





# DCMB BioComputing BootCamp Day 3, Lecture 3: Data Exploration and Visualization in R

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# Now What?

- Scenario:
  - You understand the fundamentals of R
  - You've read your data into an R data frame
- During this session we will talk about
- Basic data summarization
- Visualize data with plots



# Overview

- 1. Summarizing Data in R
- 2. Creating Plots in R Using ggplot2
  - a. Histograms
  - b. Boxplots
  - c. Scatter plots

# Example Data Set:

## **Cancer Research**

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Activation of Wnt/β-Catenin in Ewing Sarcoma Cells Antagonizes EWS/ETS Function and Promotes Phenotypic Transition to More Metastatic Cell States

Elisabeth A. Pedersen, Rajasree Menon, Kelly M. Bailey, Dafydd G. Thomas, Raelene A. Van Noord, Jenny Tran, Hongwei Wang, Ping Ping Qu, Antje Hoering, Eric R. Fearon, Rashmi Chugh, and Elizabeth R. Lawlor

DOI: 10.1158/0008-5472.CAN-15-3422 Published September 2016

Articles

- Ewing's sarcoma: rare bone and soft tissue cancer occurring in children and teenagers
  - 70-80% survival
- In vitro CHLA25-7TGP ES cells stimulated to over-express WNT3A
- RNA-Seq profiling used to quantify gene expression

Download pedersenLog2RPKM20180817.txt and pedersenLog2\_matrixRPKM20180817.txt from the Day3 course website.

# Exercise: Write a Script to Read Pedersen Gene Expression Data into a Data Frame

- 1. Download both Pedersen data files
- 2. Use setwd() to move to the data file folder
- options(stringsAsFactors=F)
- 4. Use the read.delim() function to read in "pedersenLog2RPKM20180817.txt" file into a data frame called "data1"
- 5. Use the head() and dim() function to find out about the structure of this data file

How many rows? What are the columns?

# Quickly Calculate Simple Statistics

- R has many built in statistical functions that use fast vector and matrix operations
- No need to write a for loop, sum, and then divide by n
- Just provide a vector of data to the mean() function:
  - > mean(data1\$log2RPKM)
- With one line of code you have take the mean of 97,000 values!

Exercise: Use mean(), median(), max(), min(), summary() functions on the Pedersen data

# Using the table() Function

- When exploring new datasets it is often useful to count the number of values
- table() can be used to build a contingency table of the counts of each value
  - For one column:

```
> table(data1$tx)
control WNT3A
   48585 48585
```

For multiple columns:

```
> table(data1$tx,data1$rep)

1 2 3
control 16195 16195 16195
WNT3A 16195 16195 16195
```

# Using the aggregate() Function

- Often times we want to perform a function on subsets of our data
  - example question: What is the mean expression for each sample?
- aggregate() splits data into subsets, computes summary statistics for each and returns the result
- aggregate() takes several arguments:

Exercise: Use aggregate() to calculate the maximum log2RPKM value per sample.

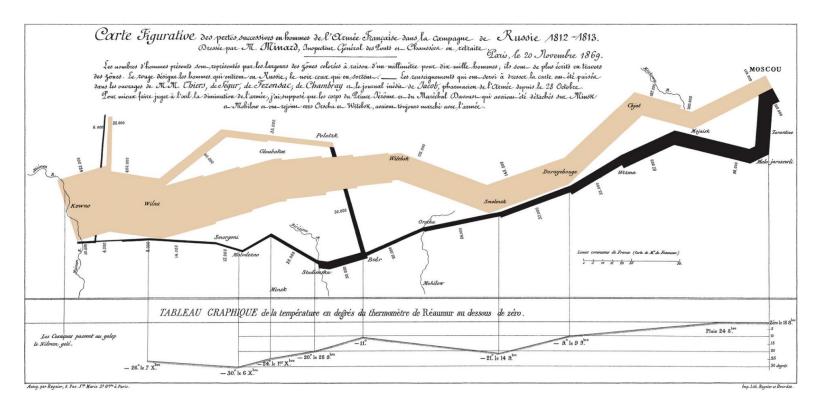
### formula format: y ~ x1

- y is a numeric value
- x1 is a grouping variable
- possible to specify multiple groups as x1 + x2 + ...

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# Data Visualization Allows Researchers to Visually Present Data



- Minard's 1869 diagram of Napoleonic France's invasion of Russia
  - Line width indicates size of army
  - Color indicates army's course to and from Russia

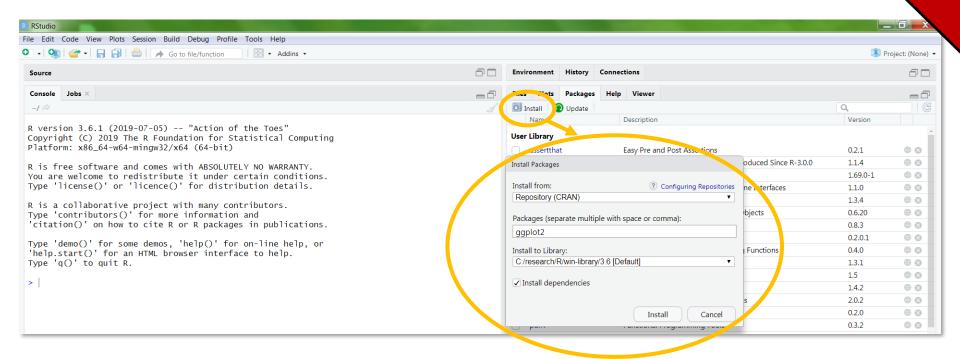
# Data Visualization Allows Researchers to Visually Present Data

- Data visualizations should:
  - Show the data
  - Avoid distorting the data
  - Present many numbers in a small space
  - Make large data sets coherent
  - Serve a reasonably clear purpose
  - Be closely integrated with the statistical and verbal descriptions of a data set

# R Base Graphics Versus ggplot2

- R comes with "base graphics" built in to support commonly used data visualizations
- Today we will focus on using an alternative data visualization framework called ggplot2
- ggplot2 is an external package that must be downloaded, installed, and loaded with the library command
- A common practice is to use ggplot2 to construct publication quality graphs but still use base graphics to quickly visualize data

# Install ggplot2 Package



## To install ggplot2:

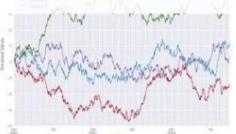
- 1. select "packages" tab
- 2. select "Install"
- 3. Type in ggplot2
- 4. Select "Install"

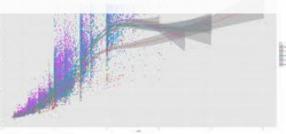
# ggplot2

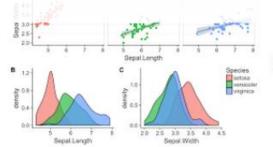
- ggplot2 is an R data visualization package created by Hadley Wickham
  - One of the most popular R packages
  - Breaks up graph construction into additive functions called layers
- ggplot2 documentation and cheat sheet:

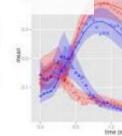
https://www.rdocumentation.org/packages/ggplot

2/versions/3.0.0









# Creating a Visualization with ggplot2

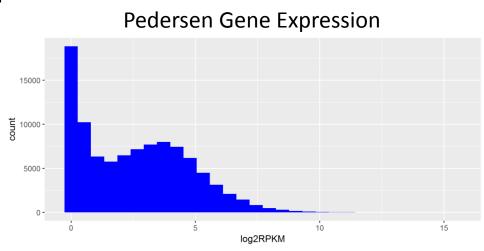
- ggplot2 visualization function calls consist of several basic components
- 1. ggplot()
- geom\_XXX()
- 3. optional layers
- 4. ggsave()
- Multiple function calls are combined together using "layers"
- aes(thetic) functions are used to map input data to plot features (e.g. x axis, y axis, colors)

# Visualizing Data Using Histograms

 histogram: a type of bar graph visualization in which data measurements are counted based on value

> For discrete measures it shows the frequency of values in each category

 For continuous measure it shows the frequency of values occurring in small intervals covering the whole range



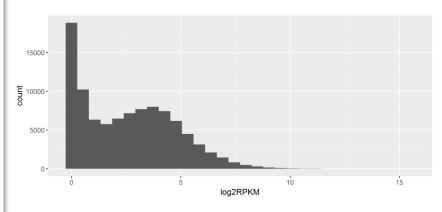
# How to Create a Basic Histogram Using ggplot2

```
options(stringsAsFactors=F)

library(ggplot2)

inFile1 =
'pedersenLog2RPKM20180817.txt'
data1 = read.delim(inFile1)

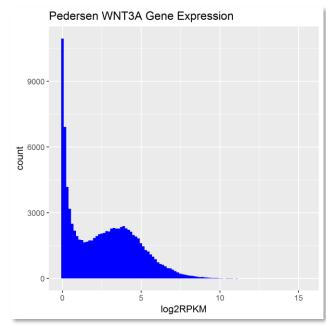
ggplot(data1,aes(x = log2RPKM)) +
   geom_histogram()
ggsave('histogram1.png')
```



In Class Exercise.

# Exercise: Create a histogram using the code from the previous page and update your visualization to:

- 1. Change the color
  - HINT: ?geom\_histogram
  - HINT: fill = "blue"
- 2. Set the image width and height to be 5 inches
  - HINT: ?ggsave
  - HINT: height = 5
- 3. Adjust the number of bins to 100
- 4. Add a title
  - HINT: ?labs

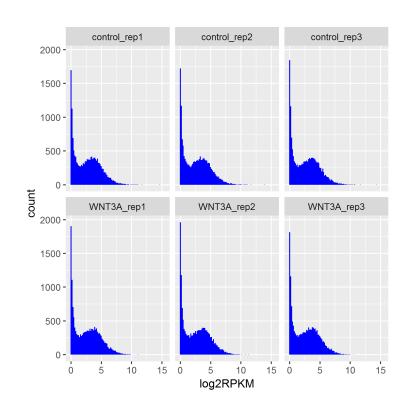


# Create Sub-plots Using Facets

- Sub-plots can easily be created using facet layers:
  - facet\_wrap()
  - facet\_grid()

# Exercise: Update your histogram visualization to facet on sample

- Add a facet\_wrap() layer
  - HINT: + facet\_wrap(~sample)

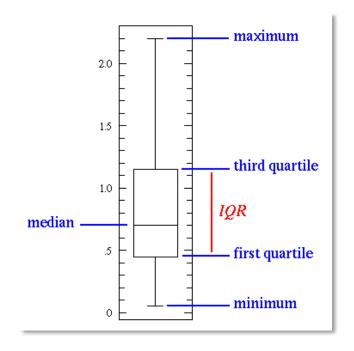


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# Visualizing Data Using Boxplots

- <u>boxplot</u>: graphically represents data distributions using quartiles
- box-and-whisker plot: includes boxplots with lines extending from boxes to indicate variability outside the upper and lower quartiles
- Why it is useful?
  - Summarize the main characteristics of the data: Mean/median, quartile, spread, symmetry and outliers.
  - Efficient less complicated than histogram
  - Allows us to represent multiple data distributions in the same graph



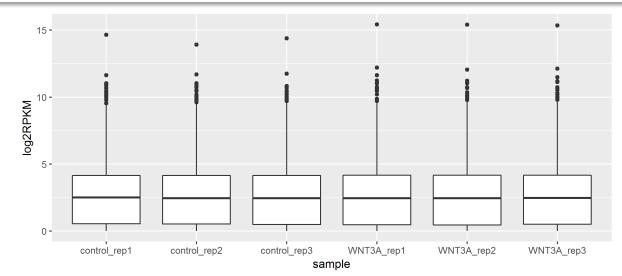
# How to Create a Boxplot Using gplot2

```
options(stringsAsFactors=F)

library(ggplot2)

inFile1 = 'pedersenLog2RPKM20180817.txt'
data1 = read.delim(inFile1)

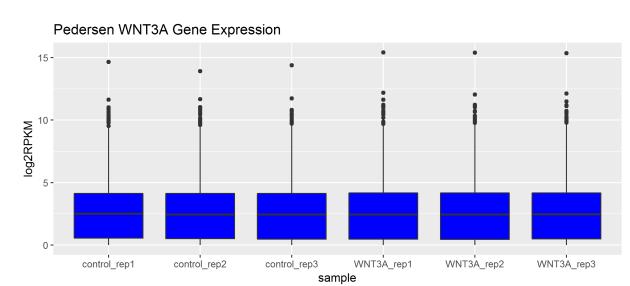
ggplot(data1,aes(x = sample, y = log2RPKM)) +
   geom_boxplot()
ggsave('boxplot1.png')
```



In Class Exercise

# Exercise: Create a boxplot using the code from the previous page and update your visualization to:

- 1. Change the fill color
  - HINT: fill = 'blue'
- 2. Add a title
  - HINT: ?labs

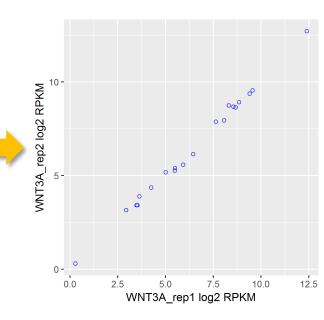


If time: Try creating a violin plot using geom\_violin()

# Visualizing Data Using Scatter Plots

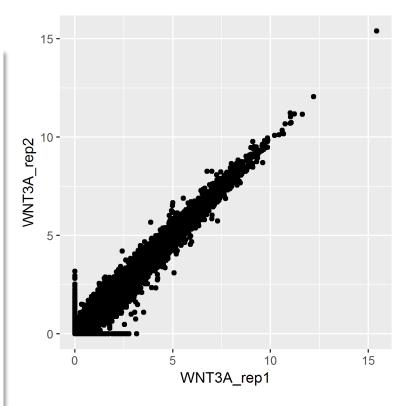
- Scatter Plots are visualizations that display two data values for the same measurement
  - <u>example</u>: two sample replicates expression values for each gene
- Data points that are not on the diagonal indicate disagreement
- We expect strong agreement between sample replicates

gene	WNT3A_rep1	WNT3A_rep2
A1BG	2.38	1.64
A1BG-AS1	0.83	0.58
A1CF	0.02	0.00
A2M	0.30	0.67
A2M-AS1	0.09	0.10
A2ML1	0.33	0.73
A2MP1	0.00	0.00
A4GALT	4.20	4.82
A4GNT	0.00	0.00
AAAS	5.57	5.53
AACS	2.70	2.52
AACSP1	0.13	0.15
AADAC	0.12	0.00
AADACL2	0.00	0.00
AADACL4	2.30	2.03
AADAT	0.94	1.19
AAED1	1.20	1.51
AAGAB	4.36	4.29
AAK1	1.68	1.97
AAMDC	4.41	3.78



# How to Create a Scatter Plot Using gplot2

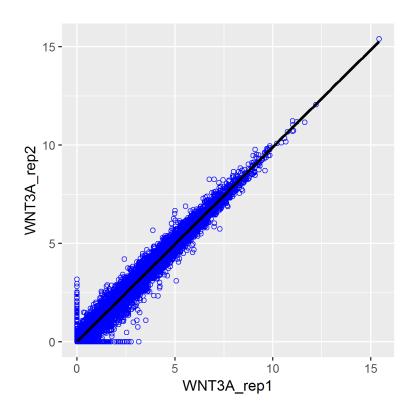
```
options (stringsAsFactors=F)
library(ggplot2)
inFile2 =
'pedersenLog2RPKM matrix20180817.txt'
data2 = read.delim(inFile2)
ggplot(data2, aes(x = WNT3A rep1, y =
WNT3A rep2)) +
 geom point()
ggsave('scatter2.png')
```

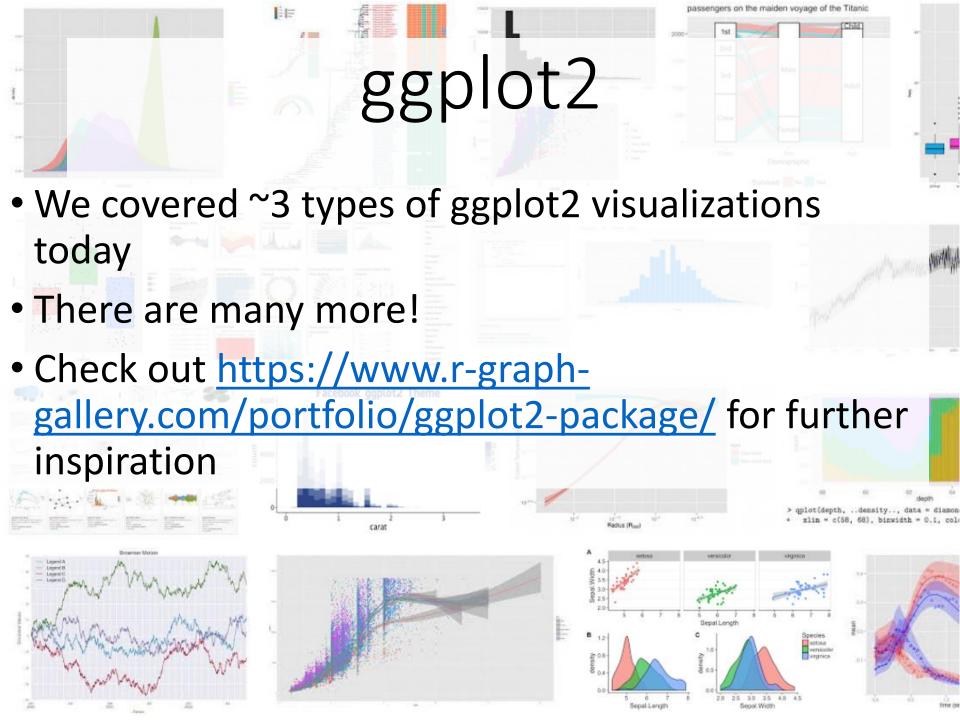


# In Class Exercise.

# Exercise: Create a boxplot using the code from the previous page and update your visualization to:

- 1. Change the color and shape of scatter plot points
  - HINT: color = 'blue'
  - HINT: shape = 1
- 2. Add a black linear regression line using a geom\_smooth layer
  - HINT: geom\_smooth(method = lm)

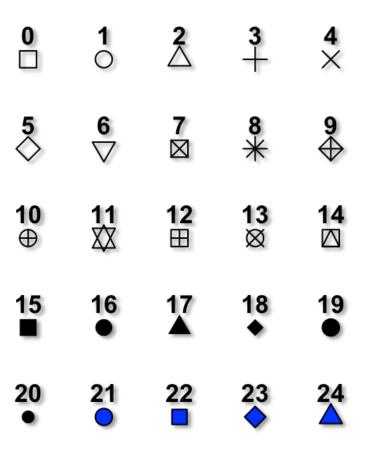




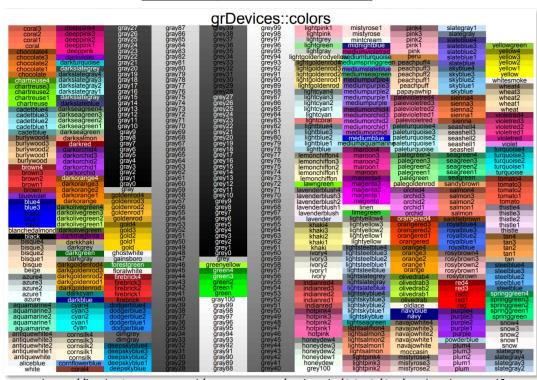
# References

- Gentleman, Robert. R Programming for Bioinformatics. CRC Press, 2009.
- Slides sourced in part from Jacob Kitzman and Barry Grant

## **R Graphics Shapes**



## **R Graphics Colors**



http://bc.bojanorama.pl/wp-content/uploads/2013/04/rcolorsheet.pdf

25 <del>V</del>

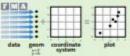
## **Data Visualization** with ggplot2

Cheat Sheet

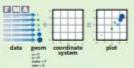


#### Basics

ggplot2 is based on the grammar of graphics, the idea that you can build every graph from the same few components: a data set, a set of geoms-visual marks that represent data points, and a coordinate system.



To display data values, map variables in the data set to aesthetic properties of the geom like size, color, and x and y locations.



Build a graph with qplot() or ggplot()



qplot(x = cty, y = hwy, color = cyl, data = mpg, geom = "point") Creates a complete plot with given data, geom, and mappings. Supplies many useful defaults.

#### ggplot(data = mpg, aes(x = cty, y = hwy))

Begins a plot that you finish by adding layers to. No defaults, but provides more control than qpiot().

ggplot(mpg, aes(hwy, cty)) + geom\_point(aes(color = cyl)) + geom\_smooth(method ="lm") + coord cartesian() + scale\_color\_gradient() + theme bw()

Add a new layer to a plot with a geom \*() or stat\_\*() function. Each provides a geom, a set of aesthetic mappings, and a default stat and position adjustment.

#### last plot()

Returns the last plot

#### ggsave("plot.png", width = 5, height = 5)

Saves last plot as 5' x 5' file named "plot.png" in working directory. Matches file type to file extension.

#### One Variable

#### Continuous

a <- ggplot(mpg, aes(hwy))



### geom\_area(stat = "bin")

x, y, alpha, color, fill, linetype, size b + geom\_area(aes(y = .density..), stat = "bin")



geom\_density(kernel = "gaussian") x, y, alpha, color, fill, linetype, size, weight b + geom\_density(aes(y = \_county..))

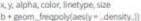


### geom\_dotplot()

x, y, alpha, color, fill



geom\_freqpoly()





geom\_histogram(binwidth = 5) x, y, alpha, color, fill, linetype, size, weight b + geom\_histogram(aes(y = ..density..))

#### Discrete

b <- ggplot(mpg, aes(fl))



geom bar() x, alpha, color, fill, linetype, size, weight

### **Graphical Primitives**

c <- ggplot(map, aes(long, lat))



geom\_polygon(aes(group = group)) x, y, alpha, color, fill, linetype, size

d <- ggplot(economics, aes(date, unemploy))</p>



- geom\_path(lineend="butt". linejoin="round", linemitre=1)
- x, v, alpha, color, linetype, size



- geom\_ribbon(aes(ymin\*unemploy 900, ymax=unemploy + 900)) x, ymax, ymin, alpha, color, fill, linetype, size
- e <- ggplot(seals, aes(x = long, y = lat))



geom\_segment(aes/ xend = long + delta\_long,

yend = lat + delta (at)) x, xend, y, yend, alpha, color, linetype, size



xmax=long+delta\_long, vmax = lat + delta (at))

xmax, xmin, ymax, ymin, alpha, color, fill, linetype, size

#### Two Variables

### Continuous X. Continuous Y

f <- ggplot(mpg, aes(cty, hwy))

Geoms - Use a geom to represent data points, use the geom's aesthetic properties to represent variables. Each function returns a layer.



geom\_jitter()

geom\_blank()



x, y, alpha, color, fill, shape, size



x, y, alpha, color, fill, shape, size

geom\_rug(sides = "bl")

alpha, color, linetype, size

geom\_quantile()



x, y, alpha, color, linetype, size, weight



geom\_smooth(model=lm)



x, y, alpha, color, fill, linetype, size, weight geom\_text(aes(label = cty))

x, y, label, alpha, angle, color, family, fontface hjust, lineheight, size, vjust

### Discrete X. Continuous Y

g < ggplot(mpg, aes(class, hwy)) geom\_bar(stat = "identity")



x, v. alpha, color, fill, linetype, size, weight



geom boxplot()



lower, middle, upper, x, ymax, ymin, alpha, color, fill, linetype, shape, size, weight geom\_dotplot(binaxis = "y",



stackdir = "center") x, y, alpha, color, fill



geom\_violin(scale = "area") x, y, alpha, color, fill, linetype, size, weight

### Discrete X. Discrete Y

h < ggplot(diamonds, aes(cut, color))



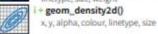
geom\_jitter()

x, y, alpha, color, fill, shape, size

Continuous Bivariate Distribution i <- ggplot(movies, aes(year, rating))



 $geom_bin2d(binwidth = c(5, 0.5))$ xmax, xmin, ymax, ymin, alpha, color, fill, linetype, size, weight



geom\_hex()

geom\_density2d()



Continuous Function <- ggplot(economics, aes(date, unemploy))</pre>



x, y, alpha, color, fill, linetype, size



x, y, alpha, color, linetype, size



geom\_step(direction = "hv") x, y, alpha, color, linetype, size

Visualizing error

df <- data.frame(grp = c("A", "B"), fit = 4:5, se = 1:2) k <- ggplot(df, aes(grp, fit, ymin = fit-se, ymax = fit+se))



geom\_crossbar(fatten = 2) x, y, ymax, ymin, alpha, color, fill, linetype,



width (also geom\_errorbarh()) geom\_linerange()



x, ymin, ymax, alpha, color, linetype, size



geom\_pointrange() x, y, ymin, ymax, alpha, color, fill, linetype,

data <- data frame/murder = USArrestsSMurder, state = tolower(rownames(USArrests))) map <- map\_data("state") | <- ggp(ot/data, aes(fill = murder))</p>



geom\_map(acs(map\_id = state), map = map) + expand\_limits(x = map\$long, y = map\$lat) map\_id, alpha, color, fill, linetype, size

#### **Three Variables**

seals5z <- with(seals, sqrt(delta\_long^2 + delta\_lat^2)) m <- ggplot(seals, aes(long, lati))



n + geom\_raster(aes(fill = z), hjust=0.5, vjust=0.5, interpolate=FALSE) x, y, alpha, fill



m + geom\_tile(aes(fill = z)) x, y, alpha, color, fill, linetype, size



geom\_contour(aes(z=z)) x, y, z, alpha, colour, linetype, size, weight

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Learn more at docs.ggplot2.org - ggplot2 0.9.3.1 - Updated: 3/15



+ stat\_quantile(quantiles = c(0.25, 0.5, 0.75), formula = y - log(x),

f + stat\_smooth(method = "auto", formula = y - x, se = TRUE, n = 80,

X. V ...X...V

method = "rg")

f + stat\_identity()

f + stat\_sum()

f+stat\_unique()

x, y, size | \_size.

dparams = list(df=5))

sample, x, y | .x., .y.

x, y | .quantile.., \_x., \_y\_

fullrange = FALSE, level = 0.95)

x, y | .se., .x., .y., \_ymin., .ymax.

 $ggplot() + stat_function(aes(x = -3:3),$ 

fun = dnorm, n = 101, args = list(sd=0.5))

f + stat\_summary(fun.data = "mean\_cl\_boot")

ggplot() + stat\_qq(aes(sample=1:100), distribution = qt,

### Color and fill scales

Scales

Scales control how a plot maps data values to the visual

values = c("skyblue", "royalblue", "blue", "navy"),

name = "fuel", labels = c("D", "E", "P", "R"))

General Purpose scales

Use with any aesthetic:

alpha, color, fill, linetype, shape, size

scale\_\*\_discrete() - map discrete values to visual values

X and Y location scales

Use with x or y aesthetics (x shown here)

breaks = date\_breaks("2 weeks")) - treat x

same arguments as scale\_x\_date().

scale\_x\_log10() - Plot x on log10 scale

values as dates. See ?strptime for label formats.

scale x datetime() - treat x values as date times. Use

scale\_\*\_identity() - use data values as visual values

manually chosen visual values

scale\_\*\_manual(values = c()) - map discrete values to

limits = c("d", "e", "p", "r"), breaks =c("d", "e", "p", "r")

values of an aesthetic. To change the mapping, add a

 $1 \le b + geom_bar(aes(fill = fl))$ 

scale\_fill\_manual(

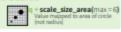
custom scale.



#### Shape scales







#### Coordinate Systems

 $r \leftarrow b + geom_bar()$ 



coord\_cartesian(xlim = c(0, 5)) xlim, ylim

The default cartesian coordinate system



+ coord\_fixed(ratio = 1/2)

ratio, xlim, ylim

Cartesian coordinates with fixed aspect ratio between x and y units



+ coord\_flip() xlim, ylim Flipped Cartesian coordinates



+ coord\_polar(theta = "x", direction=1) theta, start, direction Polar coordinates



+ coord\_trans(ytrans = "sqrt") xtrans, ytrans, limx, limy Transformed cartesian coordinates. Set extras and strains to the name of a window function.

z + coord\_map(projection = "ortho", orientation=c(41, -74, 0))

projection, orientation, xlim, ylim Map projections from the mapproj package (mercator (default), azequalarea, lagrange, etc.)

#### Faceting

Facets divide a plot into subplots based on the values of one or more discrete variables.

t <- ggplot(mpg, aes(cty, hwy)) + geom\_point()



t + facet\_grid(year ~ fl) facet into both rows and columns

t + facet\_wrap(~ fl) wrap facets into a rectangular layout

Set scales to let axis limits vary across facets

t + facet\_grid(y ~ x, scales = "free") x and y axis limits adjust to individual facets

"free\_x" - x axis limits adjust

"free\_y" - y axis limits adjust

Set labeller to adjust facet labels



#### Position Adjustments

Position adjustments determine how to arrange geoms that would otherwise occupy the same space.

s <- ggplot(mpg, aes(fl, fill = drv))



s + geom\_bar(position = "dodge") Arrange elements side by side

s + geom\_bar(position = "fill") Stack elements on top of one another, normalize height



s + geom\_bar(position = "stack") Stack elements on top of one another

f + geom\_point(position = "jitter") Add random noise to X and Y position of each element to avoid overplotting

Each position adjustment can be recast as a function with manual width and height arguments

s + geom\_bar(position = position\_dodge(width = 1))

t + ggtitle("New Plot Title") Add a main title above the plot

t + xlab("New X label") Change the label on the X axis

to update legend labels t + ylab("New Y label")

Change the label on the Y axis

t + labs(title =" New title", x = "New x", y = "New y") All of the above

#### Legends

t + theme(legend.position = "bottom") Place legend at "bottom", "top", "left", or "right"

t + guides(color = "none")

Set legend type for each aesthetic: colorbar, legend, or none (no legend)

t + scale fill discrete(name = "Title". labels = c("A", "B", "C"))

Set legend title and labels with a scale function.

#### **Themes**



theme classic() White background no gridlines

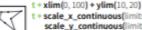
theme minimal() Minimal theme

#### Zooming

Without clipping (preferred) t + coord\_cartesian(

xlim = c(0, 100), ylim = c(10, 20)

With clipping (removes unseen data points)



t + scale\_x\_continuous(limits = c(0, 100)) + scale\_y\_continuous(limits = c(0, 100))

ggthemes - Package with additional ggplot2 themes

Learn more at docs.ggplot2.org • ggplot2 0.9.3.1 • Updated: 3/15

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