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SHORT COMMUNICATION

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Correlation between aerobic and anaerobic resistance to metronidazole in trichomonads: application of a new computer program for permutation tests

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Abstract An indispensable step of any comparative study is testing the concordance between the distribution of phenetic traits and the evolutionary history of the taxa under study. Here we present a computer program TREEPT which can perform these tasks on the basis of a permutation test. The use of the program was demonstrated on trichomonad drug sensitivity data. The program can also perform permutation tests analogous to the t-test, analysis of variance (ANOVA) and correlation analysis and is available at http://www.natur.cuni.cz/~flegr/PROGRAMS/TREEPT.ZIP and http://www.karlin.mff.cuni.cz/-zaboj/treept.

The testing of concordance between the distribution of a particular trait and the evolutionary history of a taxon is a principal task of many comparative studies (Harvey and Pagel 1991). The distribution of a trait among representatives of the taxon can reflect either the distribution of a common function and, therefore, of a common selective pressure pattern (the species subjected to the same selective pressure have the same trait) or the evolutionary history of the taxon (the phylogenetically relative species share the traits). The existence of a statistically significant association between the distribution of the trait and the position of the species (or strains) within the genealogic tree indicates that the distribution of the trait reflects simply a random process of evolutionary history of the taxon cladogenesis (Archie 1989).

Several approaches are being used for testing of concordance between the distribution of the trait and the evolutionary history of a taxon, depending on the type of data (character set/distance matrices) available for the studied trait. If the trait is described by character data, a cladistic analysis can be performed with forced tree topology (reflecting the previously known cladogenesis of the taxon). The consistency index provided by common cladistic programs can be used as a simple measure of the degree of concordance between the distribution of the trait and the phylogeny. The null model can be tested by a permutation-tail probability test (Moore et al. 1994). If the trait is described by distance data, Mantel tests can be used to test one or more hypotheses (independent variables represented as matrices) against an observed pattern (dependent matrix) using (partial) regression or correlation (Thorpe 1996).

The latter method is more universal because any character data can be transformed to distance matrices. However, before analysis the distances should be corrected for differences in the rates of evolution in different branches of the phylogram. Moreover, no integrated public-domain software exists for Mantel tests or for other important types of permutation tests.

Recently we developed the program TREEPT for various types of permutation tests, including those for analysis of concordance between the distribution of traits and a phylogeny. The program can analyze the qualitative and quantitative character data as well as the distance matrices. The phylogenetic tree can be entered in the usual bracketed format. The average distance between sister OTUs (operational taxonomic units, i.e., sister strains or sister branches of the tree) is calculated (or read from a distance matrix) and used as a measure of concordance, which is tested in a one-sided or two-sided permutation-tail test (Manly 1991). The program can generate all possible permutations of terminal branches of the tree or, alternatively, the number of trees to be generated can be user-defined. Usually, 5000 ran-

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Table 1 Susceptibility of *Trichomonas vaginalis* strains to metronidazole[a]

Strain	MLC aerobic			MLC anaerobic		
	Mean	Range	*n*	Mean	Range	*n*
IR-78	107.20	50–200	20	2.45	1.56–6.25	20
Tv79-49	1.92	1.56–6.5	10	1.46	0.78–3.125	10
Tv71-96	3.35	3.125–6.25	10	1.10	0.78–1.56	10
FF28	2.14	1.56–3.125	11	1.38	0.78–3.125	11
TALL-MT	3.125	3.125	10	1.67	1.56–3.125	10
CP-1	2.01	0.78–6.25	11	0.78	0.39–1.56	11
JH-31A	3.85	1.56–6.25	11	1.03	0.78–1.56	10
C-1:NIH	1.56	1.56	6	0.78	0.78	5
JT	2.50	1.56–3.125	6	0.67	0.39–0.78	9
Tv10-02	4.7	3.125–12.5	36	1.70	1.56–3.125	48
CDC-85	1000[b]			16.0[b]		

[a] Data represent geometric mean MLC values for metronidazole (in μg) as determined by in vitro microtiter plate assay (Tachezy et al. 1993) under aerobic and anaerobic conditions
[b] Data obtained from Müller et al. (1988)

dom trees provide a stable estimation of the *P* value and can be generated by an ordinary personal computer within seconds.

The use of the program can be demonstrated on trichomonad drug-susceptibility data. Some strains of parasitic protozoan *Trichomonas vaginalis* show either aerobic or anaerobic resistance to the main antitrichomonad drug metronidazole. The mechanisms of these two types of resistance seem to be different (Čerkasovova et al. 1988). However, the clinical isolates of *T. vaginalis* isolated from patients refractory to treatment with metronidazole that have thus far been tested have consistently displayed the aerobic type of resistance. Therefore, routine susceptibility assays are performed under aerobic conditions. Preliminary laboratory results suggest a possible correlation between the values for aerobic and anaerobic susceptibility measured in in vitro tests (Lossick et al. 1986). Unpublished drug-susceptibility data available at our department include values for the minimal lethal concentration (MLC) of metronidazole for 11 strains of *T. vaginalis*.

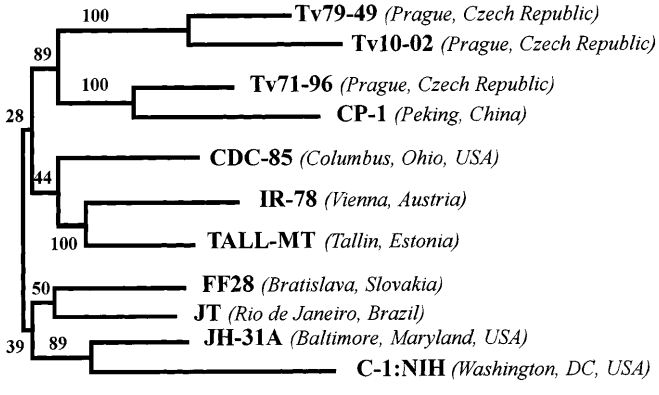

Fig. 1 Phylogenetic tree created for 11 strains of *Trichomonas vaginalis*. The geographic origins of the strains are shown in *parentheses*. The *numbers* show the OTU-based jackknifing values (in percent), which reflect the statistical support for the existence of particular branches

The MLC values were obtained both under aerobic and anaerobic conditions using a standard microplate assay (Tachezy et al. 1993; Table 1). We used these data to test the correlation between aerobic and anaerobic MLC values. The resistance data showed a non-normal distribution. Therefore, we used a nonparametric Spearman correlation test. The results [Spearman $R = 0.674$, $t(9) = 2.74$] suggested that a significant ($P = 0.022$) correlation existed between the aerobic and anaerobic resistance of the strain. The same results provided a permutation test of correlation implemented in the TREEPT program ($P = 0.01$). Both tests work well under the condition that the data for different strains represent independent observations. Because of the existence of phylogenetic relationships between different strains, this condition can be violated. Therefore, the possible concordance between the drug susceptibility and the strain phylogeny should be tested first. The phylogenetic tree generated for 11 strains of trichomonads using DNA-fingerprinting data by the neighbor-joining method (Saitou and Nei 1987) is shown in Fig. 1.

The concordance between the position of the strain within the tree and the aerobic or anaerobic MLC value was estimated by a permutation test, typing:

TREEPT –n 5000 ((((1.92 4.7)(3.35 2.01))
((107.2 3.125)1000))((2.14 2.5)(1.56 3.85)))

and

TREEPT –n 5000 ((((1.4 61.7)(1.1 0.78))((2.45 1.67)16.0))
((1.38 0.67)(0.78 1.03))),

respectively. The results suggest a significant concordance between aerobic ($P = 0.014$) and anaerobic ($P = 0.0023$) drug resistance and the strain phylogeny. Therefore, the results of the correlation tests could be positively biased and could not be used with presently available data as a proof of correlation between the two types of drug susceptibility.

The program TREEPT can also perform the permutation tests in a manner analogous to that used for the *t*-test, analysis of variance (ANOVA), and correlation analysis. As shown by Adams and Anthony (1996), the permutation tests can be used for non-normally distributed data and are generally more powerful than analogous nonparametric tests. (The program TREEPT is available at http://www.karlin.mff.cuni.cz/~zaboj/treept.)

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