

Microarray and Gene Expression Analysis

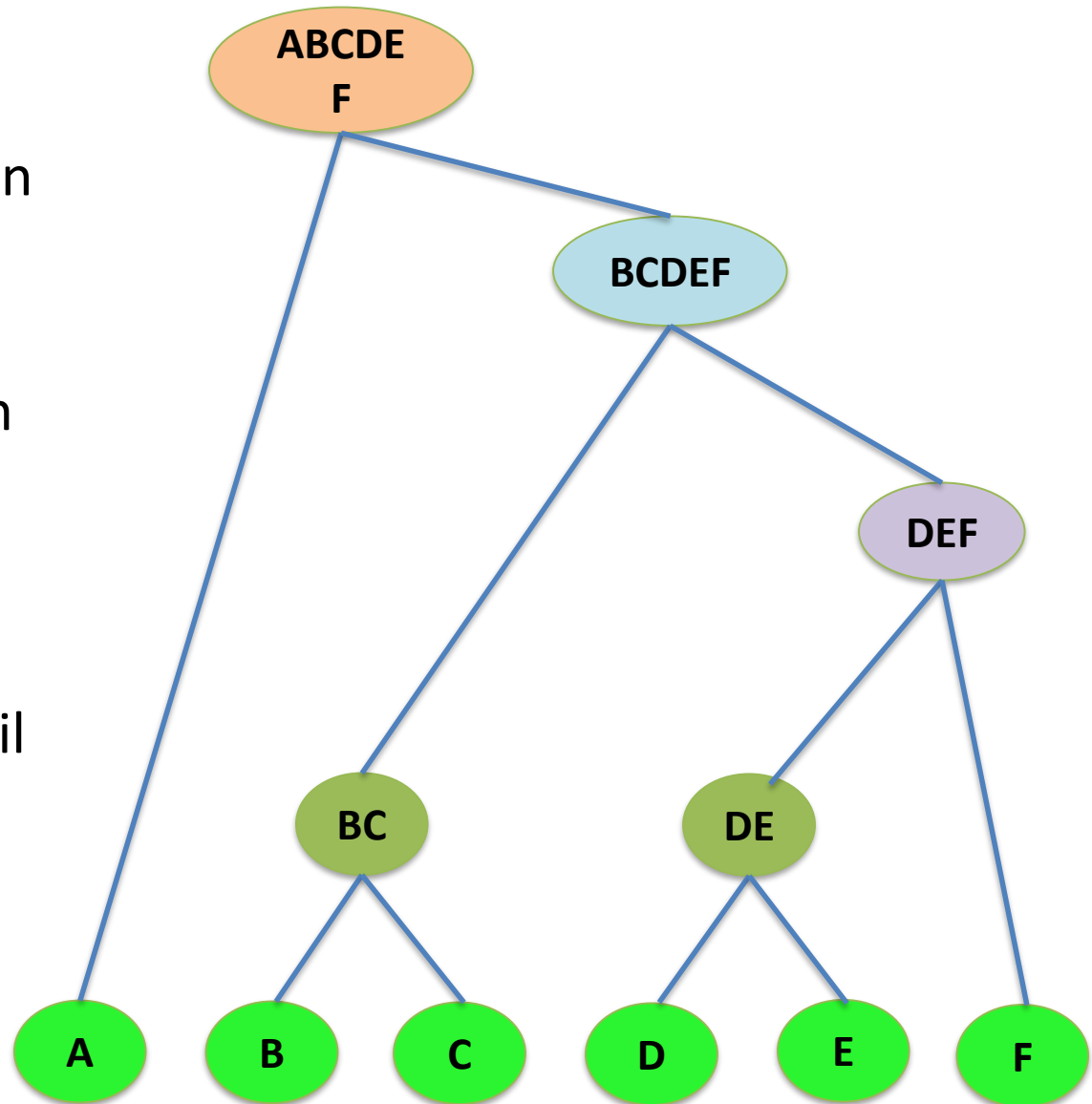
Garrett Dancik, PhD

Identification of Differentially Expressed Genes

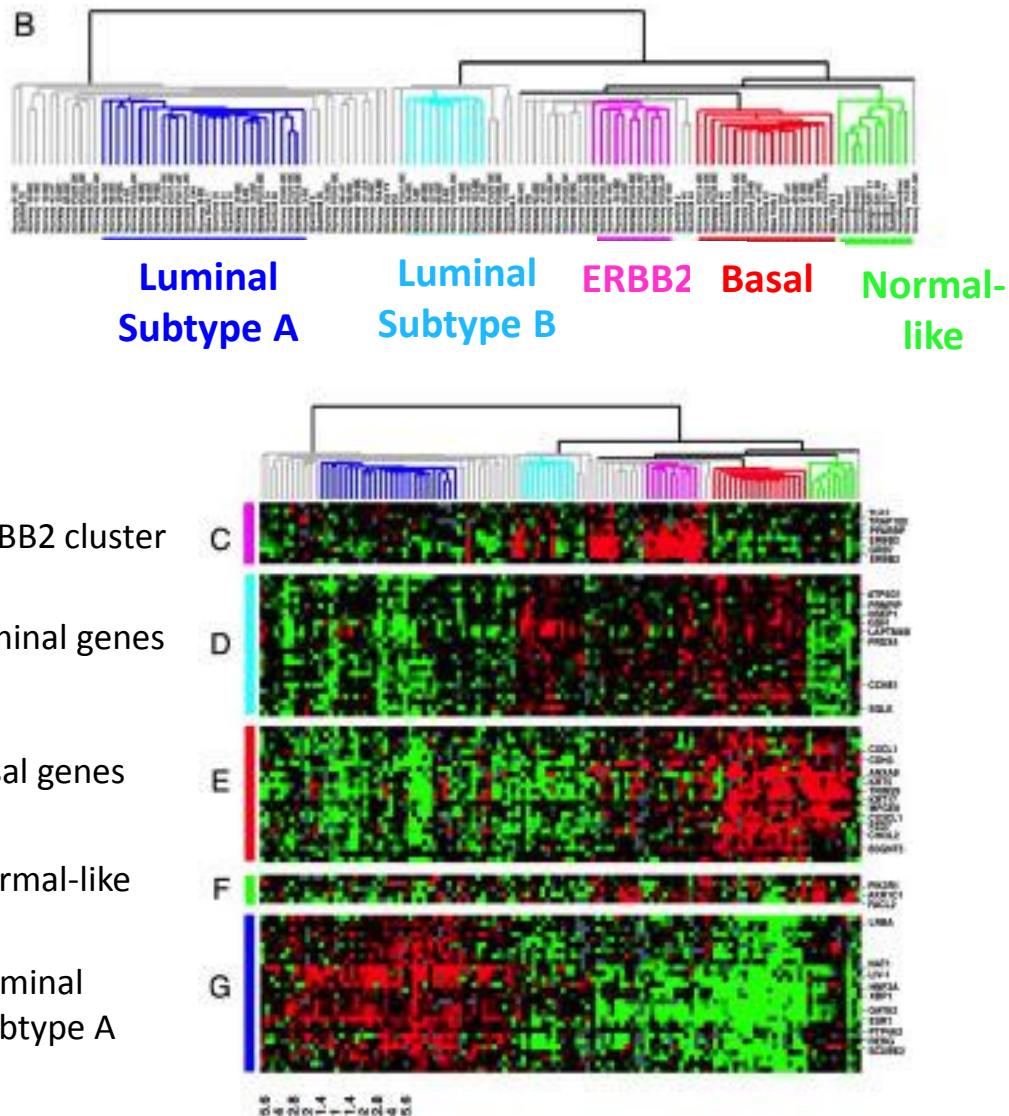
- Concern #1: Multiple comparison problem
 - Type I error probability (typically 5%) does not hold when you have multiple comparisons
 - If **no** genes are differentially expressed, and we analyzed 20,000 probes, there would be 1,000 false positives at significance level of 0.05!
 - In practice, p-values are adjusted to a false discovery rate (FDR), which is the expected proportion of false positives in list of genes with adjusted p-values \leq FDR
- Concern #2 (minor): Estimates of standard deviation are not stable
- We will use the *limma* package in R which addresses both of these concerns

Hierarchical agglomerative ("bottom up") clustering groups samples by similarity

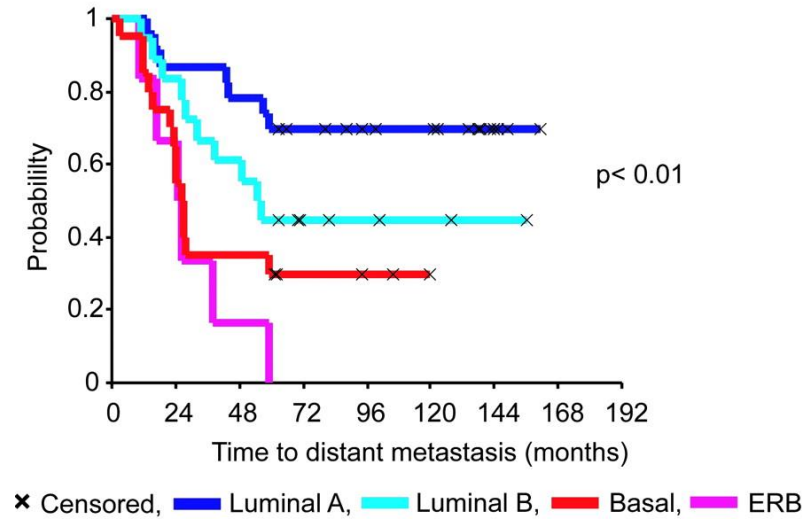
- Each observation starts in its own cluster
- Pairwise distances are calculated between each cluster
- The two most similar clusters are merged
- This process repeats until there is only one cluster



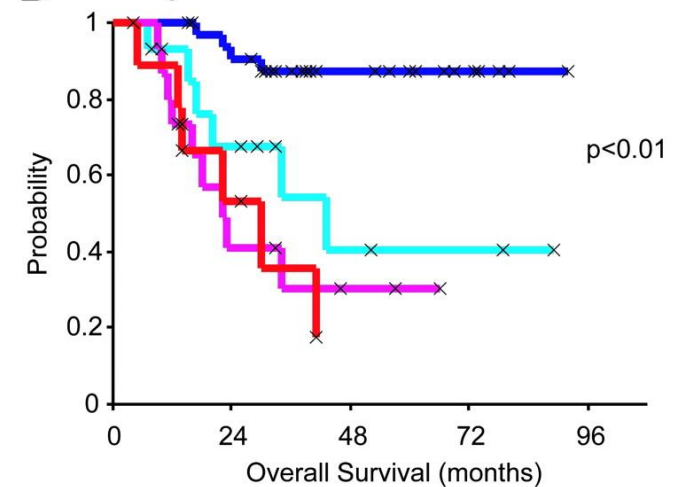
Hierarchical clustering of gene expression data identifies intrinsic breast cancer subtypes



A van't Veer data set



B Norway/Stanford data set

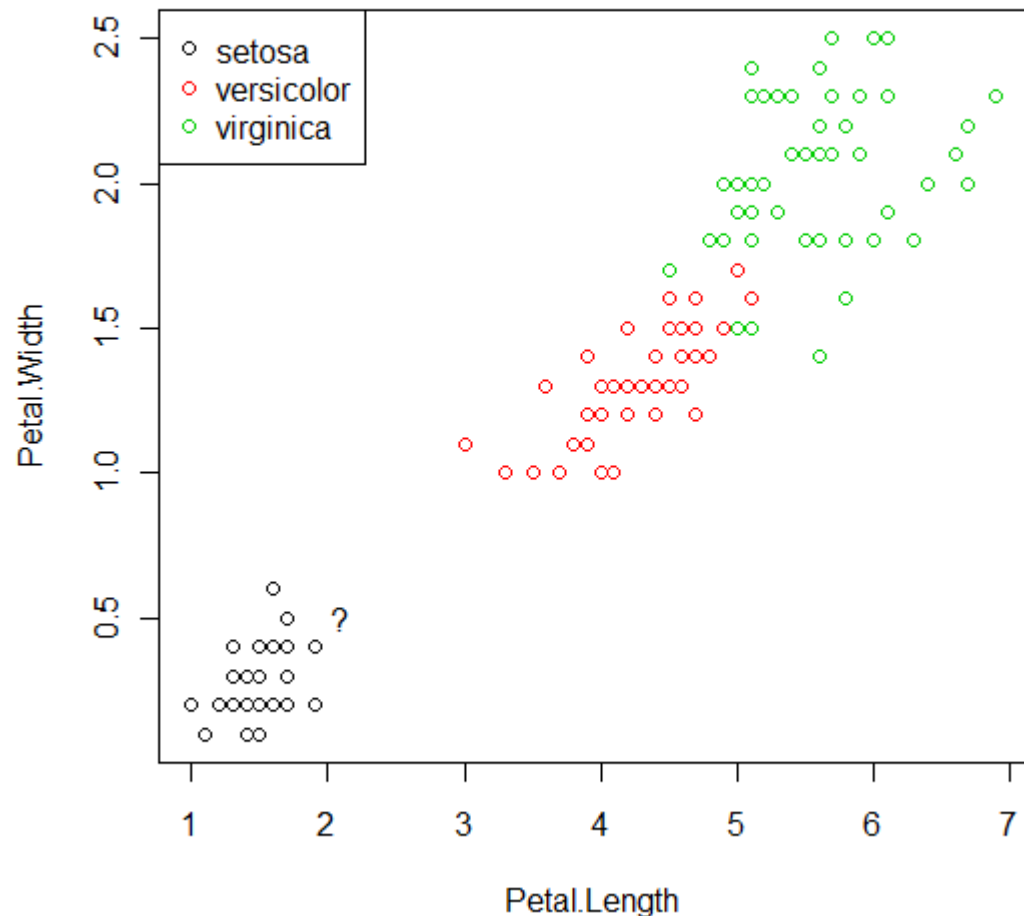


Classification Methods

- Objective: Identify the class of an individual (e.g., male or female) based on observed features (e.g., gene expression levels)
- Classes: c_1, c_2, \dots, c_m Features: x_1, \dots, x_k
- General Procedure
 - Train the classifier: Using a *training* data set, determine the mapping function $f(x) \rightarrow c$
 - Validation: assess the accuracy of the classifier by applying it to a *test* data set with known classes
 - Independent validation
 - Leave one out cross validation
 - K-fold cross validation
 - Classification / prediction of target data set

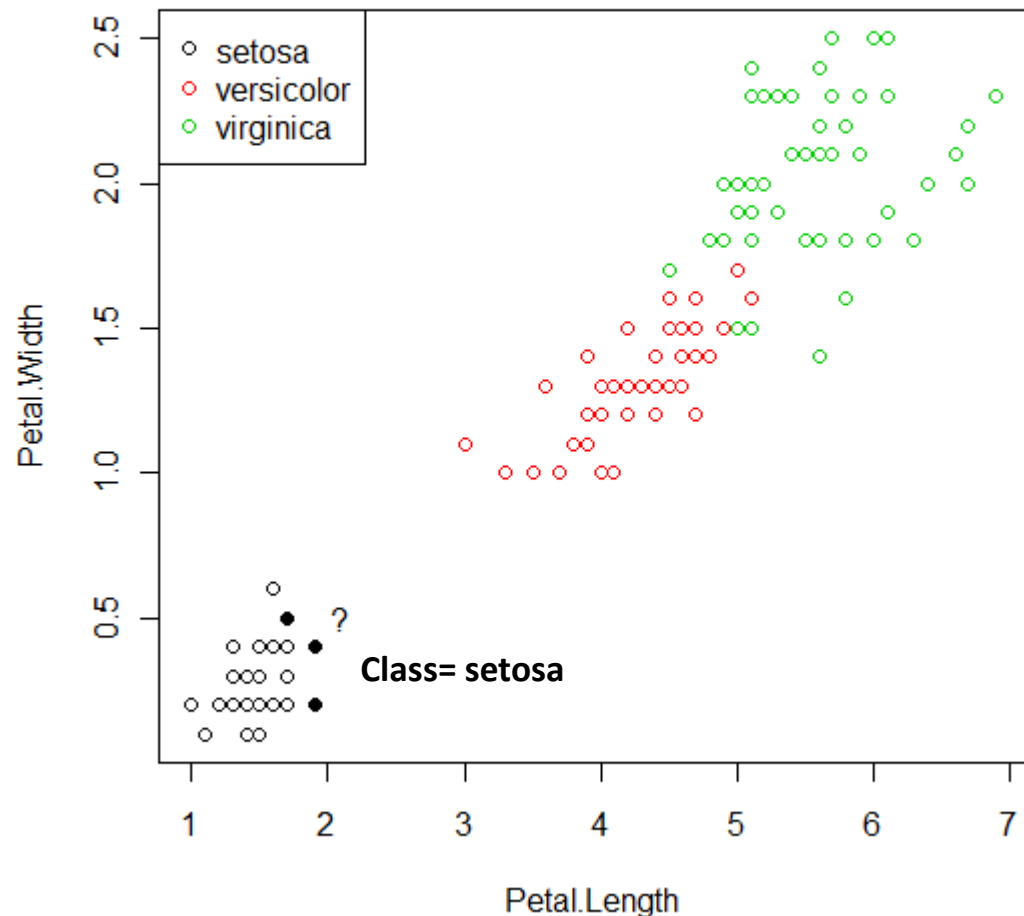
Classification Methods: K-Nearest Neighbors (KNN)

- For a test observation A , find the distance between A and every other observation in the feature space
- Classify the test observation based on the votes of its K nearest neighbors



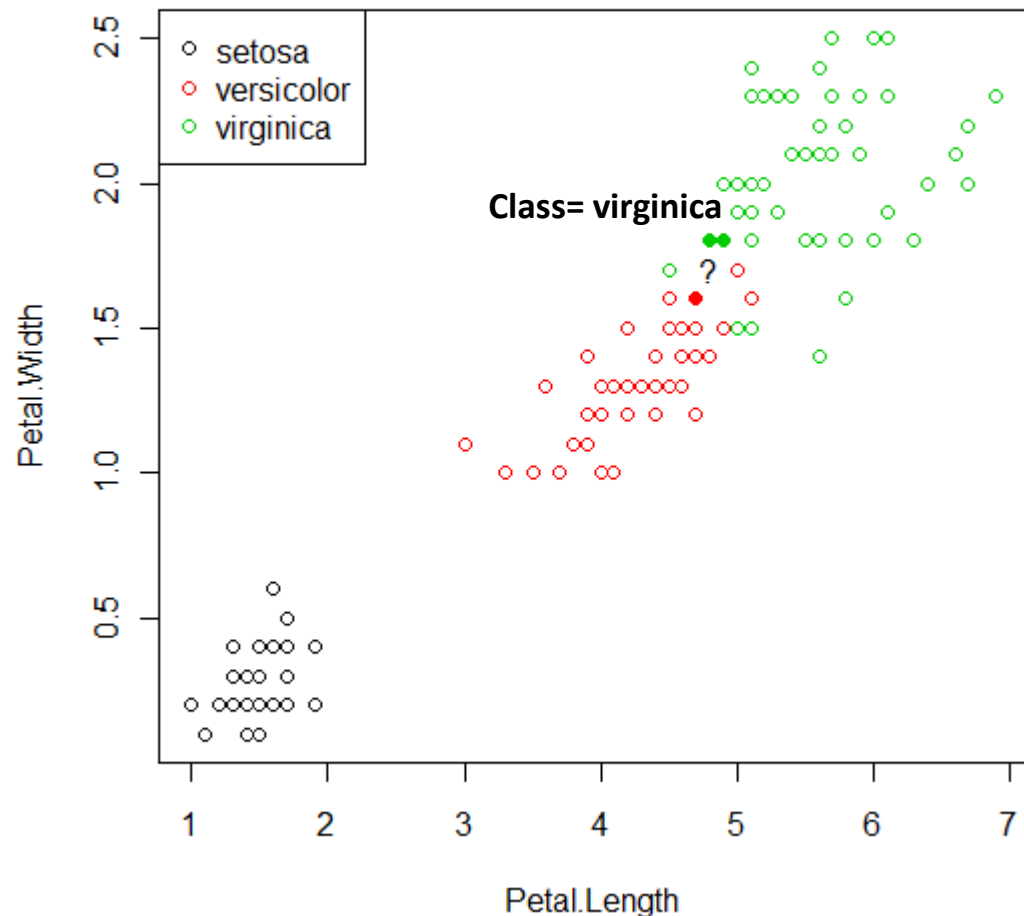
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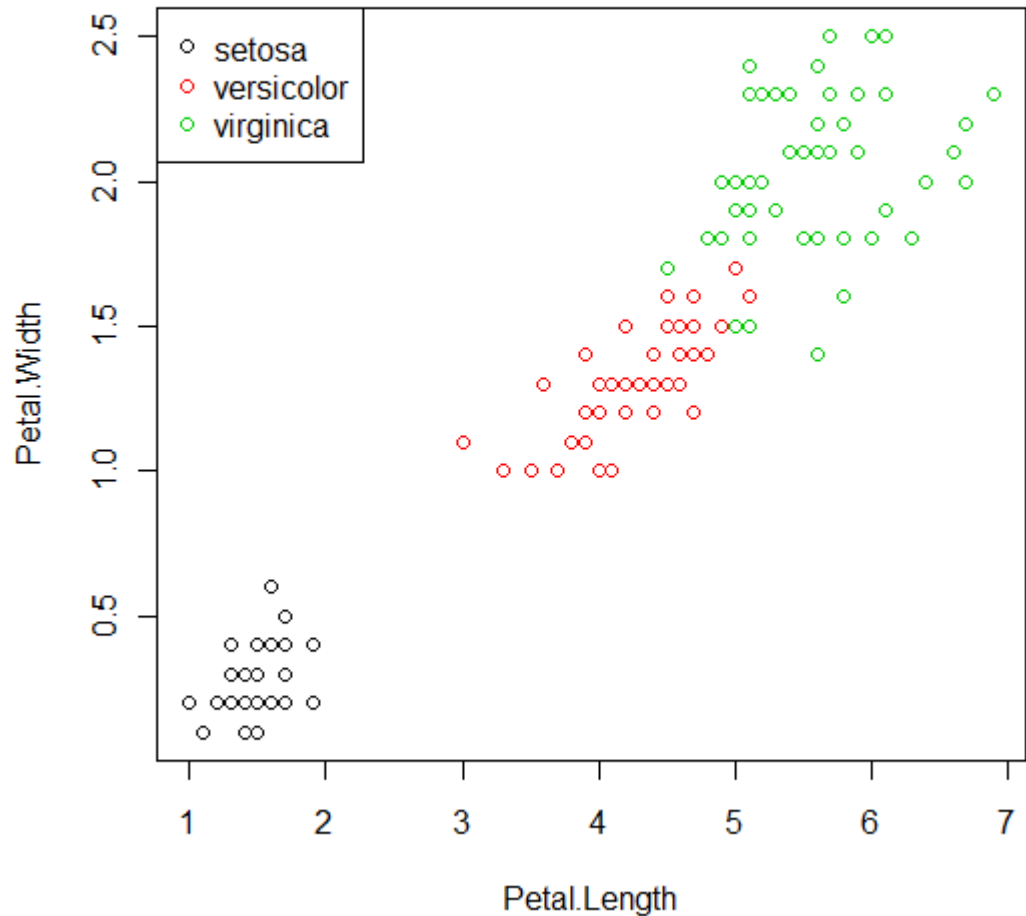
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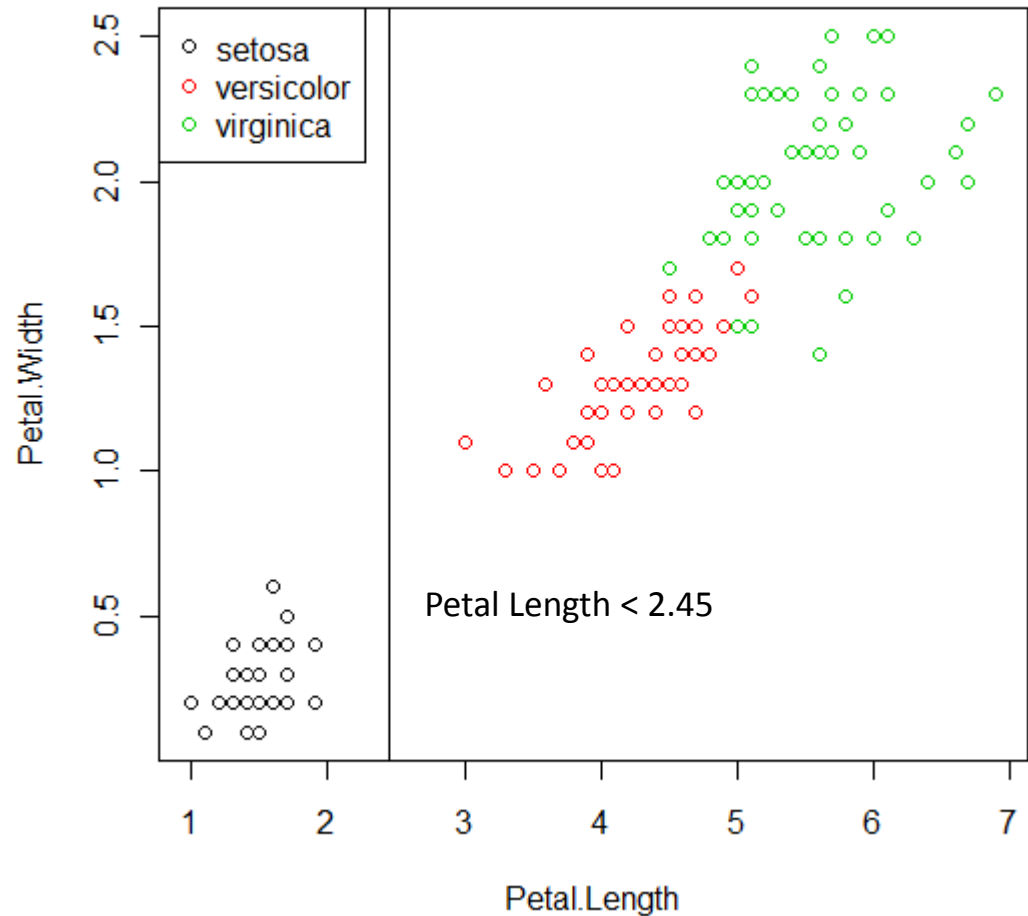
Classification Methods: Decision Trees (DT)

- Create a node by splitting the data according to a feature that optimally splits the data
- Repeat on data subsets until a stopping criterion is met
- Each leaf corresponds to a class



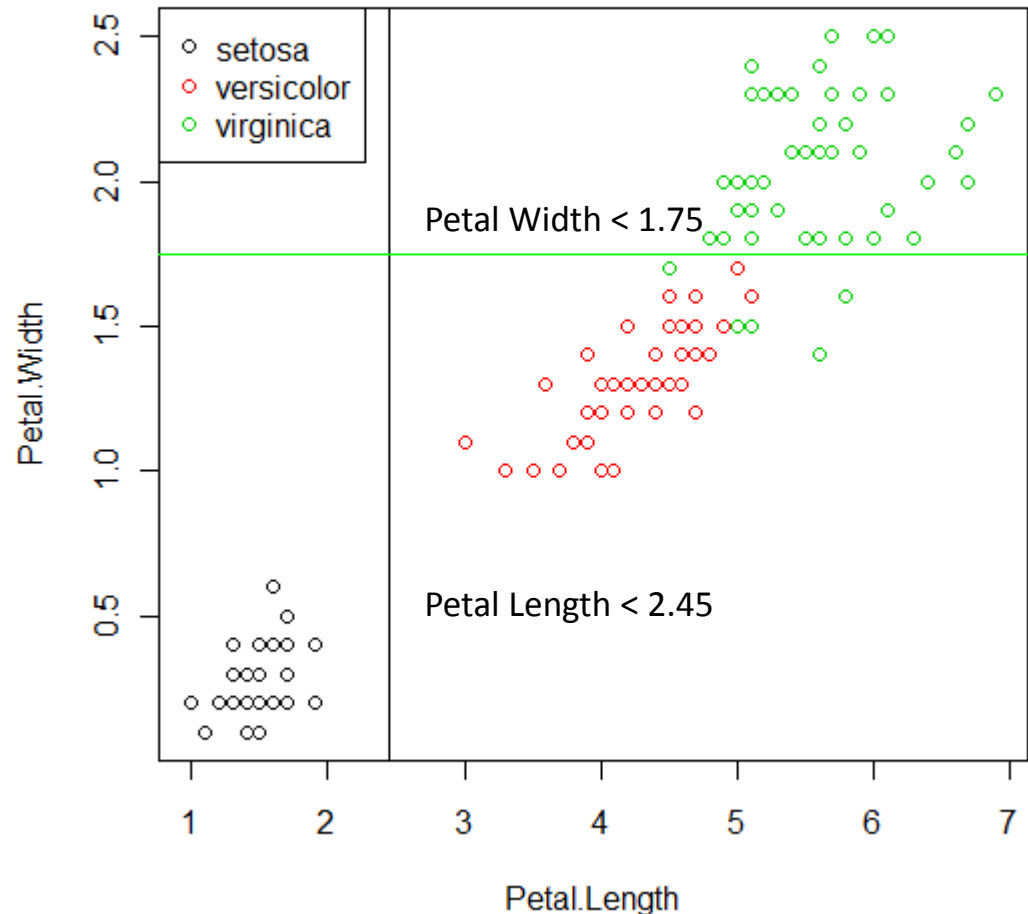
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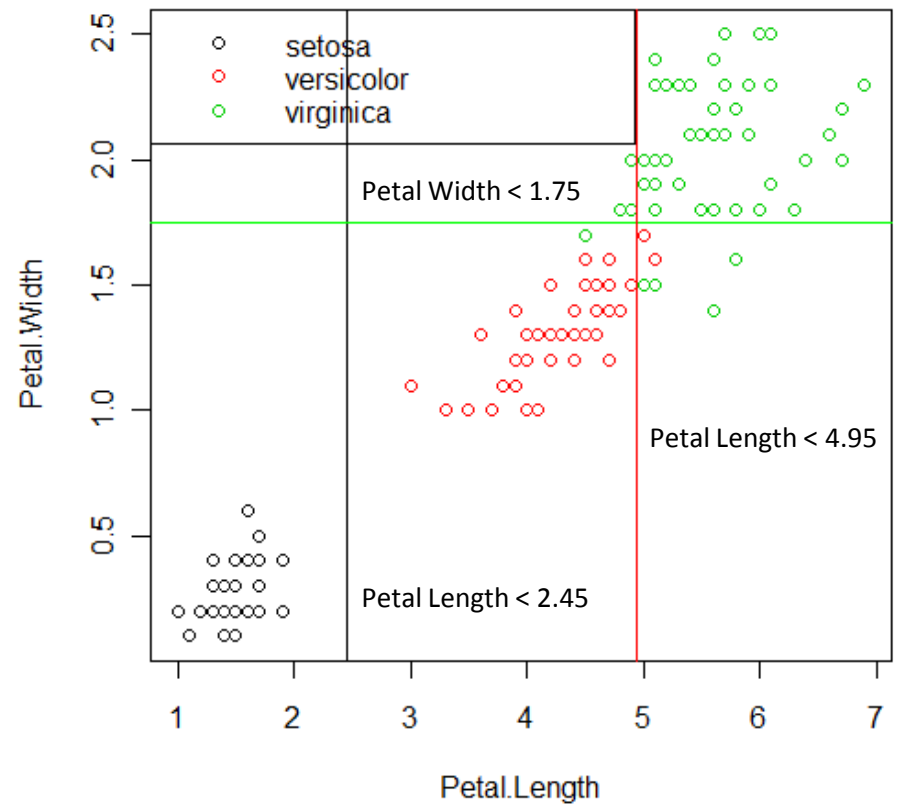
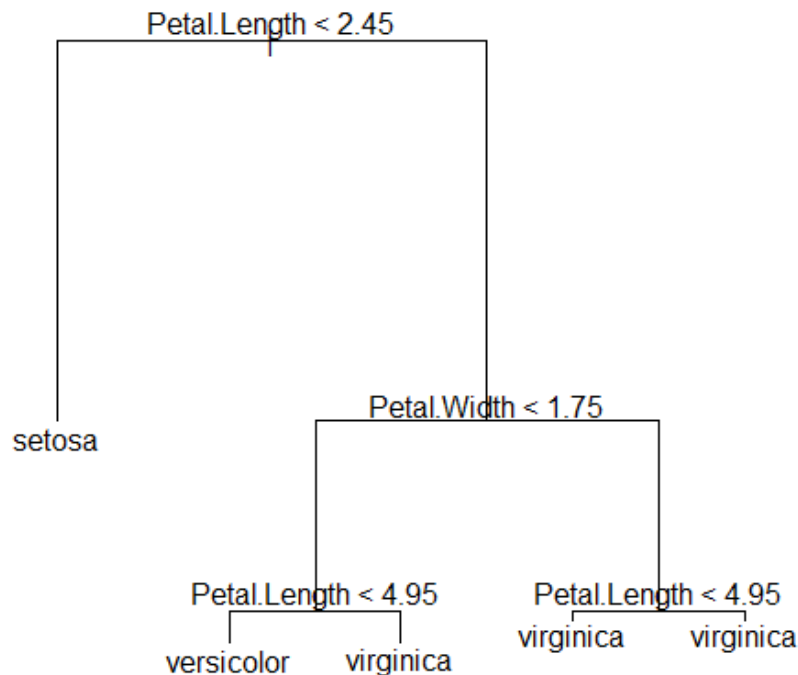


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Classification Methods: Decision Trees (DT)



Note: DT are known to overfit data. However more robust methods such as Random Forests can be used

Classification Methods: Naïve Bayes (NB)

- Based on Bayes' theorem that relates conditional probabilities

$$p(C|x_1, \dots, x_n) \propto p(x_1, \dots, x_n|C)$$

- Naïve Bayes assumes independence of features, so that

$$p(x_1, \dots, x_n|C) = p(x_1|C) \times \dots \times p(x_n|C)p(C)$$

- For quantitative features, calculate by treating

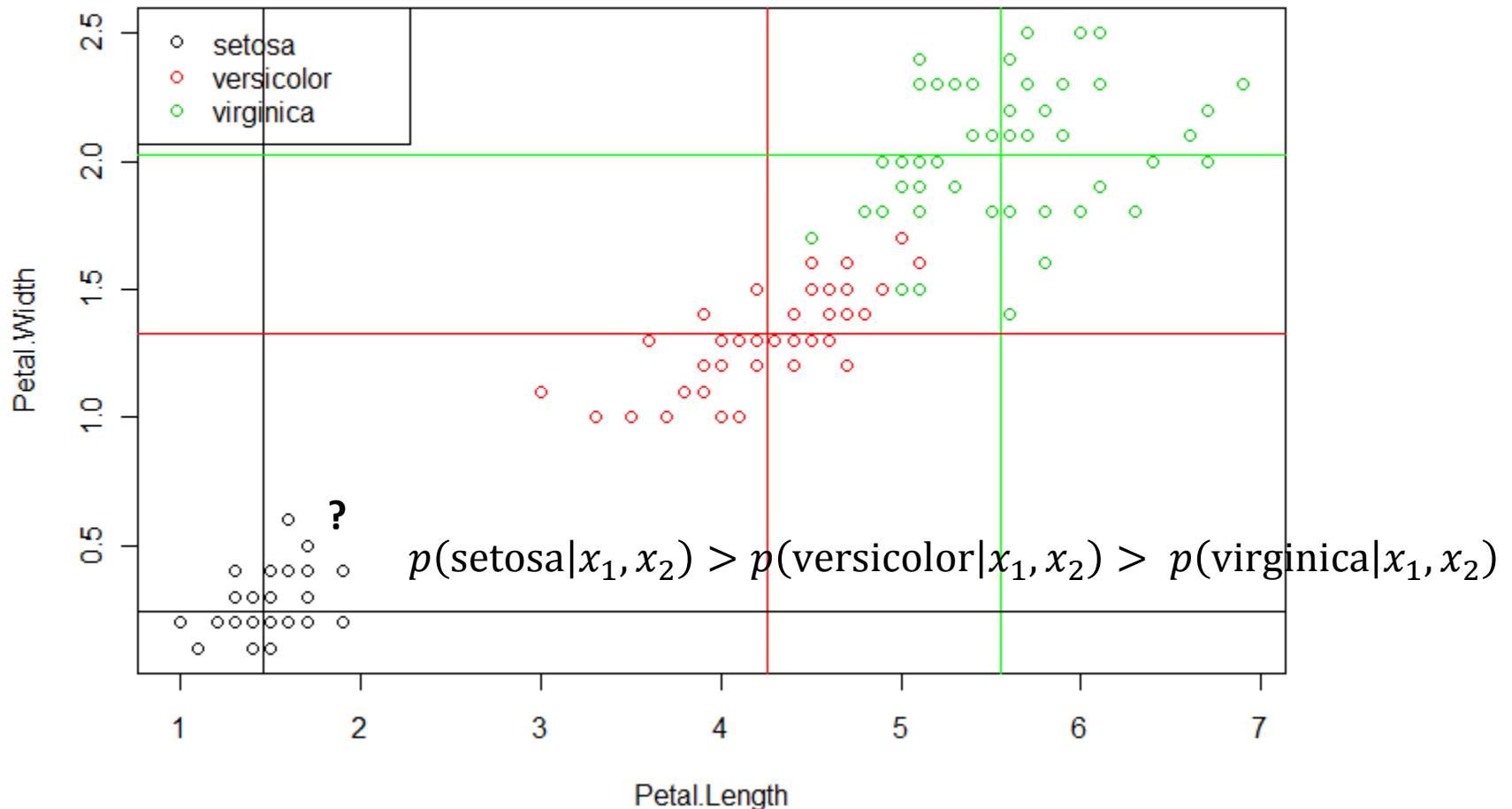
$$p(x|C) \sim N(\mu_x, \sigma_x)$$

- Select the class C that maximizes

$$p(C|x_1, \dots, x_n) \propto p(x_1|C) \times \dots \times p(x_n|C)p(C)$$

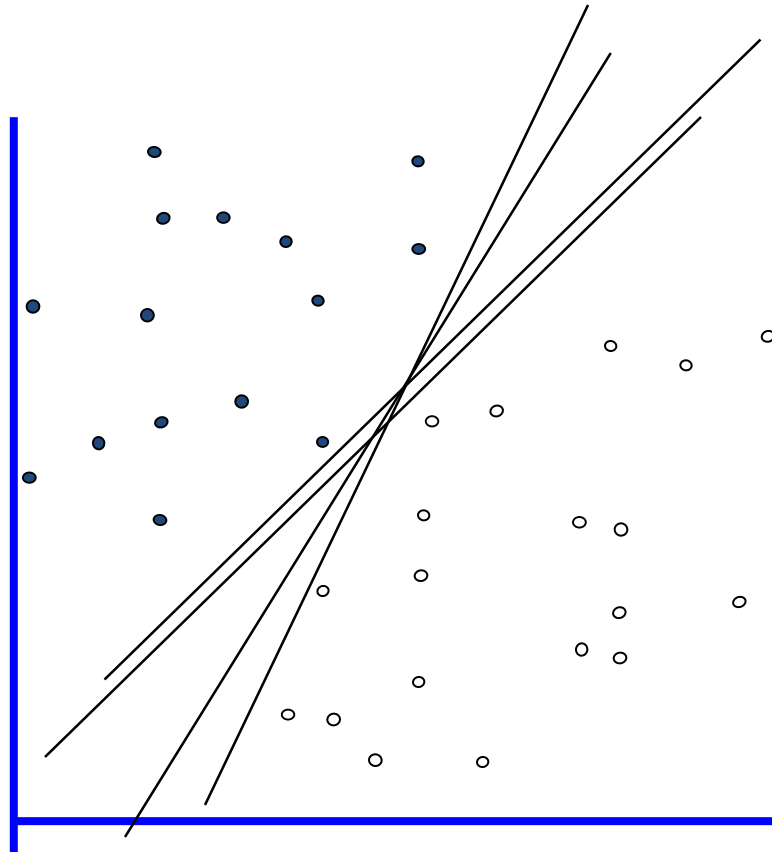
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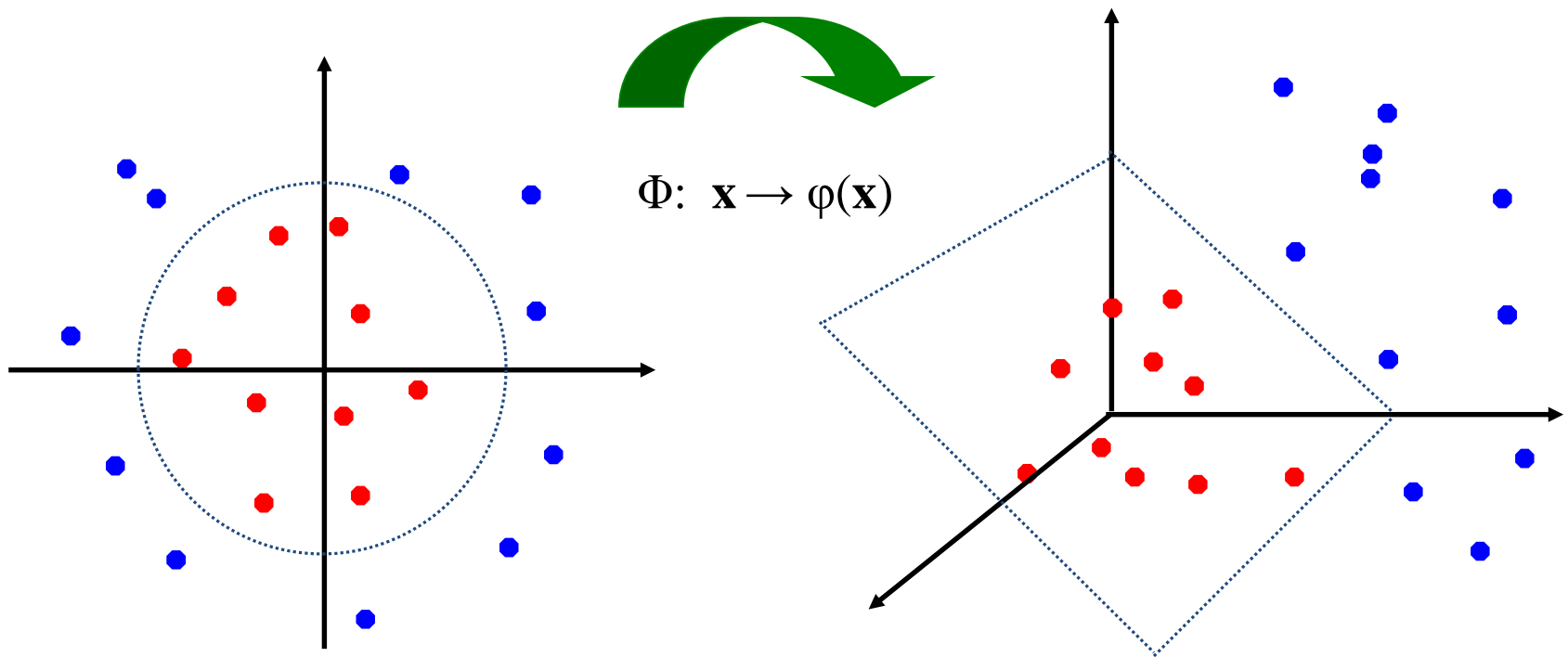


Classification Methods: Support Vector Machines (SVM)

- Find the optimum hyperplane that linearly separates the classes
- If classes are not linearly separable, map the data into a higher dimensional space through the use of a kernel function



Classification Methods: Support Vector Machines (SVM)



Caveats and strategies

- Validation
 - Overfitting is often a problem: a classifier can perform very well on a training data set but may not generalize to additional data sets
 - Validation on independent data sets are ideal
 - Cross-validation is useful when data is limited
- Basic Strategy
 - Use cross-validation to select
 - The number of features (e.g., probes/genes)
 - Optimal parameters for classification model (e.g, value of k in knn)