Clusters and Heatmaps

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Want to combine the visualization of quantitative data with clustering algorithms? Unsatisfied with the options provided by the base R packages? In this hands-on workshop, we'll use the `ggendro` package for R to make publication-quality graphics.

- Consider the `ggdendro` package? See: https://stackoverflow.com/questions/6673162/reproducing-lattice-dendrogram-gra
- ggdendro page

0. What are the data? (the otter data?)
1. Clustering approach (e.g. `hclust`)
2. Drawing just a cluster
3. Drawing just a heatmap (`geom_tile`); individuals on Y, measurement on X

Learning objectives

1. one
2. two
3. three

[DESCRIPTION OR MOTIVATION; 2-4 sentences that would be used for an announcement]

Getting started

Start by creating a new project in RStudio and creating two folders we'll use to organize our efforts. The two folders should be `data` and `output` and will store. . . data and output.

```
dir.create("data")
dir.create("output")
```

We will also need a few R packages that are not included in the standard distribution of R. Those packages are `ggplot2`, `ggdendro`, `reshape2`, and `grid` and can be installed with the `install.packages()` function. Note these packages need only be installed once on your machine.

```
install.packages("ggplot2")
install.packages("ggdendro")
install.packages("reshape2")
install.packages("ggplot2")
```

Finally, download data file from https://jcoliver.github.io/learn-r/data/otter-mandible-data.csv or http://tinyurl.com/otter-data-csv (the latter just re-directs to the former). These data are a subset of those used in a study on skull morphology and diet specialization in otters doi: 10.1371/journal.pone.0143236. **Save this file in the `data` folder we just created**.

Data preparation

For any work that we do, we want to record all the steps we take, so instead of typing commands directly into the R console, we keep all our work in an R script. These scripts are just text files with R commands; by convention, we start the script with a brief bit of information about what the script does.

```
# Cluster & heatmap on otter data
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# 2017-08-15

rm(list = ls()) # clean up the environment

################################################################################

# Read in data
otter <- read.csv(file = "data/otter-mandible-data.csv", header = TRUE)
```

If we look at the first few rows of data, we see the first three columns have information about the specimen from which the measurements were taken, while columns 4-9 have data for six mandible measurements (`m1` - `m6`):

```
head(otter)
```

```
##      species museum accession     m1     m2     m3     m4     m5     m6
## 1 A. cinerea   AMNH    101458 15.100 27.790 21.885 13.010 10.500 61.635
## 2 A. cinerea   AMNH    101461 12.740 26.750 20.265 13.255  8.340 59.370
## 3 A. cinerea   AMNH    101466 12.425 25.915 20.735 12.300  9.430 56.270
## 4 A. cinerea   AMNH    101635 13.400 28.030 22.075 10.580 10.455 58.080
## 5 A. cinerea   AMNH    101459 14.400 26.160 21.385 12.100  9.600 58.635
## 6 A. cinerea   AMNH    101462 14.525 29.020 22.305 11.905 11.070 60.655
```

For our purposes, we only want to look at the data for two species, so we subset the data, including only those rows that have either "A. cinerea" or "L. canadensis" in the `species` column. Note, this *does not* alter the data in the original file we read into R; it only alters the data object `otter` currently in R's memory.

```
two.species <- c("A. cinerea", "L. canadensis")
otter <- otter[otter$species %in% two.species, ]
```

We also want to use the specimen accession number for categorizing specimens, so we convert this column to be of type `factor`.

```
otter$accession <- factor(otter$accession)
```

Missing data can cause problems in downstream analyses, so we will just remove any rows that have missing data. Here we replace the original data object `otter` with one in which there are no missing values.

```
otter <- na.omit(otter)
```

Because R *does not* automatically re-number the rows when we drop those with `NA` values, we can force re-numbering via:

```
rownames(otter) <- NULL
```

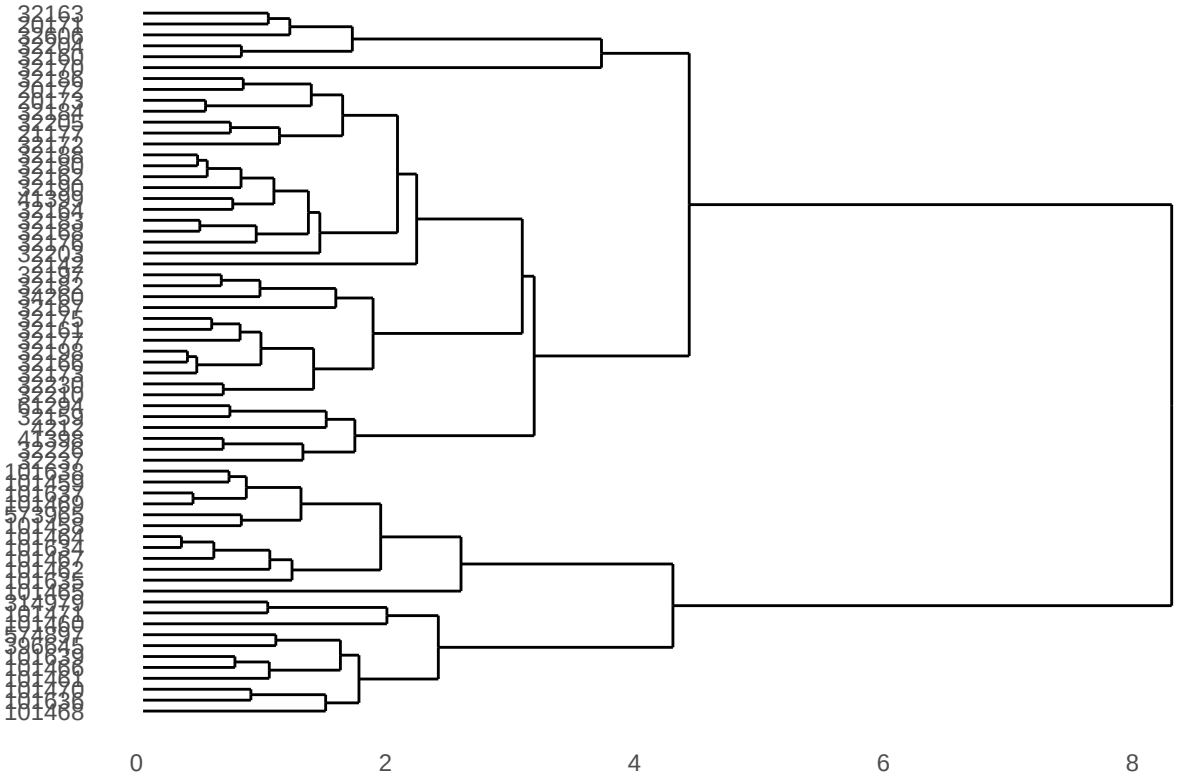
The last thing we need to do is scale the data variables (columns 4-9) so measurements have a mean value of 0 and unit variance.

```
otter.scaled <- otter
otter.scaled[, c(4:9)] <- scale(otter.scaled[, 4:9])
```

Clustering

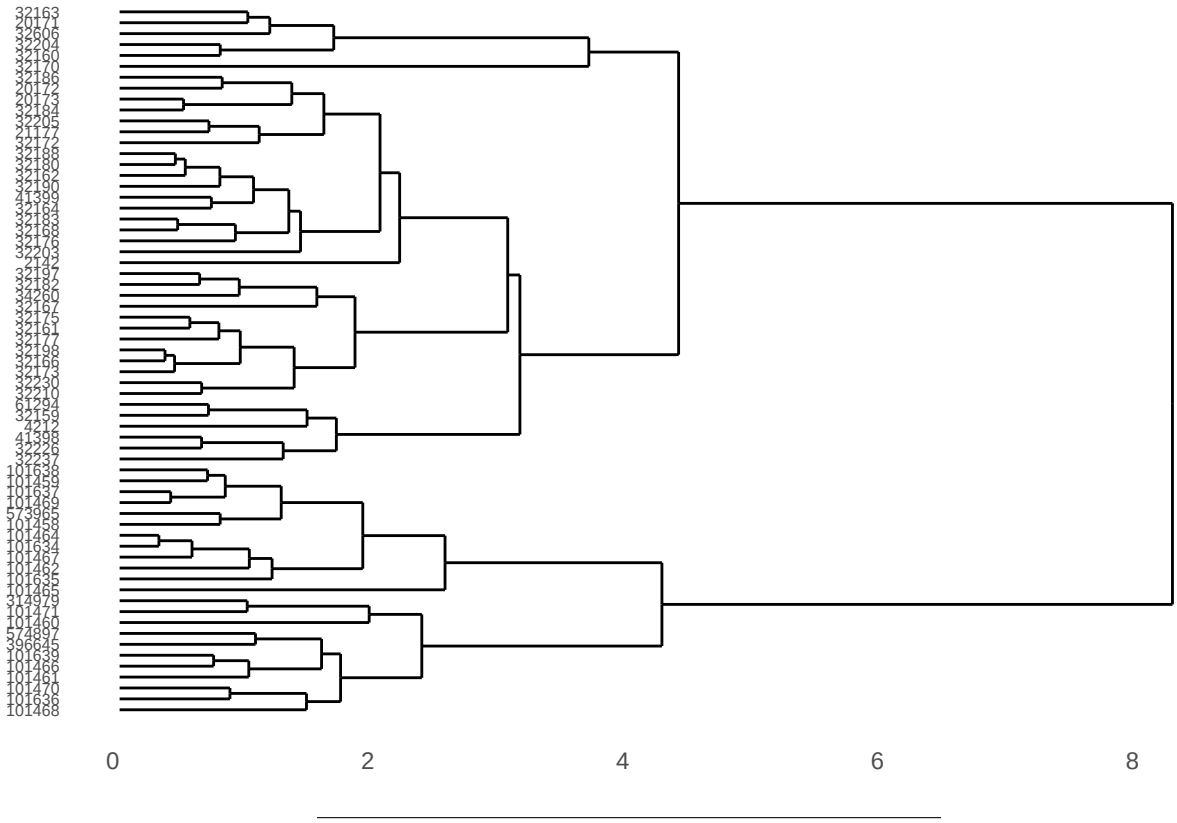
To make our figure, we will build the two plots (the cluster diagram and the heatmap) separately, then use the `grid` framework to put them together. We start by making the dendrogram (or cluster).

```
# Run clustering
library("ggdendro")
otter.matrix <- as.matrix(otter.scaled[, -c(1:3)])
rownames(otter.matrix) <- otter.scaled$accession
otter.dendro <- as.dendrogram(hclust(d = dist(x = otter.matrix)))
otter.dendro.data <- dendro_data(otter.dendro)

# Create dendro
library("ggplot2")
dendro.plot <- ggdendrogram(data = otter.dendro, rotate = TRUE)

# Preview the plot
print(dendro.plot)
```



We can make those labels a bit smaller and closer to the dendrogram:

```
dendro.plot <- dendro.plot + theme(axis.text.y = element_text(size = 6))

# Preview the plot
print(dendro.plot)
```



Heatmap

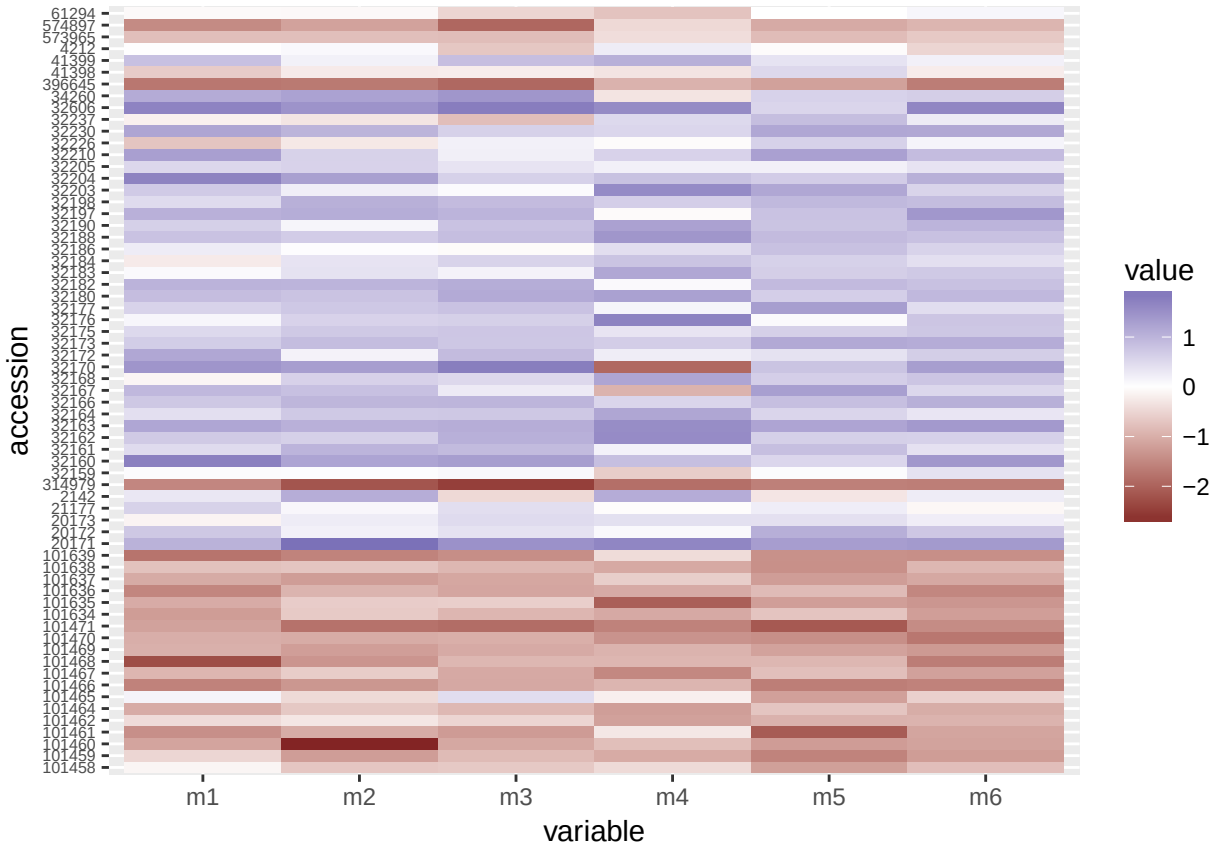
We need to start by transforming data to a "long" format, where each row only contains values for one measurement. For example, if our first two rows of data are:

insert table here

we want to transform it to this format:

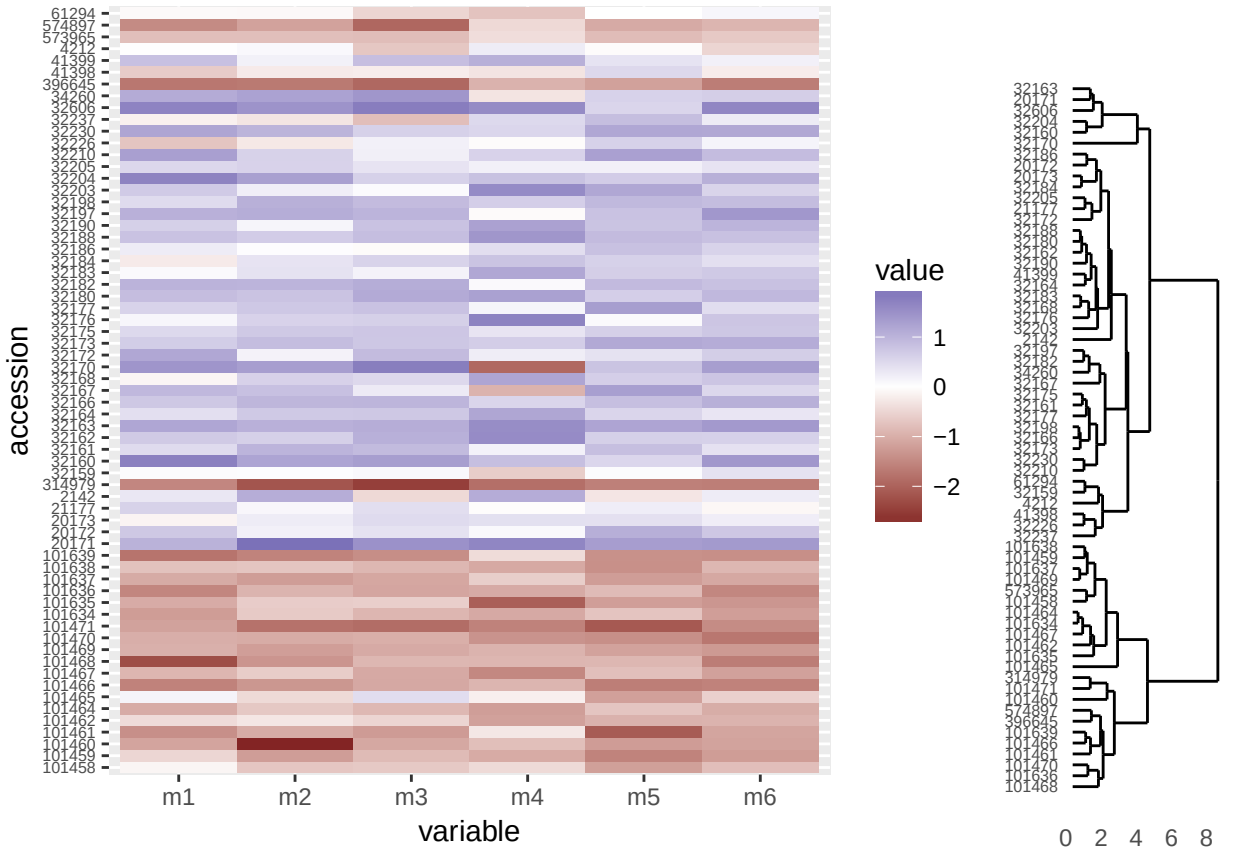
insert table here

```
# Heatmap
# Data wrangling
library("reshape2")
otter.long <- melt(otter.scaled, id = c("species", "museum", "accession"))
# Order the levels according to their position in the cluster
# otter.long$accession <- factor(x = otter.long$accession, levels = otter.scaled$accession[otter.order]
heatmap.plot <- ggplot(data = otter.long, aes(x = variable, y = accession)) +
  geom_tile(aes(fill = value)) +
  scale_fill_gradient2() +
  theme(axis.text.y = element_text(size = 6))

# Preview the heatmap
print(heatmap.plot)
```



Putting it all together

```
library("grid")
grid.newpage()
print(heatmap.plot, vp = viewport(x = 0.4, y = 0.5, width = 0.8, height = 1.0))
print(dendro.plot, vp = viewport(x = 0.90, y = 0.445, width = 0.2, height = 1.0))
```



OK, that's a start, but there are a few things we need to address:

1. Notice the tips of the dendrogram are not in the same order as the y-axis of the heatmap. We'll need to re-order the heatmap to match the structure of the clustering.
2. It would be nice to have the dendrogram and heatmap closer together, without a legend separating them.
3. The dendrogram should be vertically stretched so each tip lines up with a row in the heatmap plot.

Aligning the two plots

We'll use the order of the tips in the dendrogram to re-order the rows in our heatmap. The order of those tips are stored in the `otter.dendro` object, and we can extract it with the `order.dendro` function:

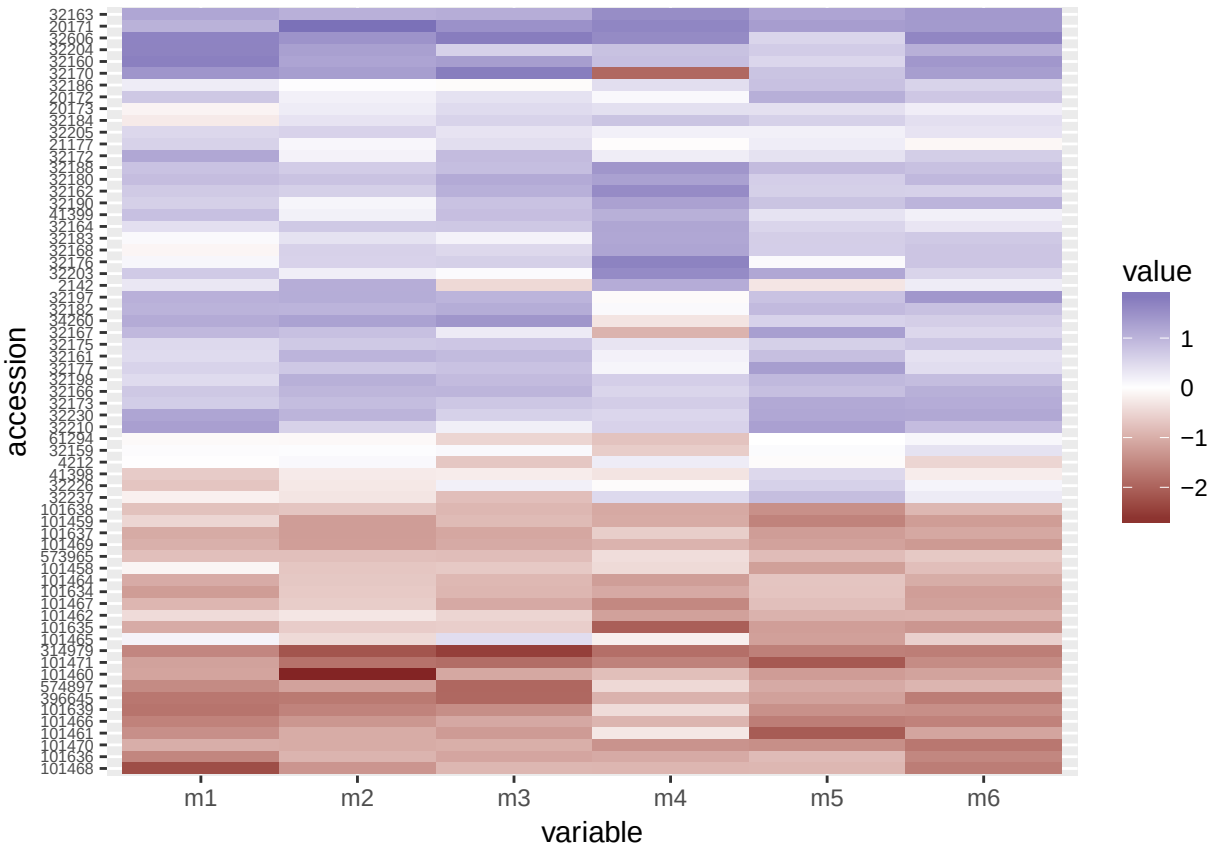
```
otter.order <- order.dendrogram(otter.dendro)
```

And now for the fun part. By default, `ggplot` use the level order of the y-axis labels as the means of ordering the rows in the heatmap. That is, level 1 of the factor is plotted in the top row, level 2 is plotted in the second row, level 3 in the third row and so on. So we can dictate the order in which these rows are plotted by *setting* the order of the levels in the column we use for the y-axis labels in the heatmap. The data we used for the heatmap was the long-format, `otter.long` object, and the column we used for the y-axis is `accession`. Using the order of the dendrogram tips (we stored this above in the `otter.order` vector), we can re-level the `otter.long$accession` column to match the order of the tips in the trees.

```
# Order the levels according to their position in the cluster
otter.long$accession <- factor(x = otter.long$accession,
                               levels = otter.scaled$accession[otter.order],
                               ordered = TRUE)
```

At this point, we need to re-create the heatmap, because the underlying data (`otter.long`) has changed:

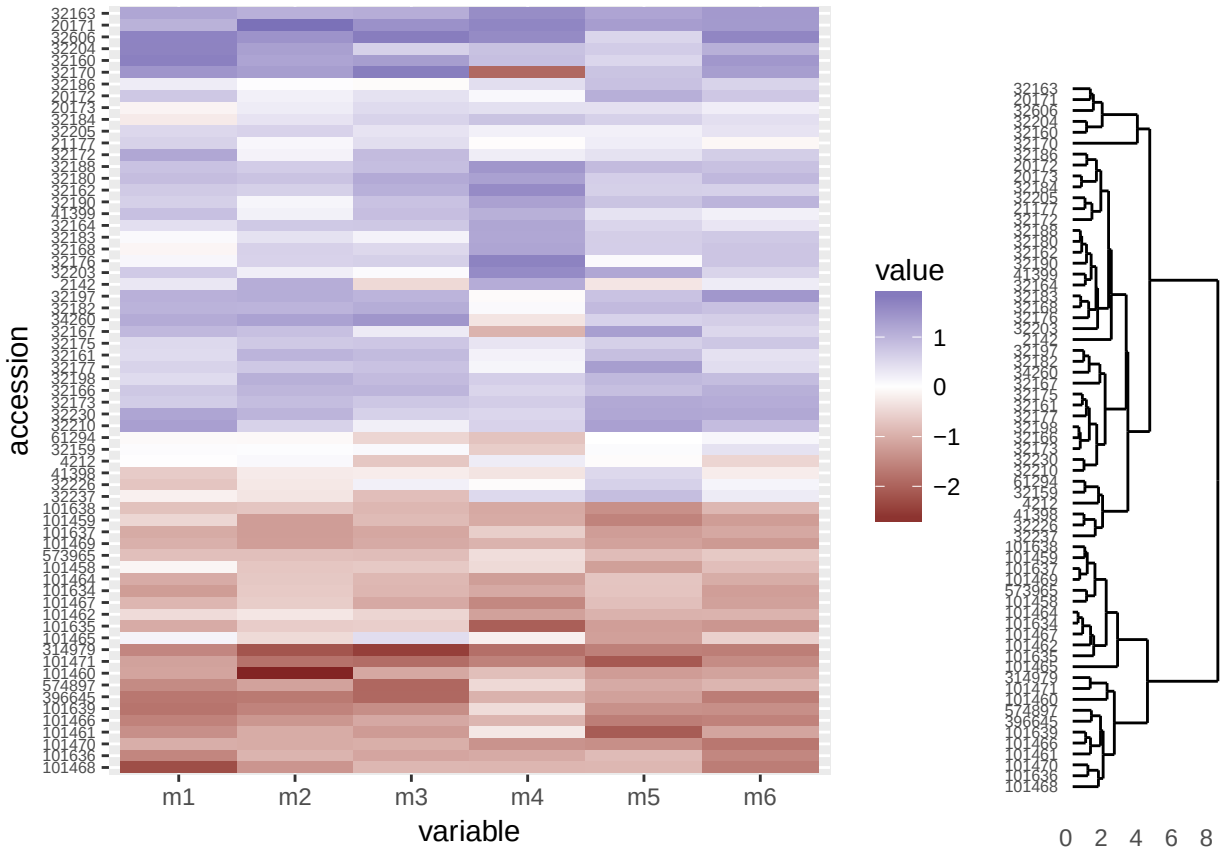
```
# Heatmap
heatmap.plot <- ggplot(data = otter.long, aes(x = variable, y = accession)) +
  geom_tile(aes(fill = value)) +
  scale_fill_gradient2() +
  theme(axis.text.y = element_text(size = 6))

# Preview the heatmap
print(heatmap.plot)
```



Now the rows have been re-arranged and we can see right away that the heatmap now does a better job of grouping "red" rows together and "blue" rows together.

Take a look at the combined figure:

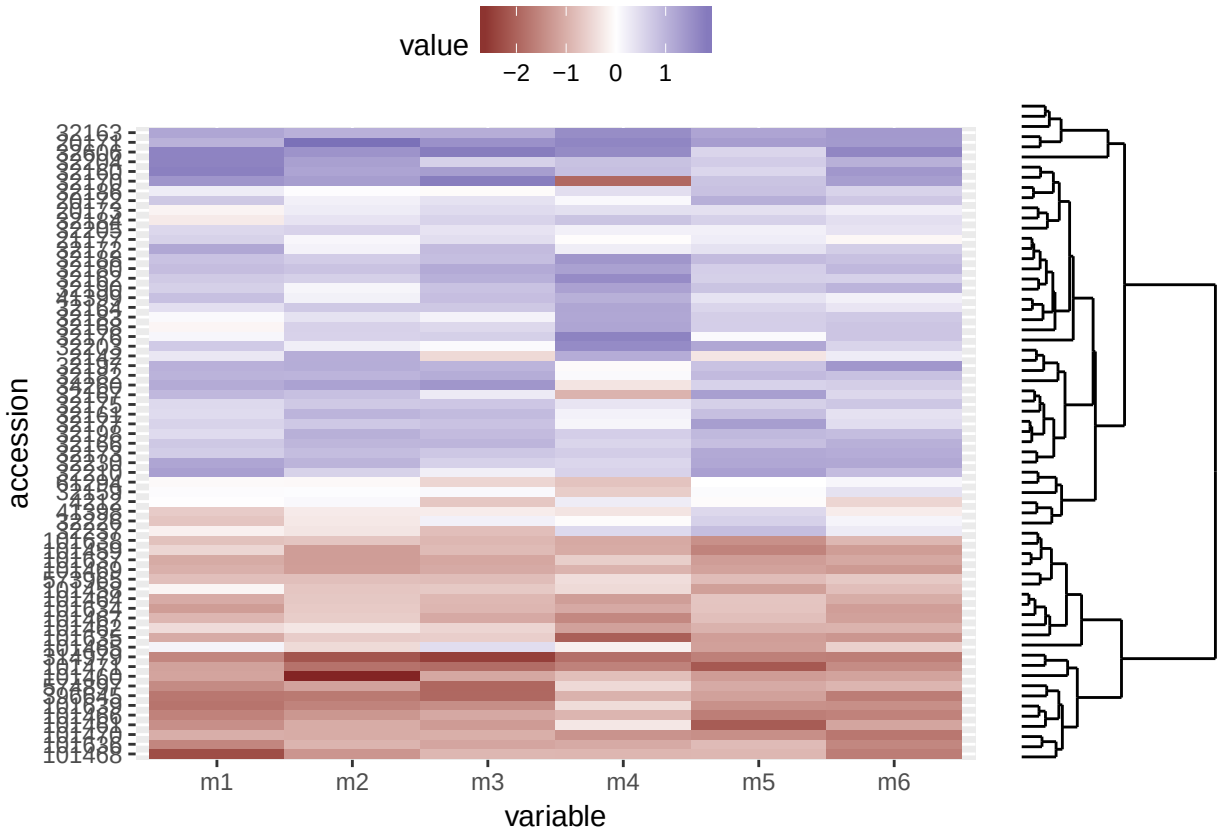
```
grid.newpage()
print(heatmap.plot, vp = viewport(x = 0.4, y = 0.5, width = 0.8, height = 1.0))
print(dendro.plot, vp = viewport(x = 0.90, y = 0.445, width = 0.2, height = 1.0))
```



Final-ish

```
otter <- read.csv(file = "data/otter-mandible-data.csv", header = TRUE)
two.species <- c("A. cinerea", "L. canadensis")
otter <- otter[otter$species %in% two.species, ]
otter <- na.omit(otter)
otter.scaled <- otter
otter.scaled[, c(4:9)] <- scale(otter.scaled[, 4:9])
otter.scaled$accession <- factor(otter.scaled$accession)

# Renumber rows
rownames(otter) <- NULL

# Run clustering
library("ggdendro")
otter.matrix <- as.matrix(otter.scaled[, -c(1:3)])
otter.dendro <- as.dendrogram(hclust(d = dist(x = otter.matrix)))
otter.dendro.data <- dendro_data(otter.dendro)
otter.order <- order.dendrogram(otter.dendro)

# Create dendro
library("ggplot2")
dendro.plot <- ggplot(data = segment(otter.dendro.data)) +
```

```
  geom_segment(aes(x = x, y = y, xend = xend, yend=yend)) +
  coord_flip() +
  theme_dendro()

# Heatmap
# Data wrangling
library("reshape2")
otter.long <- melt(otter.scaled, id = c("species", "museum", "accession"))
# Order the levels according to their position in the cluster
otter.long$accession <- factor(x = otter.long$accession, levels = otter.scaled$accession[otter.order], 
heatmap.plot <- ggplot(data = otter.long, aes(x = variable, y = accession)) +
  geom_tile(aes(fill = value)) +
  scale_fill_gradient2() +
  theme(legend.position = "top")

# All together
library("grid")
grid.newpage()
print(heatmap.plot, vp = viewport(x = 0.4, y = 0.5, width = 0.8, height = 1.0))
print(dendro.plot, vp = viewport(x = 0.90, y = 0.445, width = 0.2, height = 0.92))
```



Additional resources

- resource one

- resource two

Back to learn-r main page

Questions? e-mail me at jcoliver@email.arizona.edu.