Inferencia filogenética usando el paquete

PHYLIP

(phylogeny inference package)
por

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Inferencia filogenética usando el paquete

PHYLIP

- Distribuído como código fuente en C, desde 1980

  http://evolution.gs.washington.edu/phylip.html

- también existen ejecutables para PCs y Macs (multiplataforma)

- Infiere filogenias por MP, compatibilidad, métodos de matrices de distancias, y ML
- También calcula árboles consenso, distancias entre árboles, hace remuestreo de datos (bootstrap), imprime y edita árboles, calcula matrices de distancias
- Maneja como datos alineamientos de nt y aa, matrices de frecuencias génicas, sitios de restricción, fragmentos de restricción, caracteres discretos y contínuos
- Es de los paquetes más utilizados para inferir filogenias y ES GRATIS, con excelente documentación

PHYLIP
– conversión de formatos y técnicas de remuestreo

SEQBOOT

Reads in a data set, and produces multiple data sets from it by **bootstrap resampling**. Since most programs in the current version of the package allow processing of multiple data sets, this can be used together with the consensus tree program CONSENSE to do bootstrap (or delete-half-jackknife) analyses with most of the methods in this package. This program also allows the Archie/Faith technique of permutation of species within characters. It can also **rewrite a data set to convert it from between the PHYLIP Interleaved and Sequential forms**, and into a preliminary version of a new XML sequence alignment format which is under development and which is described in the RETREE documentation web page.

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PHYLIP - distancias

DNADIST

Computes four different **distances between species from nucleic acid sequences**. The distances can then be used in the distance matrix programs. The distances are the Jukes-Cantor formula, one based on Kimura's 2-parameter method, Jin and Nei's distance which allows for rate variation from site to site, and a maximum likelihood method using the model employed in DNAML (**F84**). The latter method of computing distances can be very slow.

PROTDIST

Computes a **distance measure for protein sequences**, using maximum likelihood estimates based on the **JTT**, Dayhoff PAM matrix, Kimura's 1983 approximation to it, or a model based on the genetic code plus a constraint on changing to a different category of amino acid. Rate variation from site to site is also allowed. The distances can be used in the distance matrix programs.

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PHYLIP - distancias

FITCH

Estimates phylogenies from distance matrix data under the "additive tree model" according to which the distances are expected to equal the sums of branch lengths between the species. Uses the Fitch-Margoliash criterion and some related least squares criteria. Does not assume an evolutionary clock. This program will be useful with distances computed from molecular sequences, restriction sites or fragments distances, with DNA hybridization measurements, and with genetic distances computed from gene frequencies.

KITSCH

Estimates phylogenies from distance matrix data under the "ultrametric" model which is the same as the additive tree model except that an evolutionary clock is assumed. The Fitch-Margoliash criterion and other least squares criteria are assumed This program will be useful with distances computed from molecular sequences, restriction sites or fragments distances, with distances from DNA hybridization measurements, and with genetic distances computed from gene frequencies.

NEIGHBOR

An implementation by Mary Kuhner and John Yamato of Saitou and Nei's "NJ Method," and of the UPGMA (Average Linkage clustering) method. Neighbor Joining is a distance matrix method producing an unrooted tree without the assumption of a clock. UPGMA does assume a clock. The branch lengths are not optimized by the least squares criterion but the methods are very fast and thus can handle much larger data sets.

PHYLIP - árboles

DRAWGRAM

Plots rooted phylogenies, cladograms, and phenograms in a wide variety of user-controllable formats. The program is interactive and allows previewing of the tree on PC or Macintosh graphics screens, and Tektronix or Digital graphics terminals. Final output can be to a file formatted for one of the drawing programs, on a laser printer (such as Postscript or PCL-compatible printers), on graphics screens or terminals, on pen plotters (Hewlett-Packard or Houston Instruments) or on dot matrix printers capable of graphics (Epson, Okidata, Imagewriter, or Toshiba).

DRAWTREE

Similar to DRAWGRAM but plots unrooted phylogenies.

CONSENSE

Computes consensus trees by the majority-rule consensus tree method, which also allows one to easily find the strict consensus tree. Is not able to compute the Adams consensus tree. Trees are input in a tree file in standard nested-parenthesis notation, which is produced by many of the tree estimation programs in the package. This program can be used as the final step in doing bootstrap analyses for many of the methods in the package.

RETREE

Reads in a tree (with branch lengths if necessary) and allows you to reroot the tree, to flip branches, to change species names and branch lengths, and then write the result out.
Can be used to convert between rooted and unrooted trees, and to write the tree into a preliminary version of a new XML tree file format which is under development and which is described in the RETREE documentation web page.

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PHYLIP - MP

PROTPARS

Estimates phylogenies from protein sequences (input using the standard one-letter code for amino acids) using the parsimony method, in a variant which counts only those nucleotide changes that change the amino acid, on the assumption that silent changes are more easily accomplished.

DNAPARS

Estimates phylogenies by the parsimony method using nucleic acid sequences. Allows use the full IUB ambiguity codes, and estimates ancestral nucleotide states. Gaps treated as a fifth nucleotide state. Can use 0/1 weights, reconstruct ancestral states, and infer branch lengths.

DNAPENNY

Finds all most parsimonious phylogenies for nucleic acid sequences by branch-and-bound search. This may not be practical (depending on the data) for more than 15 species or so.

PHYLIP - ML

DNAML

Estimates phylogenies from nucleotide sequences by maximum likelihood. The model employed allows for unequal expected frequencies of the four nucleotides, for unequal rates of transitions and transversions, and for different (prespecified) rates of change in different categories of sites, with the program inferring which sites have which rates. It also allows different rates of change at known sites.

DNAMLK

Same as DNAML but assumes a molecular clock. The use of the two programs together permits a likelihood ratio test of the molecular clock hypothesis to be made.

PROML

Estimates phylogenies from protein amino acid sequences by maximum likelihood. The PAM or JTTF models can be employed. The program can allow for different (prespecified) rates of change in different categories of amino acid positions, with the program inferring which posiitons have which rates. It also allows different rates of change at known sites.

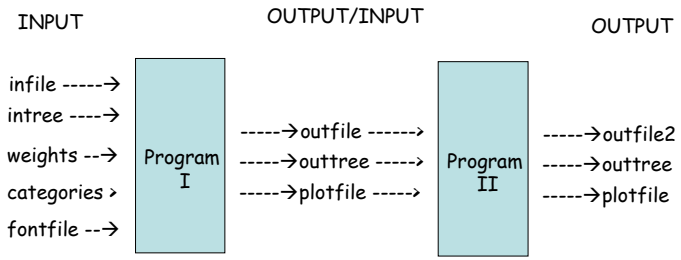
PROMLK

Same as PROML but assumes a molecular clock. The use of the two programs together permits a likelihood ratio test of the molecular clock hypothesis to be made.

Inferencia filogenética usando el paquete PHYLIP – manejo de archivos y programas

- Phylip tiene una estructura modular: consta de muchos programas, cada cual hace un(os) análisis particular(es) (similar a los programas de UNIX/LINUX)

- Para hacer unos análisis complejos hemos de construir una "pipeline" en la que la salida de un programa se usa como entrada del siguiente



Esquema de una "pipiline" de programas del paquete PHYLIP

Inferencia filogenética usando el paquete PHYLIP – manejo de archivos y programas

PHYLIP programs and documentation

PHYLIP, the PHYLogeny Inference Package, consists of 35 programs.
There are documentation files for each program, in the form of web pages in HTML 3.2. There are also documentation web pages for each group of programs, and a main documentation file that is the basic introduction to the package.
Before running any of the programs you should read it.
Below you will find a list of the programs and the documentation files.
The names of the documentation files are highlighted as links that will take you to those documentation files.

http://evolution.genetics.washington.edu/phylip/phylip.html

Esta es la liga a la documentación en formato HTML 3.2. Es una documetnación muy buena. Este URL lo encuentras también en las ligas de nuestra página del curso

Ejercicios – métodos de distancia

- Usando el archivo 5_atpD+recA_phy.phy (ya alineado y en formato phylip)

1. Vamos a calcular un árbol NJ con 1000 pseudoréplicas de bootstrap

Los ejecutables de PHILIP: vista en formato windows

PHYLIP – Seqboot


```
C:\Archivos de programa\PHYLIP3.6\exe\seqboot.exe
seqboot.exe: can't find input file "infile"
Please enter a new file name> 5_atpD+recA_phy.phy_
```

< -- infile

```
C:\Archivos de programa\PHYLIP3.6\exe\seqboot.exe

Settings for this run:
  D       Sequence, Morph, Rest., Gene Freqs?  Molecular sequences
  J  Bootstrap, Jackknife, Permute, Rewrite?  Bootstrap
  %    Regular or altered sampling fraction?  regular
  B       Block size for block-bootstrapping?  1 (regular bootstrap)
  R                    How many replicates?  1000
  W               Read weights of characters?  No
  C                 Read categories of sites?  No
  S      Write out data sets or just weights?  Data sets
  I             Input sequences interleaved?  Yes
  0       Terminal type (IBM PC, ANSI, none)?  IBM PC
  1         Print out the data at start of run  No
  2      Print indications of progress of run  Yes

  Y to accept these or type the letter for one to change
y
Random number seed (must be odd)?
33

seqboot.exe: the file "outfile" that you wanted to
     use as output data file already exists.
     Do you want to Replace it, Append to it,
     write to a new File, or Quit?
     (please type R, A, F, or Q)
r
```

OPCIONES

R – 1000 (Con la opción R (réplicas) podemos modificar el valor a 1000)
y (con Y aceptamos las condiciones para el análisis y lo iniciamos)

outfile -- > contiene 1000 pseudoréplicas de bootstrap de nuestro alineamiento original; renombramos outfile como **1000boot**

PHYLIP – dnadist


```
C:\Archivos de programa\PHYLIP3.6\exe\dnadist.exe

Nucleic acid sequence Distance Matrix program, version 3.6b

Settings for this run:
  D  Distance (F84, Kimura, Jukes-Cantor, LogDet)?  F84
  G          Gamma distributed rates across sites?  Yes
  T              Transition/transversion ratio?  0.9000
  W                    Use weights for sites?  No
  F               Use empirical base frequencies?  Yes
  L                    Form of distance matrix?  Lower-triangular
  M                 Analyze multiple data sets?  Yes, 1000 data sets
  I              Input sequences interleaved?  Yes
  0           Terminal type (IBM PC, ANSI, none)?  IBM PC
  1             Print out the data at start of run  No
  2          Print indications of progress of run  Yes

  Y to accept these or type the letter for one to change
y

Coefficient of variation of substitution rate among sites (must be positive)
 In gamma distribution parameters, this is 1/(square root of alpha)
1.4
```

Opciones:
T – 0.9 (estimado por ML)
G – yes, CV = 1.4 (α = 0.39, estimado por ML)
L – lower-triangular matrix
M – mutlitple data sets (=1000)

renombramos outfile como 1000F84+G

PHYLIP – neighbor


```
C:\Archivos de programa\PHYLIP3.6\exe\neighbor.exe

Neighbor-Joining/UPGMA method version 3.6b

Settings for this run:
  N       Neighbor-Joining or UPGMA tree?  Neighbor-joining
  O                       Outgroup root?  Yes, at species number  5
  L         Lower-triangular data matrix?  Yes
  R         Upper-triangular data matrix?  No
  S                       Subreplicates?  No
  J     Randomize input order of species?  Yes (random number seed =      35)
  M          Analyze multiple data sets?  Yes, 1000 sets
  0   Terminal type (IBM PC, ANSI, none)?  IBM PC
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                   Print out tree  Yes
  4        Write out trees onto tree file?  Yes

  Y to accept these or type the letter for one to change
```

Opciones:
O – redefinimos el taxon 5 como outgruop
L – lower-triangular matrix
J – randomizamos la entrada de taxa en el algoritmo
M – mutlitple data sets (=1000)
Y

renombramos outfile como 1000NJtrees

PHYLIP – consense


```
C:\Archivos de programa\PHYLIP3.6\exe\consense.exe
consense.exe: can't find input tree file "intree"
Please enter a new file name> 1000NJtrees
```

```
C:\Archivos de programa\PHYLIP3.6\exe\consense.exe

Consensus tree program, version 3.6b

Settings for this run:
  C          Consensus type (MRe, strict, MR, Ml):  Majority rule (extended)
  O                       Outgroup root:  Yes, at species number  5
  R            Trees to be treated as Rooted:  No
  T          Terminal type (IBM PC, ANSI, none):  IBM PC
  1              Print out the sets of species:  Yes
  2          Print indications of progress of run:  Yes
  3                         Print out tree:  Yes
  4                Write out trees onto tree file:  Yes

Are these settings correct? (type Y or the letter for one to change)
y
```

Opciones:
O – redefinimos el taxon 5 como outgruop
Y

renombramos outfile como 1000NJcns

PHYLIP – drawgram

drawgram.exe

```
C:\Archivos de programa\PHYLIP3.6\exe\drawgram.exe
drawgram.exe: can't find input tree file "intree"
Please enter a new file name> 1000NJcns
DRAWGRAM from PHYLIP version 3.6b
Reading tree ...
Tree has been read.
Loading the font ....
drawgram.exe: can't find font file "fontfile"
Please enter a new file name> font1
```

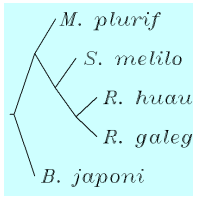
```
C:\Archivos de programa\PHYLIP3.6\exe\drawgram.exe

Rooted tree plotting program version 3.6b

Here are the settings:
 0  Screen type (IBM PC, ANSI):  IBM PC
 P       Final plotting device:  Postscript printer
 U          Previewing device:  MS Windows display
 H                 Tree grows:  Horizontally
 S                 Tree style:  Curvogram
 B          Use branch lengths:  No
 L              Angle of labels:  90.0
 R      Scale of branch length:  Automatically rescaled
 D       Depth/Breadth of tree:  0.53
 T     Stem-length/tree-depth:  0.05
 C    Character ht / tip space:  0.3333
 A             Ancestral nodes:  Weighted
 F                        Font:  Times-Roman
 M          Horizontal margins:  1.65 cm
 M            Vertical margins:  2.16 cm
 #               Pages per tree:  one page per tree

 Y to accept these or type the letter for one to change
y
```

Opciones:
O – taxon 5 como outgruop
S – curvogram
B – no
y



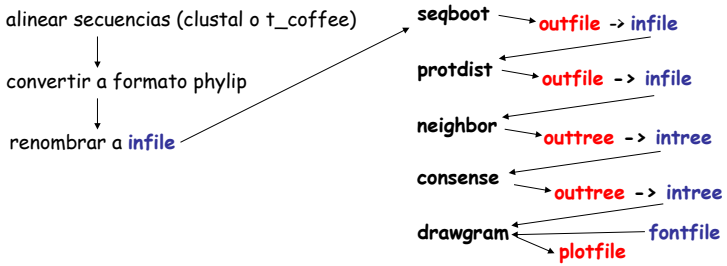
No hay outfile, sólo el display --- >

Desde luego que hay mejores editores de árboles, en particular **TreeView**

PHYLIP – una tubería de análisis desde la línea de comandos (UNIX)

- **Problema a resolver**:
  Vamos a hacer un análisis de NJ con 100 pseudoréplicas de bootstrap bajo modelo JTT de cada uno de los archivos de ejemplo de GDPs de eucariontes y procariones, así como del alineamiento de perfiles de ambos sets.

1. Debemos de pensar en la secuencia de análsis a realizar. Veamos el pseudocódigo:



PHYLIP – una tubería de análisis desde la línea de comandos (UNIX)

- Para poder correr el script necesitamos escribir además los archivos de comandos (*.cmd) requeridos por cada programa phylip

- estos archivos son muy sencillos: contienen en cada renglón el comando que usarías al manejar phylip desde el menú gráfico. Sólo anota el orden exacto de la secuencia de comandos y sus valores en un archivo de texto plano, y ponle por ej. la extensión cmd para identificarlos.

seqboot.cmd

```
r
100
y
99
```

protdist_plus100boot.cmd

```
m
d
100
y
```

neighbor_100datasets.cmd

```
j
77
m
100
99
y
```

consense.cmd

```
y
```

PHYLIP – selección del modelo de sustitución y estima del parámetro alpha para secs. de prot. bajo ML

- Existen dos programas en el paquete PHYLIP para inferir filogenias de ML a partir de secuencias de proteína: **proml** y **promlk**

- Ambos programas implementan tres matrices empíricas de sustitución:

	JTT	PMB (Blossum-like)	PAM
comando:	defecto	P	P
			P

- Se pueden construír modelos +G, +G+I (por defecto no asume HTSES)

	+G	+G+I
comando:	R	R
		R

PHYLIP – selección del modelo de sustitución y estima del parámetro alpha para secs. de prot. bajo ML

- Si quieres obtener una estima de ML del valor del parámetro alpha (α) de la distribución gamma (Γ) bajo un modelo particular (p. ej. JTT), debes de probar con una serie de valores de CV, donde **CV = 1/ $(\alpha)^{\frac{1}{2}}$**

- Por ejemplo, podemos evaluar los siguentes valores de CV (y α correspondientes):

CV	1.41	1.00	0.82	0.71	0.63	0.58	0.50
α	0.50	1.00	1.50	2.00	2.50	3.00	4.00

- Finalmente PROML (o PROMLK) les van a pedir el número de categorías con el que quieran aproximar (discretamente) la distribución gamma. Un valor de **4 categorías** es generalmente suficiente.

PHYLIP – selección del modelo de sustitución y estima del parámetro alpha para secs. de prot. bajo ML

- Este problema es nuevamente muy tedioso y tardado de hacer manualmente. Por ello vamos a usar el script **proml_modelfit_V01.pl** para ejecutarlo. El script toma todos los alineamientos *.phy de un directorio y calcluar los valores de verosimilitud global de las filogenias resultantes bajo cada uno de los tres modelos empíricos de sustitución que implementados en PROML/PROMLK bajo los siete valores de CV mostrados en la página anterior. Evaluamos **3 modelos X 7 valores de CV = 21 filogenias de ML** por alineam.

- El script **proml_modelfit_V01.pl** abre cada archivo de salida (outfile) de PROML para **parsearlo**. Es decir, capturamos de cada outfile los datos que nos interesan: (CV, alpha y –lnL). El programa **imprime en pantalla los resultados del parseo y además los escribe en archivos**. Ello va a facilitar poder hacer un **análisis gráfico de la función de verosimilitud dados alpha y –lnL**

PHYLIP – selección del modelo de sustitución y estima del parámetro alpha para secs. de prot. bajo ML

#1CV	alpha	-lnL	for JTT
1.414000	0.500151	-2867.31600	
1.000000	1.000000	-2856.13225	
0.816000	1.501826	-2852.46224	
0.707000	2.000604	-2852.25738	
0.632000	2.503605	-2853.03578	
0.577000	3.003643	-2854.04273	
0.500000	4.000000	-2855.96898	
#2CV	alpha	-lnL	for PMB
1.414000	0.500151	-2867.00406	
1.000000	1.000000	-2856.74978	
0.816000	1.501826	-2849.46126	
0.707000	2.000604	-2846.65591	
0.632000	2.503605	-2845.50671	
0.577000	3.003643	-2845.05301	
0.500000	4.000000	-2844.91106	
#3CV	alpha	-lnL	for PAM
1.414000	0.500151	-2895.76260	
1.000000	1.000000	-2882.49693	
0.816000	1.501826	-2878.60925	
0.707000	2.000604	-2878.58367	
0.632000	2.503605	-2879.62446	
0.577000	3.003643	-2880.89181	
0.500000	4.000000	-2883.26476	

PHYLIP – selección del modelo de sustitución y estima del parámetro alpha para secs. de prot. bajo ML