Hotelling’s test for highly correlated data

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Abstract: This paper is motivated by the analysis of gene expression sets, especially by finding differentially expressed gene sets between two phenotypes. Gene log_2 expression levels are highly correlated and, very likely, have approximately normal distribution. Therefore, it seems reasonable to use two-sample Hotelling’s test for such data. We discover some unexpected properties of the test making it different from the majority of tests previously used for such data. It appears that the Hotelling ’s test does not always reach maximal power when all marginal distributions are differentially expressed. For highly correlated data its maximal power is attained when about a half of marginal distributions are essentially different. For the case when the correlation coefficient is greater than 0.5 this test is more powerful if only one marginal distribution is shifted, comparing to the case when all marginal distributions are equally shifted. Moreover, when the correlation coefficient increases the power of Hotelling’s test increases as well.

1 Introduction

In many situations statisticians need to test multidimensional hypotheses. In a lot of cases components of observed random vectors are highly dependent, which may change the properties of the tests used. One of the examples of such data is provided by gene expression levels. Gene expressions are highly correlated between genes (see for example [Klebanov and Yakovlev (2007)]). Moreover, often the genes are investigated not just separately, but also as a set of dependent genes. Therefore one has to deal with multidimensional hypotheses and in order to test such hypotheses, gene sets should be expressed differentially. The most popular tests for gene sets are Hotelling’s test, N-test and tests derived from marginal t-statistics. In the papers [Ackermann and Strimmer (2009), Glazko and Emmert-Streib (2009)], an approach to comparing these test in various situations was made. Our goal is not to make another comparison, but rather to describe some interesting properties of the Hotelling’s test which seems to be unexpected.

2 Hotelling’s test

One of the most well known tests is t-test. Hotelling’s test is a multidimensional extension of t-test. Similar to t-test, we can consider both one-sample and two-sample Hotelling’s test. One-sample case deals with the hypothesis that the expected value of a sample from multidimensional normal distribution is equal to some given vector. In the two-sample case it deals with the hypothesis of the equality of expected values of two samples from multidimensional normal distributions (with the equal covariance structure). In this paper we will focus on the two-sample Hotelling’s test.

Suppose we have two independent samples (of sizes $n_x$ and $n_y$, respectively) from two $n$-dimensional normal distributions with identical covariance matrices equal to $\Sigma$. In other words, we consider $X_1, \ldots, X_{n_x}$ as i.i.d random vectors having $N_n(\mu_x, \Sigma)$ and $Y_1, \ldots, Y_{n_y}$ as i.i.d random vectors having $N_n(\mu_y, \Sigma)$ ($X_i$ and $Y_j$ are independent for all $i = 1, \ldots, n_x; j = 1, \ldots, n_y$). For simplicity we assume that $n < n_x + n_y - 1$. Our goal is to test the hypothesis $H : \mu_x = \mu_y$ against alternative $A : \mu_x \neq \mu_y$. For this we use Hotelling’s test based on the statistic

$$T^2 = \frac{n_x n_y}{n_x + n_y} (\bar{X} - \bar{Y})^T S^{-1}(\bar{X} - \bar{Y}),$$

(1)
where \( \bar{X} = \frac{1}{n_x} \sum_{i=1}^{n_x} X_i; \bar{Y} = \frac{1}{n_y} \sum_{i=1}^{n_y} Y_i \), and \( S = \frac{\sum_{i=1}^{n_x} (X_i - \bar{X})(X_i - \bar{X})^T + \sum_{i=1}^{n_y} (Y_i - \bar{Y})(Y_i - \bar{Y})^T}{n_x + n_y - 2} \). \( T^2 \) is related to the \( F \)-distribution by

\[
\frac{n_x + n_y - n - 1}{n(n_x + n_y - 2)} T^2 \sim F(n, n_x + n_y - n - 1).
\]

For more details about Hotelling’s test see, for example, [Chatfield and Collins (1980)]. We made the assumption \( n < n_x + n_y - 1 \) for two reasons. For \( n \geq n_x + n_y - 1 \) the estimate \( \hat{S} \) of \( \Sigma \) results in an irregular matrix, so \( \hat{S}^{-1} \) does not exist and moreover numerator of (2) is non-positive as well as the degree of freedom of the \( F \)-distribution. In such situations it is possible to use some pseudo-inversion of \( S \) and in order to estimate \( p \)-value of \( H \), we can use permutations of \( (X_1, ..., X_n, Y_1, ..., Y_m) \).

### 3 Hotelling’s test for strongly dependent data

As it was mentioned above, genes are highly dependent and we will suppose that their \( \log_2 \) expression levels have approximately normal distributions. Many papers work with gene sets (for example, \([Barry et al. (2008)](\text{Barry et al. (2008)})\)) instead of genes alone and therefore deal with multidimensional hypotheses. It seems to be reasonable to use Hotelling’s test in this situation.

Assume that we have two multidimensional samples and need to test the hypothesis suggesting the equality of expected values in these two samples. Assume for simplicity that all elements on the main diagonal of the covariance matrix \( \Sigma \) for both samples are equal to 1 and all other elements are equal to \( \rho > 0 \), i.e.

\[
\Sigma = \begin{pmatrix}
1 & \rho & \rho & \cdots & \rho \\
\rho & 1 & \rho & \cdots & \rho \\
\vdots & \vdots & \ddots & \ddots & \vdots \\
\rho & \cdots & \rho & 1
\end{pmatrix}.
\]

Further on, we assume that \( \mu_x = (0, ..., 0)^T \), but \( \mu_y \) has first \( m \) elements equal to 1 and the others equal to 0, i.e.

\[
\mu_y = \left(1, ..., 1, 0, ..., 0\right)^T.
\]

For large \( n_x \) and \( n_y \) the matrix \( \Sigma \) and its estimate \( \hat{S} \) are approximately the same as well as the differences between the expected values \( (\mu_x - \mu_y) \) and between the mean values \( (\bar{X} - \bar{Y}) \). When dialing with real data, \( n_x \) and \( n_y \) might not be large enough, but for theoretical reasons we may use the approximations \( \hat{S} \approx \Sigma \) and \( \bar{X} - \bar{Y} \approx \mu_x - \mu_y \). In this case \( \hat{S}^{-1} \approx \Sigma^{-1} \), that is

\[
\hat{S}^{-1} \approx \Sigma^{-1} = \begin{pmatrix}
\alpha & -\beta & -\beta & \cdots & -\beta \\
-\beta & \alpha & -\beta & \cdots & -\beta \\
-\beta & \cdots & \ddots & \ddots & \vdots \\
-\beta & \cdots & -\beta & \alpha
\end{pmatrix},
\]

where \( \alpha = \frac{(1+\rho)(n_x-1)}{(1-\rho)(1+\rho)(n_x-2)} \) and \( \beta = \frac{\rho}{(1-\rho)(1+\rho)(n_x-1)} \). For fixed \( n_x \) and \( n_y \) we can consider the fraction \( \frac{n_x n_y}{n_x n_y} = k \) of Hotelling’s statistic (1) as a normalizing constant. Let us denote \( T^2 \) Hotelling’s statistic with \( \Sigma^{-1} \) instead of \( S^{-1} \) and \( \mu_x - \mu_y \) instead of \( \bar{X} - \bar{Y} \) divided by the constant \( k \). Therefore, we have

\[
T^2/k \approx T^* = (\mu_x - \mu_y)^T \Sigma^{-1} (\mu_x - \mu_y)
\]
It can be seen from figure 1 as well. Moreover, the statistic alternative with only one marginal shift than for alternative that all marginal distributions are equally shifted. Suppose that

\[ T^* \text{ is not very small we have maximal power for } \]

Hotelling’s test for two-dimensional data

\[ \text{Since the denominator is greater than zero, then } \]

\[ m \alpha - (m^2 - m)\beta = \frac{m(1 + (n - 2)\rho) - m(m - 1)\rho}{(1 - \rho)(1 + (n - 1)\rho)} = \frac{m(1 + (n - m - 1)\rho)}{(1 - \rho)(1 + (n - 1)\rho)}. \]

Let us note that it does not matter if \( \mu_i \) consists of ones and zeros or equals to a constant and zeros. In the latter case, statistic \( T^{*2} \) would be multiplied by \( a^2 \). Now we will work with statistic \( T^{*2} \) and investigate its behavior.

If we expected that \( T^{*2} \) is an increasing function of \( m \) then \( \alpha - m^2\beta \) should be greater then zero. But we have

\[ \alpha - 2m\beta = \frac{1 + (n - 2)\rho}{(1 - \rho)(1 + (n - 1)\rho)} - \frac{2m\rho}{(1 - \rho)(1 + (n - 1)\rho)} = \frac{1 + (n - 2m - 2)\rho}{(1 - \rho)(1 + (n - 1)\rho)}. \]

Since the denominator is greater than zero, then \( \alpha - 2m\beta > 0 \) only if \( \frac{1}{2m + 2 - n} > \frac{\rho}{2n - n} \). It means that for not very small values of \( \rho \)'s and \( m > \frac{n}{2} - 1 \) the statistic \( T^{*2} \) is a decreasing function of \( m \). This means that maximal power of Hotelling’s test (as a function of \( m \)) is not always attained for \( m = n \) but for \( \rho \)'s which are not very small we have maximal power for \( m \) near \( \frac{n}{2} \). Some examples of the behavior of \( T^{*2} \) as a function of \( m \) are illustrated on figure 1.

However, this issue is not the only one that is surprising about Hotelling’s test. Now we look if \( T^{*2} \) is always lower than \( T_n^{*2} \). It is the case when one different marginal distribution influences more than all \( n \) different distributions. So we need to compare \( \alpha \) with \( n\alpha - n(n - 1)\beta \). We have

\[ T_1^{*2} - T_n^{*2} = \alpha - n\alpha + n(n - 1)\beta = (n - 1)\frac{(1 - 2\rho)}{(1 - \rho)(1 + (n - 1)\rho)}. \]

So \( T_1^{*2} - T_n^{*2} > 0 \) only if \( \rho < 0.5 \). Therefore we can say that for \( \rho > 0.5 \) Hotelling’s test has better power for alternative with only one marginal shift than for alternative that all marginal distributions are equally shifted. It can be seen from figure 1 as well. Moreover, the statistic \( T^{*2} \) is an increasing function of \( \rho \), that may seem surprising as well.

### 4 Hotelling’s test for two-dimensional data

Let us look at Hotelling’s test in the two-dimensional case. As in the previous case, we will consider the two-sample problem, but now we will generalize the difference of expected values of these two samples. Suppose that \( \mu_i - \mu_j = (a_1, a_2) \) and that the covariance matrix is

\[ \Sigma = \begin{pmatrix} 1 & \rho \\ \rho & 1 \end{pmatrix}. \]
Then inverse of $\Sigma$ is the matrix with diagonal elements $\alpha = \frac{1}{(1 - \rho)(1 + \rho)}$ and off-diagonal elements $-\beta = -\frac{\rho}{(1 - \rho)(1 + \rho)}$. Then

$$T^{*2} = \alpha a_1^2 + \alpha a_2^2 - 2\beta a_1 a_2.$$  

First we consider that $a_1 = 1$ and $a_2 = 0$. Then $T^{*2} = \alpha$. Now we will investigate for which $a_1, a_2 \in \mathbb{R}$ statistic $T^{*2} = \alpha$. That is, we need to solve an equation

$$\alpha a_1^2 + \alpha a_2^2 - 2\beta a_1 a_2 = \alpha.  \tag{4}$$

After dividing both sides of equation (4) by $\alpha$ we get

$$a_1^2 + a_2^2 - 2\rho a_1 a_2 - 1 = 0. \tag{5}$$

For fixed $a_1$ equation (5) is quadratic in $a_2$ with the roots

$$a_{21,2} = \frac{2\rho a_1 \pm \sqrt{(2\rho a_1)^2 - 4(a_1^2 - 1)}}{2}.$$
It is defined only if \((2\rho a_1)^2 - 4(a_1^2 - 1) \geq 0\), i.e. for \(|a_1| \leq \sqrt{1/\rho}\). Some plots of the solutions of the equation \(5\) for different values of the correlation coefficient \(\rho\) are given on figure [2]. We can see that the plots of these solutions produce elliptic curves. Let us rotate these ellipses by the angle \(\varphi = \pi/4\) clockwise. To do this, we use transformation
\[
a_1 = x \cos \varphi - y \sin \varphi = \frac{\sqrt{2}}{2} x - \frac{\sqrt{2}}{2} y,
\]
\[
a_2 = x \sin \varphi + y \cos \varphi = \frac{\sqrt{2}}{2} x + \frac{\sqrt{2}}{2} y,
\]
where \(x\) and \(y\) are new rotated coordinates. After substitution into \(5\) it gives
\[
\left(\frac{\sqrt{2}}{2} x - \frac{\sqrt{2}}{2} y\right)^2 + \left(\frac{\sqrt{2}}{2} x + \frac{\sqrt{2}}{2} y\right)^2 - 2\rho \left(\frac{\sqrt{2}}{2} x - \frac{\sqrt{2}}{2} y\right)\left(\frac{\sqrt{2}}{2} x + \frac{\sqrt{2}}{2} y\right)
\]
\[= x^2(1 - \rho) + y^2(1 + \rho) = \frac{x^2}{a^2} + \frac{y^2}{b^2} = 1,
\]
where \(a = \sqrt{1/\rho}\) and \(b = \sqrt{1/\rho}\) are respectively the major radius and the minor radius of the ellipse. Since \(a > b\), the Hotelling’s test has the weakest power in the direction of \(a_1 = a_2\), while the fastest increase of its power is observed towards the direction of \(a_1 = -a_2\). For example, for \(\rho = 0.9\) we have \(a = 3.162\) and \(b = 0.725\). It means that for \(a_1 = a_2 = \sqrt{\frac{3.1632}{2}} = 2.236\) Hotelling’s test has approximately the same power as for \(a_1 = 1, a_2 = 0\) (or for \(a_1 = -a_2 = \sqrt{0.725^2} = 0.513\) as well). So, if there is only one marginal distribution shifted by one unit, then the power of Hotelling’s test is approximately the same as if both marginal distribution were equally shifted (in the same direction) by 2.236 units (for the shift in opposite direction it should be only 0.513 unit). These results are in contradiction with other multidimensional tests. For example, consider the test based on marginal \(t\)-statistics. The power of this test is higher if both distributions are shifted by the same amount (both \(t\)-statistics are "large", not depending on direction of shift) than if there was only one marginal distribution shifted (one \(t\)-statistic is "near" zero).

5 Theory and reality

The analytical results obtained above should be verified by checking if actual Hotelling’s test outcomes correspond to the analytical results regarding real data. In this section we will compare the behavior of theoretical Hotelling’s statistic \(T^2\) with real Hotelling’s statistic \(T^2\). For large \(n_x\) and \(n_y\) we assumed that \(T^2 \approx T^2/k\), where \(k = \frac{n_x n_y}{n_x + n_y}\). Constant \(k\) changes as \(n_x\) and \(n_y\) change. It is reasonable to divide Hotelling’s statistic \(T^2\) by \(k\) instead of multiplying \(T^2\) by \(k\) in order to be able to compare how do \(T^2\) and \(T^2\) differ for various \(n_x\) and \(n_y\).

In order to compare the actual results with the analytical ones, we did the following simulations. All data were simulated from \(n\)-dimensional normal distributions. Consider three different values for the number of genes in a gene set. We take \(n = 10, n = 15\) and \(n = 25\). All simulations were performed for three different values of the correlation coefficient \(\rho:\ \rho = 0.1, \rho = 0.5\) and \(\rho = 0.9\). In order to compare the behavior of Hotelling’s test for various sizes of samples we took three choices of \(n_x\) and \(n_y:\ n_x = n_y = n, n_x = n_y = 1.4n\) and \(n_x = 2.4n\). The value \(m\) which is the number of false marginal distributions varies from one to \(n\). The shift value for each of the different marginal distributions is set to one. The theoretical Hotelling’s statistic is calculated according to \(3\). Real Hotelling’s statistic is estimated from 1000 simulations for each
Plots of solutions of equation (??) for two-dimensional case for $\rho = 0.25; 0.5; 0.9$. Notice: each plot is differently scaled!

Plots of our simulated cases are shown on figure [3]. We can see that for all simulated situations, the shapes of real and theoretical Hotelling’s statistics are similar. The only difference is in the heights of these curves. For small $n_x$ and $n_y$, statistic $T^2$ has higher values than for large $n_x$ and $n_y$. The reason for that stems from the inaccurate estimates of the expected values and of the covariance matrix. However, we observe that with the increase of $n_x$ and $n_y$, statistic $T^2/k$ goes to $T^{*2}$ relatively fast. Therefore, the behavior of Hotelling’s test for real data is expected to be very similar to the behavior of statistic $T^{*2}$.

In previous section we saw that for the two-dimensional case the plotted shifts with equal values of the power of theoretical Hotelling’s test form elliptic curves. Hotelling’s statistics $T^2$ are random variables. Therefore, we can only estimate if their expected values form elliptic curves when plotted. To check this we did following simulations. Instead of calculating the shifts for which Hotelling’s test has equal powers, we took the points provided by the elliptic curves observed for theoretical Hotelling’s statistics. For each pair of these points $(a_1, a_2)$ we did 1000 simulations and calculated Hotelling’s statistic. We estimated the expected value $E(T^2/k)$ as the mean for these 1000 repetitions. We divided Hotelling’s statistics by $k$ for better understanding how fast these statistics go to $T^{*2}$. We did this simulation for the values of the correlation coefficient $\rho = 0.3$ and $\rho = 0.9$ and as the number of observations in each sample we took $n_x = n_y = 5$, $n_x = n_y = 10$ and $n_x = n_y = 20$. Results of our simulation are given in Table 1. We observe that estimated mean values of $T^2/k$ are not very different, that they go to $T^{*2}$ and that their variance decreases with increasing number of observations. Clearly, these points form elliptic curves. Hence, we can claim that the real Hotelling’s test behaves very similar to the theoretical one and the theory derived for the theoretical test holds for the real Hotelling’s test as well.

## 6 Discussion

In this paper we have discovered that two-sample Hotelling’s test (for testing the equality of the expected values of two samples from multidimensional normal distribution with equal covariance structure) has some unexpected properties. At first sight, one could expect that with a larger number of false marginal distribu-
Figure 3: Comparisons of theoretical statistics $T^2$ and real Hotelling’s statistic $T^2/k$ for number of genes $n = 10, 15, 25$ (from the top to the bottom); for correlation coefficient $\rho = 0.1, 0.5, 0.9$ (from the left to the right) and number of observations in each sample $n_x = n_y = n$ (denoted by ‘+’), $n_x = n_y = 1.4n$ (denoted by ‘x’) and $n_x = n_y = 2.4n$ (denoted by ‘●’). The theoretical statistic $T^2$ is denoted by ‘◦’. Number of different marginal distribution $m$ is set from one to $n$. Notice: each plot is differently scaled!

...
Table 1: Results of simulations of two-dimensional adjusted Hotelling’s statistics $T^2/k$ with $n_x = n_y = n_s$ observations for each sample and correlation coefficient $\rho$. $T^*^2$ stands for theoretical Hotelling’s statistics and $(a_1, a_2)$ is difference between expected values $\mu_x - \mu_y$ of these samples. On bottom line is the estimate of variance of each column.

| $T^*^2 = 1.0989$ | $\rho = 0.3$ | $T^*^2 = 5.2632$ | $\rho = 0.9$ |
|-------------------|-------------|-------------------|-------------|
| $a_1$ | $a_2$ | $n_x = 5$ | $n_x = 10$ | $n_x = 20$ | $a_1$ | $a_2$ | $n_x = 5$ | $n_x = 10$ | $n_x = 20$ |
| -0.84 | 0.35 | 3.12 | 1.74 | 1.35 | -1.83 | -1.05 | 9.58 | 6.72 | 5.96 |
| -0.63 | 0.61 | 3.03 | 1.81 | 1.42 | -1.38 | -0.44 | 9.55 | 6.51 | 5.96 |
| -0.42 | 0.79 | 3.04 | 1.82 | 1.39 | -0.92 | 0.09 | 9.55 | 6.65 | 5.99 |
| -0.21 | 0.92 | 3.00 | 1.75 | 1.42 | -0.46 | 0.57 | 9.62 | 6.93 | 5.98 |
| 0.00 | 1.00 | 3.03 | 1.72 | 1.42 | 0.00 | 1.00 | 9.10 | 6.99 | 5.83 |
| 0.21 | 1.04 | 3.04 | 1.74 | 1.36 | 0.46 | 1.39 | 9.74 | 6.78 | 5.99 |
| 0.42 | 1.04 | 3.01 | 1.87 | 1.39 | 0.92 | 1.74 | 10.11 | 6.75 | 5.86 |
| 0.63 | 0.99 | 3.00 | 1.79 | 1.40 | 1.38 | 2.04 | 9.36 | 6.87 | 5.85 |
| 0.84 | 0.85 | 3.32 | 1.81 | 1.41 | 1.83 | 2.25 | 10.21 | 6.87 | 5.96 |
| 1.05 | 0.35 | 3.35 | 1.85 | 1.36 | 2.29 | 2.09 | 9.94 | 6.85 | 5.97 |

| var: | 0.0176 | 0.0025 | 0.0007 | var: | 0.1133 | 0.0202 | 0.0039 |

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References

Ackermann, M and Strimmer, K. (2009), A general modular framework for gene set enrichment analysis, *BMC Bioinformatics*, 10, 47.

Barry, W., T., Nobel, A., B., and Wright, F., A. (2008), A statistical framework for testing functional categories in microarray data, *The Annals of Applied Statistics*, 2 No.1, 286-315.

Chatfield, C. and Collins, A., J. (1980), Introduction To Multivariate Analysis, *Chapman&Hall/CRC*.

Glazko, G. and Emmert-Streib, F. (2009), Unite and conquer: univariate and multivariate approaches for finding differentially expressed gene sets, *Bioinformatics*, 25 No. 18, 2348-2354.

Klebanov, L. and Yakovlev, A. (2007), Diverse correlation structures in gene expression data and their utility in improving statistical inference, *The Annals of Applied Statistics*, 1 No.2, 538-559.