From the Department of Applied Economics and Statistics 204, University of Nevada, Reno, NV 89557. Address correspondence to George C. J. Fernandez at the address above.

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DAMBE: Software Package for Data Analysis in Molecular Biology and Evolution

X. Xia and Z. Xie

DAMBE (data analysis in molecular biology and evolution) is an integrated software package for converting, manipulating, statistically and graphically describing, and analyzing molecular sequence data with a user-friendly Windows 95/98/2000/NT interface. DAMBE is free and can be downloaded from http://web.hku.hk/~xxia/software/software.htm. The current version is 4.0.36.

DAMBE (data analysis in molecular biology and evolution) is an integrated computer program for descriptive and comparative analysis of molecular data (including nucleotide and amino acid sequence data, as well as allele frequency and distance matrix data). It has features either not available or poorly implemented in other programs. These features are grouped into (1) sequence format conversion and manipulation supporting 20 commonly used molecular data formats; (2)

Table 1. Common data file formats used in DAMBE

Sequence format	Read in	Convert to
PHYLIP	+	+
PAUP	+	+
MEGA	+	+
CLUSTAL	+	–
FASTA	+	+
GenBank	+	+
GCG	+	+
MSF	+	+
DNA strider	+	+
PAML	+	+
RST,MP[a]	+	–
PHYLTEST	–	+
IG/Stanford	+	+
NBRF	+	+
EMBL	+	+
FITCH	+	+
PIR/CODATA	+	+
Plain text[b]	+	+
Allele frequency	+	–
Distance matrix	+	+

[a] Sequence formats for storing original sequences and reconstructed ancestral sequences from the original sequences.

[b] The one-sequence-per-file text format from programs such as Sequence Navigator and DNA Star.

descriptive statistics such as nucleotide, amino acid, and codon frequencies, dinucleotide and diamino acid frequencies, analysis of codon usage and amino acid usage bias; and (3) comparative sequence analysis such as phylogenetic reconstruction of trees and ancestral sequences with distance, maximum parsimony and maximum-likelihood methods, bootstrapping and jackknifing, significance tests of molecular clock and alternative phylogenetic hypotheses (i.e., how much better is the best tree compared to alternative trees), fitting statistical distributions to substitution data over sites including Poisson, negative binomial, and gamma distributions. DAMBE features a user-friendly Windows interface with extensive on-line help.

Sequence Input and Sequence Format Conversion

DAMBE can read and convert almost all commonly used molecular data formats (Table 1). In particular, DAMBE can take advantage of the rich information contained in the FEATURES table of GenBank files and extract specific segments such as CDS, exons, introns, rRNA, etc., by a few mouse clicks. The user can also use "custom splicing" to extract sequence segments that are not specified in the GenBank sequences.

DAMBE can read sequence files directly from a networked computer, such as a remote UNIX workstation, in the same way as one would read a file from a local hard

disk. Extensive network functions have also been implemented for retrieving sequences directly from GenBank, either by LOCUS name or accession number, or by keyword search.

DAMBE features a color-coded sequence editor for either sequence input or visual alignment. Sequences can be as long as 32,768 bp.

Sequence Manipulation

We will only highlight two of the many sequence manipulation features in DAMBE.

Sequence Alignment

DAMBE can align nucleotide and amino acid sequences as most other alignment programs do. However, one particular feature that is not available in most other alignment programs is the ability to align protein-coding nucleotide sequences against aligned amino acid sequences. Other programs often introduce frameshift indels in the aligned protein-coding sequences, even if the protein genes are known to be functional and do not have these frame-shifting indels. In other words, the introduced frame-shifting indels in the aligned sequences are alignment artifacts, and the correctly aligned sequences should have complete codons, not one or two nucleotides, inserted or deleted.

DAMBE solves this problem by aligning the protein-coding nucleotide sequences against aligned amino acid sequences. One can read in the protein-coding nucleotide sequences, translate them into amino acid sequences, align the amino acid sequences, and then align the original nucleotide sequences against the aligned amino acid sequences.

Translation

DAMBE implements all 12 different genetic codes and can therefore translate protein-coding nucleotide sequences from any organism to amino acid sequences. The implementation of these genetic codes greatly facilitates amino acid-based and codon-based analyses.

Descriptive Sequence Analysis

This includes nucleotide, amino acid and codon usage analysis, compositional analysis based on dinucleotide and diamino acid frequencies, quantification of the effect of GC and T_pA frequencies on exon and CDS lengths, and the methylation effect on codon usage bias.

A substitution model used in compara-

tive sequence analysis, such as phylogenetic reconstruction using the maximum-likelihood method, typically has two categories of parameters, the frequency parameters and the rate ratio parameters. The descriptive sequence analysis helps to understand the factors affecting the frequency parameters and to select which substitution model to use in phylogenetic reconstruction.

Comparative Sequence Analysis

Quantification of Substitution Patterns
A substitution model is characterized by frequency parameters and rate ratio parameters, and it is important to know the empirical substitution patterns in order to decide which substitution model to use in analyzing sequences. The quantification of empirical substitution patterns requires pairwise comparisons. However, when the comparison is done between all possible sequence pairs, the resulting substitution pattern may be biased because the comparisons are not independent (Felsenstein 1992; Nee et al. 1996; Xia et al. 1996). For example, if there is one species that has recently experienced a large number of A→G transitions and few other substitutions, then all pairwise comparisons between this species and the other species will each contribute one data point with a large A→G transition bias. One way to avoid such a problem of nonindependence is to reconstruct ancestral states of DNA sequences and estimate the number of substitutions between neighboring nodes along the phylogenetic tree (Tamura and Nei 1993; Xia 1998; Xia and Li 1998). DAMBE automates this process.

Phylogenetic Reconstruction
DAMBE implements most commonly used phylogenetic methods such as distance-based (UPGMA, Fitch-Margoliash, and neighbor-joining methods), maximum parsimony, and maximum-likelihood methods. A variety of genetic distances are implemented. Nucleotide-based distances include the one-parameter (Jukes and Cantor 1969) and two-parameter (Kimura 1980) distances, the paralinear distance (Lake 1994), as well as distances based on the F84 model (Felsenstein 1993) and TN93 model (Tamura and Nei 1993). Codon-based distances include Li's (1993) synonymous and nonsynonymous distances. The UPGMA and neighbor-joining methods can handle tied values in the matrix and generate all possible alternative trees. Most other distance-based pro-

grams output only one of the possible trees.

All phylogenetic analyses for protein-coding genes can be performed on individual codon positions or combinations of codon positions, for example, the first and second codon positions when the third codon position experienced substitution saturation. Alternatively, one can also perform analysis on translated amino acid sequences. DAMBE can translate nucleotide sequences from any organism into amino acid sequences because it implements all known genetic codes.

Phylogenetic Analysis Involving Bootstrapping and Jackknifing
Bootstrapping and delete-half jackknifing are implemented in DAMBE in conjunction with the phylogenetic methods mentioned above. Resampling can be nucleotide based, amino acid based, or codon based. The last is necessary for doing bootstrapping and jackknifing with codon-based methods such as Li's (1993) synonymous and nonsynonymous distances. Consensus trees are displayed with bootstrapping values at internal nodes. The branch lengths of a consensus tree can be evaluated.

Testing Alternative Phylogenetic Hypotheses
It is often necessary to evaluate the relative statistical support for alternative phylogenetic hypotheses such as alternative phylogenetic trees. Such hypothesis tests can be carried out in DAMBE with the distance, maximum parsimony, or maximum-likelihood methods. The significance tests make proper multiple comparisons involving multiple trees.

For the distance methods, the test is similar to that detailed in Xia (2000), except that the following equation

$$\text{var } E = \frac{\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} (x_{ij} - y_{ij})^2}{n(n-1)/2 - m - 1} \quad (1)$$

replaces equation (21.2) in Xia (2000).

For the maximum parsimony method, a rooted tree is required to represent alternative topologies. DNAPARS in PHYLIP has already provided a significance test if you include user trees in the input file. In short, DNAPARS computes the number of steps (changes in character states) for each topology, the difference in the number of steps between the best and each alternative topology, and the associated (large sample) variance of the differences.

The z score is computed and declared as significant if it is larger than 1.96 (Felsenstein 1985). The main problem with this test is that the result can be interpreted probabilistically only when you have just two topologies and is not appropriate with multiple comparisons. DAMBE takes the same approach but uses the Newman–Keuls test that is better for multiple comparisons.

For the maximum-likelihood method, the Kishino–Hasegawa test (Kishino and Hasegawa 1989), which is also called the RELL test, is implemented as in PAML (Yang 2000) from which I have taken part of the code. The Kishino–Hasegawa test, as is practiced in literature, is analogous to the test in DNAPARS mentioned above, except that the test is based on the likelihood values rather than on the number of steps. In short, one calculates the log-likelihood for each topology, the difference in log-likelihood between the best tree and each of the alternative topologies, and the variance of the differences estimated by resampling methods such as bootstrapping. The z score is then calculated and declared as significant if it is larger than 1.96. Again, such interpretation is heuristic and is not appropriate probabilistically if there are more than two topologies being compared. DAMBE does the same computation but uses the Newman–Keuls test which is more appropriate for multiple comparisons.

Phylogenetic Tree Viewing and Manipulation
DAMBE can graph and print publication-quality trees. The tree-displaying window is scrollable and therefore can accommodate very large trees. The displayed tree can also be copied to presentation programs such as Microsoft PowerPoint.

Fitting Statistical Distributions to Substitutions Over Sites
It is important to know if the substitution rates vary among sites, because such rate heterogeneity, according to a comparative study based on simulated data (Kuhner and Felsenstein 1994), results in failure to recover the true phylogenetic relationships in virtually all commonly used phylogenetic programs (or algorithms), including the maximum-likelihood method (e.g., PHYLIP), maximum parsimony (e.g., PAUP), or neighbor-joining (e.g., MEGA) methods. DAMBE can fit the Poisson, negative binomial, and gamma distributions to substitution data over sites. The maximum-likelihood estimator for k in the neg-

ative binomial distribution is from Johnson et al. (1992), and that for the shape parameter in the gamma distribution is from Evans et al. (1993).

Graphics

In addition to graphically displaying and printing trees, DAMBE also produces a variety of graphic outputs including plotting one or more amino acid properties along amino acid sequences (e.g., polarity plot), saturation plots (i.e., transitions and transversions over divergence), variability-over-site plots, substitution-over-site plots, etc.

In short, DAMBE is a user-friendly program for the Windows platform that features a suite of unique features as well as the capability of performing most routine data analyses in molecular biology, ecology, and evolution.

From the Bioinformatics Laboratory, Department of Ecology and Biodiversity, University of Hong Kong, Pokfulam Road, Hong Kong. DAMBE has incorporated codes from PHYLIP with permission from J. Felsenstein, and from the BASEML program in PAML with permission from Z. Yang. Part of the codes for sequence alignment in DAMBE are taken from the program CLUSTAL by D. Higgins, J. Thompson, and T. Gibson. DAMBE development is supported by a CRCG

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