



DnaSP, DNA polymorphism analyses by the coalescent and other methods

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ABSTRACT

Summary: DnaSP is a software package for the analysis of DNA polymorphism data. Present version introduces several new modules and features which, among other options allow: (1) handling big data sets (~5 Mb per sequence); (2) conducting a large number of coalescent-based tests by Monte Carlo computer simulations; (3) extensive analyses of the genetic differentiation and gene flow among populations; (4) analysing the evolutionary pattern of preferred and unpreferred codons; (5) generating graphical outputs for an easy visualization of results.

Availability: The software package, including complete documentation and examples, is freely available to academic users from: <http://www.ub.es/dnasp>

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INTRODUCTION

Recent advances in DNA sequencing and polymorphism detection methodologies are generating huge data sets of DNA sequence variation and of single nucleotide polymorphisms (SNPs). Analysis of such DNA polymorphism data will definitively enhance our understanding of both the evolutionary significance of DNA polymorphisms and of the evolutionary history of populations and species (Nordborg and Innan, 2002). Additionally, DNA polymorphism information has a wide range of applications, including pharmacogenomics, animal and plant breeding, conservation genetics, epidemiology genetics, medicine and forensics.

Current massive data sets are stimulating the development of numerous methods to interpret DNA polymorphism data. These methods capture different features of the data (SNP frequency, association among variants, haplotype structure, synonymous and non-synonymous changes, recombinational events, codon usage, etc.) (Rosenberg and Nordborg, 2002; Bamshad and Wooding, 2003). In this context, the coalescent

theory (see Hudson, 1990; Rosenberg and Nordborg, 2002) has become the primary framework to analyse the data. Indeed, coalescent-based methods are critical for detecting the signature of positive natural selection, in the identification of haplotype blocks across the genome, or for inferring the effect of intragenic recombination. Here, we describe version 4 of the DnaSP software package (Rozas and Rozas, 1999). Present version largely extends the capabilities of the software allowing extensive DNA polymorphism analyses on a user-friendly interface.

SYSTEMS AND METHODS

DnaSP version 4 is written in Microsoft Visual Basic v. 6.0 and runs on ix86 compatible processors under Microsoft Windows®. DnaSP can also run on Apple Macintosh, Linux and Unix-based platforms using Windows emulator software with one of the required Microsoft Windows® versions.

MAIN NEW FEATURES

DnaSP provides a user-friendly Microsoft Windows graphic interface and can read (and export) five multiple-aligned nucleotide sequence file formats: FASTA, MEGA, NBRF/PIR, NEXUS and PHYLIP. DnaSP allows the analysis of polymorphism, divergence, genetic differentiation, gene flow, gene conversion, linkage disequilibrium, recombination, codon usage and also conducts a number of neutrality tests. The analyses can be performed in a subset of sites (including synonymous, non-synonymous, non-coding, i-fold degenerate sites) or in a subset of DNA sequences. Coding region analysis can be performed using a number of predefined genetic codes and codon usage tables.

Coalescent-based methods

DnaSP has extensively increased the capabilities of the coalescent-based analyses. Present DnaSP version allows conducting most of the developed neutrality tests (with and without outgroup) and linkage disequilibrium statistics, including—among others—(1) Tajima's, Fu's and Fu and Li's tests (Tajima, 1989; Fu and Li, 1993; Fu, 1997);

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(2) Depaulis and Veuille's haplotype-based tests (Depaulis and Veuille, 1998); (3) *B*- and *Q*-tests (Wall, 1999); (4) *H*-test (Fay and Wu, 2000); (5) Z_{NS} , Z_Z and Z_A linkage disequilibrium based-statistics (Kelly, 1997; Rozas *et al.*, 2001). DnaSP also computes a number of statistical tests for detecting population growth including the recently developed R_2 test (Ramos-Onsins and Rozas, 2002). The Monte Carlo computer simulation module allows generating the empirical distribution for a very large number of test statistics. Simulations can be conducted for different recombination rates.

Gene flow and genetic differentiation

The *Gene Flow* module has been completely rewritten. Present version allows performing a number of gene flow and genetic differentiation among population analyses with different options for treating alignment gaps. To detect genetic differentiation among subpopulations DnaSP implements several statistics based both on the number of haplotypes and on the number of nucleotide changes (i.e. sequence-based statistics) (Hudson *et al.*, 1992a; Hudson, 2000). DnaSP also estimates several parameters of the standardized measure of the genetic diversity among populations (F_{ST} , and the related statistics G_{ST} , N_{ST}) (see Hudson *et al.*, 1992b). From these F_{ST} based estimators, the migration rates (in terms of Nm ; where m is the migration rate) are obtained. The outcome values can be exported as a distance data file (PHYLIP and MEGA formats) for further phylogenetic analyses. DnaSP incorporates two methods to test for genetic differentiation: (1) the standard χ^2 homogeneity test and (2) a Monte Carlo permutation (randomization) test (Hudson *et al.*, 1992a).

Analysis of preferred and unpreferred codons

Present version implements a number of algorithms and methods to analyse the impact of natural selection and mutational processes on codon usage bias. In addition to the standard codon usage bias estimators (CBI, ENC, Scaled χ^2 etc.), DnaSP also implements an algorithm to identify preferred (P) and unpreferred (U) synonymous changes. This information is critical for determining the effect of natural selection (weak selection) on synonymous codons (see Akashi, 1999). DnaSP allows estimating the numbers of preferred and unpreferred changes within species (which requires the availability of one outgroup to polarize the mutations), and also those changes polymorphic within species and fixed between species (which requires the availability of two outgroups). DnaSP also provides several predefined codon usage tables. The user, additionally, can also define his own codon usage table; this user-defined information can be stored on a private block of the NEXUS file format.

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