Clustering II

With application to gene-expression profiling technology

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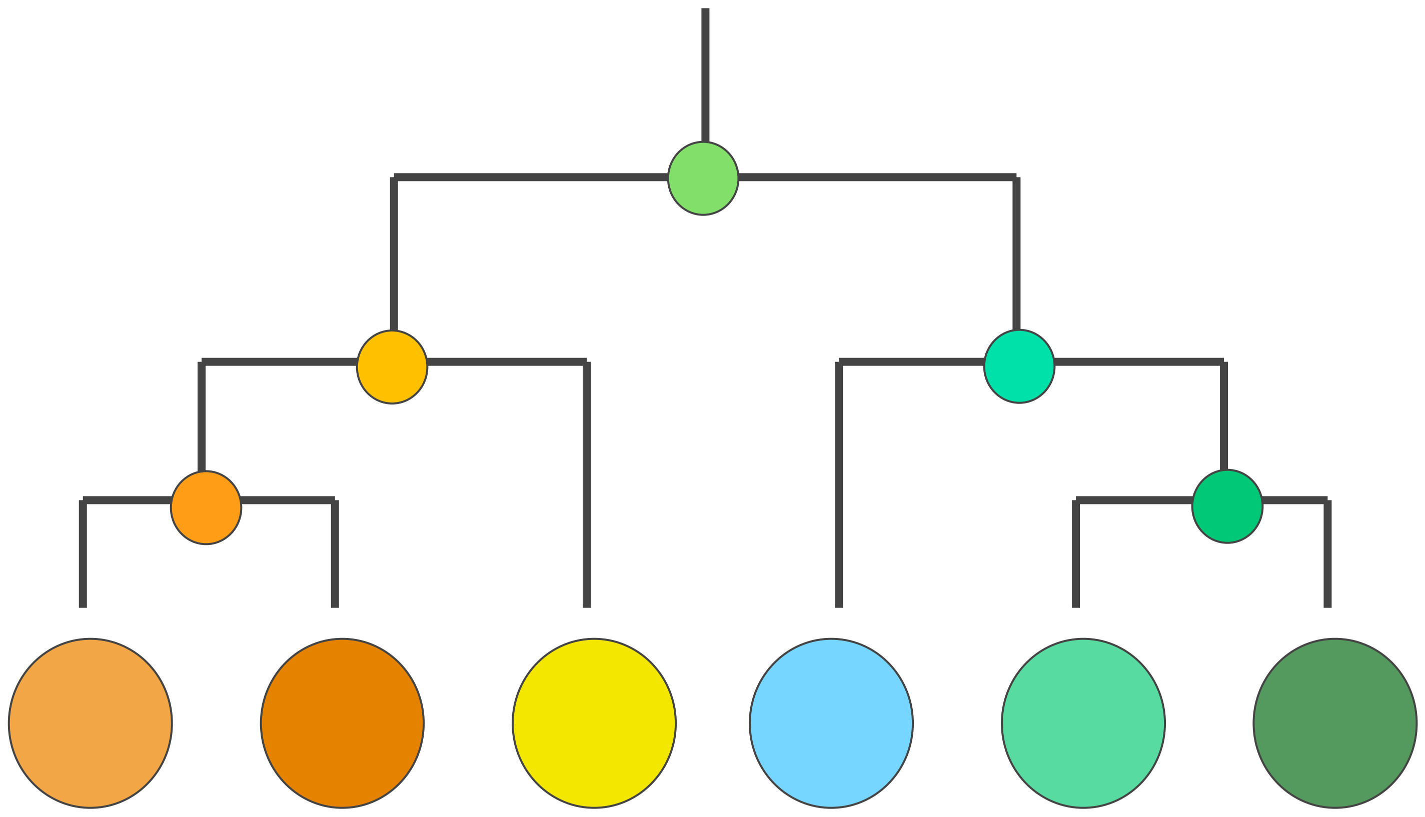
Thanks to Kevin Wayne, Matt Hibbs, & SMD for a few of the slides

Hierarchical Clustering

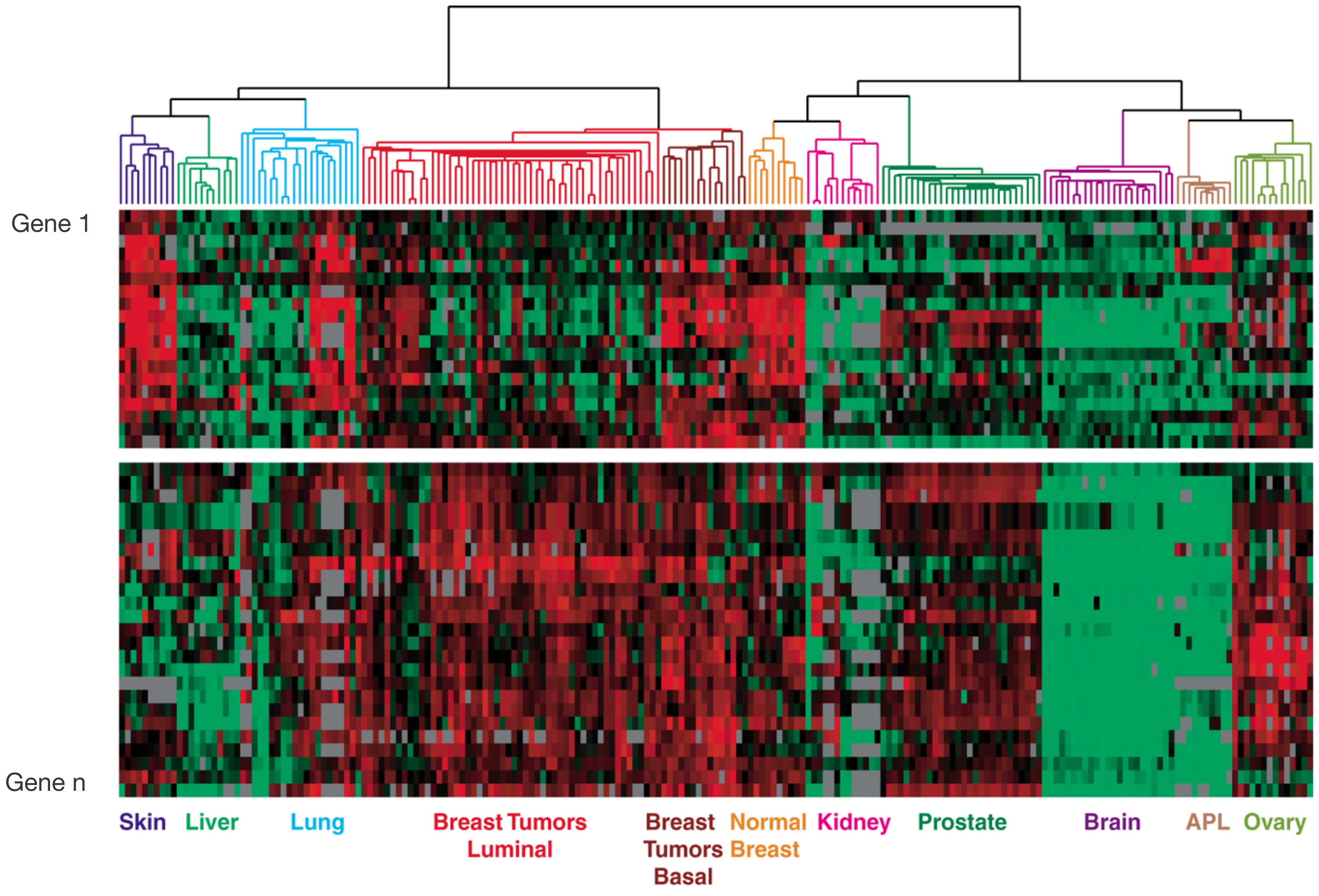
Thanks to Kevin Wayne for many of the slides

Hierarchical clustering



Hierarchical clustering

Tumors in similar tissues cluster together

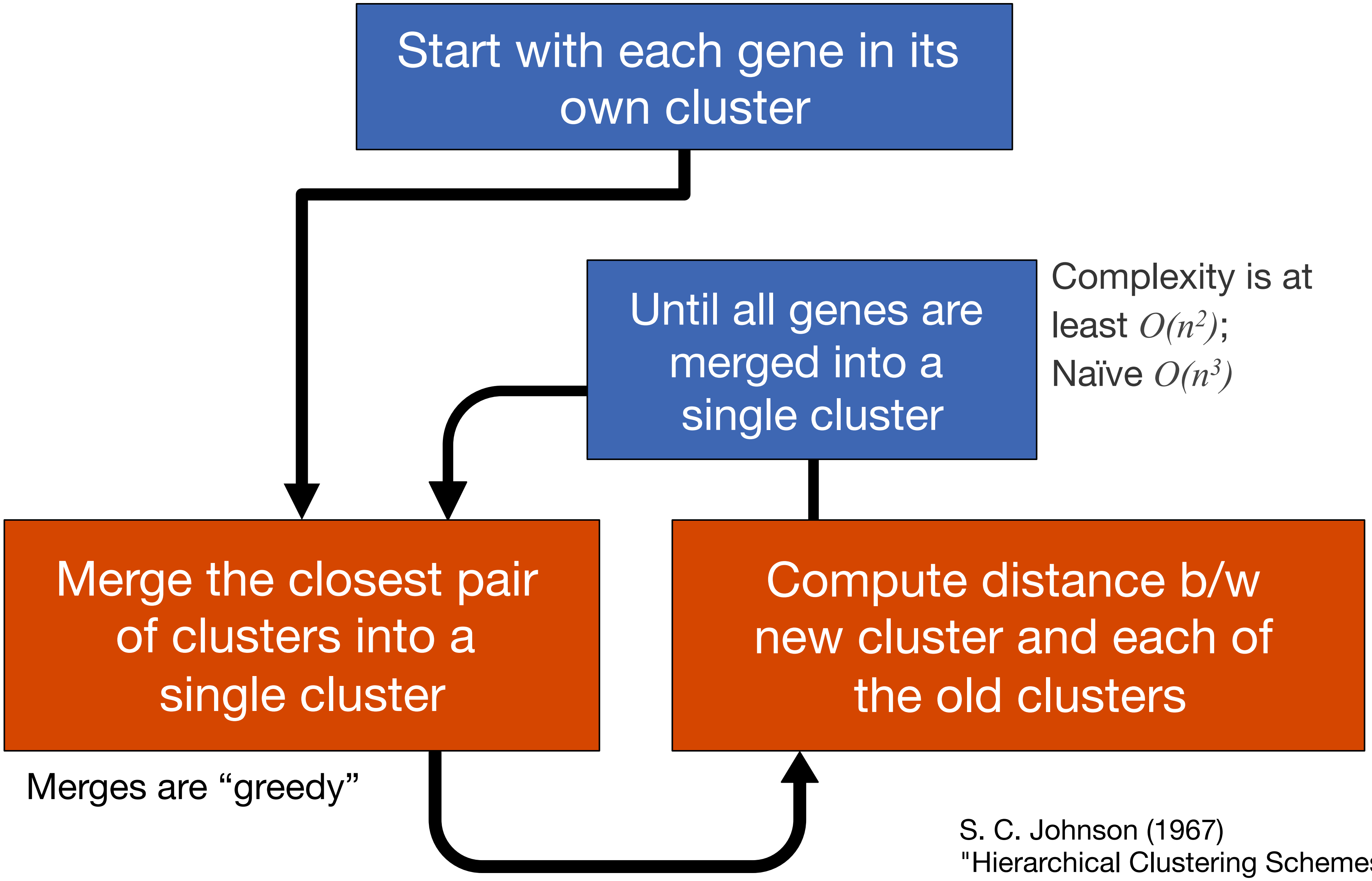


🟧 gene over expressed

🟩 gene under expressed

Reference: Botstein & Brown group

Hierarchical clustering


Start with each gene in its own cluster
Until all genes are merged into a single cluster
Merge the closest pair of clusters into a single cluster
Compute distance b/w new cluster and each of the old clusters

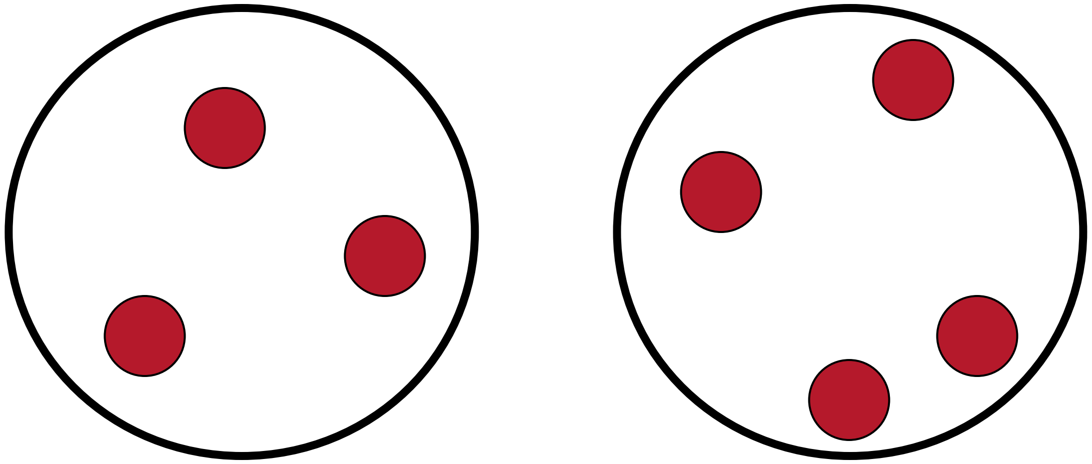
Complexity is at least $O(n^2)$; Naïve $O(n^3)$

Merges are “greedy”

S. C. Johnson (1967)
"Hierarchical Clustering Schemes”
Psychometrika, 2:241-254

Hierarchical clustering

- Distance metric
- Linkage criteria
  - Single/Minimum linkage (nearest neighbors)
  - Complete/Maximum linkage (farthest neighbors)
  - Average linkage (average of all pairs)



Single-Link Hierarchical Clustering

Input. Pre-computed matrix of distances between all pairs of genes (i, j).

Scheme. Erase rows and columns in the distance matrix as old clusters are merged into new ones.

Begin.

Each gene in its own cluster.

For each cluster i, create/maintain index dmin[i] of closest cluster.

	dmin	dist
0	1	5.5
1	3	2.14
2	4	5.6
3	1	2.14
4	3	5.5

	0	1	2	3	4
gene0	-	5.5	7.3	8.9	5.8
1	5.5	-	6.1	2.14	5.6
2	7.3	6.1	-	7.8	5.6
3	8.9	2.14	7.8	-	5.5
4	5.8	5.6	5.6	5.5	-

Single-Link Hierarchical Clustering

Iteration.

- Find closest pair of clusters (i1, i2).
- Replace row i1 by min of row i1 and row i2.
- Infinity out row i2 and column i2.
- Update dmin[i] and change dmin[i'] to i1 if previously dmin[i'] = i2.

- Merging into a cluster
- Updating matrix with dist between new cluster & old clusters

Closest pair

	dmin	dist
0	1	5.5
1	3	2.14
2	4	5.6
3	1	2.14
4	3	5.5

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3	8.9	2.14	7.8	-	5.5
4	5.8	5.6	5.6	5.5	-

gene1 closest to gene3, dist = 2.14

	dmin	dist
0	1	5.5
1	0	5.5
2	4	5.6
3	-	-
4	1	5.5

	0	1	2	3	4
0	-	5.5	7.3	-	5.8
node1	5.5	-	6.1	-	5.5
2	7.3	6.1	-	-	5.6
3	-	-	-	-	-
4	5.8	5.5	5.6	-	-

New min distance

Single-Link Clustering: Main Loop

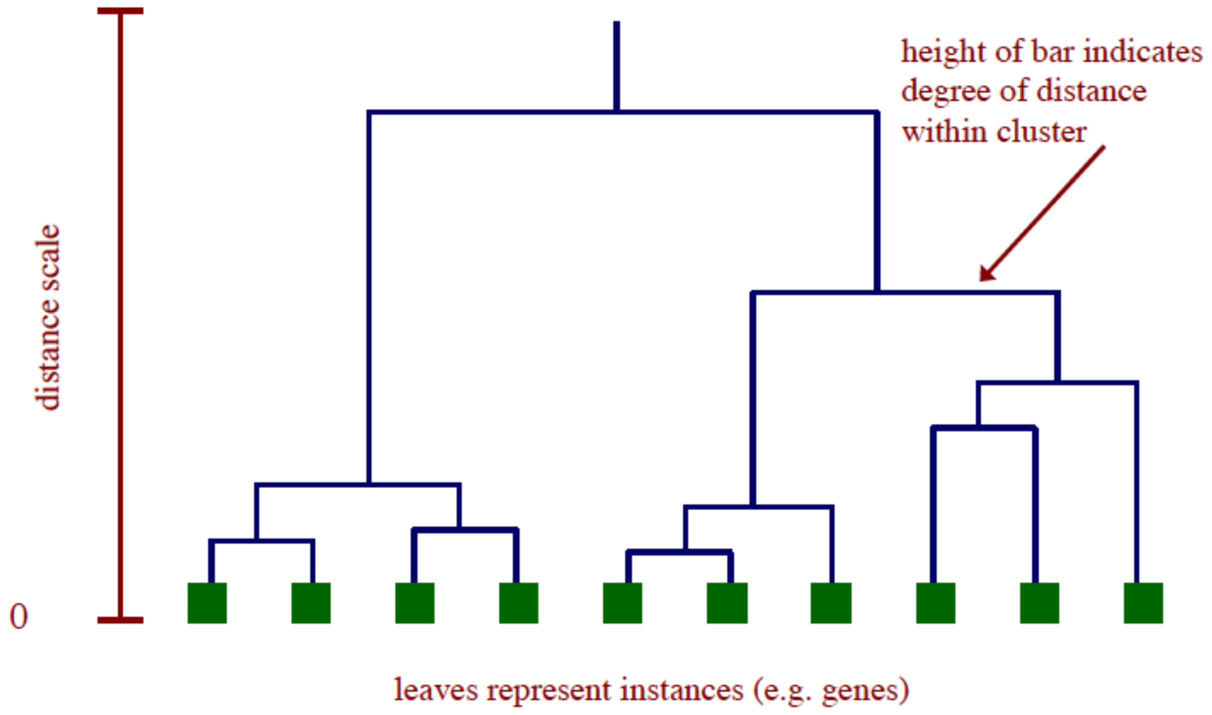
```
for (int s = 0; s < ? ; s++) {

            Find closest pair of clusters (i1, i2).

        Replace row i1 by min of row i1 and row i2.

            Infinity out row i2 and column i2.

                     Update dmin[i]
     Change dmin[i'] to i1 if previously dmin[i'] = i2.

}
```

Single-Link Clustering: Main Loop

```
for (int s = 0; s < N-1; s++) {
   // find closest pair of clusters (i1, i2)
   int i1 = 0;
   for (int i = 0; i < N; i++)
      if (d[i][dmin[i]] < d[i1][dmin[i1]]) i1 = i;
   int i2 = dmin[i1];

   // overwrite row i1 with minimum of entries in row i1 and i2
   for (int j = 0; j < N; j++)
      if (d[i2][j] < d[i1][j]) d[i1][j] = d[j][i1] = d[i2][j];
   d[i1][i1] = INFINITY;

   // infinity-out old row i2 and column i2
   for (int i = 0; i < N; i++)
      d[i2][i] = d[i][i2] = INFINITY;

   // update dmin and replace ones that previous pointed to
   // i2 to point to i1
   for (int j = 0; j < N; j++) {
      if (dmin[j] == i2) dmin[j] = i1;
      if (d[i1][j] < d[i1][dmin[i1]]) dmin[i1] = j;
   }
}
```

Dendrogram

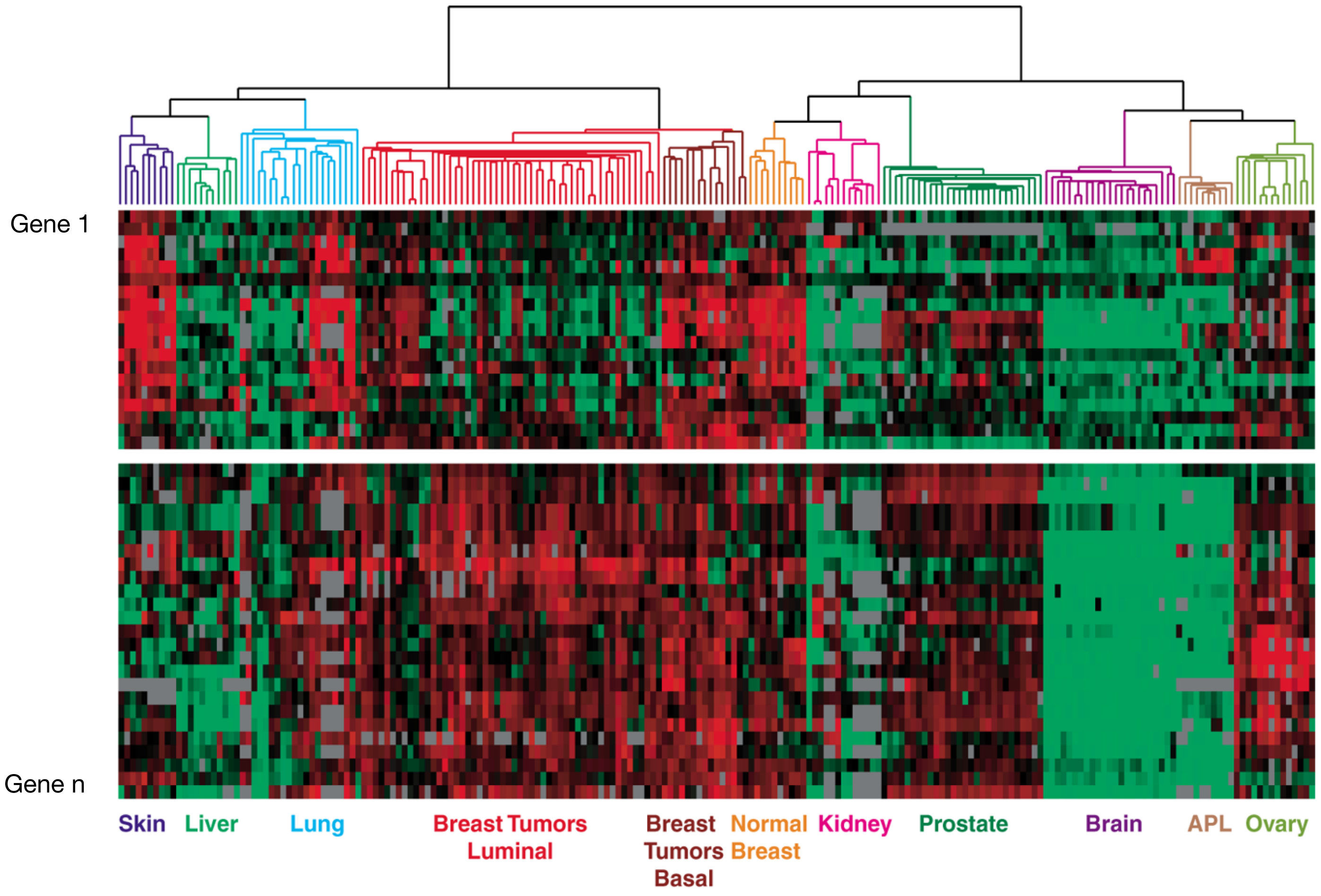
- Leaves = genes.
- Internal nodes = hypothetical ancestors.


height of bar indicates degree of distance within cluster
distance scale
0
leaves represent instances (e.g. genes)

Reference: http://www.biostat.wisc.edu/bmi576/fall-2003/lecture13.pdf

Dendrogram of Human tumors

Tumors in similar tissues cluster together.



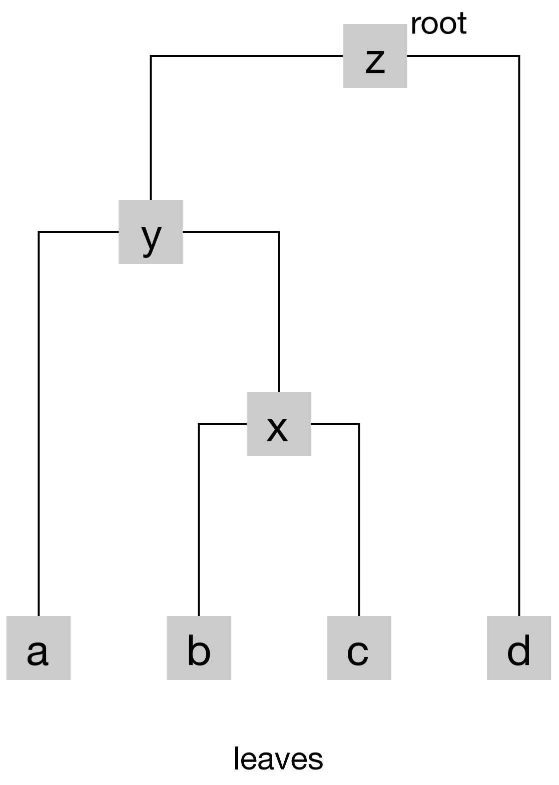
Reference: Botstein & Brown group

🟥 gene over expressed

🟩 gene under expressed

Ancestor Tree

Root. Node with no parent.
Leaf. Node with no children.
Depth. Length of path from node to root.
Least Common Ancestor. Common ancestor with largest depth.



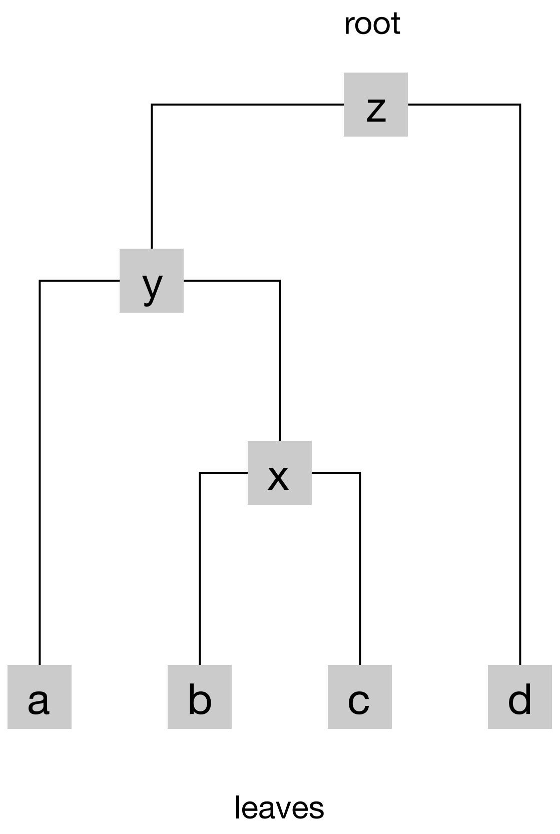
```
public static void main(String[] args) {
   TreeNode a = new TreeNode("GENE1");
   TreeNode b = new TreeNode("GENE2");
   TreeNode c = new TreeNode("GENE3");
   TreeNode d = new TreeNode("GENE4");

   TreeNode x = new TreeNode("NODE1", b, c);
   TreeNode y = new TreeNode("NODE2", a, x);
   TreeNode z = new TreeNode("NODE3", d, y);

   System.out.println(a.lca(b));
   a.lca(b).showLeaves();
}
```

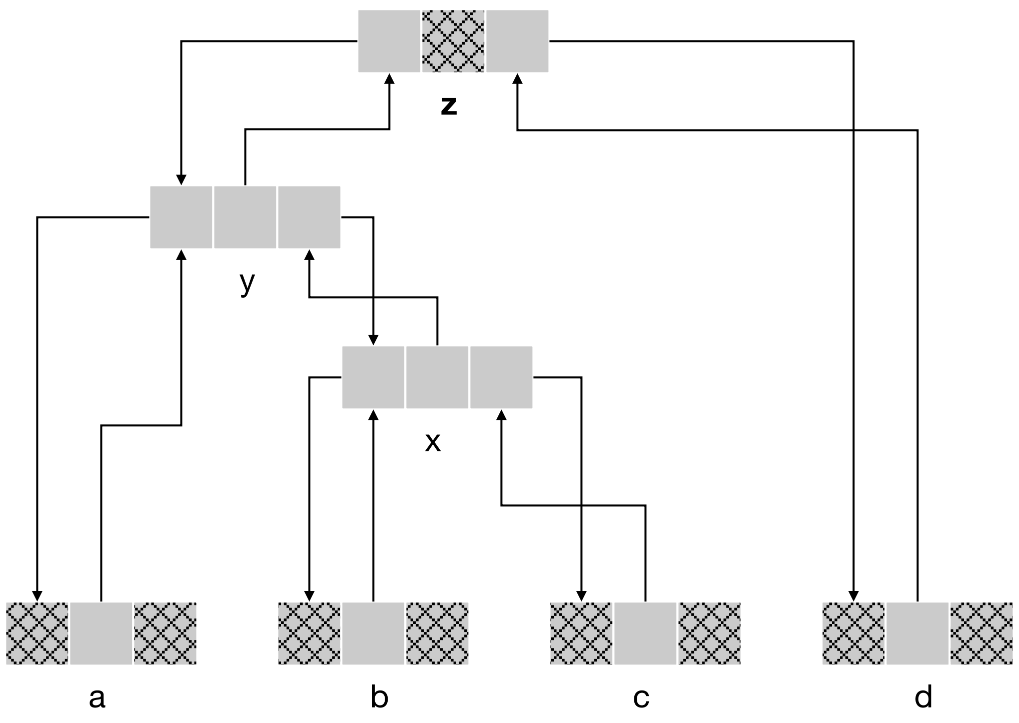
lca(a, b) = y, leaves of y = { a, b, c }

Ancestor Tree: Implementation

Node. Left pointer, right pointer, parent pointer.

Consequence. Can go up or down the tree.





Ancestor Tree

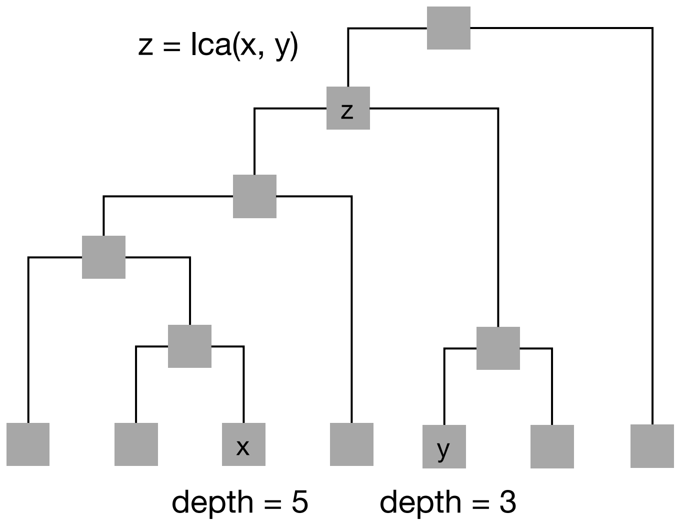
```
public class TreeNode {
    private TreeNode parent;          // parent
    private TreeNode left, right;     // two children
    private String name;              // name of node

    // create a leaf node
    public TreeNode(String name) {
        this.name = name;
    }

    // create an internal node that is the parent of x and y
    public TreeNode(String name, TreeNode x, TreeNode y) {
        this.name  = name;
        this.left  = x;
        this.right = y;
        x.parent = this;
        y.parent = this;
    }
```

Ancestor Tree: Helper Functions

```
// return depth of this node in the tree
// depth of root = 0
public int depth() {
   int depth = 0;
   for (TreeNode x = this; x.parent != null; x = x.parent)
      depth++;
   return depth;
}

// return root
public TreeNode root() {
   TreeNode x = this;
   while (x.parent != null)
      x = x.parent;
   return x;
}
```

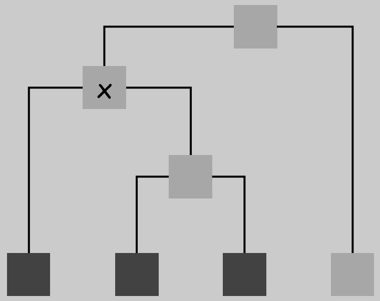
Ancestor Tree: Least Common Ancestor

```
// return the lca of node x and y
public TreeNode lca(TreeNode y) {
        TreeNode x = this;
        int dx = x.depth();
        int dy = y.depth();
        if (dx < dy) {
            for (int i = 0; i < dy-dx; i++) y = y.parent;
        }
        else {
            for (int i = 0; i < dx-dy; i++) x = x.parent;
        }
        while (x != y) {
            x = x.parent;
            y = y.parent;
        }
        return x;
    }
```



Ancestor Tree: Tree Traversal

```
// return string representation
public String toString() { return name; }

// print all leaves in tree rooted at this node
public void showLeaves() {
   if (left == null && right == null) System.out.println(this);
   else {
      left.showLeaves();
      right.showLeaves();
   }
}

// print the tree rooted at this node
public void show() {
   if (left == null && right == null) return;
   System.out.println(name + " " + left.name + " " + right.name);
   left.show();
   right.show();
}
```



Hierarchical clustering implementation

Single-Link Clustering: Java Implementation

Single-link clustering.

- Read in the data.

```
public static void main(String[] args) {
   int M = StdIn.readInt();
   int N = StdIn.readInt();

   // read in N vectors of dimension M
   Vector[] vectors = new Vector[N];
   String[] names    = new String[N];
   for (int i = 0; i < N; i++) {
   names[i] = StdIn.readString();
   double[] d = new double[M];
   for (int j = 0; j < M; j++)
      d[j] = StdIn.readDouble();
   vectors[i] = new Vector(d);
   }
```

Single-Link Clustering: Java Implementation

Single-link clustering.

- Read in the data.
- Precompute d[i][j] = distance between cluster i and j.
- For each cluster i, maintain index dmin[i] of closest cluster.

	dmin	dist
0	1	5.5
1	3	2.14
2	4	5.6
3	1	2.14
4	3	5.5

	0	1	2	3	4
gene0	-	5.5	7.3	8.9	5.8
1	5.5	-	6.1	2.14	5.6
2	7.3	6.1	-	7.8	5.6
3	8.9	2.14	7.8	-	5.5
4	5.8	5.6	5.6	5.5	-

Single-Link Clustering: Main Loop

```
for (int s = 0; s < N-1; s++) {

                 Find closest pair of clusters (i1, i2).

             Replace row i1 by min of row i1 and row i2.

                  Infinity out row i2 and column i2.

                            Update dmin[i]
          Change dmin[i'] to i1 if previously dmin[i'] = i2.

}
```

Single-Link Clustering: Main Loop

```
for (int s = 0; s < N-1; s++) {
```

- Closest pair of clusters (i, j) is one with the smallest dist value.
- Replace row i by min of row i and row j.
- Infinity out row j and column j.
- Update dmin[i] and change dmin[i'] to i if previously dmin[i'] = j.

Closest pair

	dmin	dist
0	1	5.5
1	3	2.14
2	4	5.6
3	1	2.14
4	3	5.5

	0	1	2	3	4
gene0	-	5.5	7.3	8.9	5.8
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2	7.3	6.1	-	7.8	5.6
3	8.9	2.14	7.8	-	5.5
4	5.8	5.6	5.6	5.5	-

gene1 closest to gene3, dist = 2.14

---

	dmin	dist
0	1	5.5
1	0	5.5
2	4	5.6
3	-	-
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	0	1	2	3	4
0	-	5.5	7.3	-	5.8
node1	5.5	-	6.1	-	5.5
2	7.3	6.1	-	-	5.6
3	-	-	-	-	-
4	5.8	5.5	5.6	-	-

New min dist

```
}
```

Store Centroids in Each Internal Node

Cluster analysis.

Centroids distance / similarity.

Easy modification to TreeNode data structure.

- Store Vector in each node.
  - leaf nodes: directly corresponds to a gene
  - internal nodes: centroid = average of all leaf nodes beneath it
- Maintain count field in each TreeNode, which equals the number of leaf nodes beneath it.
- When setting z to be parent of x and y,
  - set z.count = x.count + y.count
  - set z.vector = αp + (1-α)q, where p = x.vector and q = y.vector, and α = x.count / z.count

Analysis and Micro-Optimizations

Running time. Proportional to MN^2 (N genes, M arrays)
Memory. Proportional to N^2.

Ex. [M = 50, N = 6,000] Takes 280MB, 48 sec on fast PC.

$\longleftarrow$ input size proportional to MN

Some optimizations.

- Use float instead of double
- Store only lower triangular part of distance matrix
- Use squares of distances instead of distances.

How much do you think would this help?

Hierarchical clustering: problems

- Hard to define distinct clusters
- Genes assigned to clusters on the basis of all experiments
- Optimizing node ordering hard (finding the optimal solution is NP-hard)
- Can be influenced by one strong cluster – a problem for gene expression b/c data in row space is often highly correlated

Distance Metrics

- Choice of distance measure is important for most clustering techniques
- Linear measures: Euclidean distance, Pearson correlation
- Non-parametric: Spearman correlation, Kendall’s tau

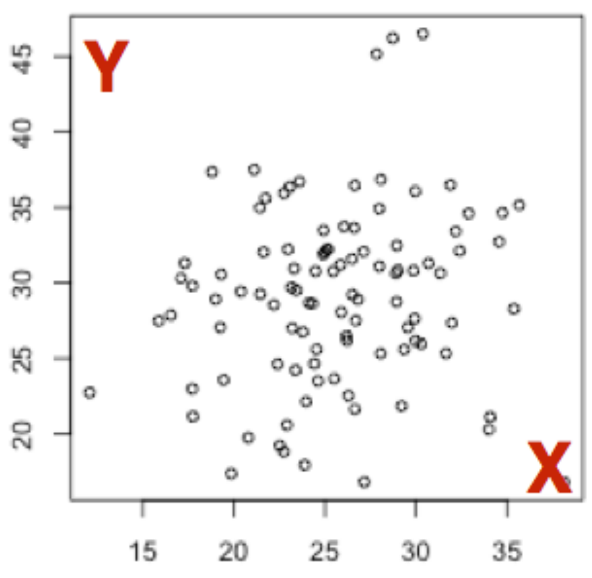
$$d = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - y_i)^2}$$

$$r = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{x_i - \bar{x}}{\sigma_x}\right)\left(\frac{y_i - \bar{y}}{\sigma_y}\right)$$

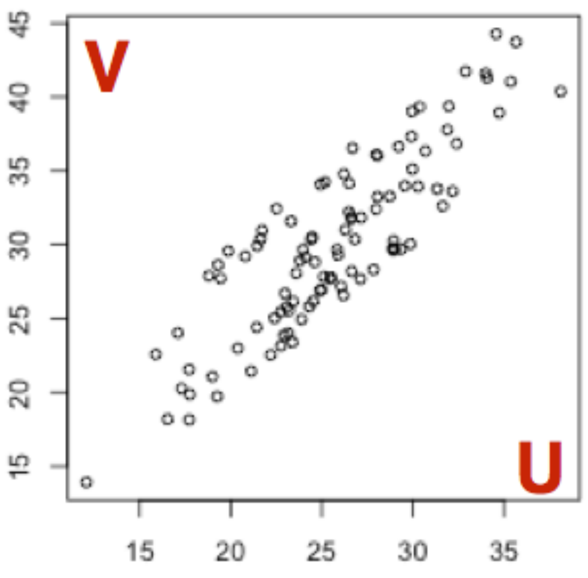
$$\rho = 1 - \frac{6\sum_{i=1}^{n}[rank(x_i) - rank(y_i)]}{n(n^2 - 1)}$$

Distance Metrics

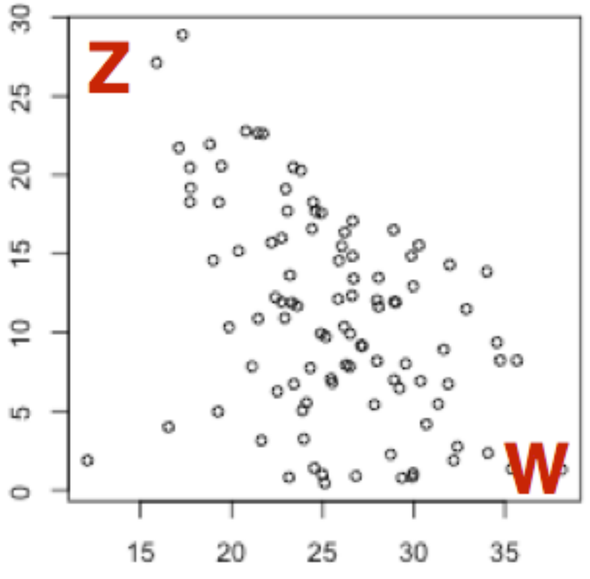
Consider the following plot of 3 pairs of genes



No correlation



Positive correlation

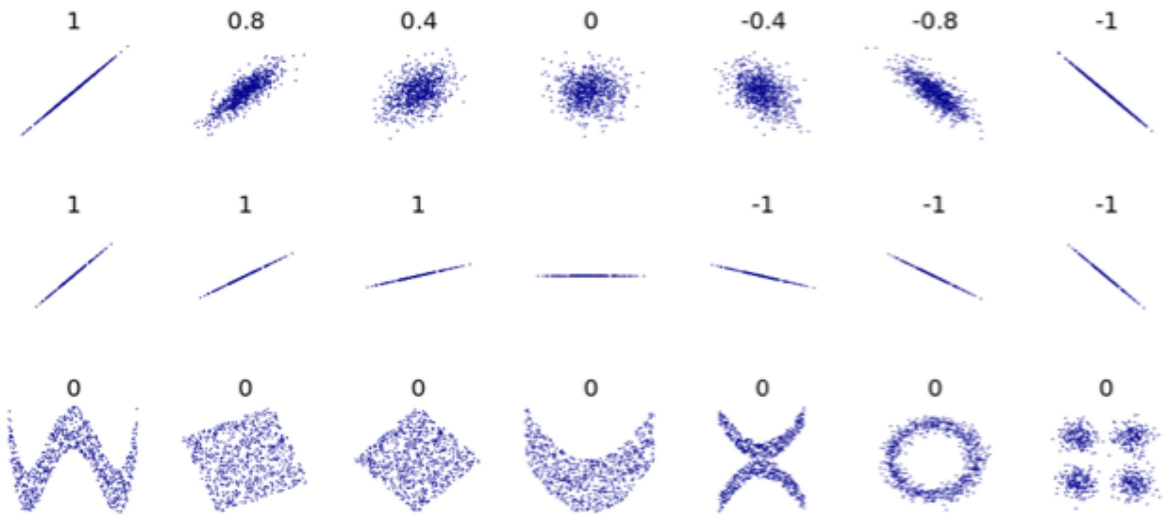


Negative correlation

Distance Metrics

Pearson correlation (r) is a measure of the linear correlation (dependence) between two variables X and Y.

$$r = \frac{1}{n-1}\sum_{i=1}^{n}\left(\frac{X_i - \bar{X}}{s_X}\right)\left(\frac{Y_i - \bar{Y}}{s_Y}\right)$$



+1 ≤ r ≤ –1

+1 is total positive correlation

0 is no correlation

–1 is total negative correlation.

Distance Metrics

11 datapoints

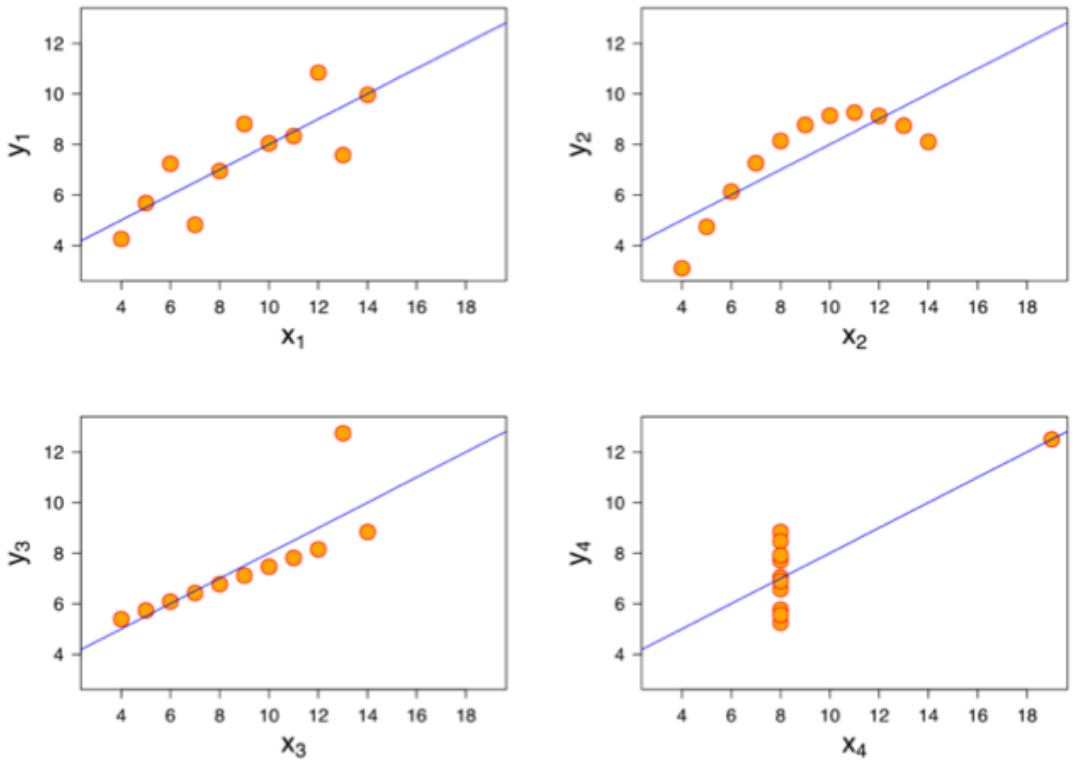
Mean (x) = 9
Var (x) = 11

Mean (y) = 7.50
Var (y) ~ 4.12

Cor (x, y) = 0.816

Linear regression line:
y = 3.00 + 0.500x

Anscombe's quartet



Anscombe, F. J. (1973). "Graphs in Statistical Analysis". American Statistician 27 (1): 17–21.

Distance Metrics

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- Linear measures: Euclidean distance, Pearson correlation
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$$r = \frac{1}{n}\sum_{i=1}^{n}\left(\frac{x_i - \bar{x}}{\sigma_x}\right)\left(\frac{y_i - \bar{y}}{\sigma_y}\right)$$

$$\rho = 1 - \frac{6\sum_{i=1}^{n}[rank(x_i) - rank(y_i)]}{n(n^2 - 1)}$$

Distance Metrics

- Choose your distance measure carefully.

- In general, before you *begin analysis*:
  - *Explore* your data by:
    - Doing simple **sanity-checks**: “Is the mean, variance, and range of values as expected?”, “Are there too many missing values?”
    - **Looking** at your data [There is no substitute for this]: Plot small portions of the data in different ways and visualize trends, shapes, relationships, etc.

The End