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

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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 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 statistically significant association between the distribution of the trait and the position of the species (or strains) within the genealogical tree indicates that the distribution of the trait simply reflects a random process of evolutionary history of the taxon cladogenesis (Archie 1989).

Several approaches are being used for testing concordance between the distribution of the trait and evolutionary history of a taxon depending on the type of data (character set/distance matrices) of the studied trait. If the trait is described by character data, a cladistic analysis can be performed with forced tree topology (reflecting the already known cladogenesis of the taxon). The consistency index provided by common cladistic programs can be used as simple measure of the degree of concordance between 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 (dependant matrix) using (partial)

regression or correlation (Thorpe 1996).

The later 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 rates of evolution in different branches of phylogram. Moreover, no integrated public domain software exists neither for Mantel tests nor for other important types permutation tests.

Recently we developed program TREEPT for various types of permutation tests, including those for analysis of concordance between 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 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 one-sided or two sided permutation tail test (Manly 1991). The program can either generate all possible permutations of terminal branches of the tree or the number of trees to be generated can be user-defined. Usually 5000 random trees provide stable estimation of p-value and can be generated by ordinary PC 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 seems to be different (erkasovova et al. 1988). However, the clinical isolates of *T. vaginalis* isolated from patients refractory to treatment with metronidazole tested so far consistently displayed the aerobic type of resistance. Therefore the routine susceptibility assays are performed under aerobic conditions. Preliminary laboratory results suggested possible correlation between the values of aerobic and anaerobic susceptibility measured in *in vitro* tests (Lossick et al. 1986). Unpublished drug-susceptibility data available at our department included values of minimal lethal concentration (MLC) of metronidazole for 11 strains of *T. vaginalis*. The MLC values were obtained both under aerobic and anaerobic conditions by using a standard microplate assay (Tachezy et al. 1993) (Tab 1). We used these data for testing 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. Same results provided a permutation test of correlation implemented in TREEPT program (p=0.01). Both tests work well under the condition that the data for different strains represents independent observations. Because of the existence of phylogenetical 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 of 11 strains of trichomonads obtained from DNA-fingerprinting data by

Neighbor Joining method (Saitou and Nei 1987) is shown in the Fig. 1. The concordance between position of the strain within the tree and the aerobic or anaerobic MLC 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 ((((1.46 1.7)(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 with presently available data cannot be used as a proof of correlation between both types of drug susceptibility.

The program TREEPT can also perform the permutation tests analogous to t-test, analysis of variance (ANOVA) and to 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 analogical non-parametric tests. The program TREEPT is available at http://www.karlin.mff.cuni.cz/~zaboj/treept.

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References

Adams DC, Anthony CD (1996) Using randomization techniques to analyse behavioural data. Anim Behav 51:733-738

Archie JV 1989: A randomization test for phylogenetic information in systematic data. Syst. Zool. 38: 239-252

erkasovova A, erkasov J, Kulda J (1988) Resistance of trichomonads to metronidazole. Acta Univ Carol Biol 30:485-503

Harvey PH & Pagel MD 1991: The comparative method in evolutionary biology. Oxford University Press, Oxford

Lossick JG, Müller M, Gorrell TE (1986) In vitro drug susceptibility and doses of metronidazole required for cure in cases of refractory vaginal trichomoniasis. J Infect Dis 153:948-955

Manly BFJ (1991) Randomization and Monte Carlo methods in biology. Chapman and Hall, London

Moore J, Freehling M, Gotelli NJ (1994) Altered behavior in two species of blattid cockroaches infected with *Moniliformis moniliformis* (*Acanthocephala*). J Parasitol 80:220-223

Müller M, Lossick JG, Gorrell TE (1988) In vitro susceptibility of *Trichomonas vaginalis* to metronidazole and treatment outcome in vaginal trichomonasis. Sex Transm Dis 15:17-24

Saitou N & Nei M (1987) The neighbor-joining method: A new method for

reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425

Tachezy J, Kulda J, Tomkova E (1993) Aerobic resistance of *Trichomonas vaginalis* to metronidazole induced in vitro. Parasitology 106:31-37

Thorpe RS (1996) The use of DNA divergence to help determine the correlates of evolution of morphological characters. Evolution 50:524-531

Table 1

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 *			16.0*		

*data obtained from literature (Müller et al. 1988)

Table 1. Susceptibility of *Trichomonas vaginalis* strains to metronidazole. The table shows the geometric means of minimal lethal concentrations for metronidazole (in µg) as determined by *in vitro* microtitre plate assay (Tachezy et al. 1993) under aerobic and anaerobic conditions.

Fig. 1

Phylogenetic tree for eleven strains of *Trichomonas vaginalis*.The geographic origins of 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.