ICQB

Introduction to Computational & Quantitative Biology (G4120)
Fall 2022
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Department of Microbiology & Immunology

Lecture 6: Quantitative Analysis and Presentation of Visual Data
October 18, 2022

Visual Display of Quantitative Data

Effective Visual Display of Data

Reveals data, does not conceal or distort it.

Clearly communicates complex, multivariate ideas.

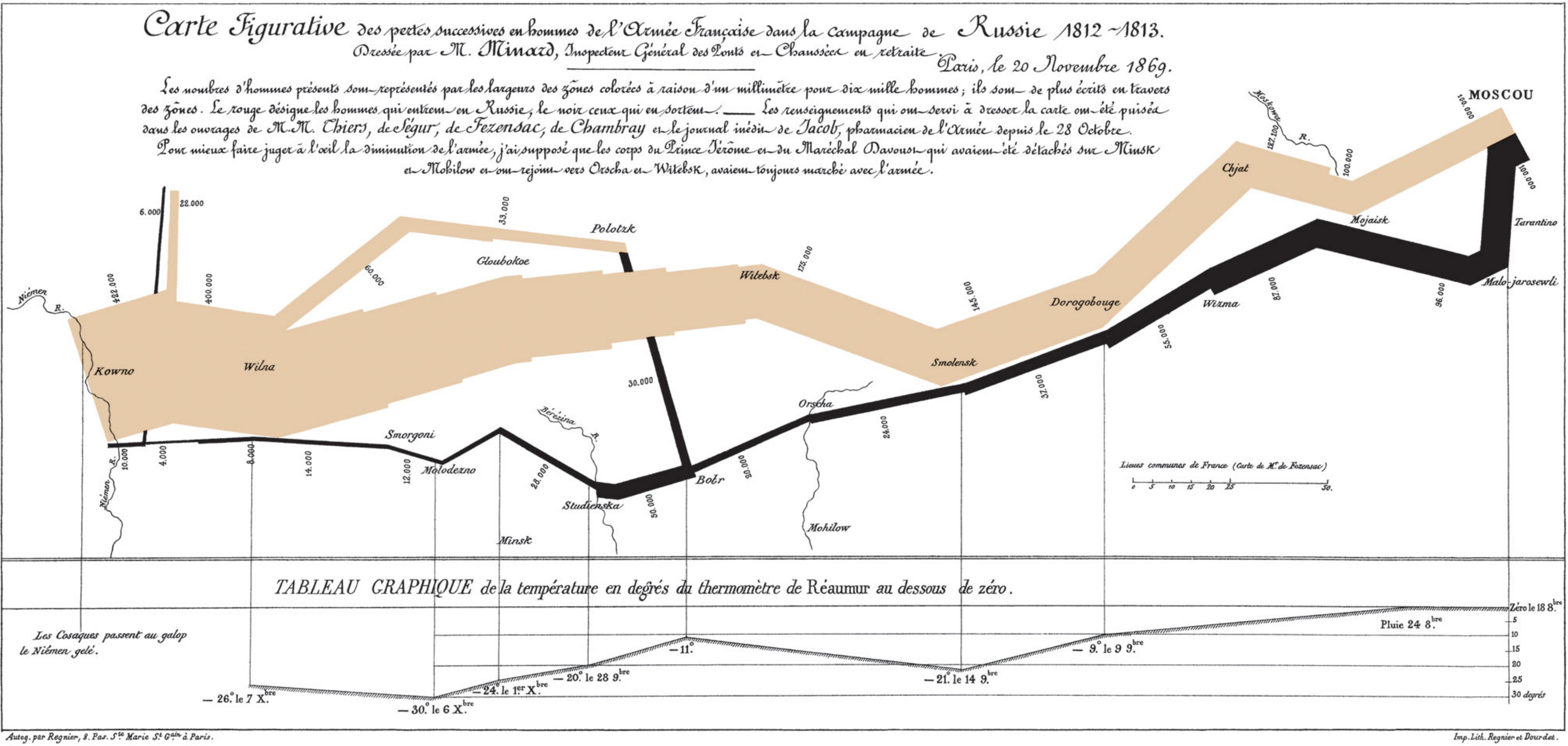
Encourages exploring the data at multiple levels.

Efficiently presents many numbers in a small space.

Has purpose, not “chartjunk”, and should focus the viewer on the substance of the data, not distract them.

Source: The Visual Display of Quantitative Information by Edward R. Tufte

Minard’s Chart of Napoleon’s Russian Campaign



Carte Figurative des pertes successives en hommes de l'Armée Française dans la campagne de Russie 1812–1813.
Dressée par M. Minard, Inspecteur Général des Ponts et Chaussées en retraite. Paris, le 20 Novembre 1869.

Les nombres d'hommes présents sont représentés par les largeurs des zônes colorées à raison d'un millimètre pour dix mille hommes; ils sont de plus écrits en travers des zônes. Le rouge désigne les hommes qui entrent en Russie, le noir ceux qui en sortent. —— Les renseignements qui ont servi à dresser la carte ont été puisés dans les ouvrages de M.M. Thiers, de Ségur, de Fezensac, de Chambray et le journal inédit de Jacob, pharmacien de l'Armée depuis le 28 Octobre.

Pour mieux faire juger à l'œil la diminution de l'armée, j'ai supposé que les corps du Prince Jérôme et du Maréchal Davoust qui avaient été détachés sur Minsk et Mobilow et ont rejoint vers Orscha et Witebsk, avaient toujours marché avec l'armée.

TABLEAU GRAPHIQUE de la température en degrés du thermomètre de Réaumur au dessous de zéro.

Les Cosaques passent au galop le Niémen gelé.

Source: Charles Joseph Minard, 1861

Typography

Typography is the art of communicating with letter forms. Decisions have to be made about the typeface to be used, the size (e.g. 12 point size), weight (e.g. light, semi-bold, bold, extra-bold) and style (e.g. italic). In modern use, a font is a typeface. Fonts are typically classified by form (Serif or Sans Serif), era (Old Style, Transitional, Modern, etc.) and spacing (Fixed Width or Variable Width).

Serif (Roman) fonts have decorative lines at the end of a stroke:
Caslon, Garamond, Goudy, Sabon (Old Style)
Baskerville, Georgia, Times New Roman (Transitional)
Bodoni (Modern)
Trajan (Incised)

Sans Serif (Gothic or Grotesque) fonts lack serifs:
Gill Sans, Myriad, Optima (Humanist)
Arial, Franklin Gothic, Helvetica (Grotesque)
Futura, Proxima Nova (Geometric)

Garamond

Gill Sans

Fixed Width vs. Variable Width

Each character in a fixed width (monospaced) font takes up the same amount of horizontal space, like a typewriter, allowing multiple sequence alignments to properly align. Variable width fonts can throw off multiple sequence alignments.

	Commonly Used Fixed Width (Monospaced) Fonts
Mac OS X	Courier, Courier New, Monaco, Letter Gothic
Windows	Courier New, Lucida Sans Typewriter

Fixed Width Font Alignment (Courier)

```
.   .   .   m   s   h   N   q   f   q   f   i   G   n   L   t   r   D
M   A   s   R   G   v   N   K   V   I   L   V   G   n   L   G   q   D
M   A   v   R   G   I   N   K   V   I   L   V   G   R   L   G   k   D
```

Variable Width Font Alignment (Times)

. . . m s h N q f q f i G n L t r D
M A s R G v N K V I L V G n L G q D
M A v R G I N K V I L V G R L G k D

Elements of Design

Alignment
Nothing should be placed arbitrarily. Every element should have some visual connection with another element. Guide lines or an underlying design grid can assist with this.

Proximity
Related items should be grouped in close proximity.

Hierarchy
Create a clear hierarchy of elements (e.g. header, sub-header, body text, legends).

Contrast
Avoid displays where everything blends together or lacks contrast. Make important items **very different.** Bold weights or italic style can help, or a using a san serif font for headers and a serif font for body text (or vice-versa).

Consistency
Repeat visual elements of the design throughout, and use them consistently. Use fonts and color carefully and consistently, and avoid overuse or arbitrary use of either.

Source: The Non-Designer’s Design Book by Robin Williams

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Comprehension

ALL CAPS vs. Mixed or lower case

Text set in ALL CAPS has a significantly lower level of comprehension than text set in mixed or lower case, even in relatively short text, such as headlines.

Line Length

Text set in lines that are too short or too long can increase reading time and decrease comprehension. Lines of 40 to 75 characters are considered ideal.

Open Counters vs. Closed Counters

Fonts with more open counters (the space enclosed by the letter form) are considered more comprehensible.

Text and Background Color

Black text or dark colored text blocks on white backgrounds or lightly tinted (10% to 20%) color backgrounds have the highest level of comprehension. Avoid using lighter color text blocks, or backgrounds with more color when possible, as these can have poor comprehension.

ALL CAPITALS

ALL CAPS VS. MIXED OR LOWER CASE
TEXT SET IN ALL CAPS HAS A SIGNIFICANTLY LOWER LEVEL OF COMPREHENSION THAN TEXT SET IN MIXED OR LOWER CASE, EVEN IN RELATIVELY SHORT TEXT, SUCH AS HEADLINES.

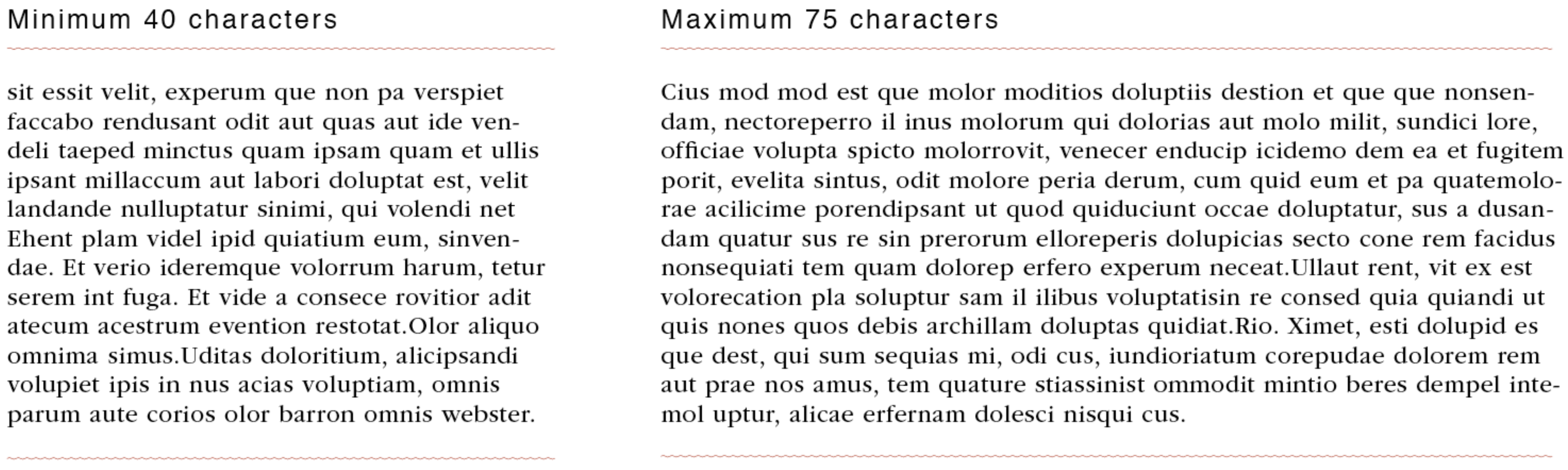
LINE LENGTH
TEXT SET IN LINES THAT ARE TOO SHORT OR TOO LONG CAN INCREASE READING TIME AND DECREASE COMPREHENSION. LINES OF 40 TO 75 CHARACTERS ARE CONSIDERED IDEAL.

OPEN COUNTERS VS. CLOSED COUNTERS
FONTS WITH MORE OPEN COUNTERS (THE SPACE ENCLOSED BY THE LETTER FORM) ARE CONSIDERED MORE COMPREHENSIBLE.

TEXT AND BACKGROUND COLOR
BLACK TEXT OR DARK COLORED TEXT BLOCKS ON WHITE BACKGROUNDS OR LIGHTLY TINTED (10% TO 20%) COLOR BACKGROUNDS HAVE THE HIGHEST LEVEL OF COMPREHENSION. AVOID USING LIGHTER COLOR TEXT BLOCKS, OR BACKGROUNDS WITH MORE COLOR WHEN POSSIBLE, AS THESE CAN HAVE POOR COMPREHENSION.

Line Length

Anything from **40 to 75 characters** in a line of text is widely regarded as an optimal length of line for a single-column page of printed text. That translates to roughly **7 to 13 words** per line. For multiple columns on a page, lines should be **40 to 50 characters.**



Minimum 40 characters

sit essit velit, experum que non pa verspiet faccabo rendusant odit aut quas aut ide vendeli taeped minctus quam ipsam quam et ullis ipsant millaccum aut labori doluptat est, velit landande nulluptatur sinimi, qui volendi net Ehent plam videl ipid quiatium eum, sinvendae. Et verio ideremque volorrum harum, tetur serem int fuga. Et vide a consece rovitior adit atecum acestrum evention restotat.Olor aliquo omnima simus.Uditas doloritium, alicipsandi volupiet ipis in nus acias voluptiam, omnis parum aute corios olor barron omnis webster.

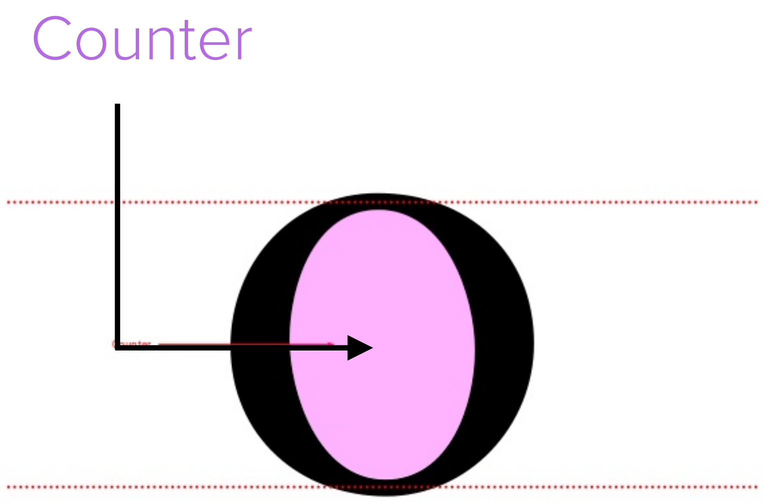
Maximum 75 characters

Cius mod mod est que molor moditios doluptiis destion et que que nonsendam, nectoreperro il inus molorum qui dolorias aut molo milit, sundici lore, officiae volupta spicto molorrovit, venecer enducip icidemo dem ea et fugitem porit, evelita sintus, odit molore peria derum, cum quid eum et pa quatemolorae acilicime porendipsant ut quod quiduciunt occae doluptatur, sus a dusandam quatur sus re sin prerorum elloreperis dolupicias secto cone rem facidus nonsequiati tem quam dolorep erfero experum neceat.Ullaut rent, vit ex est volorecation pla soluptur sam il ilibus voluptatisin re consed quia quiandi ut quis nones quos debis archillam doluptas quidiat.Rio. Ximet, esti dolupid es que dest, qui sum sequias mi, odi cus, iundioriatum corepudae dolorem rem aut prae nos amus, tem quature stiassinist ommodit mintio beres dempel intemol uptur, alicae erfernam dolesci nisqui cus.

Source: The Elements of Typographic Style by Robert Bringhurst, image by J. Rinker Design

Open vs. Closed Counters

Typeface choice: Open Counters
Counters, the white space within letters such as ‘o,’ ‘e,’ ‘c,’ etc., help to define a character. Typographers believe that large, open counters aid legibility.



Open

Lorem ipsum dolor sit amet, consectetuer adipiscing elit

✓ Legible

Closed

Lorem ipsum dolor sit amet, consectetuer adipiscing elit

✗ Not-so-legible

Source: It’s About Legibility by Allan Haley

A Study of Text and Background Color

Black Text on White Background
Black text on a white background had the highest level of comprehension (70% good).

Black or Dark Text on Lightly Tinted Backgrounds
Black or dark colored text on 10% to 20% tinted backgrounds had acceptable comprehension (32% to 68% good).

Lighter Color Text
Lighter colored text on white or 10% to 20% tinted backgrounds had poor comprehension (0% to 29% good).

Colored or Black Backgrounds
Any text on a 40% or more tinted background had poor comprehension (0% to 22% good). White text on a black background had the worst comprehension (0% good).

Preference Does Not Mean Comprehension
Despite the potential for lower comprehension, 81% of the subjects stated they found a colored background more attractive and interesting than black on white text.

If you must set type on a dark background, lighter translucent backgrounds, heavier font weights (bold or extra-bold) and simpler, more geometric fonts may help.

Source: Type & Layout by Colin Wheildon

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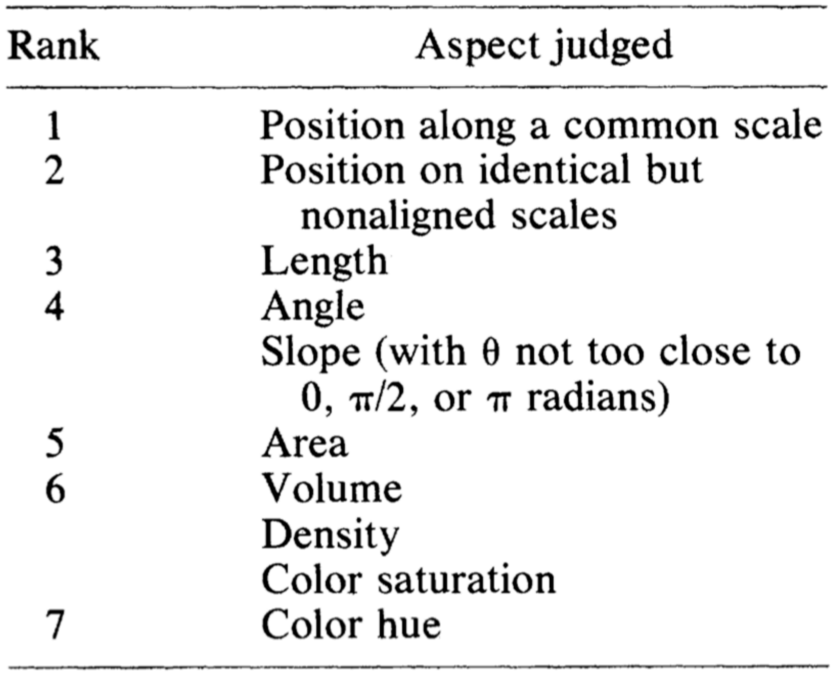
Source: Type & Layout by Colin Wheildon

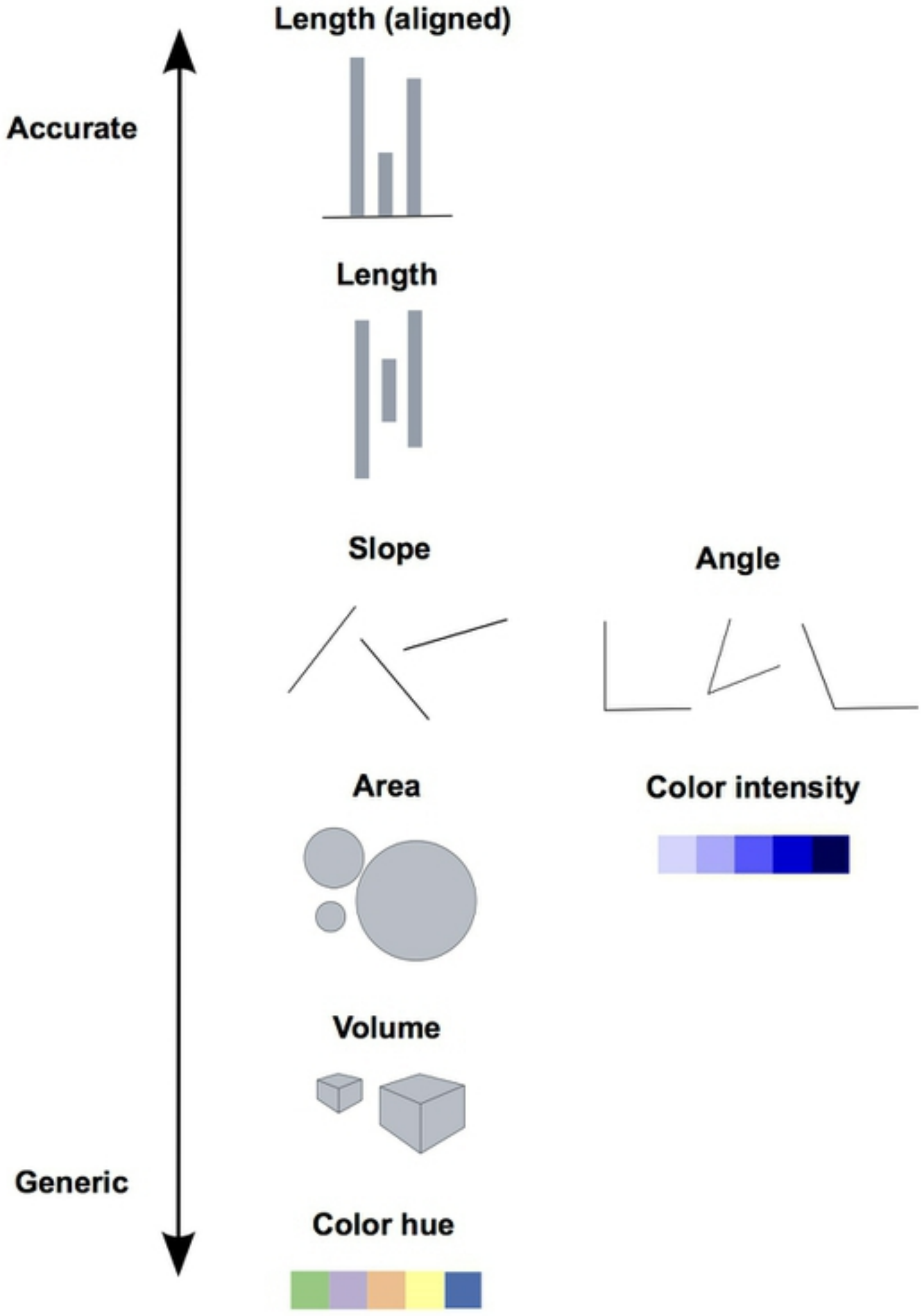
Visual Encoding of Information

There are multiple ways of using visual cues to encode data. Slope in line charts, area in bar and pie charts, color in heat maps, etc. Certain visual cues are easier to accurately interpret than others.



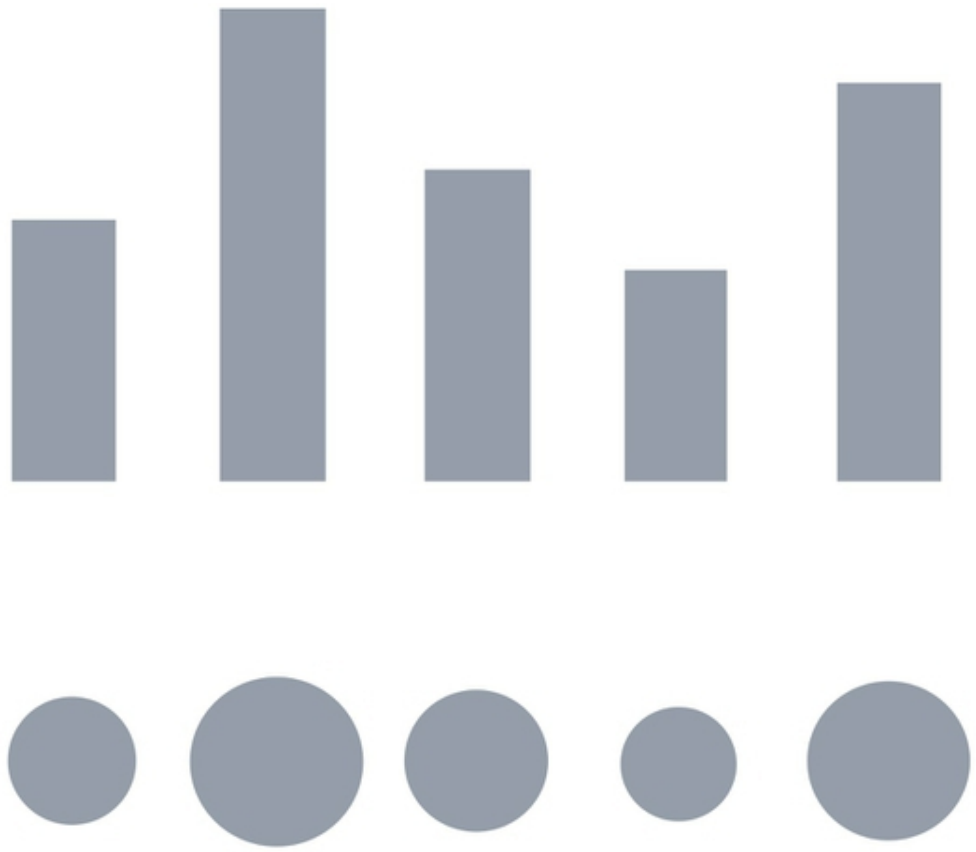
Rank	Aspect judged
1	Position along a common scale
2	Position on identical but nonaligned scales
3	Length
4	Angle
	Slope (with θ not too close to 0, π/2, or π radians)
5	Area
6	Volume
	Density
	Color saturation
7	Color hue

Source: Cleveland, W.S. and McGill, M. (1985) Science 229: 828-833 and Data Visualization by Peter Aldhous



Encoding Information in Form

Human eyes are better at accurately interpreting some forms of encoded information than others. Length aligned along a common scale is easier to accurately interpret than area or volume.



Above, the same data are presented in two ways: **aligned length** (top) and **area** (bottom). Which visual encoding is easier to compare?

Source: Data Visualization by Peter Aldhous

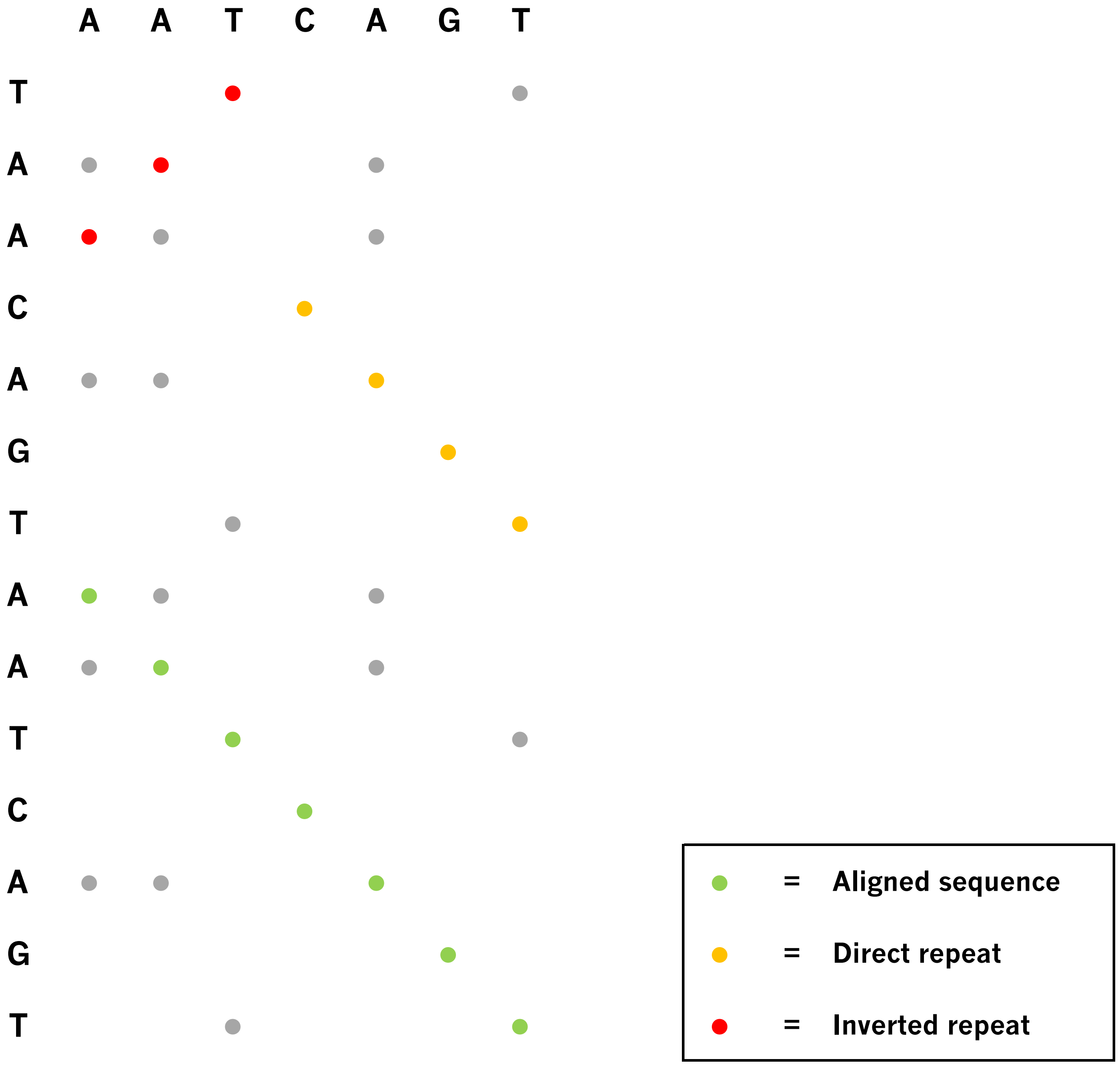
Dot Matrix Visualization of DNA

A dot matrix shows all possible matches between two sequences with a dot placed at every match. For longer sequences, a window with a specified stringency for placing a dot can be used instead.

	A	A	T	C	A	G	T
T			●				●
A	●	●			●		
A	●	●			●		
C				●			
A	●	●			●		
G						●	
T			●				●
A	●	●			●		
A	●	●			●		
T			●				●
C				●			
A	●	●			●		
G						●	
T			●				●

Dot Matrix Analysis of DNA

In dot matrix alignments, regions of sequence identity show up as diagonals. Closely related sequences will display a long diagonal corresponding to the aligned sequences. Shorter direct repeats will show up as shorter parallel diagonals. Shorter inverted repeats will show up as shorter perpendicular diagonals.



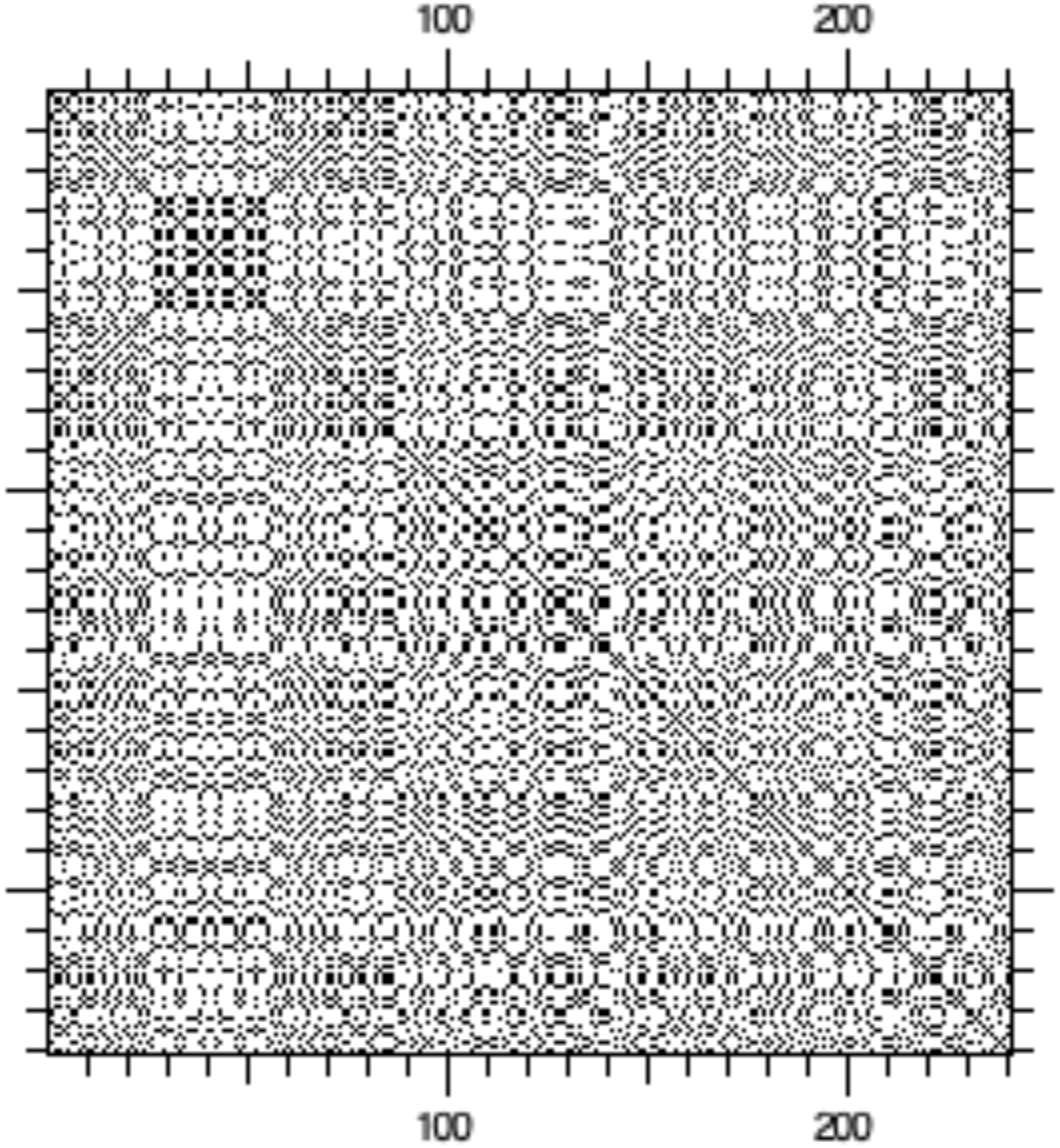
	A	A	T	C	A	G	T
T			🔴				●
A	●	🔴			●		
A	🔴	●			●		
C				🟡			
A	●	●			🟡		
G						🟡	
T			●				🟡
A	🟢	●			●		
A	●	🟢			●		
T			🟢				●
C				🟢			
A	●	●			🟢		
G						🟢	
T			●				🟢

🟢 = **Aligned sequence**

🟡 = **Direct repeat**

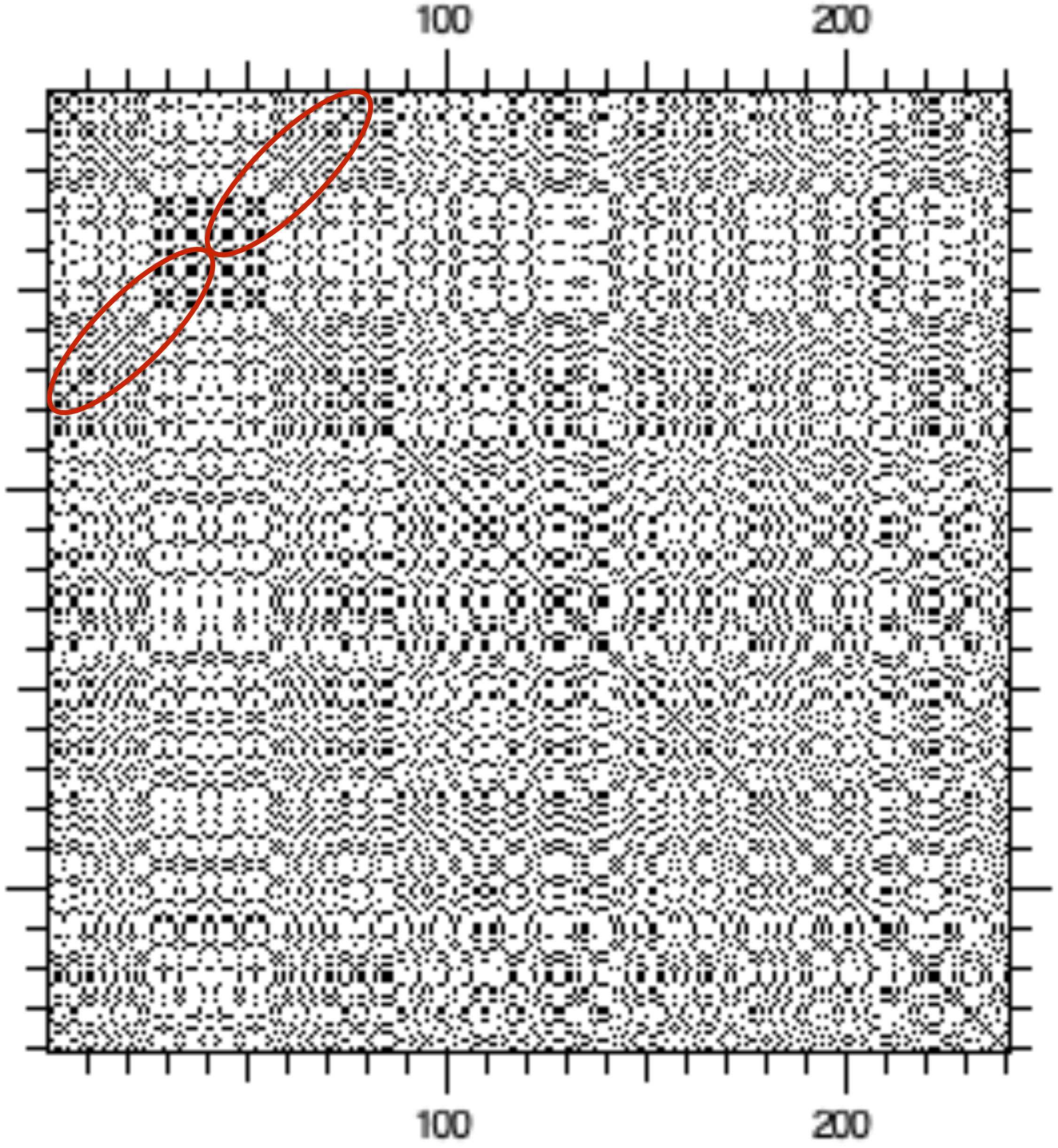
🔴 = **Inverted repeat**

Dot Matrix Self Analysis of DNA



A DNA Strider DNA Self Matrix (Window Size 1, Stringency 1) of 200 nucleotides of pBR322 sequence with a 40 nucleotide inverted repeat added to the beginning of the sequence.

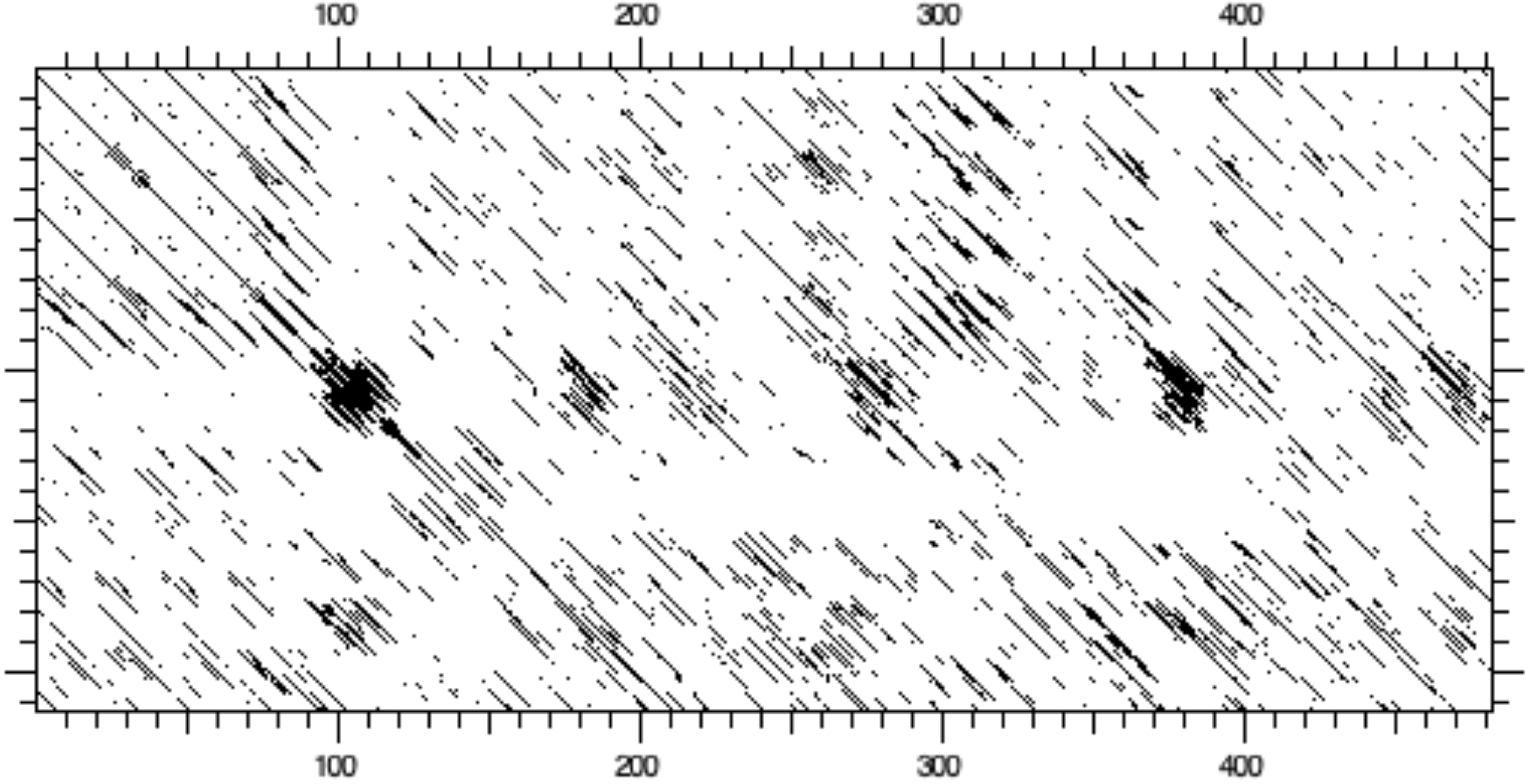
Dot Matrix Self Analysis of DNA



The 40 nucleotide inverted repeat appears as short diagonals perpendicular to the diagonal of the sequence alignment.

A DNA Strider DNA Self Matrix (Window Size 1, Stringency 1) of 200 nucleotides of pBR322 sequence with a 40 nucleotide inverted repeat added to the beginning of the sequence.

Dot Matrix Pairwise Analysis of DNA



A DNA Strider DNA Matrix (Window Size 15, Stringency 7) comparison of plasmid RSF1010 and plasmid R1162 (antiparallel) oriV regions, which contain direct repeats.

ImageJ

ImageJ is a free, public domain image processing program written in Java, with distributions available for OS X, Windows and Linux (**https://imagej.nih.gov/ij/download.html**).

ImageJ can display, edit, analyze and process 8 to 32 bit images and image series in stacks, and can import numerous image file formats, including TIFF, GIF, PNG, JPEG, BMP, DICOM, FITS and "raw" files. In addition to standard image processing functions, ImageJ can calculate area and pixel value statistics of user-defined selections, measure distances and angles and create density histograms and line profile plots.

ImageJ is multithreaded and highly optimized for image processing, able to process over 40 million pixels per second even on older computers. ImageJ has built in macro support, including a macro recorder, with over 300 macros available (**https://rsb.info.nih.gov/ij/macros/**) and an extensible plugin architecture, with over 500 plugins that add functionality to ImageJ (**https://imagej.nih.gov/ij/plugins/**). A version of ImageJ called Fiji based on the ImageJ2 core provides additional features, bundled plugins and automatic updating (**https://fiji.sc**).

Source: https://imagej.nih.gov/ij/

ImageJ Plug In Architecture

ImageJ was designed with an open architecture that provides extensibility via Java plugins. Custom acquisition, analysis and processing plugins can be developed using ImageJ's built in editor and Java compiler. User-written plugins make it possible to solve almost any image processing or analysis problem.

Acquisition: TWAIN, SensiCam Long Exposure Camera, µmanager, etc.

Analysis: Cell Counter, Colony Counter, Microscope Scale, Cell/Multi Cell Outliner, Read Plate, etc.

Color: RGB Stack Splitter and Merge, Color Counter, RGB Measure, etc.

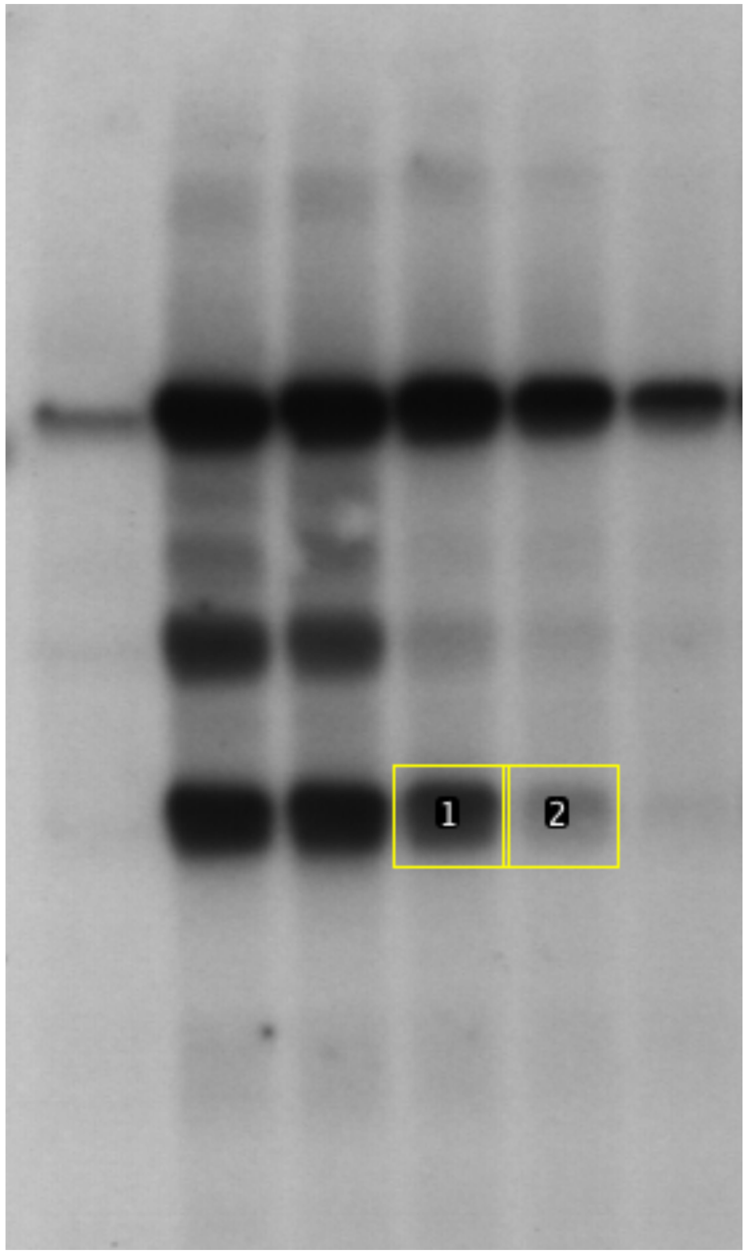
Filters: Background Correction, Subtraction and Normalization, Contrast Enhancer, Linearize Gel Data, Convolver, etc.

Graphics: Arrow, Image Slice Macro, Image Layering Toolbox, Interactive 3D Surface Plots, etc.

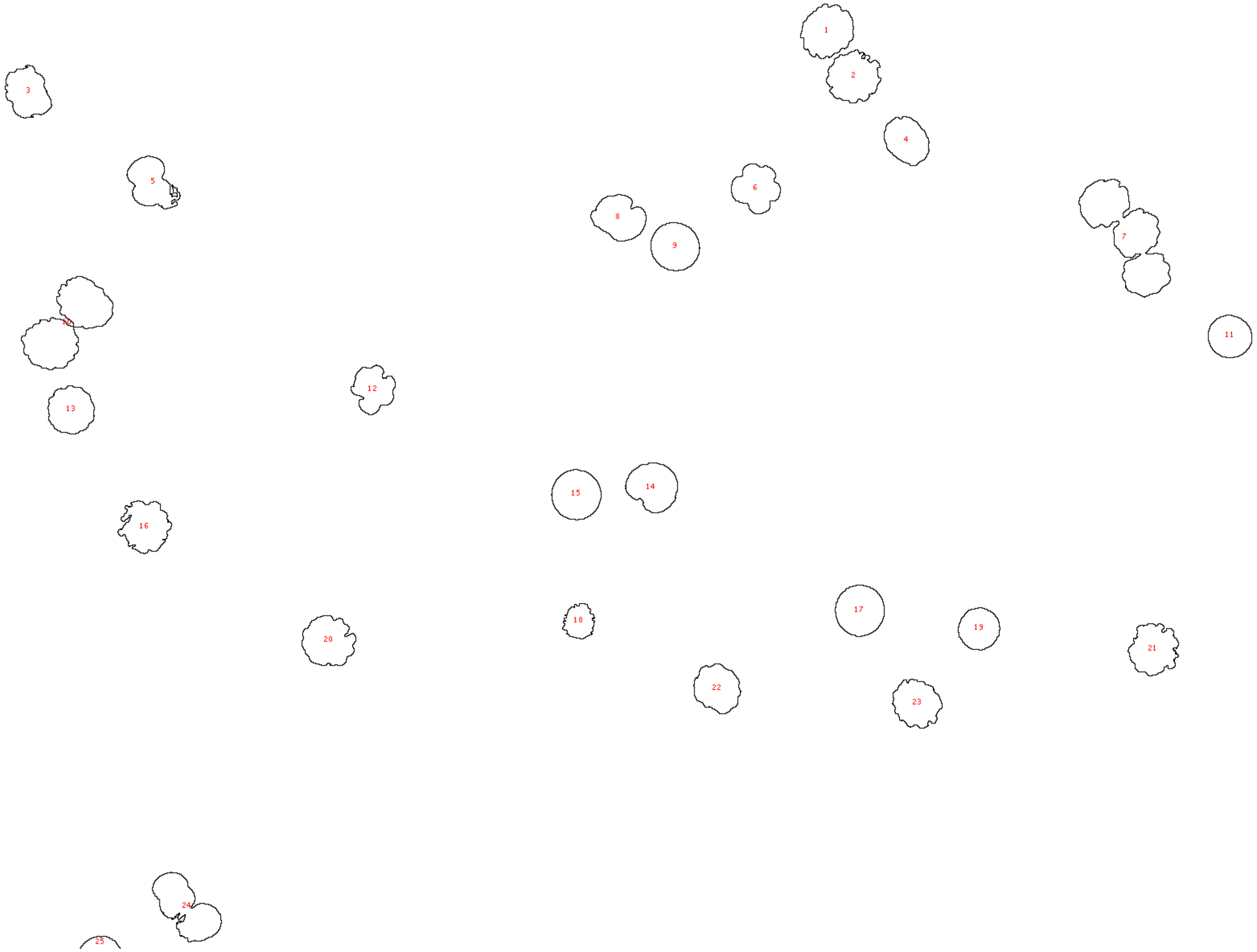
Stacks: Concatenate Images or Stacks, Depth From Focus, Object Tracker, Time Series Analyzer, etc.

Source: https://imagej.nih.gov/ij/

ImageJ and Density Analysis

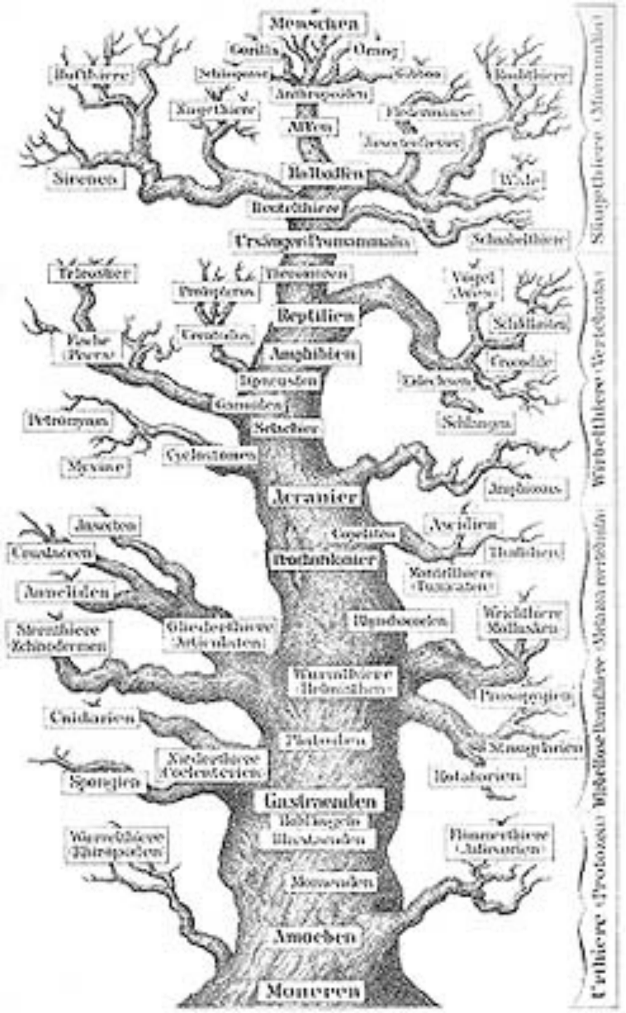


ImageJ and Particle Analysis



Phylogenetic Analysis

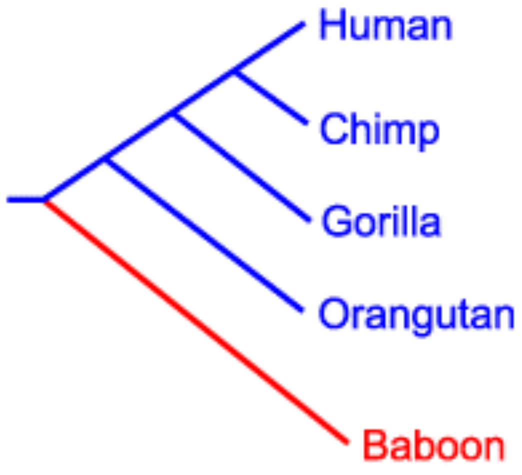
Phylogeny is the sequence of events involved in the evolutionary development of a species or taxonomic group. The physician Ernest Haeckel (1834-1919) coined the term “phylogeny,” the phrase “ontogeny recapitulates phylogeny,” and created the first phylogenetic trees after reading Darwin’s Origin of Species.



Phylogenetic Trees

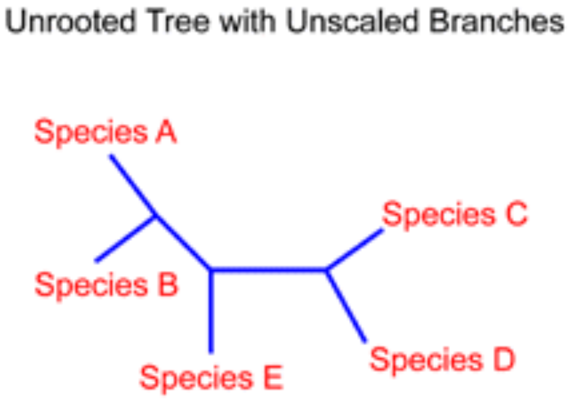
Rooted Trees

In a rooted tree, a single node is designated as a common ancestor, and a unique path leads from it through evolutionary time to all other nodes. It thus provides information about the common ancestry of sequences and the direction of evolution, and is the most common type of tree used to study evolutionary relationships.



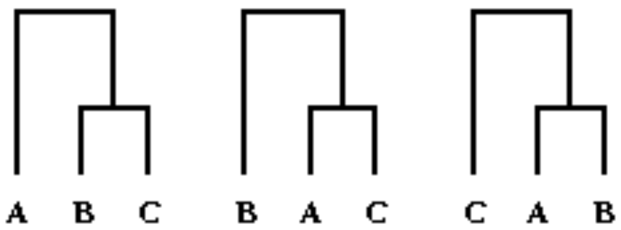
Unrooted Trees

Unrooted trees specify only the relationship between nodes, and nothing about the direction in which evolution occurred. A root can be assigned to an unrooted tree through the use of an **outgroup**, for example a species that unambiguously previously separated from the other species being compared (e.g. baboon, when comparing humans and gorillas).

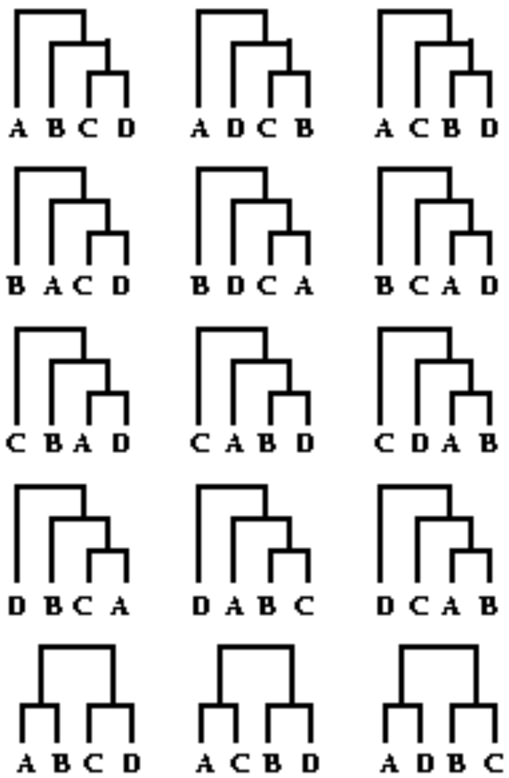


Source: Krane & Raymer, Fundamental Concepts of Bioinformatics, NCBI

Rooted Tree Reconstruction



3 species > 3 possible trees



4 species > 15 possible trees

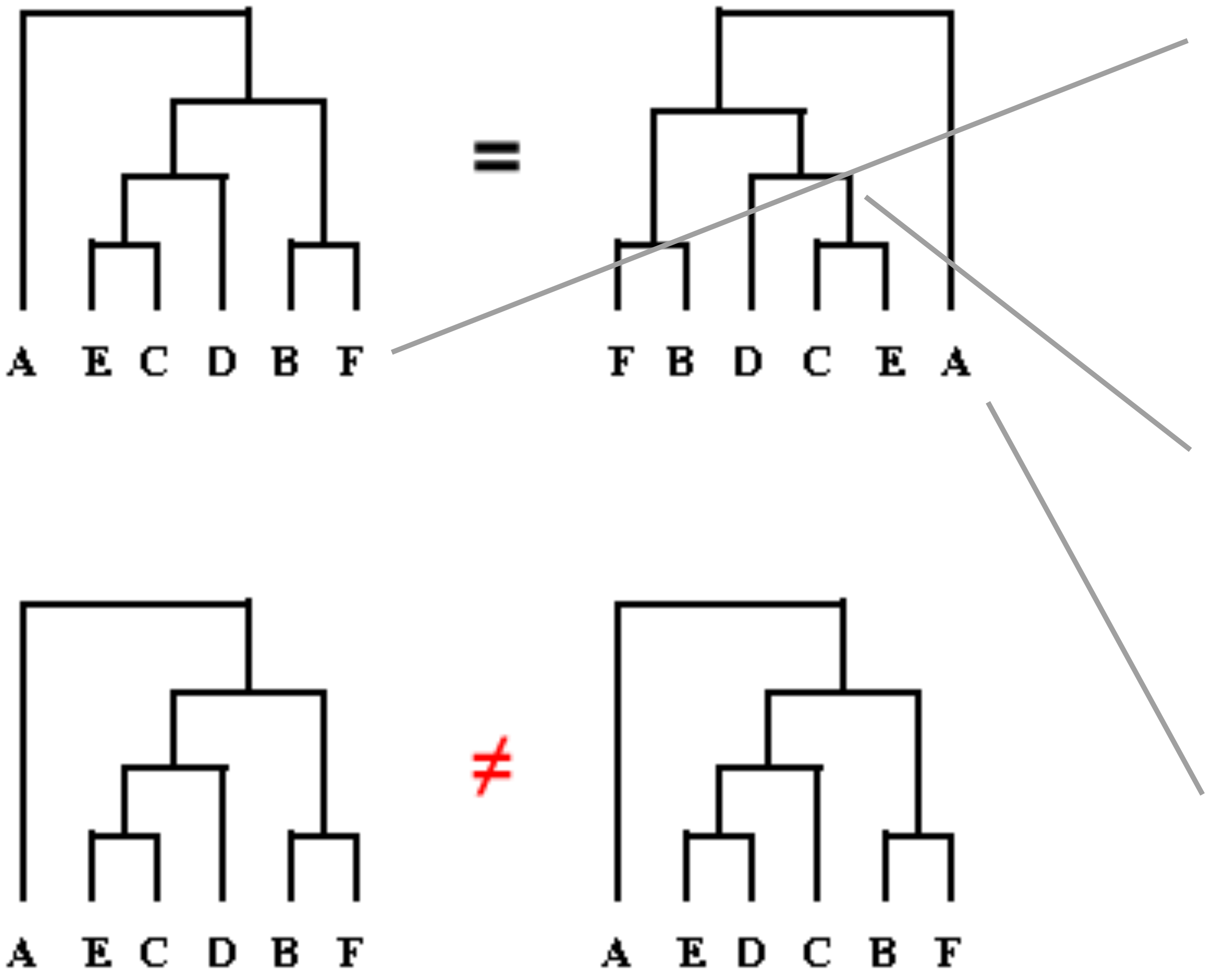
5 species > 105 possible trees

15 species > 213,458,046,676,875 possible trees

20 species > 8,200,794,532,637,891,559,375 possible trees!

The possible number of unrooted trees is one step less (i.e. 5 species or OTUs → 15 trees, still an enormous number with many species or OTUs). The number of possible trees for **n** OTUs can be estimated by **(2n-3)!/(2n-2(n-2)!)** for bifurcating rooted trees and **(2n-5)!/(2n-3(n-3)!)** for bifurcating unrooted trees.

Tree Topology



Operational Taxonomic Unit (OTU)
This corresponds to the terminal nodes of a phylogenetic tree (also known as leaves, tips or external nodes). They represent the genes, organisms, families, species or populations, as appropriate, for which you have data.

Internal Node
This corresponds to points within a phylogenetic tree where interior branches meet (also known as vertices). These represent inferred ancestors.

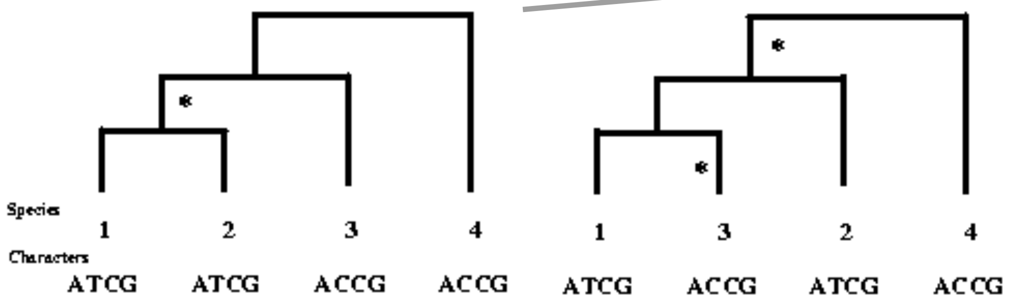
Outgroup
An OTU or taxa included for the purpose of rooting a tree.

The Principle of Parsimony

Occam's Razor

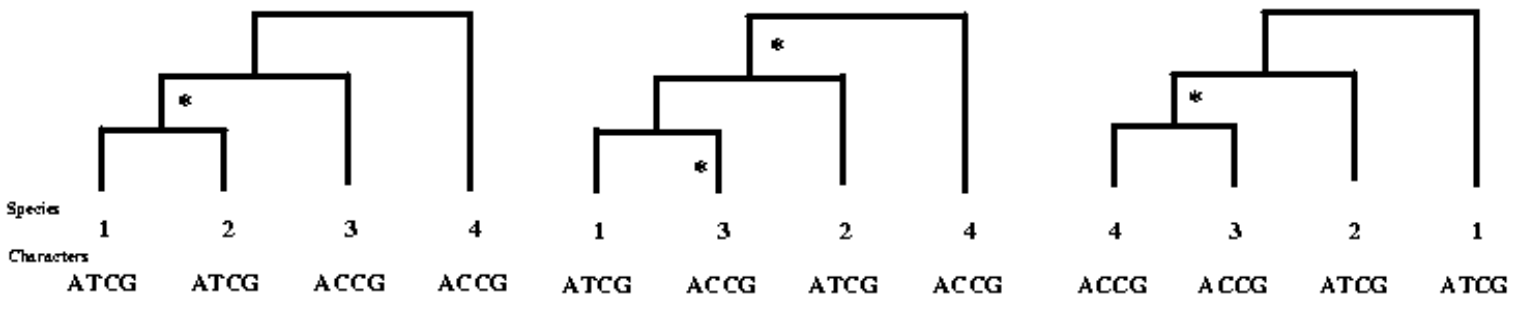
"Pluralitas non est ponenda sine necessitas" (Do not increase the number of entities required to explain anything beyond what is strictly necessary)

– William of Occam (or Ockham) (1284-1347)



Most parsimonious: requires less changes than its neighbor

These two trees are equally parsimonious



Practical Phylogeny

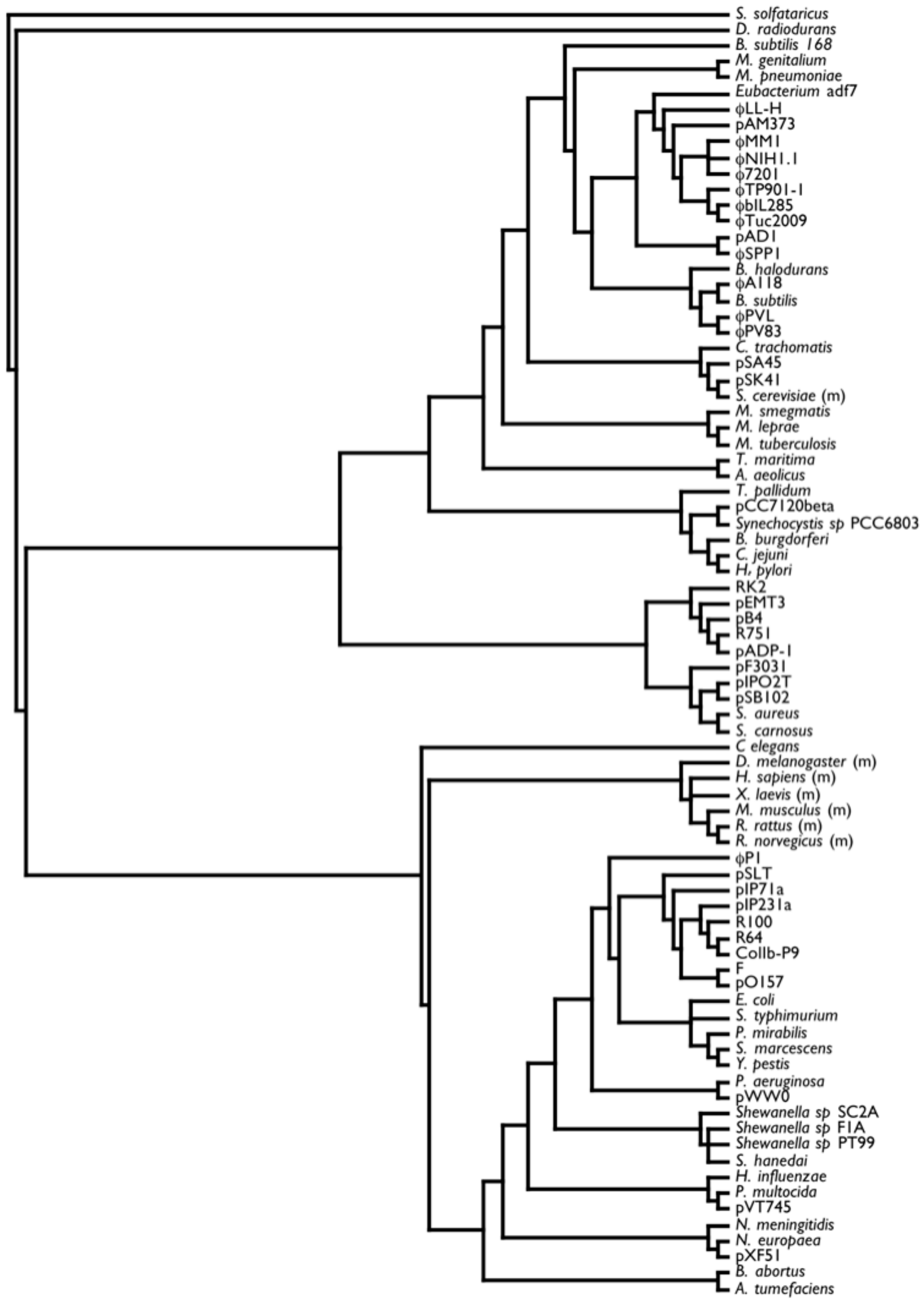
Practically speaking, when trying to build a phylogenetic tree, you will want to use the most accurate multiple sequence alignment (MSA) algorithm you can to align your sequences, then use a heuristic algorithm to build your tree, followed by a bootstrap analysis to evaluate the quality of the tree.

DNAStar MegAlign
The MegAlign package performs MSA with the ClustalW algorithm and then builds an automatically rooted tree using the Neighbor-Joining algorithm, and can perform bootstrap analysis.

DNAStar MegAlign Pro
MegAlign Pro offers additional MSA algorithms, including Clustal Omega, MUSCLE and MAFFT. It creates unrooted trees using the BioNJ algorithm.

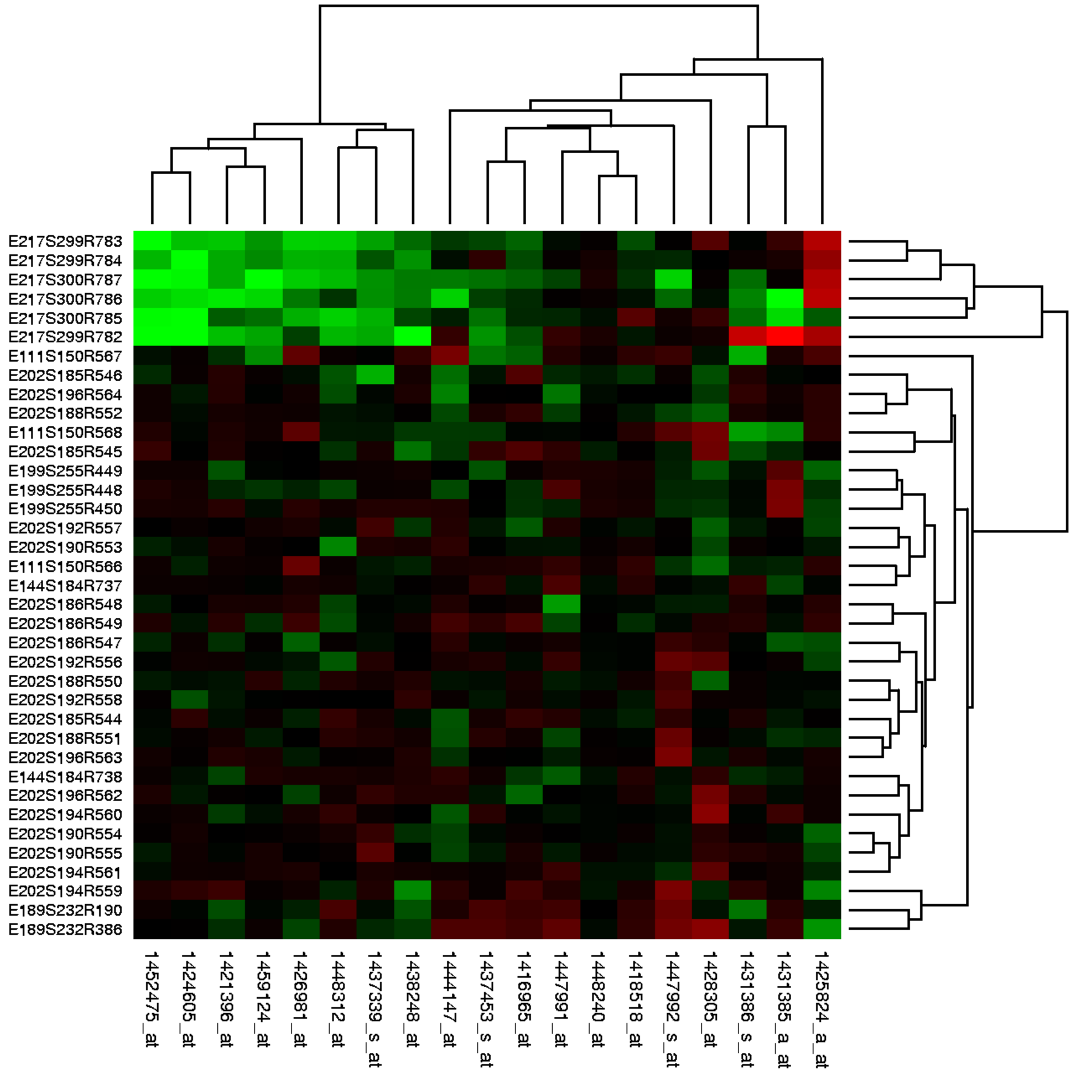
Other phylogeny packages include Clustal, MEGA (simple), MrBayes (Bayesian inference and Markov chain Monte Carlo methods), RAxML (Randomized Axelerated Maximum Likelihood), PAUP and PHYLIP. The R programming language also offers many packages for phylogenetic analysis.

Phylogenetic Tree of SSBs



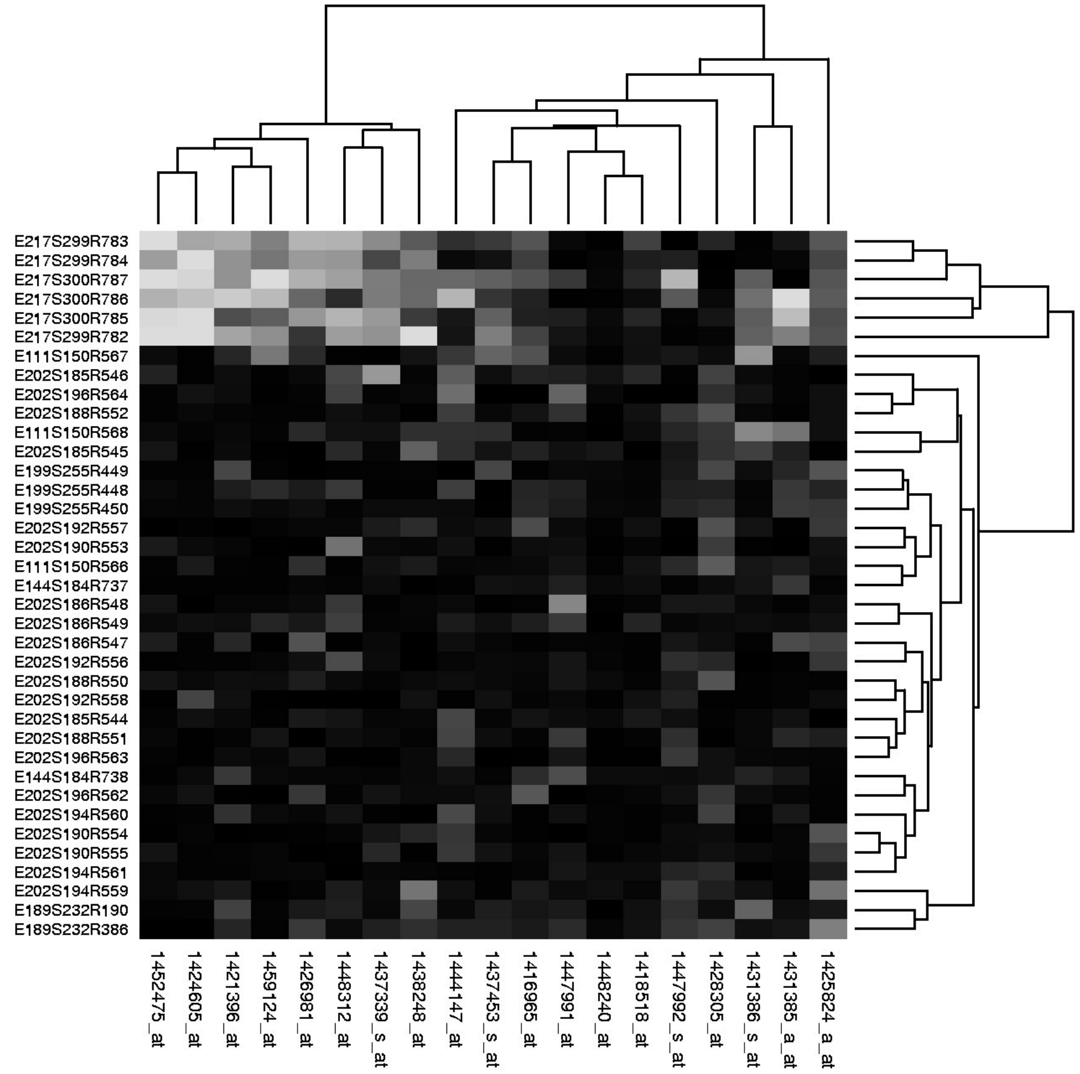
Heat Maps

Heat maps are graphical representations of data in which values in a matrix are displayed as colors. The R programming language is often used to handle the analysis and visualization of heat map data. In bioinformatics, heat maps are commonly used to represent gene expression in microarray data.



Color Blindness and Heat Maps

Red-green color blindness can have a prevalence as high as 15% in certain populations, and is generally more common in males than females. Thus, be cautious when using reds and greens with similar contrast, hue, saturation or brightness.



R

The R programming language was released in 1993 by Ross Ihaka and Robert Gentleman, statisticians at the University of Aukland in New Zealand. Their original goal was to develop a statistics language for teaching in their Mac computer lab. The name R was a reference to the S programming language for statistics as well as the first names of the authors.

The reference implementation of R is primarily written in C, Fortran and R and and is free and open source, released under the GNU General Public License, and supported by a community of open source developers at the Comprehensive R Archive Network (CRAN), which serves as a repository for R and free third party R software, and currently contains over 18,500 packages.

R is an interpreted language, and primarily supports procedural programming with functions, but has some object oriented functionality. It supports matrix arithmetic, a wide variety of data structures useful in math and statistics, math symbols, and a variety of graphing functions.

R has become one of the most popular programming languages used by statisticians and data miners, and is becoming well established in bioinformatics. The Bioconductor repository currently contains 2,140 free, open source bioinformatics and genomics packages for R.

Source: https://www.r-project.org and **https://cran.r-project.org** and **https://bioconductor.org**

RStudio

RStudio is a free, open source, Integrated Development Environment (IDE) for the R programming language that provides R with a simple graphical user interface and useful development tools. It runs on Mac, Windows and Linux.

The source editor features R specific highlighting, code completion, and smart indentation, and allows you directly run R code from it. Help and documentation are built in, along with the ability to quickly jump to function definitions. Additional support for development is provided by an interactive debugger, support for version control systems (Git and Subversion), and package authoring and documentation tools.

RStudio can simultaneously display multiple panes, typically a source code editor pane, an interactive console pane (like Terminal), a workspace pane, and a plotting pane. Interactive graphics can be created using the **manipulate** package.

Source: https://www.rstudio.com

Working with R and RStudio

Install the appropriate version of R from **https://cran.rstudio.com** and then download and install the appropriate version of the free RStudio Desktop from **https://rstudio.com**.

The RStudio Source editor pane (upper left by default) is useful for larger projects, otherwise the interactive Console pane (by default below it) can be used directly. In both the Source editor and interactive Console, **Tab** acts as an auto-complete function, suggesting file or function names and **Alt** and **-** is a shortcut for the frequently used **<-** assignment characters.

In the Source editor, **Command** and **enter** (Mac) or **Control** and **enter** (Win) sends the current line of code to the Console and runs it. Multiple lines of text can be selected beforehand. The Run button also serves the same function. In the interactive console, **Command** and **up arrow** (Mac) or **Control** and **up arrow** (Win) brings up a historical list of previous commands.

The Workspace pane (upper right by default) has an Environment tab which displays anything created during an R session, including values, objects or functions, a History tab that stores all previous commands run, and a Connections tab that displays supported data sources.

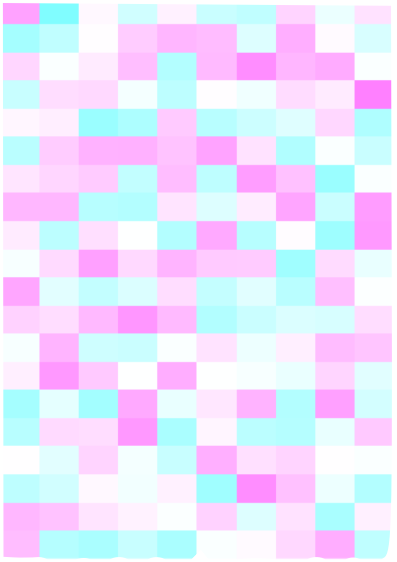
The View pane (lower left by default) has a Files tab for displaying the directory structure, a Plots tab for displaying graphs (the arrows move between multiple graphs), a Packages tab that shows loaded packages, a Help tab for displaying documentation and a Viewer tab that displays local HTML and web content.

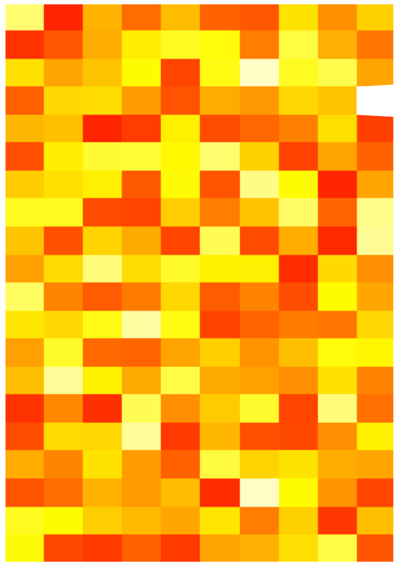
Heat Maps with R

The following R source code generates a random matrix of 10 columns and 20 rows containing 200 random integers between 1 and 100, then views the randomly generated data. It then creates a heat map using the default cyan to purple **heatmap** colors (note that there is no line break in the third line).

```
hm <- matrix(sample(1:100, 200, replace=T), ncol=10)
hm
hm_heatmap <- heatmap(hm, Rowv=NA, Colv=NA, col =
cm.colors(256), scale="column", margins=c(10,20))
```

Use **col = heat.colors(256)** for more temperature-like colors. Third-party packages such as **ggplot2** or **RColorBrewer** offer far more options.





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ImageJ User Guide free at:
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Phylogenetic Trees Made Easy by Barry G. Hall

An Introduction to R free at:
https://cran.r-project.org/doc/manuals/r-release/R-intro.pdf

A Little Book of R for Bioinformatics free at:
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Phylogentic Analysis Appendix

Phenetic vs. Cladistic Phylogeny

Phylogenetic Reconstruction
Phylogenetic reconstruction attempts to estimate the phylogeny for some data. Any collection of sequences will share some ancestral relationship, and the data within the sequences contains information that can be used to reconstruct or infer these ancestral relationships. A phylogenetic tree is a branching structure which illustrates the relationships between the sequences.

Phenetic Approach
Phenetic taxonomy was invented in 1750 by Michel Adanson. In the phenetic approach, a tree is constructed by considering the phenotypic similarities of the species without trying to understand the evolutionary pathways of the species, and thus may or not be the correct phylogeny. Trees constructed by this method are called phenograms or dendrograms.

Cladistic Approach
Cladistic taxonomy was invented by the German entomologist Willi Hennig in 1950. It involves the rigorous application of the concept of evolution to taxonomy. Taxa are defined by what distinctive features their members have, not what features they share with others. In the cladistic approach, a phylogentic tree is reconstructed by considering the various possible pathways of evolution and choosing from amongst these the best possible tree, that is, the tree that involves the fewest changes, and thus the least amount of convergent evolution. Trees reconstructed by this method are called cladograms.

Tree Terminology

Monophyletic
A group descended from a single common ancestor that contains only and all descendants from that ancestor.

Paraphyletic
A group descended from a single common ancestor that does not contain all the descendants from that ancestor.

Polyphyletic
A group whose members are not descended from a single common ancestor.

Gene Tree
A phylogenetic tree based on divergence observed within a single homologous gene in different species. It may accurately represent the evolutionary history of that gene, but not necessarily the species. Species trees should be based on comparison of numerous genes.

Bootstrapping
A method for checking the robustness of a given phylogentic tree by checking whether every portion of the alignment equally supports the structure of the tree.

Phylogenetic Terminology

Homologs
Genes with a common ancestral sequence. They may have been separated by speciation (orthologs) or duplication (paralogs).

Orthologs
Homologous genes in different species that arose from a common ancestor. They tend to have similar structure and function.

Paralogs
Similar genes within a single species that are the result of a gene duplication. They tend to have different but related functions.

Xenologs
Genes acquired by horizontal transfer between species, typically mediated by a plasmid, transposable element, or virus.

Symplesiomorphy
Having characters that are both derived from a common ancestor and uniquely shared by a group. This is essential to clearly establishing a phylogeny. Having only derived or shared characters is not sufficient to establish a phylogeny.

Homoplasies
Convergences of a particular character at a particular site. These typically pose the most difficulty in attempting to reconstruct the ancestral phylogenetic tree.

Distance Matrix Methods

Distance Method
Distance based methods attempt to construct trees based on measures of distance between OTUs (i.e. genes or species). In contrast, character based methods evalute particular features (i.e. DNA sequence, amino acid sequence, # of legs, etc.).

Unweighted-Pair-Group Method with Arithmetic Mean (UPGMA)
A clustering algorithm which constructs a distance matrix, then clusters together the least distant pair of Operational Taxonomic Units (OTUs), followed by successively more distant OTUs. At each step of the algorithm, the number of OTUs declines by one, replaced by a joint OTU, from which subsequent distances from other OTUs are calculated, until the algorithm finishes by clustering the last pair of OTUs. This method assumes that the rate of evolutionary change between all branches of the tree is the same, which is generally not a valid assumption. In nature, examples of rates of evolution varying between taxa are common. As a result, corrections to this assumption are often used with this approach.

Neighbor Joining Method
This attempts to correct for the assumption made by UPGMA that the same rate of of evolutionary change applies to all branches of the tree. It is otherwise similar to UPGMA, but generally gives better results. It yields an unrooted tree.

Fitch and Margoliash
This method attempts to find an optimal tree of minimal distance. It yields an unrooted tree.

Maximum Parsimony Methods

Maximum Parsimony
The maximum parsimony method involves evaluating as many trees as possible, giving each a score that is used to choose between different trees. The highest scoring, or most parsimonious tree is the one with the minimum number of evolutionary changes. A number of different methods can be used to calculate scoring.

Fitch Parsimony
For a particular tree, traverse from the leaves toward the root of the tree. At each internal node, determine the set of possible states (e.g. nucleotides). Then, traverse the tree from the root towards the leaves, picking ancestral states for each internal node to minimize the number of changes required. The Fitch algorithm assumes position independence, and that any state is equally likely to change to any other state. Variations which weight the costs of changes differently exist.

Dollo Parsimony
Assumes that derived states are irreversible, that is, a derived character state cannot be lost and then regained. Hence, the state can evolve and be lost many times throughout evolution, but cannot be inferred to have evolved twice. The tree with maximum parsimony is the one in which derived characters have been lost the fewest number of times.This method has been used with restriction fragment length polymorphism (RFLP) data, since restriction sites are difficult to gain, but easy to lose. It may be more useful when dealing with non-sequence data, for example, complex phenotypes, which are unlikely to have evolved more than once.

Source: Brian Golding, Reconstructing Phylogenies

Other Methods

Maximum Likelihood

The method of maximum likelihood attempts to reconstruct a phylogeny using an explicit model of evolution. It specifies values for the likelihood of a given trait evolving within a lineage, and chooses the most likely tree, given these values. It attempts to predict the most likely interior nodes given the OTUs, then the most likely tree. Theoretically, this may be the most powerful method available. For a given model of evolution, no other method will perform as well nor provide you with as much information about the tree. Unfortunately, this is computationally difficult to do and hence, the model of evolution must be a simple one. Even with simple models of evolutionary change the computational task is enormous and this is the slowest of all methods.

Compatibility

Compatibility methods recode data involving multi-state characters to include knowledge of the ancestral states of characters, and from this determine what changes are compatible. Compatibility methods are more accurate when there are slow rates of evolutionary change. Both compatibility and parsimony assume that homoplasies will be rare.

Source: Brian Golding, Reconstructing Phylogenies

Phylogeny Rules of Thumb

- Use more than one method. Each one will provide a phylogenetic history biased by that method's assumptions.
- Bootstrap or jackknife your data to test the quality of your tree. When bootstrapping, use at least several hundred iterations of resampling and tree generation.
- Run your analysis with different subsets of taxa to see if the trees thus generated are congruent. Dropping a single OTU should not dramatically change your tree.
- Treat long branches with caution. They tend to attract each other.
- Beware of non-orthologous genes, horizontal gene transfers, or recombinant sequences. Standard phylogenetic methods do not handle them well.
- When using outgroups, consider including more than one outgroup taxa, and choose outgroup species that are evenly spaced on the tree.
- Including intermediate taxa can help resolve even the relationship of a few taxa.
- When the number of substitutions per site is unusually high or low, distance methods may perform better than parsimony methods.
- If you expect homoplasies to be scattered at random throughout the sequence data, then a parsimony method will perform best. If homoplasies are expected to be concentrated in a few characters, whose identities are known in advance, then compatibility methods will perform better.