Introduction to R for Biologists

Day 4 – Data analysis

*Several slides have been contributed by or modified from slides by Dr. Claus Wilke

Day 4 Outline

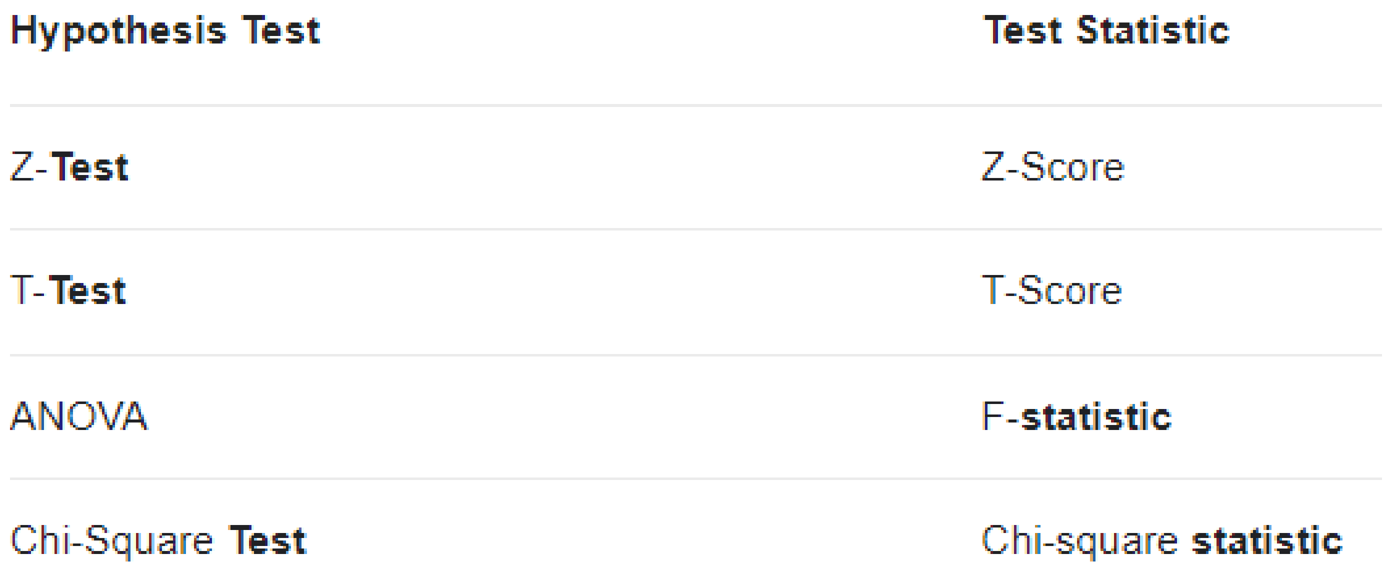
1. Hypothesis testing
   1. Test statistics
   2. p-values
   3. False discovery rate
2. Exploratory analysis
   A. Dimensionality reduction
   B. Clustering

Many, many types of statistical tests

Your choice will depend on your experiment:

http://www.biostathandbook.com/testchoice.html

But generally these are the work horses:



Hypothesis Test	**Test Statistic**
Z-**Test**	Z-Score
T-**Test**	T-Score
ANOVA	F-**statistic**
Chi-Square **Test**	Chi-square **statistic**

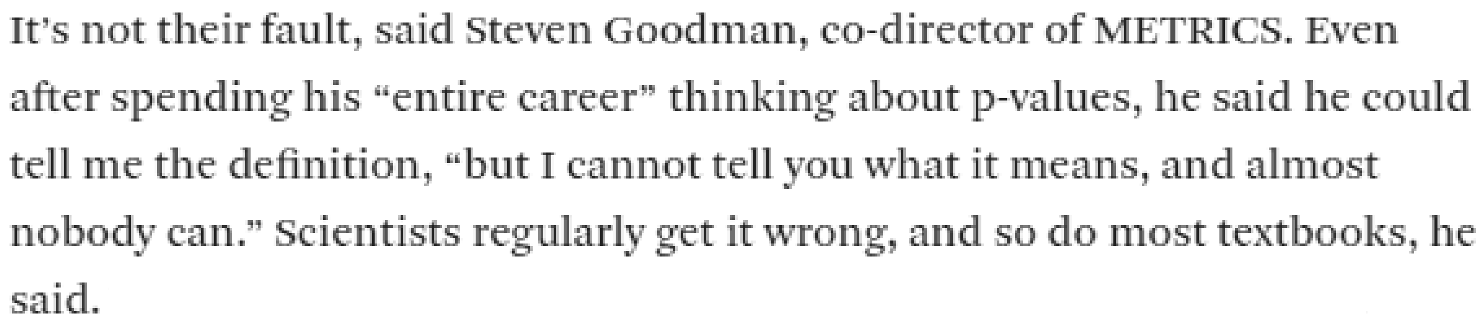
But they all follow the same pattern

1. Calculate a sample statistic (δ) from your real data
   - Mean, difference in means, median, proportion, difference in proportions, chi-squared value, etc
2. Use simulation to create a null distribution
3. Compare δ to the null distribution – how does it fit?
4. Calculate probability (p-value) that δ could exist in a null world
5. Decide if δ is statistically significant

Conceptualizing the p-value

Technical definition:

- Probability of observing the results by chance given that the null hypothesis is correct



It's not their fault, said Steven Goodman, co-director of METRICS. Even after spending his "entire career" thinking about p-values, he said he could tell me the definition, "but I cannot tell you what it means, and almost nobody can." Scientists regularly get it wrong, and so do most textbooks, he said.

Conceptualizing the p-value

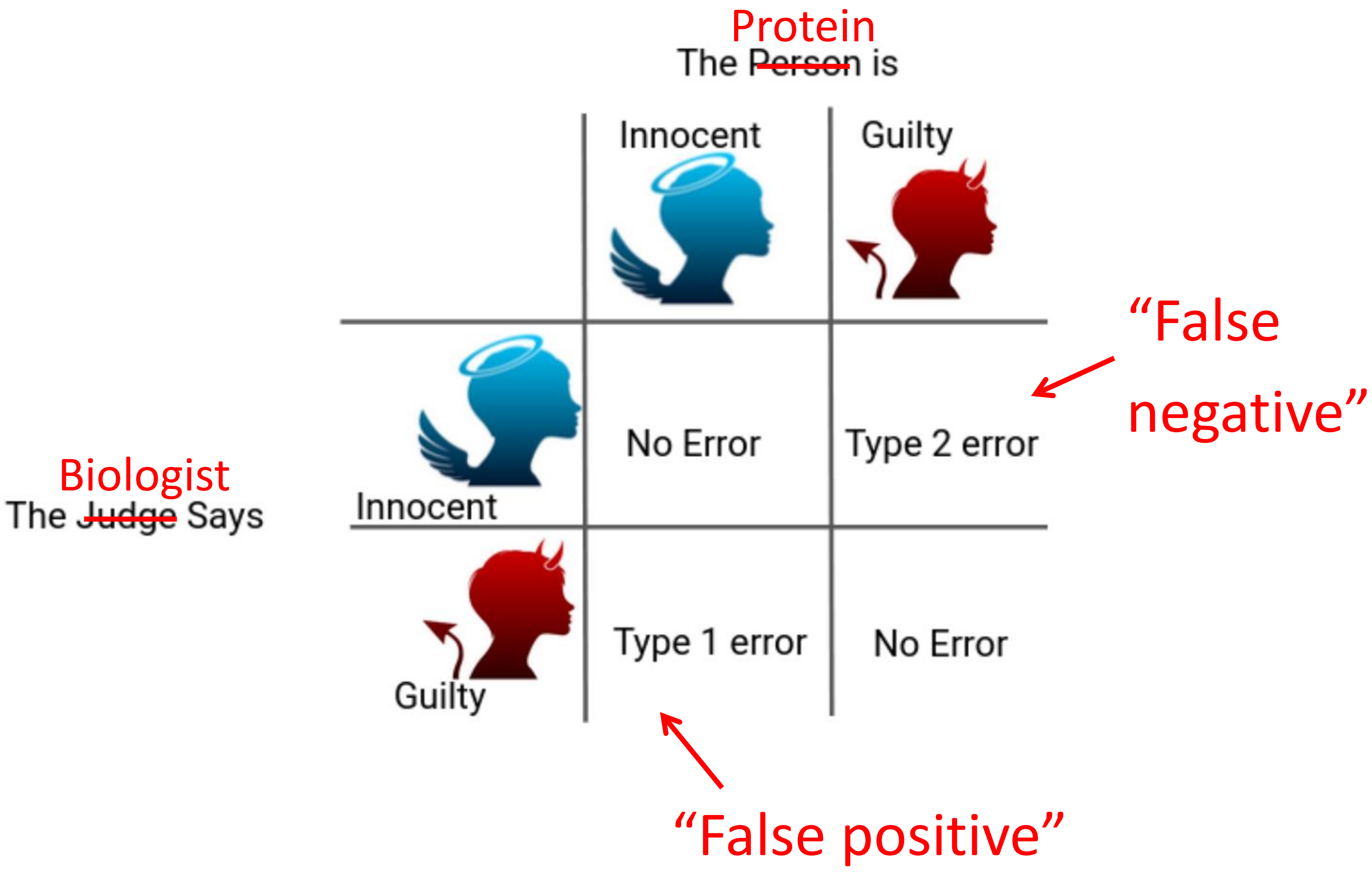
The Presumption of Innocence:

All molecules are innocent until proven guilty $\underline{\text{beyond a reasonable doubt.}}$

$\downarrow$

Significance level
or p-value cutoff

Conceptualizing the p-value

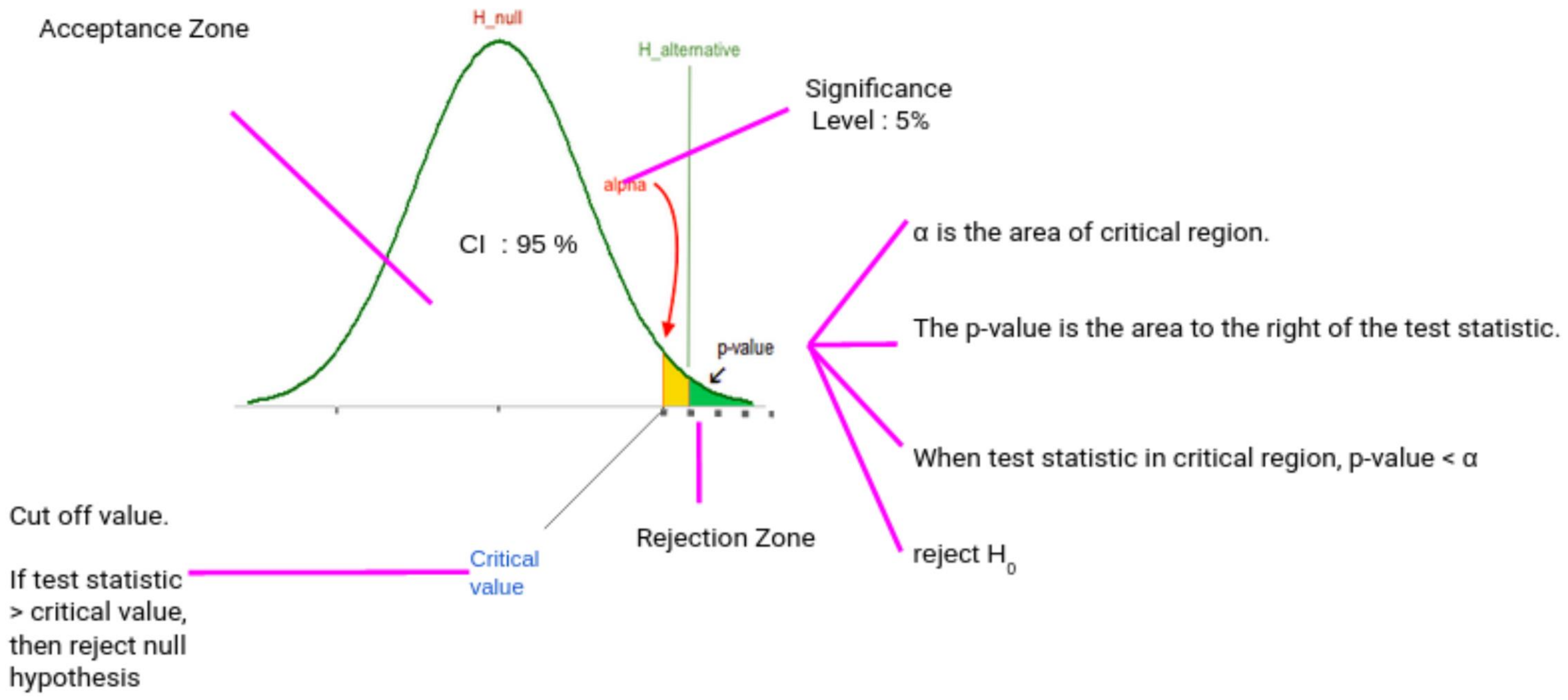


		The ~~Person~~ Protein is	
		Innocent	Guilty
The ~~Judge~~ Biologist Says	Innocent	No Error	Type 2 error
	Guilty	Type 1 error	No Error

"False negative"

"False positive"

Conceptualizing the p-value



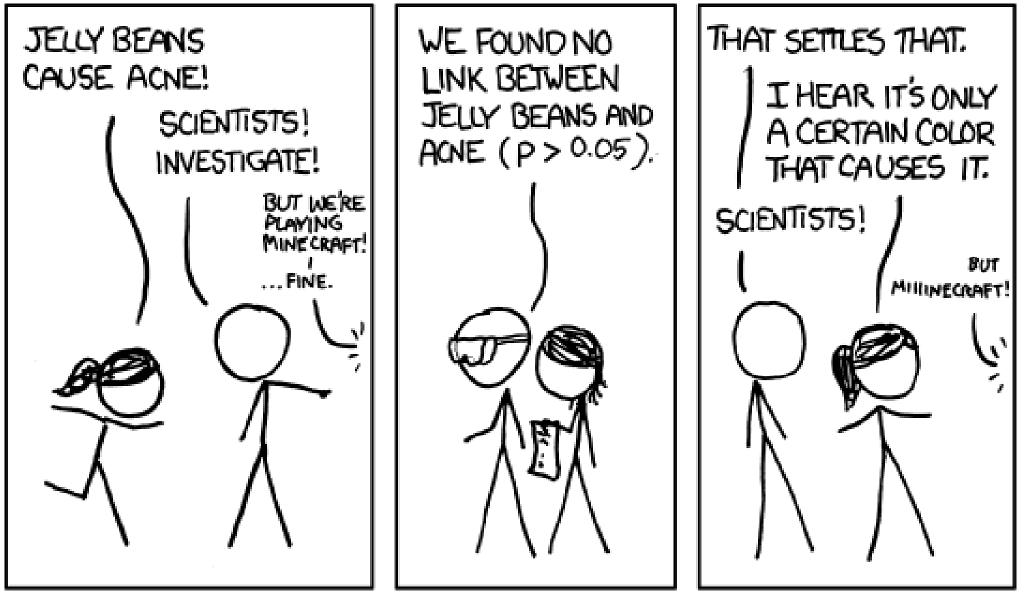
Acceptance Zone
H_null
H_alternative
Significance Level : 5%
alpha
CI : 95 %
p-value
α is the area of critical region.
The p-value is the area to the right of the test statistic.
When test statistic in critical region, p-value < α
reject H_0
Cut off value.
If test statistic > critical value, then reject null hypothesis
Critical value
Rejection Zone

How good is your evidence?

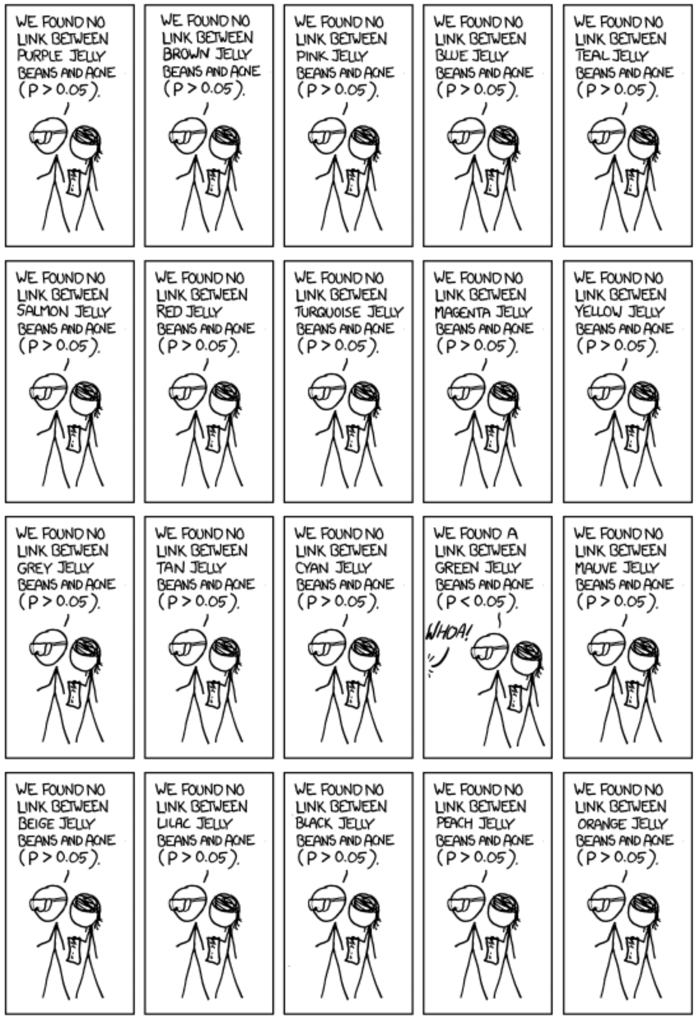
- i.e., how much statistical power does your experiment have?
- Two factors:
  - Effect size
  - Sample size

Multiple comparisons problem

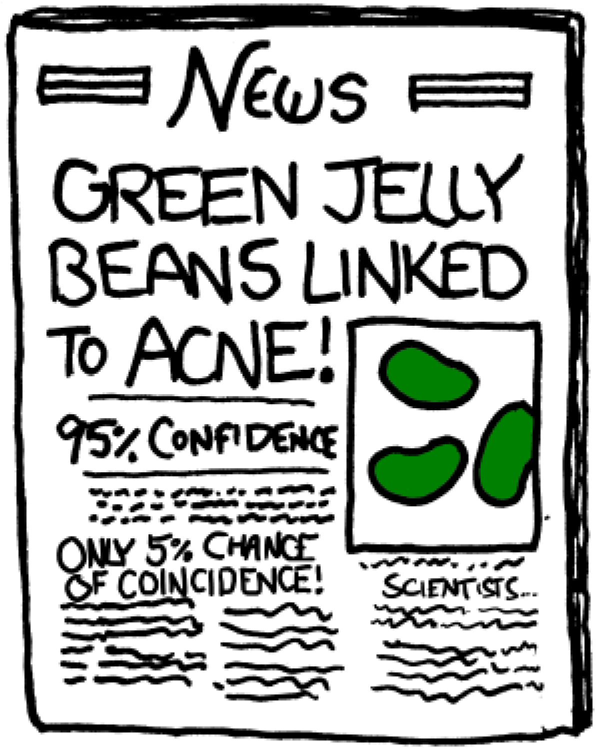
As the # of comparisons go up → probability of a false positive goes up


JELLY BEANS CAUSE ACNE!
SCIENTISTS! INVESTIGATE!
BUT WE'RE PLAYING MINECRAFT!
...FINE.
WE FOUND NO LINK BETWEEN JELLY BEANS AND ACNE (P > 0.05).
THAT SETTLES THAT.
I HEAR IT'S ONLY A CERTAIN COLOR THAT CAUSES IT.
SCIENTISTS!
BUT MIIIINECRAFT!

Multiple comparisons problem


WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TURQUOISE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN MAGENTA JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN YELLOW JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN GREY JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN TAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN CYAN JELLY BEANS AND ACNE (P > 0.05).
WE FOUND A LINK BETWEEN GREEN JELLY BEANS AND ACNE (P < 0.05).
WHOA!
WE FOUND NO LINK BETWEEN MAUVE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BEIGE JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN LILAC JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN BLACK JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN PEACH JELLY BEANS AND ACNE (P > 0.05).
WE FOUND NO LINK BETWEEN ORANGE JELLY BEANS AND ACNE (P > 0.05).

So if you perform the same test a bunch of times, eventually you're going to get a false positive


NEWS
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...

Multiple comparisons problem

- Consider an RNA-seq differential expression experiment in HEK293 cell lines, +/- drug treatment

Multiple comparisons problem

- You measure 12,000 transcripts in both the control and treated samples

$$\downarrow$$

- You perform 12,000 statistical tests for mRNA in control vs treated (p-value <= 0.05)

$$\downarrow$$

- Probability of **at least** 1 false positive increases to essentially 100%

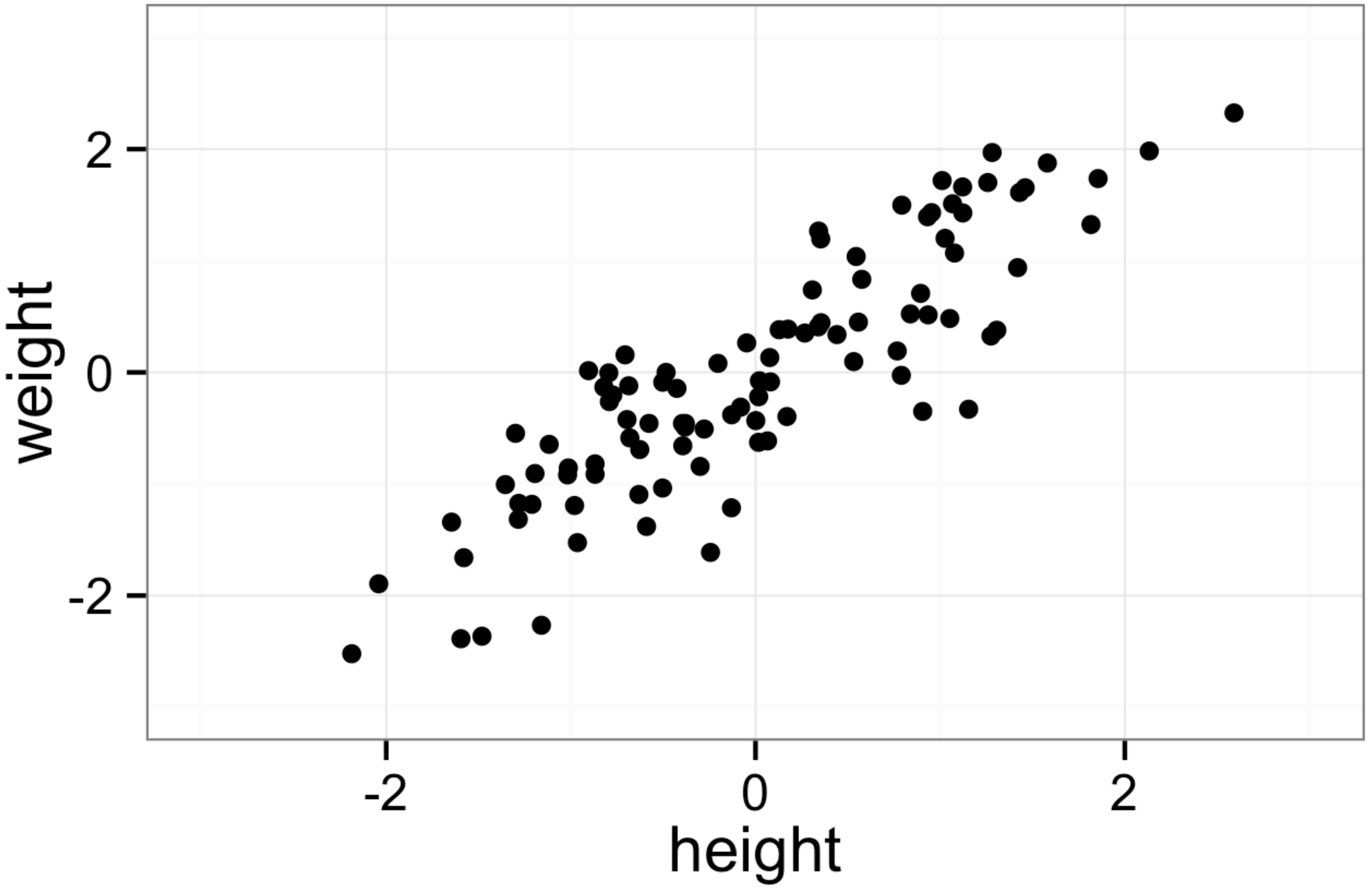
Multiple comparisons problem

- Active area of research
- No universally accepted approach
- Corrective algorithms span the extremely conservative (e.g., Bonferroni correction) to the less conservative (e.g., Benjamini-Hochberg procedure)

Principal Components Analysis (PCA)

- Dimension reduction
- Useful for exploratory data analysis of high-dimensional data sets.

Example: Consider a data set of heights and weights of people



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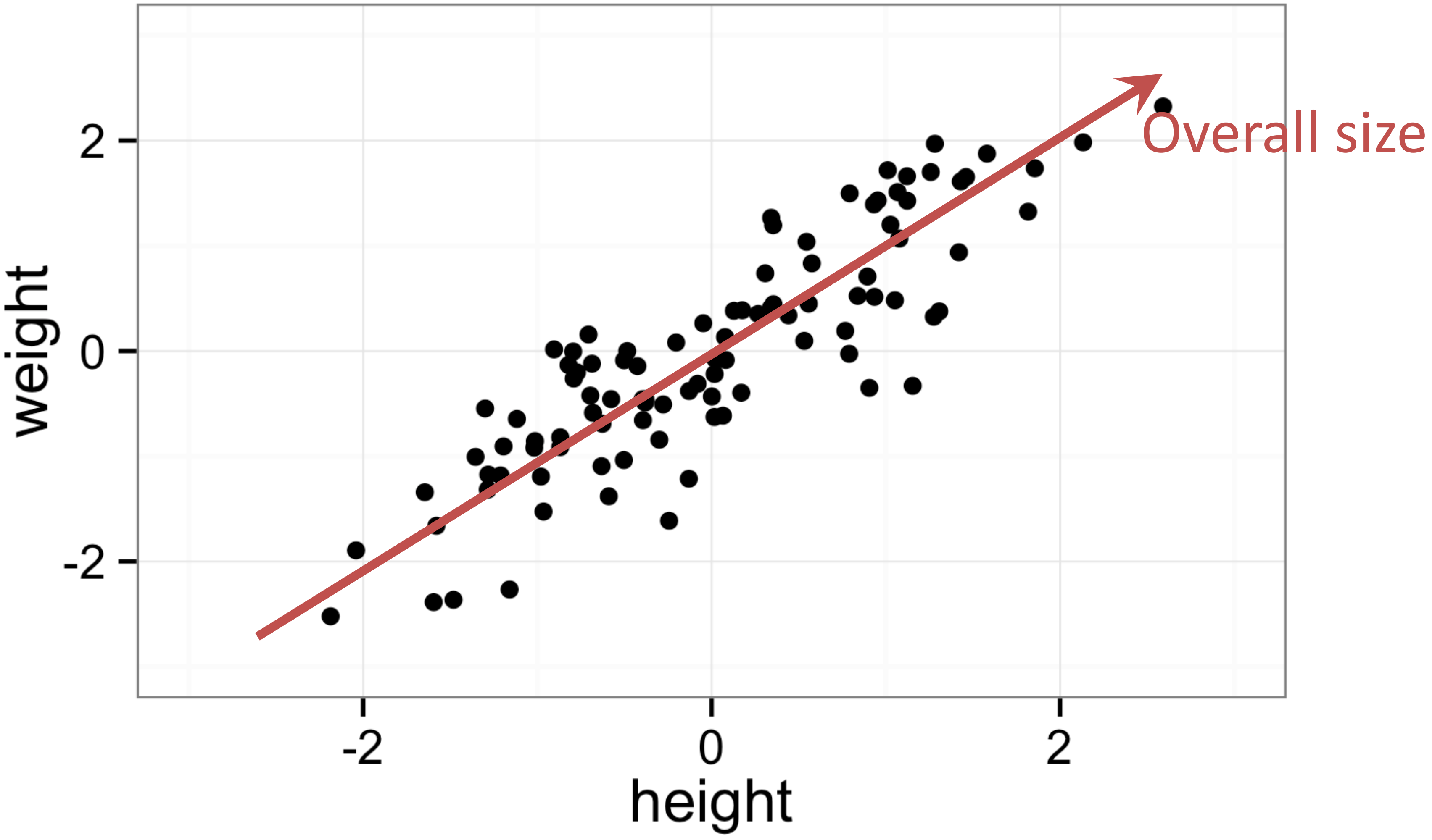

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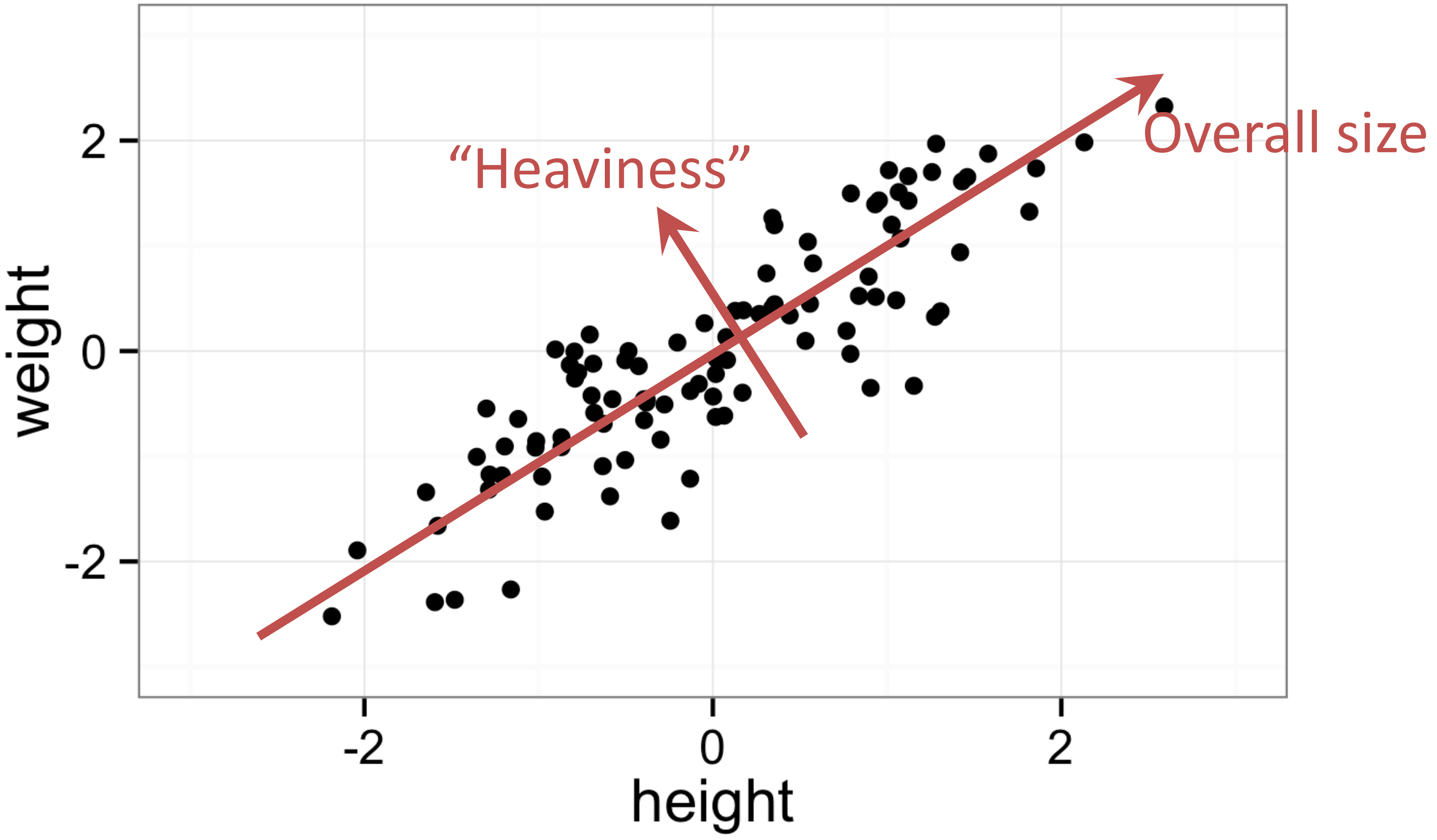
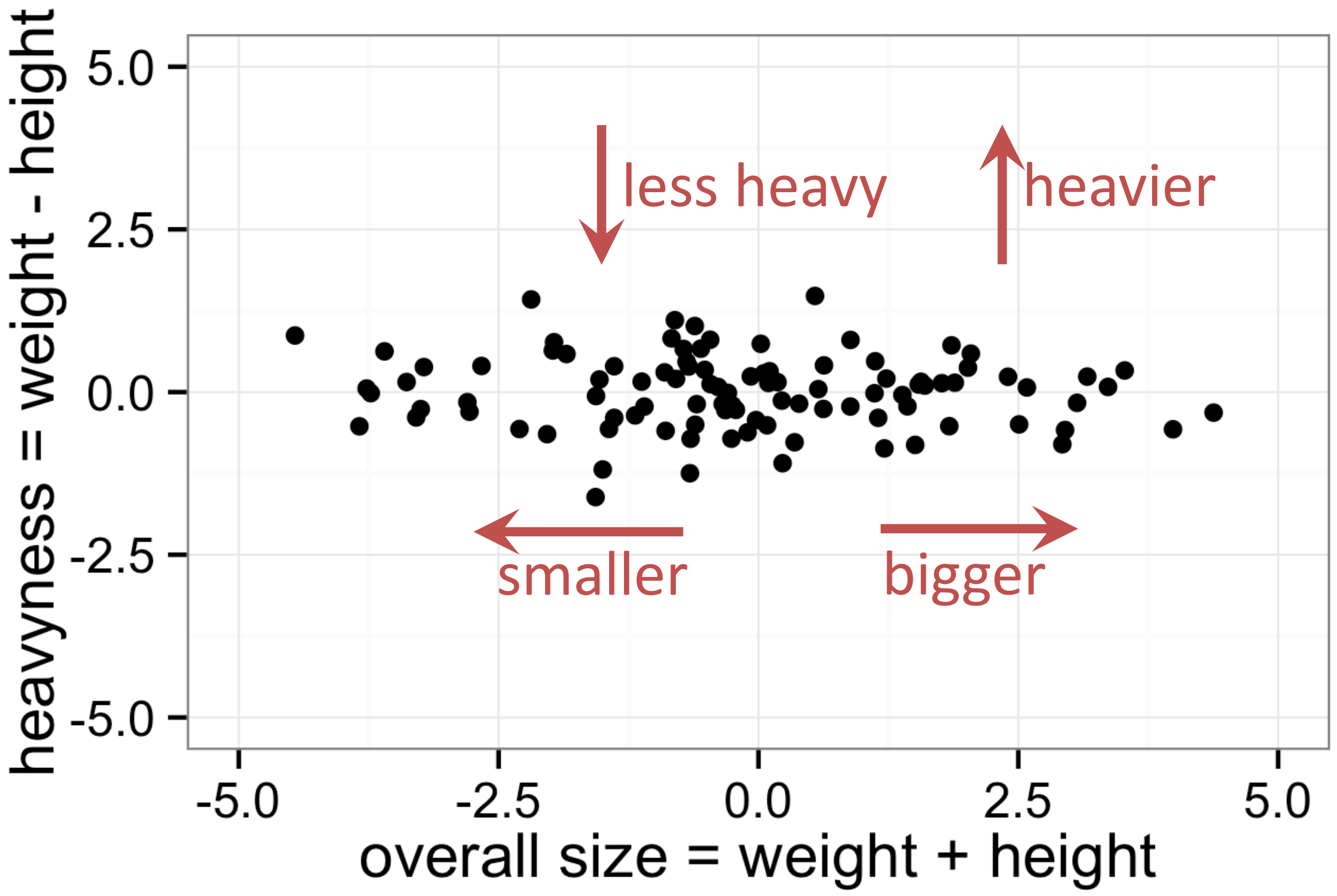
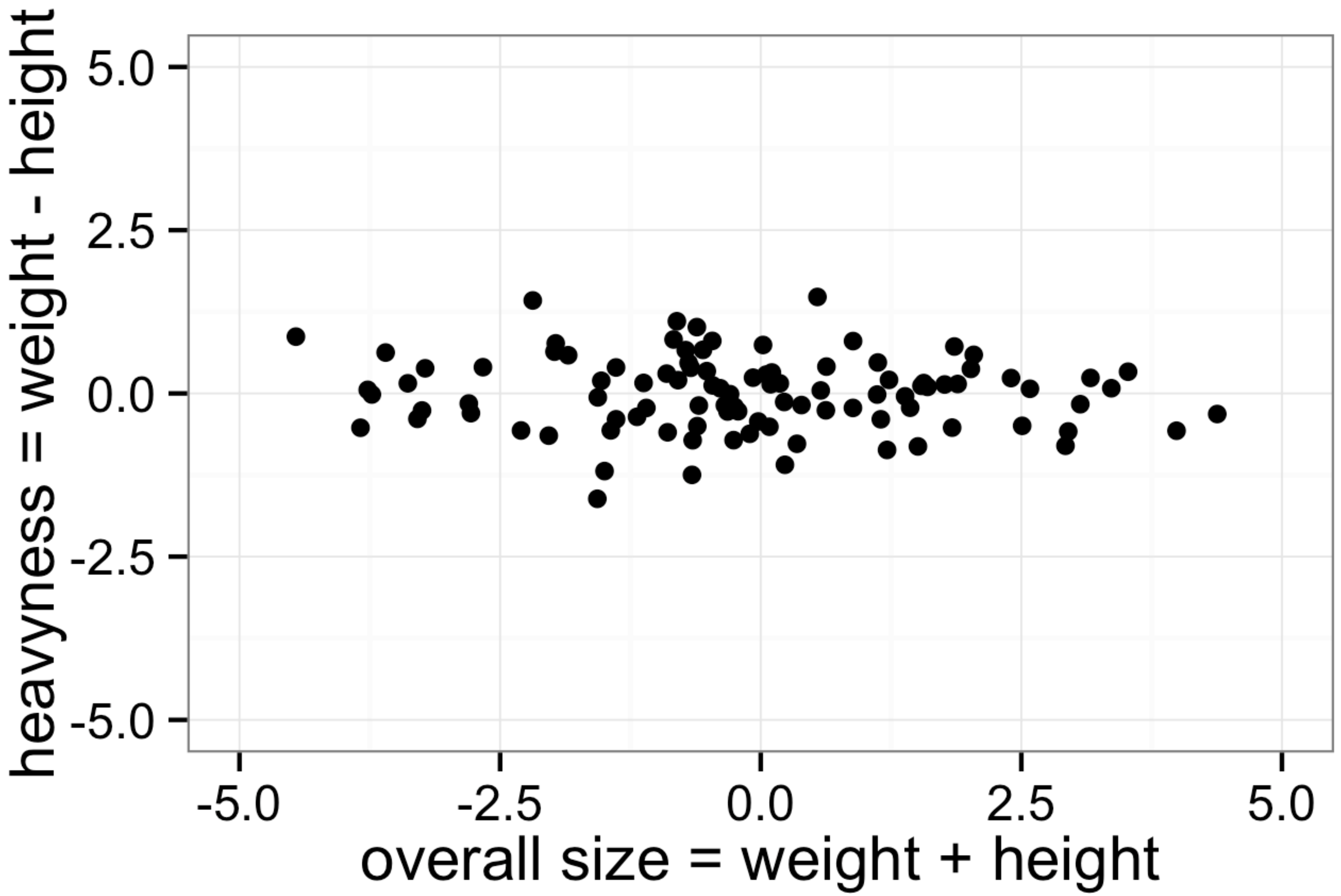


PCA on this data set reframes data in terms of overall size and heavyness



In our earlier example, overall size and heaviness are uncorrelated



Doing a PCA in R

```
iris %>%
     select(-Species) %>%    # remove Species column
     scale() %>%             # scale to zero mean
                             # and unit variance
     prcomp() ->             # do PCA
     pca                     # store result
                             # in variable “pca”
```

Doing a PCA in R

```
> pca
Standard deviations:
[1] 1.7083611 0.9560494 0.3830886 0.1439265

Rotation:
                    PC1         PC2        PC3        PC4
Sepal.Length  0.5210659 -0.37741762  0.7195664  0.2612863
Sepal.Width  -0.2693474 -0.92329566 -0.2443818 -0.1235096
Petal.Length  0.5804131 -0.02449161 -0.1421264 -0.8014492
Petal.Width   0.5648565 -0.06694199 -0.6342727  0.5235971
```

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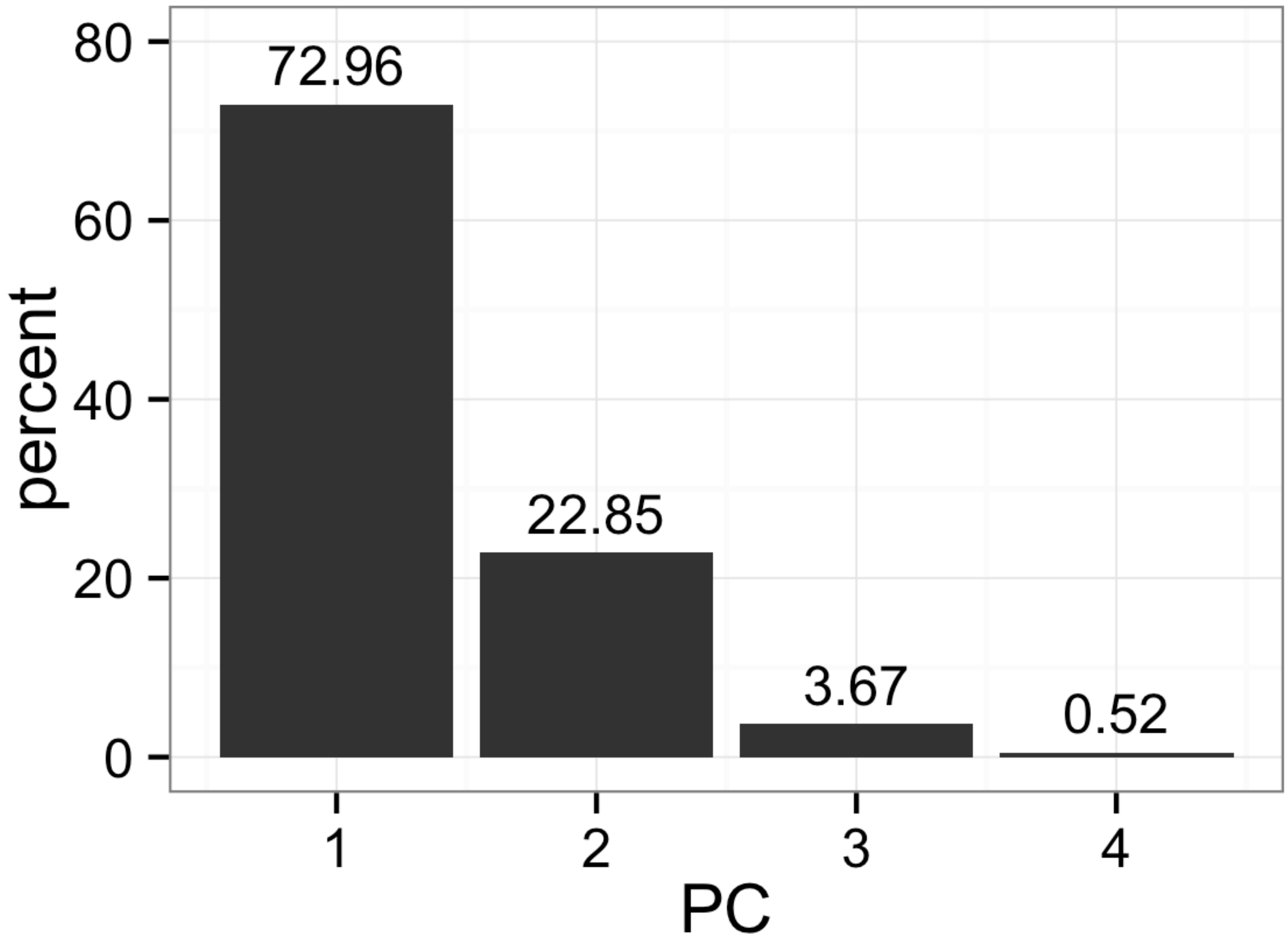
```

Squares of the std. devs represent the % variance explained by each PC



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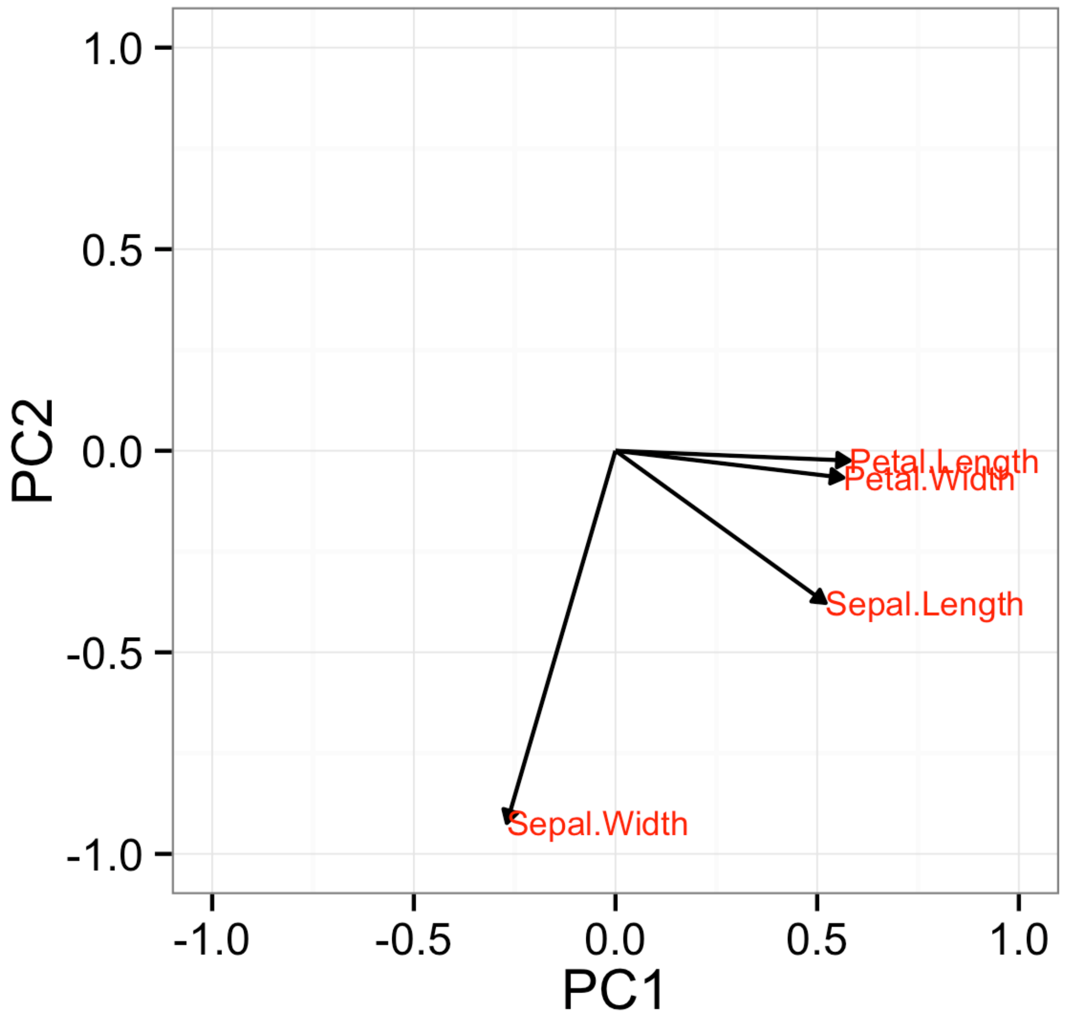
Petal.Width   0.5648565 -0.06694199 -0.6342727  0.5235971
```

The rotation matrix tells us which variables contribute to which PCs



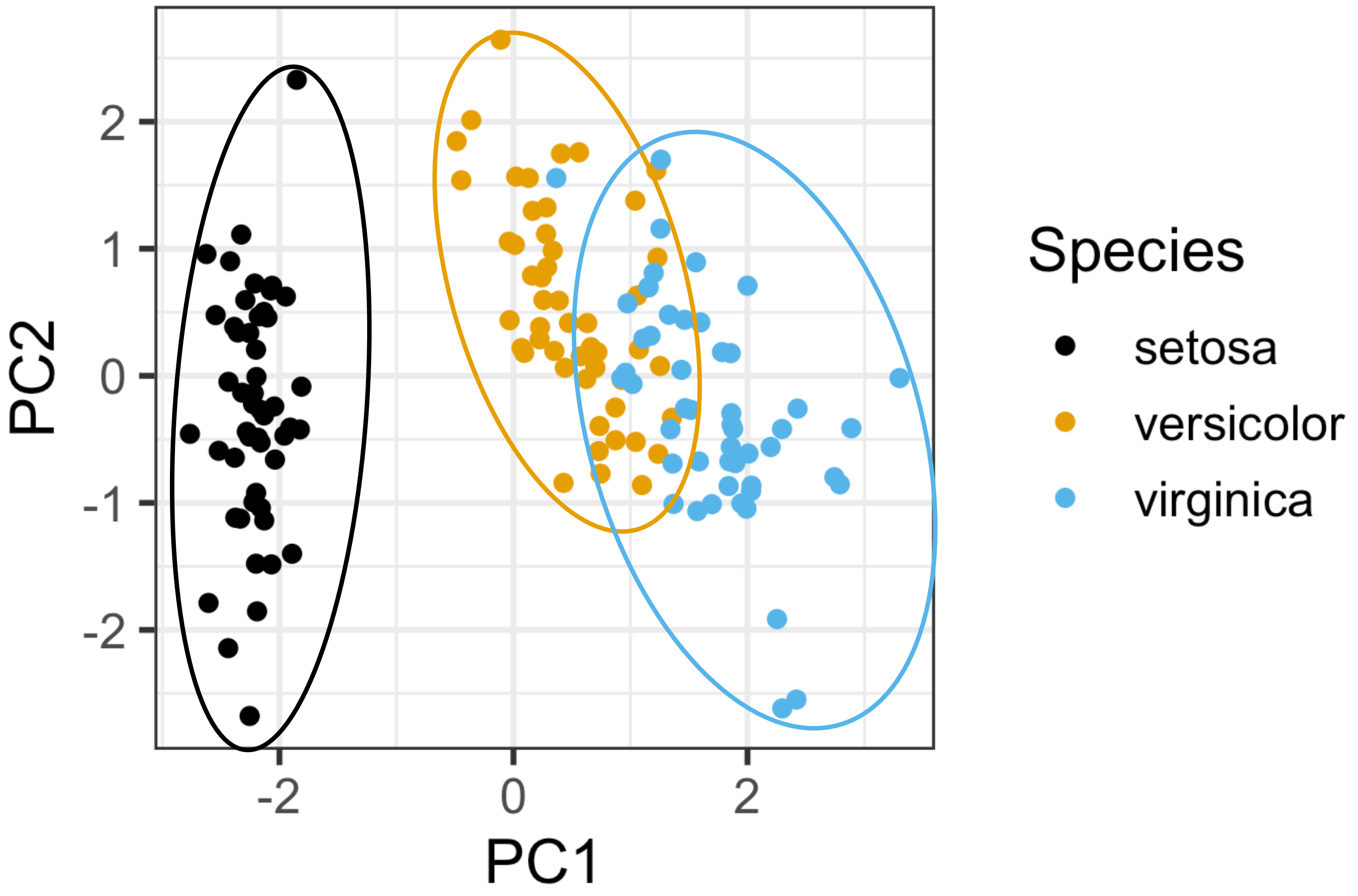
We can also recover each original observation expressed in PC coordinates

```
> pca$x
```

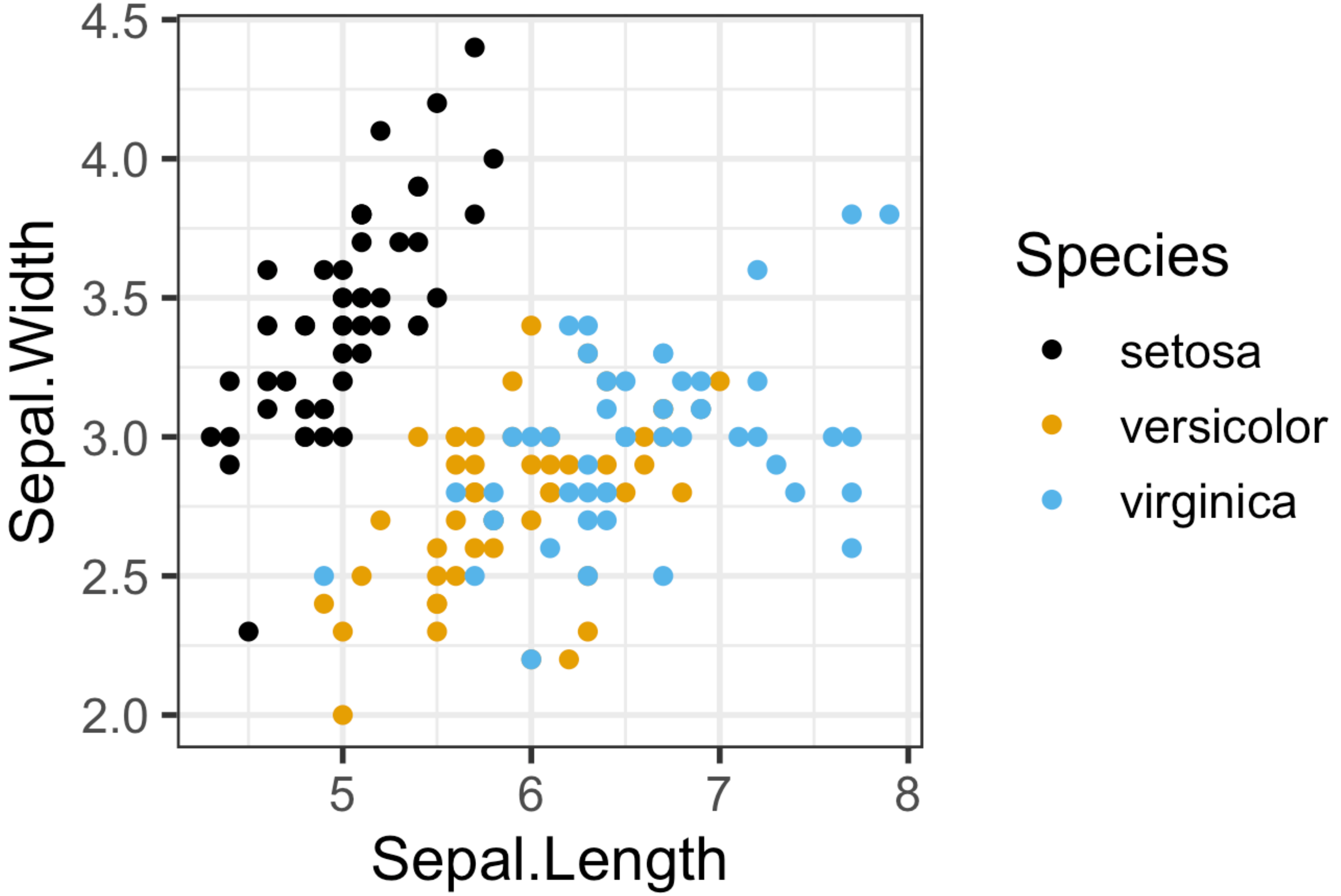
We can also recover each original observation expressed in PC coordinates

```
> pca$x
             PC1          PC2          PC3          PC4
 [1,] -2.25714118 -0.478423832  0.127279624  0.024087508
 [2,] -2.07401302  0.671882687  0.233825517  0.102662845
 [3,] -2.35633511  0.340766425 -0.044053900  0.028282305
 [4,] -2.29170679  0.595399863 -0.090985297 -0.065735340
 [5,] -2.38186270 -0.644675659 -0.015685647 -0.035802870
 [6,] -2.06870061 -1.484205297 -0.026878250  0.006586116
 [7,] -2.43586845 -0.047485118 -0.334350297 -0.036652767
 [8,] -2.22539189 -0.222403002  0.088399352 -0.024529919
 [9,] -2.32684533  1.111603700 -0.144592465 -0.026769540
[10,] -2.17703491  0.467447569  0.252918268 -0.039766068
[11,] -2.15907699 -1.040205867  0.267784001  0.016675503
[12,] -2.31836413 -0.132633999 -0.093446191 -0.133037725
[13,] -2.21104370  0.726243183  0.230140246  0.002416941
```

Plot of iris plants in PC coordinates reveals differences among species



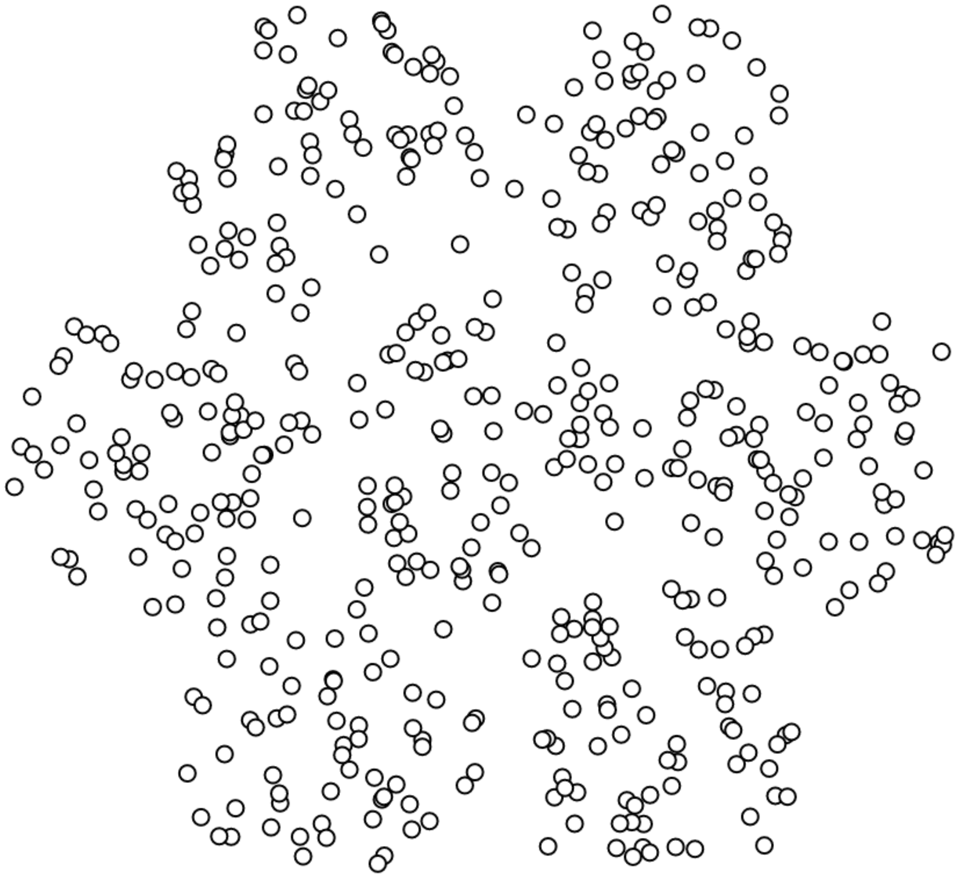
These differences are much harder to see in the original variables



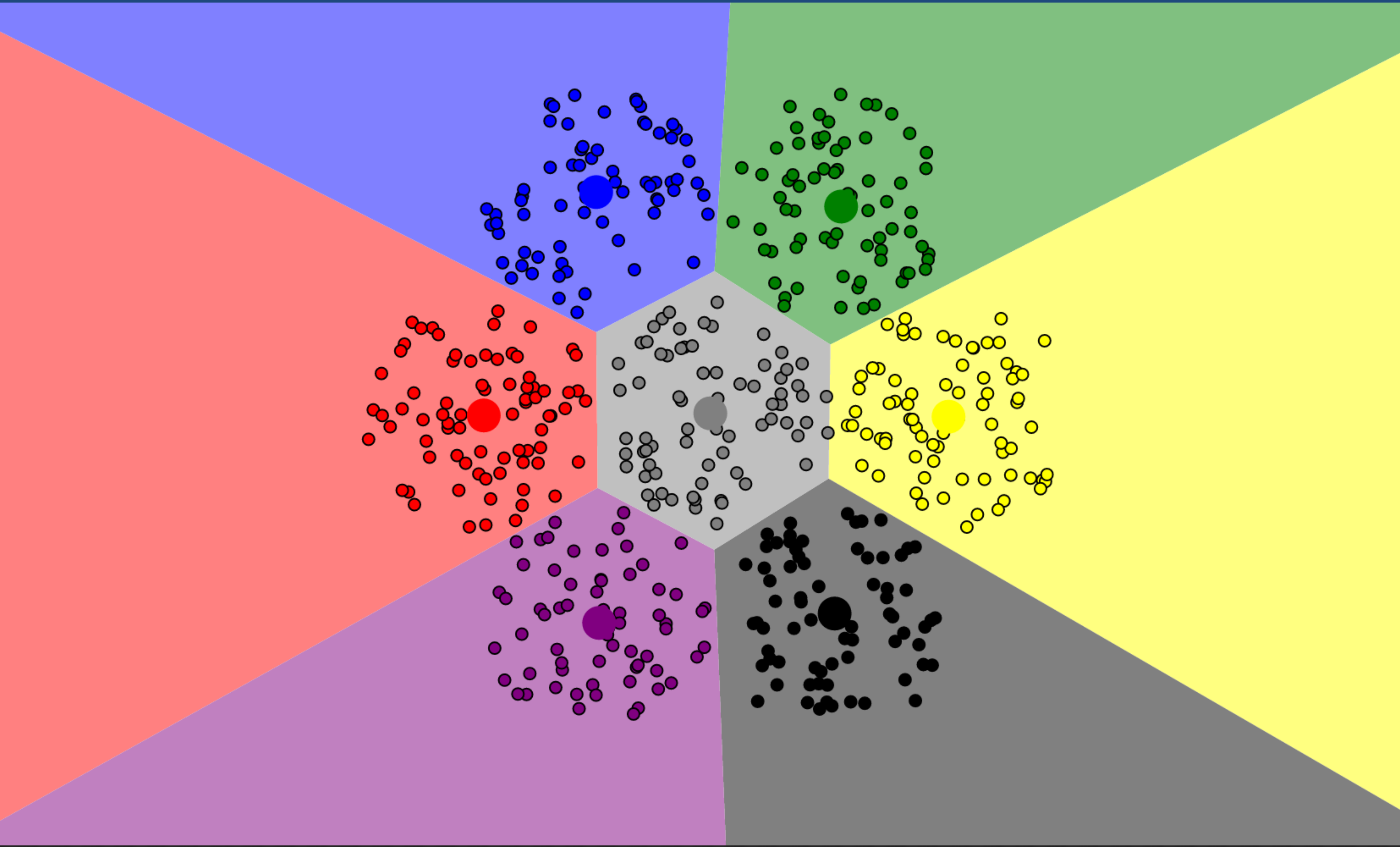
k-means clustering

Method to automatically separate data sets into distinct groups.

Clustering example



Clustering example

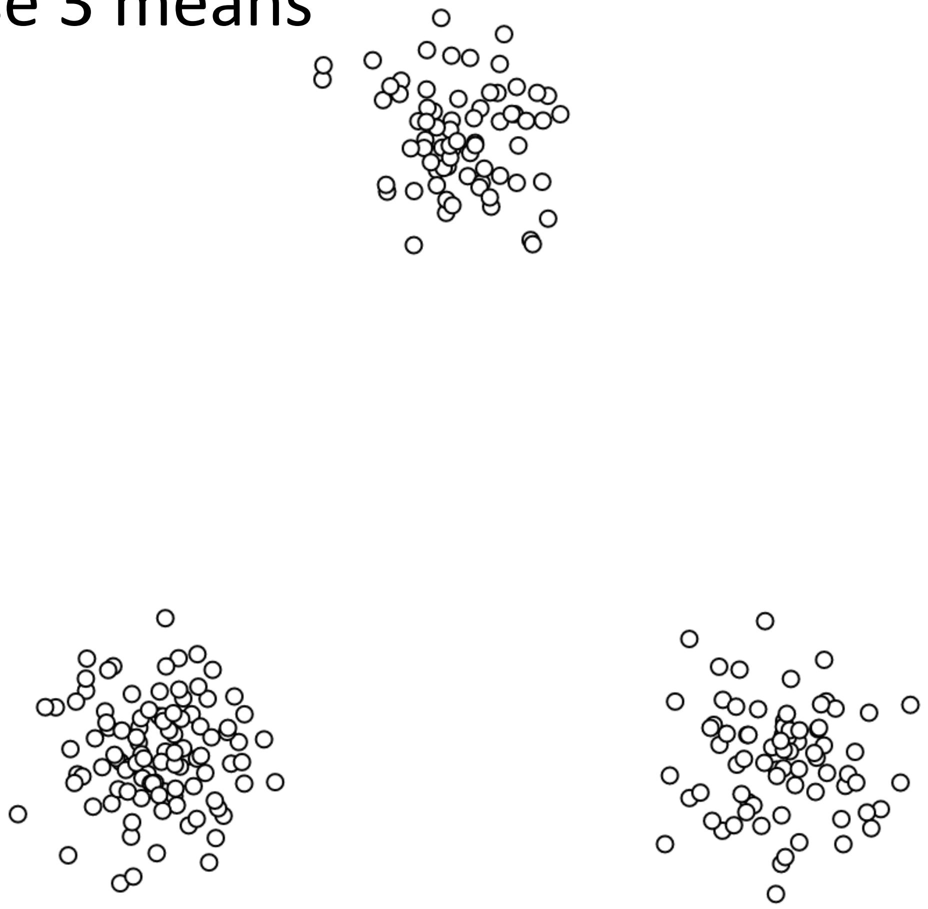


k-means clustering algorithm

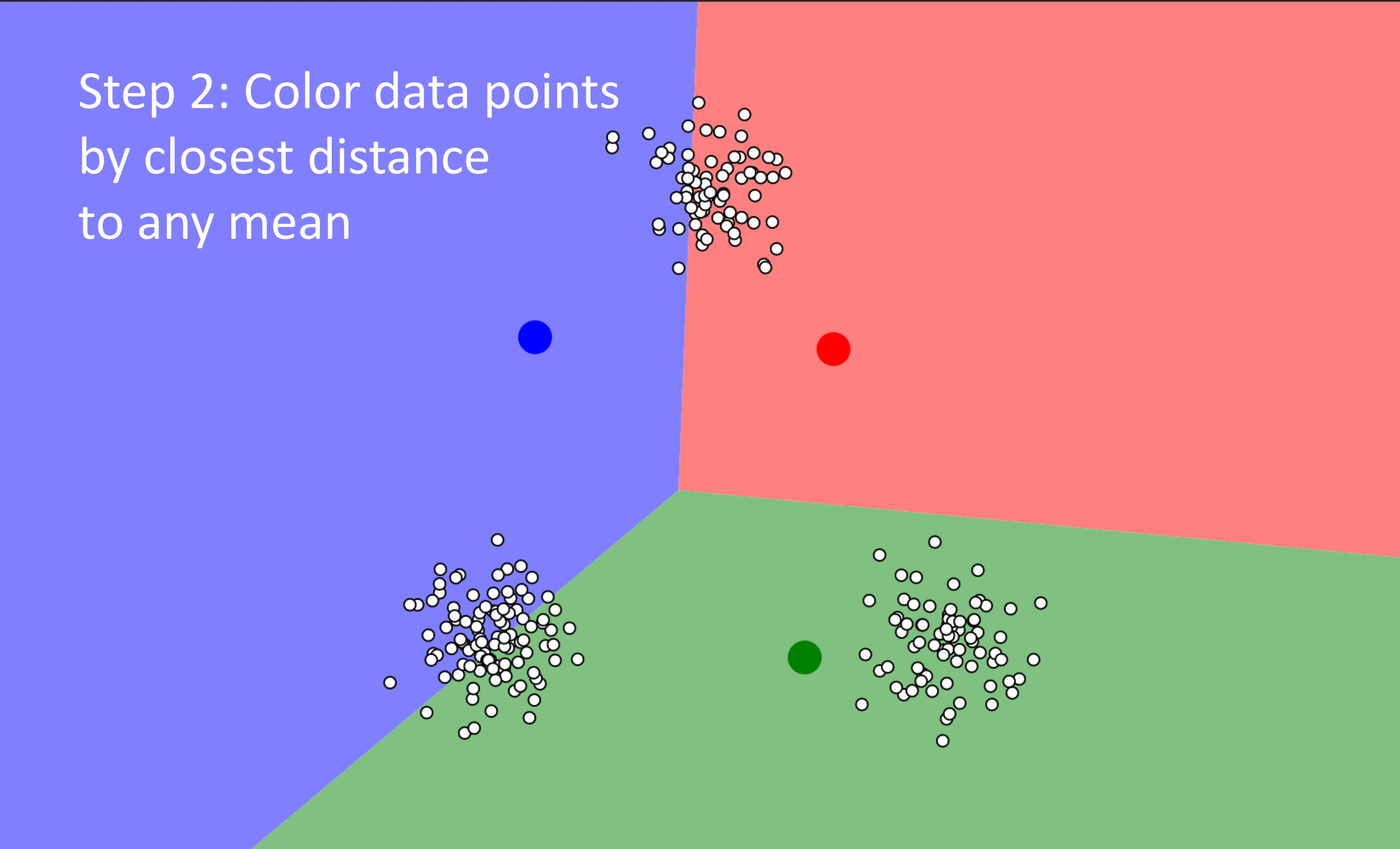
1. Start with *k* randomly chosen means
2. Color data points by the shortest distance to any mean
3. Move means to centroid position of each group of points
4. Repeat from step 2 until convergence

Algorithm example (k = 3)

Step 1: Choose 3 means at random

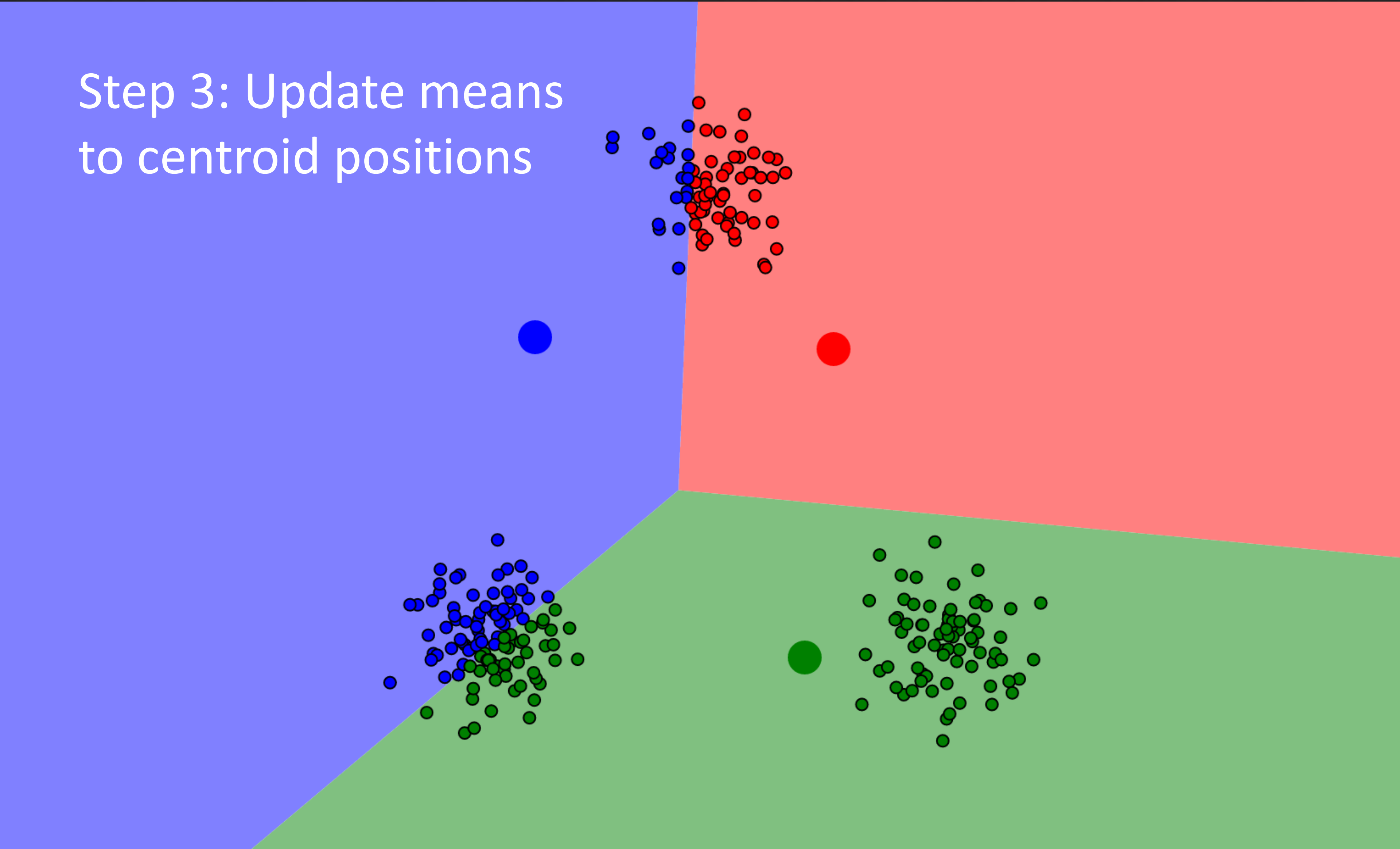


Algorithm example ($k = 3$)



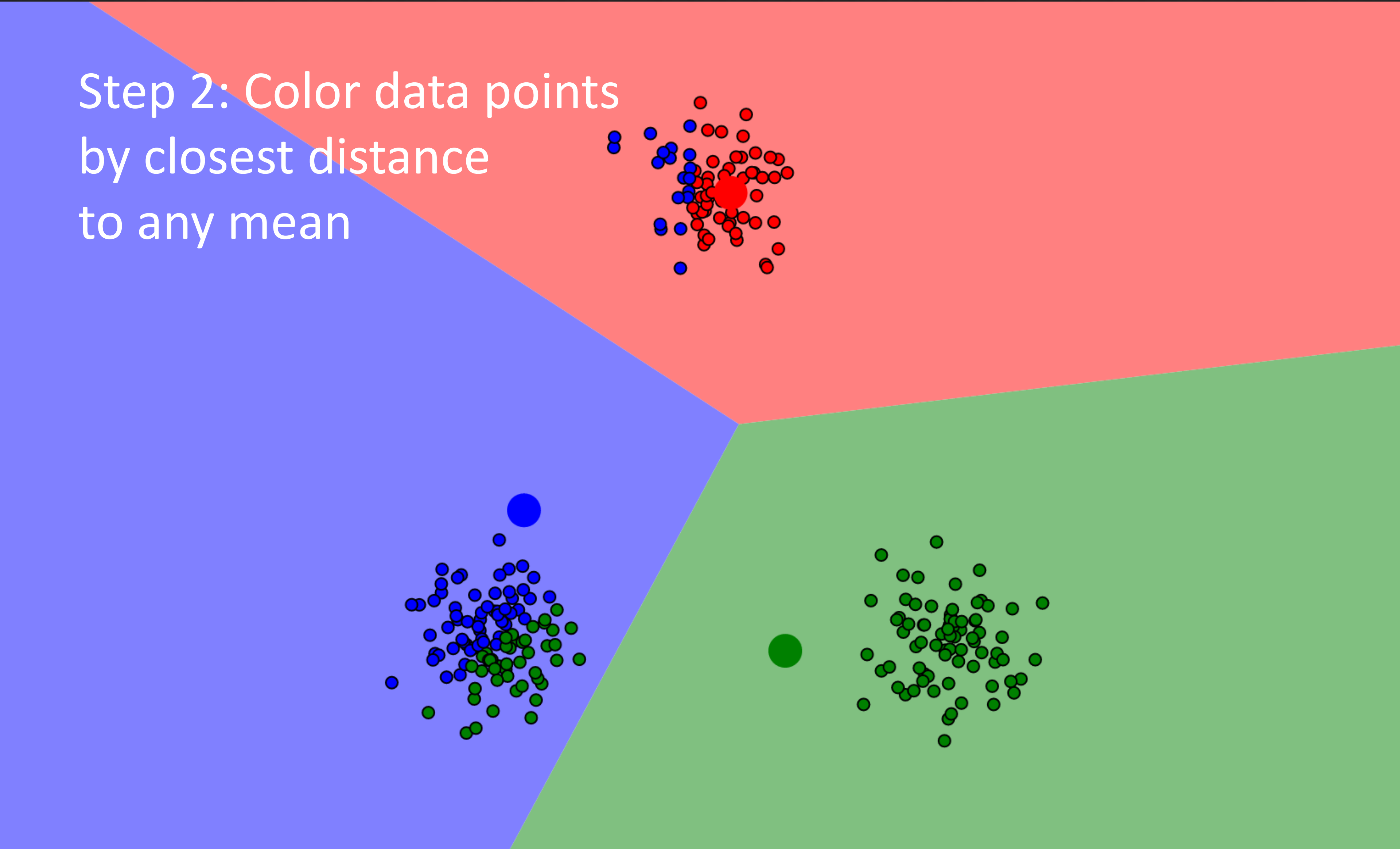
Step 2: Color data points by closest distance to any mean

Algorithm example (k = 3)



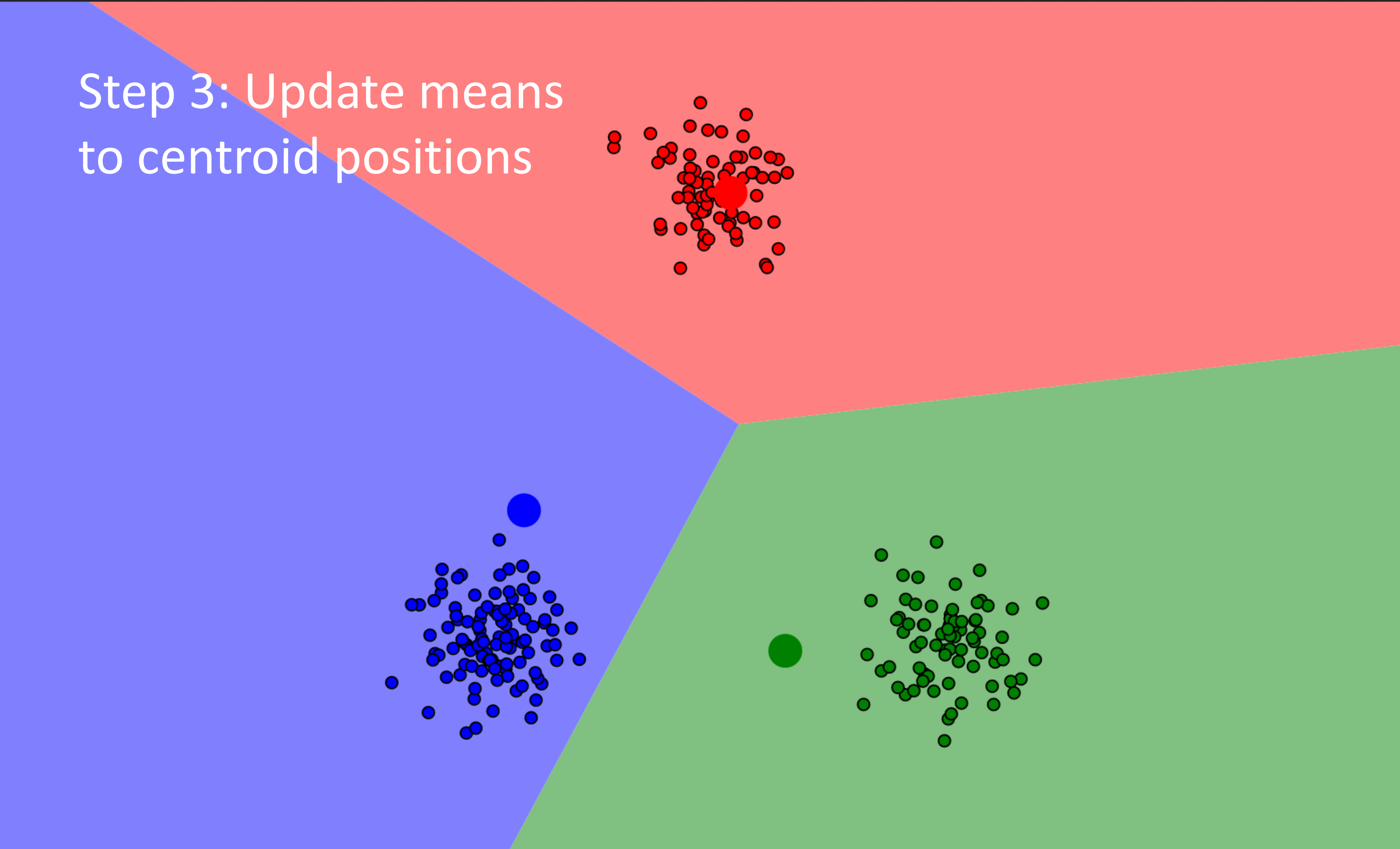
Step 3: Update means to centroid positions

Algorithm example ($k = 3$)



Step 2: Color data points by closest distance to any mean

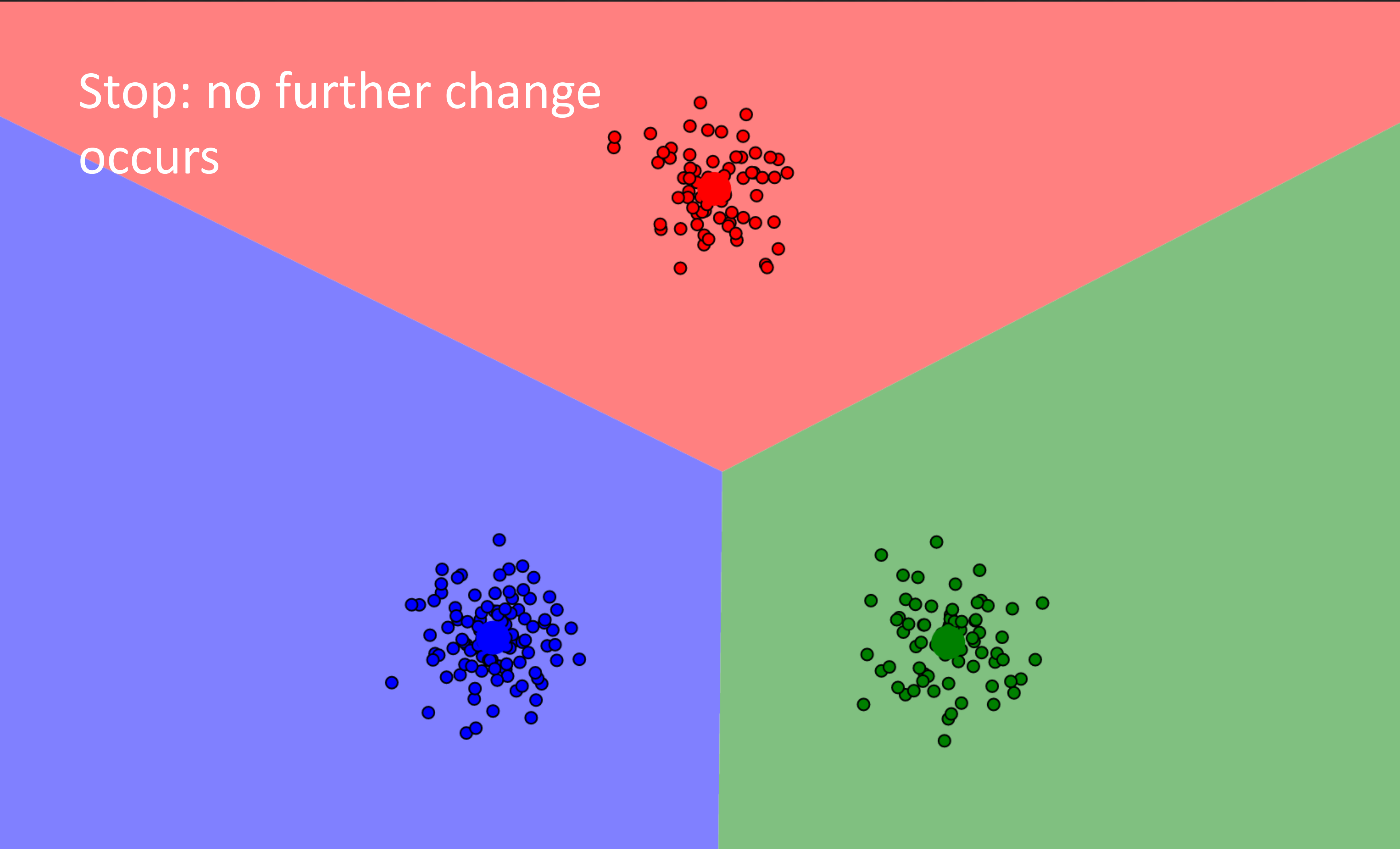
Algorithm example (k = 3)



Step 3: Update means to centroid positions

Algorithm example ($k = 3$)

Stop: no further change occurs



k-means in R (example: iris data set)

```
iris %>%
  select(-Species) %>%       # remove Species column
  kmeans(centers=3) ->       # do k-means clustering
                             # with 3 centers
  km                         # store result as "km"
```

k-means in R (example: iris data set)

```
> km
K-means clustering with 3 clusters of sizes 38, 62, 50

Cluster means:
  Sepal.Length Sepal.Width Petal.Length Petal.Width
1     6.850000    3.073684     5.742105    2.071053
2     5.901613    2.748387     4.393548    1.433871
3     5.006000    3.428000     1.462000    0.246000

Clustering vector:
  [1] 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
 [38] 3 3 3 3 3 3 3 3 3 3 3 3 3 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
 [75] 2 2 2 1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 1 2 1 1 1 1 2 1 1 1 1
[112] 1 1 2 2 1 1 1 1 2 1 2 1 2 1 1 2 2 1 1 1 1 1 2 1 1 1 1 2 1 1 1 2 1 1 1 2 1
[149] 1 2

Within cluster sum of squares by cluster:
[1] 23.87947 39.82097 15.15100
 (between_SS / total_SS =  88.4 %)
```

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[149] 1 2

Within cluster sum of squares by cluster:
[1] 23.87947 39.82097 15.15100
 (between_SS / total_SS =  88.4 %)
```

Cluster means: the location of the final centroids

k-means in R (example: iris data set)

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 [149] 1 2

Within cluster sum of squares by cluster:
[1] 23.87947 39.82097 15.15100
 (between_SS / total_SS =  88.4 %)
```

Clustering vector: provides the cluster to which each observation belongs

k-means in R (example: iris data set)

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  Sepal.Length Sepal.Width Petal.Length Petal.Width
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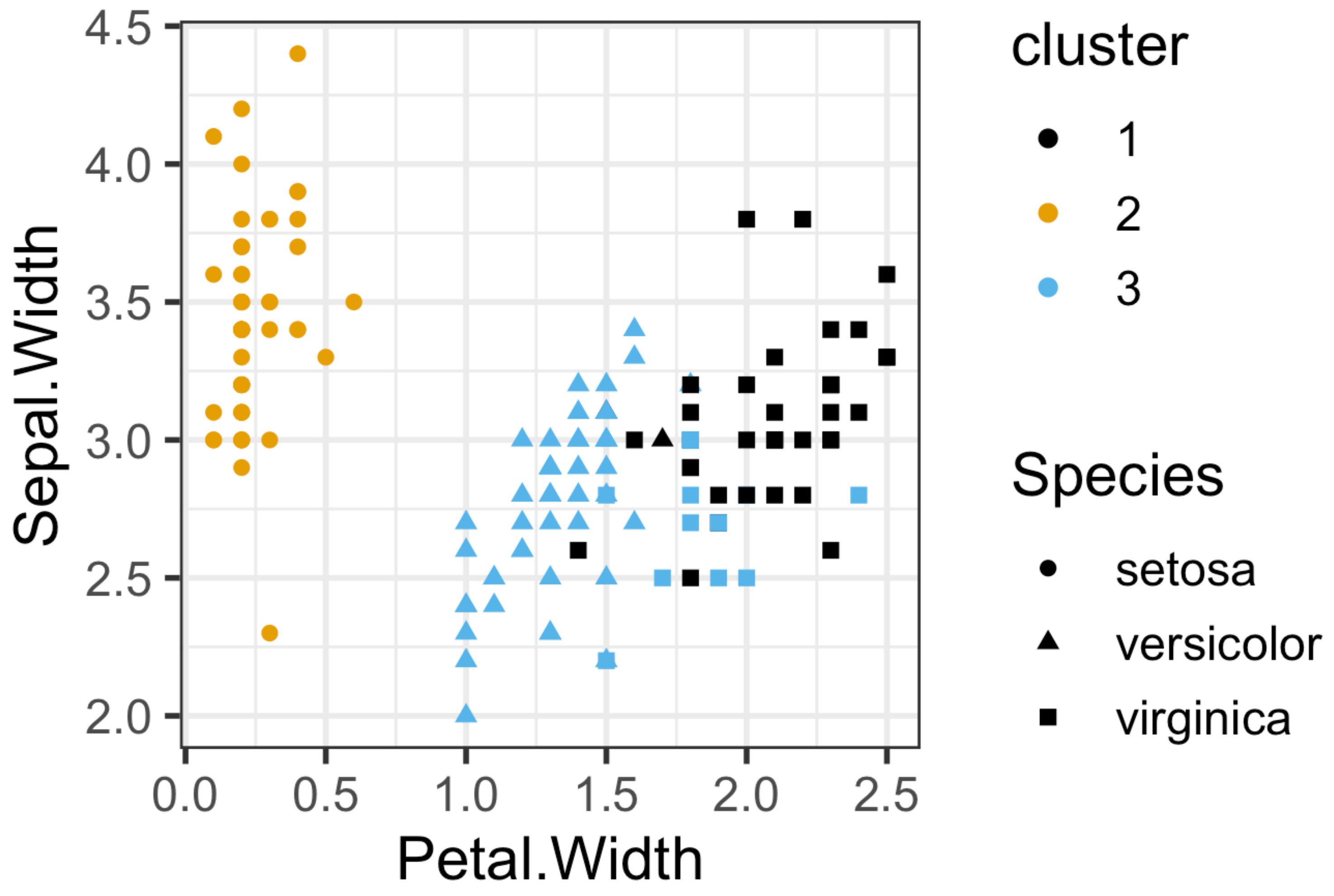
Within cluster sum of squares by cluster:
[1] 23.87947 39.82097 15.15100
 (between_SS / total_SS =  88.4 %)
```

Within cluster sum of squares: measures quality of the clustering (lower is better)

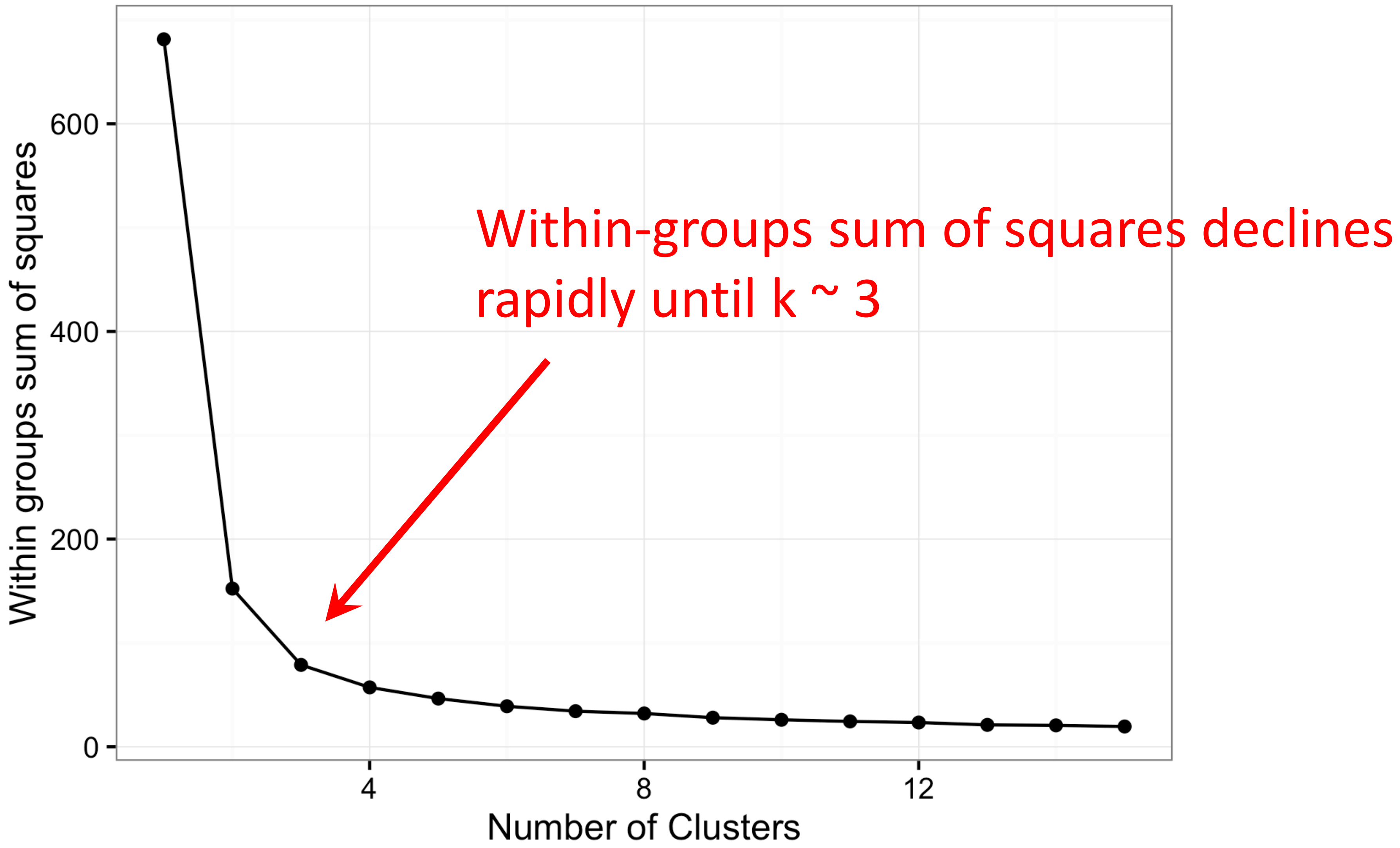
The clusters mostly but not exactly recapitulate the species assignments



How do we determine the right number of means *k*?

- Many different methods, see e.g.: http://stackoverflow.com/a/15376462/4975218
- Simplest: plot within-sum-of-squares against *k*

A bend in within-sum-of-squares indicates the ideal number of clusters


Within-groups sum of squares declines rapidly until k ~ 3
Within groups sum of squares
Number of Clusters