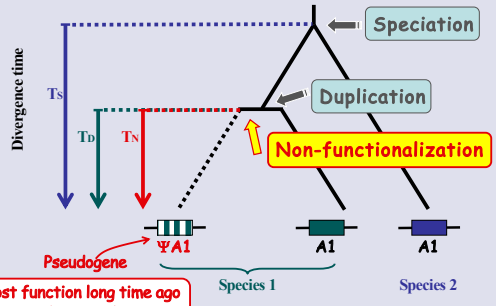
Pseudogene



Lost function long time ago ($d_N/d_S \approx 1$)

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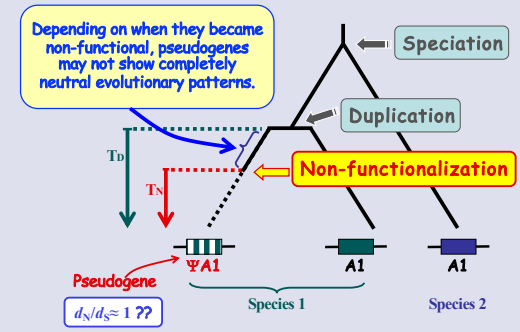
Pseudogene

Depending on when they became non-functional, pseudogenes may not show completely neutral evolutionary patterns.



$d_N/d_S \approx 1$??

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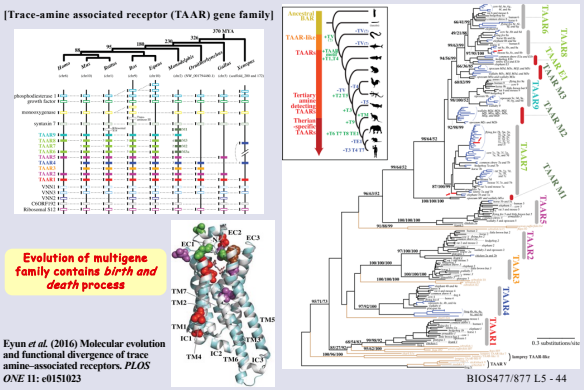
Gene duplication creates gene family: multigene family

[Trace-amine associated receptor (TAAR) gene family]



Evolution of multigene family contains *birth and death* process

Eyun *et al.* (2016) Molecular evolution and functional divergence of trace amine–associated receptors. *PLOS ONE* 11: e0151023

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Homology vs. Similarity

- **Similarity**: the extent to which sequences are related.
  → makes no statement about descent from a common ancestor
- **Homology**: sequence similarity that can be attributed to descent from a common ancestor

Homology ≠ Similarity !!

- Sequences can be either homologous or non-homologous, but not in between (*e.g.*, you cannot say two genes are 10% homologous!)
- Homology is not directly measurable or observable.
- Similarity is a direct measurement.

Higgs and Attwood (2005) Chapter 1 page 8
Read also Petsko (2001), Koonin (2001), Jensen (2001)

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Next time ...

- Pairwise sequence comparison by dotplot
  - Dotlet JS https://dotlet.vital-it.ch
  - Dotlet (old Java version) https://myhits.isb-sib.ch/cgi-bin/dotlet
    [For security reasons, old Java programs are not available within UNL network]
  - DotMatcher (A program in EMBOSS)
    http://emboss.bioinformatics.nl/cgi-bin/emboss/dotmatcher
    (see course Web for other EMBOSS links)
  - JDotter: Java Dot Plot Alignments
    http://pgrc.ipk-gatersleben.de/jdotter/
  - YASS: Visualization of local pairwise alignments
    https://bioinfo.univ-lille.fr/yass/index.php

See the Course website for more programs

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