**MetATT: a web-based metabolomics tool for analyzing time-series and two-factor datasets**

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**ABSTRACT**

**Summary:** Time-series and multifactor studies have become increasingly common in metabolomic studies. Common tasks for analyzing data from these relatively complex experiments include identification of major variations associated with each experimental factor, comparison of temporal profiles across different biological conditions, as well as detection and validation of the presence of interactions. Here we introduce MetATT, a web-based tool for time-series and two-factor metabolomic data analysis. MetATT offers a number of complementary approaches including 3D interactive principal component analysis, two-way heatmap visualization, two-way ANOVA, ANOVA-simultaneous component analysis and multivariate empirical Bayes time-series analysis. These procedures are presented through an intuitive web interface. At the end of each session, a detailed analysis report is generated to facilitate understanding of the results.

**Availability:** Freely available at http://metatt.metabolomics.ca

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**1 INTRODUCTION**

Metabolomics involves the study of multiple small-molecule compounds in a biological system. Biofluids (i.e. urine, blood) are by far the most commonly used sample type in metabolomic studies. These samples can be collected conveniently and relatively non-invasively, and are often used in longitudinal studies such as monitoring disease progression, tracking nutritional interventions or observing drug toxicity. Biofluids have also been increasingly used in cross-sectional studies for biomarker discovery. One important lesson learned from these studies is that metabolomic profiles of many biofluids are very sensitive to multiple factors such as diet, age, gender, etc. (Paloggius et al., 2008; Slupsky et al., 2007). Therefore, one often needs to consider other factors together with the biological condition of interest during data analysis. Specially designed statistical approaches have been developed to deal with the multifactor issues as well as for time-series studies, such as parallel factor analysis (PARAFAC) (Harshman and Lundy, 1994), ANOVA-simultaneous component analysis (ASCA) (Smilde et al., 2005), multivariate empirical Bayes time-series analysis (MEBA) (Tai and Speed, 2006) and multivariate multivariate time analysis of multisource data (Huopaniemi et al., 2010). However, most of these methods are only available in the form of command-line scripts written in MATLAB (The MathWorks Inc., Natick, MA, USA) or R (http://www.r-project.org/). Consequently, researchers need to have a deep understanding of the programming languages in order to use these methods. To help overcome this barrier, we have created MetATT, an easy-to-use web-based tool designed to perform common tasks involved in time-series and two-factor metabolomic data analysis, including identification of major patterns associated with main experimental factors, comparison of temporal profiles across biological conditions, as well as detection and validation of the presence of interactions. MetATT is available at http://metatt.metabolomics.ca. It has also been incorporated to MetaBAnalyt (Xia et al., 2009, http://www.metaboanalyst.ca).

**2 METHODS**

**2.1 Data visualization**

To facilitate data exploration and pattern discovery, MetATT offers two visualization approaches—3D interactive PCA (iPCA) and two-way heatmaps. The iPCA supports rotation, zooming and highlighting. Clicking on any data point will display the characteristic variables of the corresponding sample. The heatmap supports a variety of options on clustering, coloring and sorting. Users can adjust the display by using a combination of the available options from the drop-down menus.

**2.2 Statistical algorithms**

MetATT supports the classical two-way ANOVA and the two recent multivariate inventions—ASCA and MEBA. The two-way ANOVA method supports within- and between-subjects analysis for time-series and independent sample measures, respectively. The ASCA approach first decomposes the variation of the entire data into individual variations induced by each experimental factor and their interactions; it then applies PCA to each variation to get a simple and interpretable result. MetATT also provides ASCA model validation (Vis et al., 2007) and variable selection (Nueda et al., 2007). The MEBA method assesses treatment differences by comparing time-course mean profiles while allowing for variability both within and between time points, thereby reducing false positives and false negatives.

**2.3 Implementation of web application**

MetATT’s web interface was implemented using the JavaServer Faces technology. The 3D interactive visualization tools are based on the LiveGraphics3D technology (Glaab et al., 2010). The statistical algorithms were written in R. MetATT is hosted on GlassFish (version 3.1) using a Linux operating system (Ubuntu 10.04 LTS). The web application has been successfully tested on all major web browsers with a Java plug-in installed.
ANOV A. As shown in Figure 1A, the interaction effects are
Accepted data formats are described in the ‘Data Format’ section
variables associated with Time and (C) two variables with distinctive temporal profiles identified by MEBA.

3 EXAMPLE ANALYSIS
Here, we present the analysis on a subset of the data from a recently
published time-course metabolomics study (Meinicke et al., 2008).
Accepted data formats are described in the ‘Data Format’ section
of the MetATT online documentation. The study was designed to
compare the metabolic profiles of two Arabidopsis thaliana lines—
wide type (WT) and dde 2-2 mutant (MT) during a wounding
time-course. Samples were collected at four time points. This dataset
is available as the test data on MetATT’s data upload page. In this
example, we use the default parameters for data uploading and
normalization.

Data visualization: click the ‘iPCA’ or ‘Heatmap2’ node on the
navigation tree on the left panel to access these two methods.
Both show clear clustering patterns with regard to time points or
phenotypes. Two-way ANOVA: click the ‘ANOVA2’ node to access
this function. As the data consists of time-series repeated sample
measures, the ‘within-subject ANOVA’ is used. The results are
summarized in a Venn diagram (Fig. 1A). It is clear that many
variables are significant with regard to each experimental effect and
their interactions. Click the ‘View details’ link to view a detailed
table of these variables. Detection of major trends: click the ‘ASCA
node on the navigation tree. Accept the default parameters for
component selection. Click the ‘Major Patterns’ tab to view the
major trends associated with each experimental factor as well as
the interaction effect. Click the ‘Sig. Features’ tab to view variables
that follow these trends (well modeled) as well as those that clearly
deviate (outliers) (Fig. 1B).

Testing the presence of interactions: for individual variables,
the interaction effect can be assessed by univariate two-way
ANOVA. As shown in Figure 1A, the interaction effects are
significant for more than half of the variables. The overall interaction
effect can be assessed by permutation tests on the ASCA model
for interaction. Click the ‘Model Validation’ tab on the ASCA
page. The result indicates the overall interaction effect is very
significant.

Identification of differential temporal profiles: click the ‘MEBA
node to perform multivariate empirical Bayes analysis. The result is
a list of all variables ranked by Hotelling’s $T^2$. Clicking any variable
name will display the corresponding temporal profiles (Fig. 1C).

4 CONCLUSIONS
The growing number of metabolomics being applied to various
time-course and multifactor studies has increased the need for user-
friendly bioinformatics tools to handle these types of data. MetATT
is a full-featured, easy-to-use web-based tool that is designed to
perform common tasks for time-series as well as general two-factor
metabolomic data analysis and visualization.

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