

Multivariate Statistics in Ecology and  
Quantitative Genetics  
**Analyzing gene expression data or  
other data with more parameters than  
observatioios**

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Background Correction and Normalization for Affymetrix  
Microarrays

RMA

VSN

Stability

Regularization

Gene Ontology

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## Background Correction and Normalization for Affymetrix Microarrays

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# Affymetrix Microarrays

- ▶ Probe set for each gene
- ▶ Probe sets are spread over the chip
- ▶ For each probe (pm) a control probe (mm) where the

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# Robust Multi-Array Average (RMA)

Irizarry et al., 2003

- ▶ Assume that probe expression value consists of exponential signal + normally distributed random noise.
- ▶ Each array has its own mean background level of random noise.
- ▶ After background correction, probe expression values are normalized
- ▶ Then pm expression values are summarized to gene expression values

# RMA

Model to summarize probe sets:

$$Y_{ijn} = \mu_{in} + \alpha_{jn} + \varepsilon_{ijn}$$

where  $n$  is the probe set,  $Y_{ijn}$  is the log scaled probe expression values,  $\alpha_{jn}$  is the probe affinity effect with  $\sum_j \alpha_{jn} = 0$ , and  $\mu_{in}$  is the log expression measure for gene (probe set)  $n$  on array  $i$ .

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# Variance Stabilizing Normalization (VSN)

Huber et al., 2002

- ▶ Uses error model of Rocke and Durbin (2001)

$$Y_{ijn} = \alpha_i + \beta_{ij} \cdot e^{\eta_{ijn}} + \nu_{ijn}$$

where  $\eta_{ijn} \sim \mathcal{N}(0, 1)$  and  $\nu_{ijn} \sim \mathcal{N}(0, s_\nu^2)$

- ▶ This leads to the transformation

$$h_i(y_{ijn}) = \operatorname{arsinh}(a_i + b_i y_{ijn})$$

where  $a_i$  and  $b_i$  have to be estimated from the data, and

$$\operatorname{arsinh}(x) = \log \left( x + \sqrt{x^2 + 1} \right)$$

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# Stability of gene lists

For the selection of most promising genes from gene expression data different criteria may be applied, which may lead to different list.

One way to decide which criteria are useful is to assess their stability: Do they lead to similar gene lists if part of the data is changed?

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## Ridge Regression

If covariables are correlated, regression coefficients  $\beta_i$  can become very large and cancel each other. A way to avoid this:

$$\hat{\beta} = \arg \min_{\beta} \left\{ \sum_i \left( y_i - \beta_0 - \sum_j x_{ij} \beta_j \right)^2 + \lambda \sum_j \beta_j^2 \right\}$$

or, in other words:

$$\hat{\beta} = \arg \min_{\beta: \sum_i \beta_i^2 \leq s} \left\{ \sum_i \left( y_i - \beta_0 - \sum_j x_{ij} \beta_j \right)^2 \right\}$$

Caution: depends on scale! Normalize the data before applying this.

Solution for Ridge Regression:

$$\hat{\beta} = (\mathbf{X}^T \mathbf{X} + \lambda \mathbf{I})^{-1} \mathbf{X}^T \mathbf{y}$$

(usual linear regression if  $\lambda = 0$ )

Alternative Motivation of Ridge Regression:

$\mathcal{N}(0, \tau^2)$ -Prior on  $\beta_j$

$$y_i \sim \mathcal{N}(\beta_0 + \sum_j x_{ij}\beta_j, \sigma^2)$$

Then the  $\hat{\beta}$  are means of the posterior distribution, where  $\lambda = \sigma^2/\tau^2$ .

## Geometric Interpretation of Ridge Regression:

All principal components are shrunk, the shorter they are, the more.

If  $d_j$  is the eigenvalue of the  $j$ -th principal component, shrink it by factor

$$\frac{d_j^2}{d_j^2 + \lambda}$$



Similar method: **LASSO** (least absolute shrinkage and selector operator)

$$\hat{\beta} = \arg \min_{\beta: \sum_i |\beta_i| \leq s} \left\{ \sum_i \left( y_i - \beta_0 - \sum_j x_{ij} \beta_j \right)^2 \right\}$$

the coefficients are set to 0.

“kind of continuous subset selection”

(Hastie, Tibshirani, Friedman, 2001, *The Elements of Statistical Learning*)

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