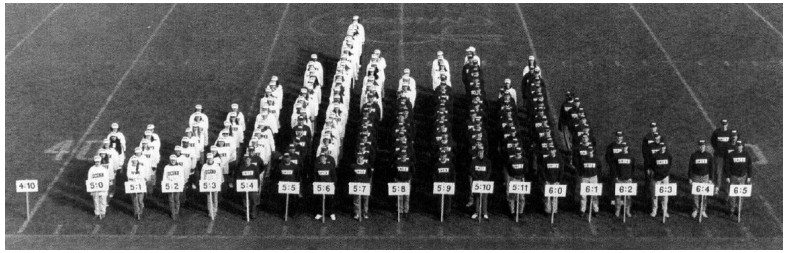
Introduction to Biostatistics



Arranged by Linda Strausbaugh (Genetics 147:5, 1997)

Introduction to Biostatistics
Lecture 1B and 2

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Contents of Lecture 1-2

Data and descriptive statistics

Probability theory and models

Sampling, SE(M) and CLT.

Visual tests (of normality)

What shall we learn today?

- Data description
  - Graphs
  - Tables and summary measures
- Probability Models
  - Glimpse at theory (models/distributions)
  - The Normal distribution
  - Some properties of samples and the Central Limit Theorem.

Types of data

A data set contains one or more *variables* for each unit of study

ID	Gender	Age	Children	Albumin	Diabetes	Happiness
1	M	67	0	3.92	0	⌣
2	F	71	3	4.12	0	⌢
3	F	49	1	4.75	1	—
⋮	⋮	⋮	⋮	⋮	⋮	⋮

Data categories:

- Categorical
  - nominal, e.g. `Gender`, `Diabetes`, *or*
  - ordinal, e.g. `Happiness`: ⌢, –, ⌣.
- Numerical
  - discrete; typically integer valued 0, 1, 2, . . . , like `Children`, *or*
  - continuous; i.e. any value in an interval, like `Albumin`.

The category determines what analyses are available.

Data management

Data might be stored in the wrong format.

Check this prior to analysis!
This is especially important if data has been transferred, e.g. between formats or operating systems.

Common problems:

- date- and categorical data exported as integers
- numerical values stored as text (due to ',' vs. '.')

"Table 1"

It is useful to provide a summary table of the variables you are working with. Choice of descriptive measures may be context dependent.

variable	`Diabetes: No`		`Diabetes: Yes`	
value	*mean*	*sd*	*mean*	*sd*
`Age`	32.0	15.9	32.5	14.1
`Albumin`	4.20	0.37	3.80	0.50
	percent	*n*	*percent*	*n*
`Gender`				
M	64%	27	52%	22
F	36%	15	48%	20
`Happiness`				
⌢	61%	19	36%	15
–	23%	7	36%	15
⌣	16%	5	28%	12

What about missing data?

'Good' scenario: Suppose in an experiment a batch of samples are destroyed throught some random accident. Typically this only leads to a smaller sample size, but there is no problem running the analysis as planned.

'Bad' scenario: Suppose we study severity of myocardial infarctions with a model that includes gender, age, BMI (some missing) and smoking status (some missing). Worry: the reason for missing depends on the value, and possibly some outcome measure.

The statistical software default is to include only those individuals with complete case data on all variables in the analysis.

This **complete case analysis** will only give an unbiased result if the reason that a variable is missing has nothing to do with the actual value (and/or the outcome).

Solutions. . . ish

There is no trick that guarantees a non-biased analysis.

Single imputation e.g. replace missing value with a "typical" value for that variable (e.g. mean or median). This method underestimates the variance in the variable and will give overly optimistic results.

Multiple imputation create multiple imputed data sets where the missing values are replaced differently in each iteration (e.g. drawn at random from the non-missing values).

> *"It is not that multiple imputation is so good; it is really that other methods for adressing missing data are so bad."*
>
> *(Donald Rubin)*

N.B. we typically do not impute our outcome data.

Visualization of (continuous) data

A sufficiently small data set might not need visualization.

The level (g/dL) of the protein albumin was recorded in a sample (of size 8) of mice (56 days old):

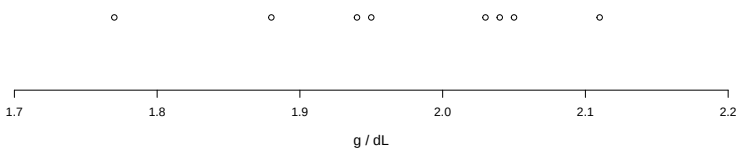
1.88 2.03 2.11 1.77 2.04 2.05 1.94 1.95

One simple way to get some handle on data is to order it:

1.77 1.88 1.94 1.95 2.03 2.04 2.05 2.11

Dotplot of albumin data

A dotplot is a one dimensional plot of the data.



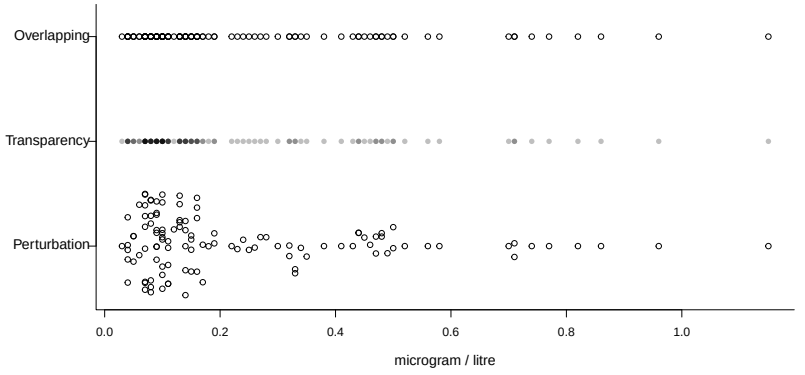
If there are non-unique (or close) points, the data set may appear smaller than it really is.

This can be alleviated by

- perturbation, or,
- (alpha) transparency.

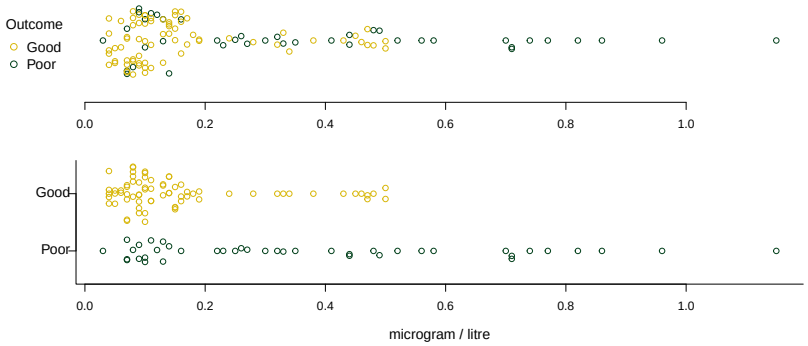
Subarachnoidal bleeding

A biomarker - the protein S100β - was measured for 113 individuals with aneurysmal subarachnoid hemorrhage.



Dotplot and groups

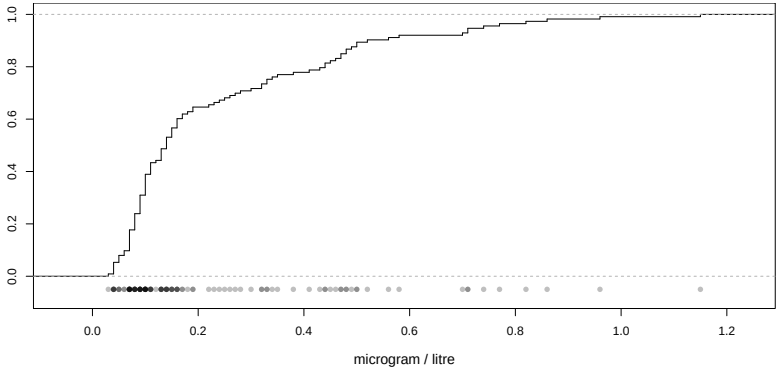
Dotplots can display groups.



Percentiles (Measure of location)

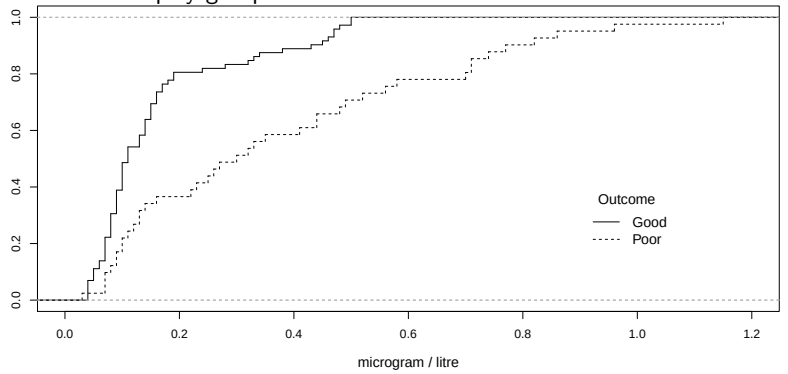
- The kth percentile is a value v such that k percent of your data lies below (or at) v. (Usually not uniquely defined.)
- The 50th percentile (the *median*) is the point which divides your ordered sample equally. (Only 'unique' if sample is odd, else use mean of the two midpoints.)
- *The Quartiles:* Q1 is the 25th percentile, Q2 is the 50th percentile and Q3 is the 75th percentile.
- We can describe all percentiles with the *cumulative frequency graph* (CF)
  When the sample size is 113 the jumps in the CF will be multiples of $1/113 \approx 0.01$.

Cumulative frequency for S100β



Cumulative frequency function

CF's can also display group differences.



Survival curves

A survival curve is a CF. Survival (time-to-event) data is typically *right censored* and the curve thus needs to be estimated (Kaplan-Meier) - more on that later in the course.

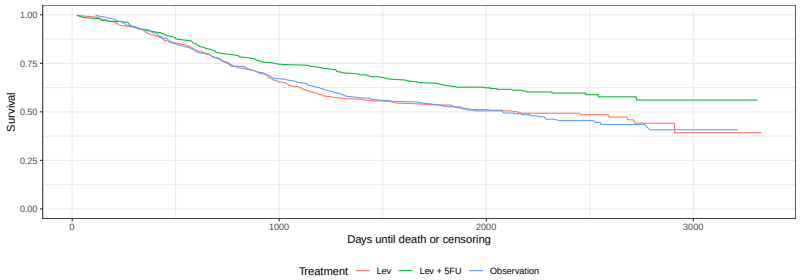
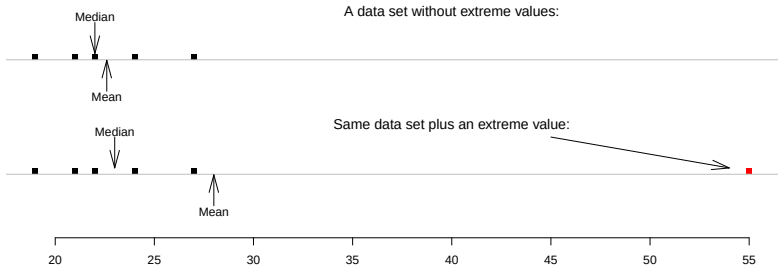


Figure: Survival curves for 3 different treatments of colon cancer; observation only, Levasimole, or Levasimole and 5-FU. (Moertel 1991)

Average value (Measure of location)

An average value should be representative of the entire data set.

- **The median:** is the midpoint of the ordered numerical sample when one iteratively cancelles the smallest and largest points.
- **The mean:** is the center of gravity of a data set.
  Note: unlike the median, it is sensitive to extreme values.



Mean or median?

Ex: A small company has 5 employees, who earns 19, 21, 22, 24, 27 (K SEK) and a boss who earns 55. (The numbers from the previous plot.)

Salaries	Excluding boss	Including boss
Median	22	23
Mean	22.6	28

Some points:

- Small data sets might not need summary measures.
- Symmetric data has mean $\approx$ median.
- (Easy enough to calculate both.)

Measuring of spread

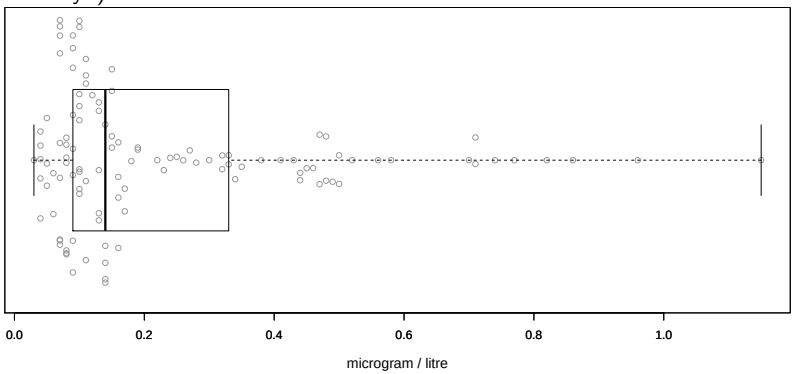
- **Range** The difference between the maximum and the minimum value.
- **Interquartile range (IQR)**: Q3-Q1.
- **Standard deviation (sd)** is given by the formula,

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2}.$$

Where $x_1, x_2, \ldots, x_n$ is the sample and $\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$ is the (sample) mean.
It is (*approximately only*) the mean distance to the mean value.
Note: the sd in the previous example is 3.0 and 13.5 if the boss is excluded or included, respectively.

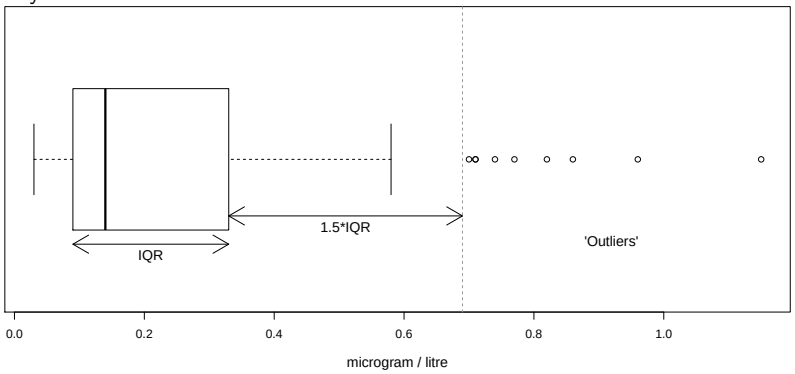
Boxplot of S100β

The boxplot usually show min, Q1, med, Q3 and max (the "5-point summary")...



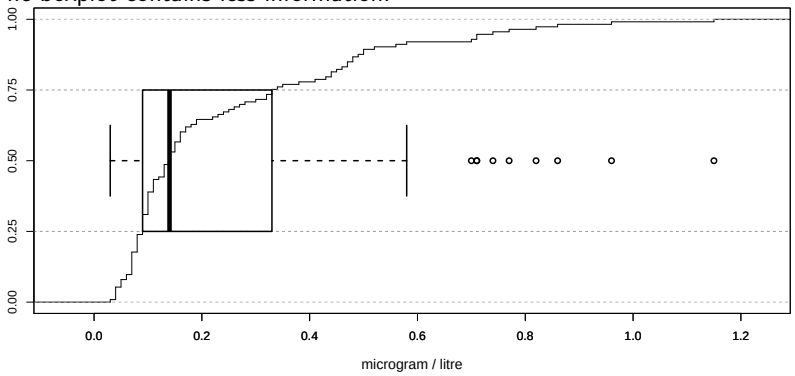
Boxplot

...but most software mark points that are more than 1.5 times the IQR away from 'the box'.



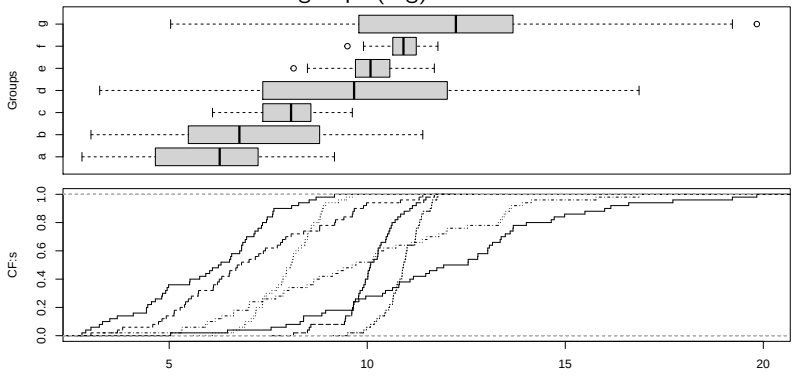
Connection between boxplot and cumulative frequency

The boxplot contains less information.



Pattern or detail?

Here is fake data whith 7 subgroups (a-g).



Some rules of thumb for tables and graphs

Both:

- Table/graph + caption should be self-contained.

Tables:

- Captions *above* the table.
- Avoid excessive precision and use adequate measures of location and spread.

Graphs:

- Captions *below* the graph
- 'Economy' Do not make a graph which is more easily expressed in text or a small table, e.g. graph with a single boxplot.
- Avoid 2D graphs shown in 3D.

Probability theory and models

Note: this is not a theoretical course in any mathematical sense.

Most lectures will be driven by applications.

However, a view towards theory and some fundamental (mathematical) results can give a better understanding of some of the methods employed in this course.

So here goes...

Probability theory studies models of random data. A **model** is a way of specifying the range of possible values and the probability with which these occur.

- **Probability functions** describe discrete numeric/categorical data
- **Density functions** describe continuous (numeric) data

Probability theory: given model (model parameters, or other aspects) - describe how data behave. E.g.

- specific results: how likely are specific deviations
- general results: Law of Large Numbers, Central Limit Theorem, etc.

Inference theory: given data, what is a likely model/parameters or other aspects of the underlying distribution (without specifying model = non-parametric statistics).

Probability models for categorical or integer-valued data

A yet undetermined random value is called a *random variable* (RV).

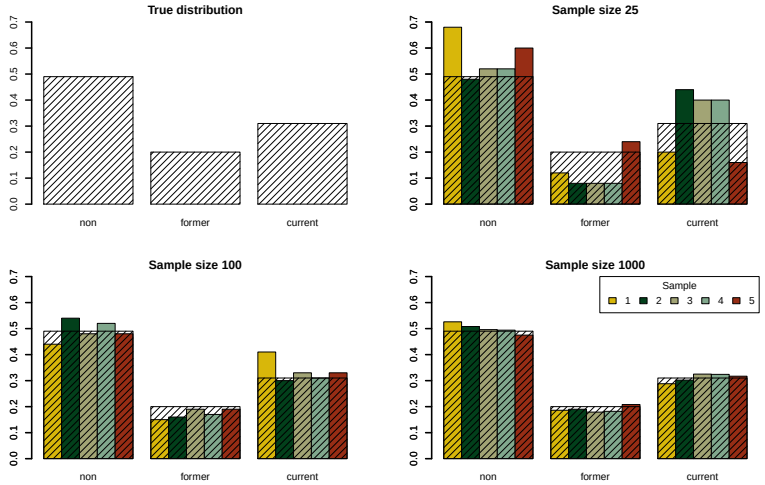
Let Z ='the outcome of the throw of a die'. Then $\text{Prob}(Z = k) = 1/6$ for all $k = 1, 2, \ldots, 6$, or, equivalently

Value k	1	2	3	4	5	6
$\text{Prob}(Z = k)$	1/6	1/6	1/6	1/6	1/6	1/6

Suppose that in the population there are 49 % non-smokers, 20 % former smokers and 31% current smokers. Then the smoking status X of a person selected at random is a RV with a probability function

Value v	non	former	current
$\text{Prob}(X = v)$	0.49	0.20	0.31

Five samples from three different sampling sizes from previous distribution



FEV data set

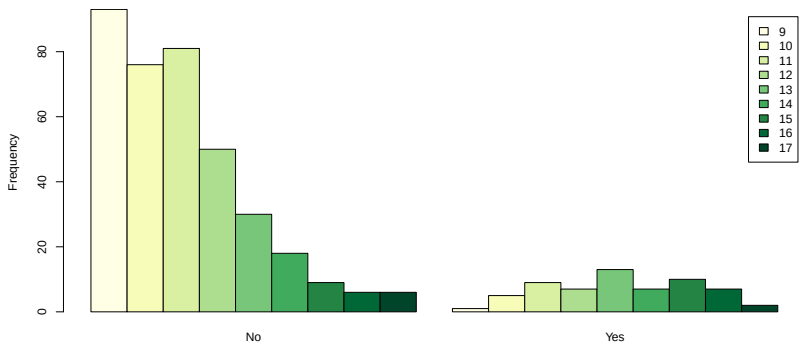
430 children (9-17 years of age) had their age, forced expiratory volume in 1 second (FEV) and smoking status (!) recorded.

A barchart is a way to visualize a variable with a small number of unique values (often categorical). They are visual analogous of tables.

Ex: how do the ages distribute over smoking status?

Smoking	**Age**								
	9	10	11	12	13	14	15	16	17
No	93	76	81	50	30	18	9	6	6
Yes	1	5	9	7	13	7	10	7	2

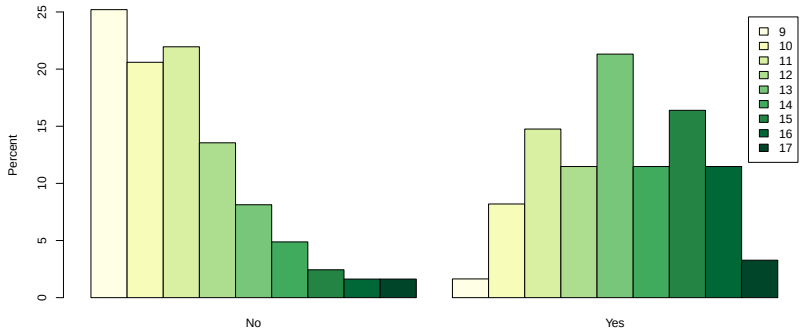
Visualizing 'age' versus 'smoking'



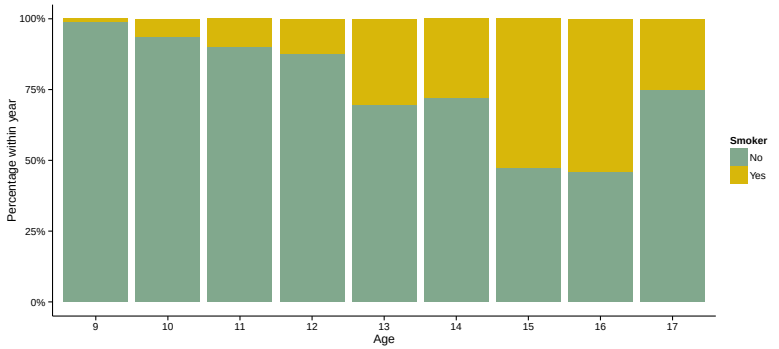
If the groups (smokers/non-smokers) aren't balanced it is difficult to compare the distributions.
Tabulate/plot the percentages within groups:

Age	**Smoking (%)**	
	No	Yes
9	0.252	0.016
10	0.206	0.082
11	0.220	0.148
12	0.136	0.115
13	0.081	0.213
14	0.049	0.115
15	0.024	0.164
16	0.016	0.115
17	0.016	0.033
Sum	1.000	1.000

Visualizing 'age' versus 'smoking' as percentages within smoking groups



Visualizing 'smoking' versus 'age' as percentages within age groups, stacked



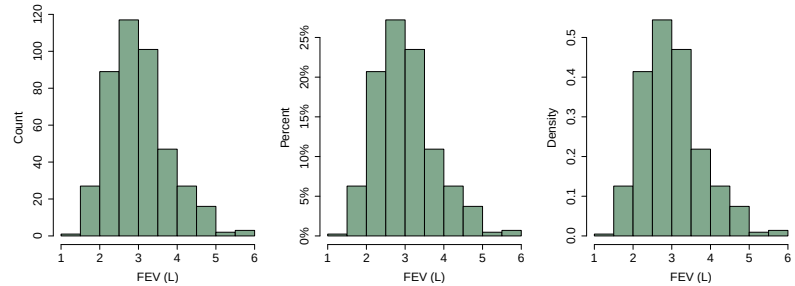
Probability model for continuous data

A continuous random variable is described by its *density function*.

If X has density function f as above, then we compute probabilities as

$$\text{Prob}(a \leq X \leq b) = \text{ Area(a,b).}$$

The shape of a histogram estimates the shape of the density function.

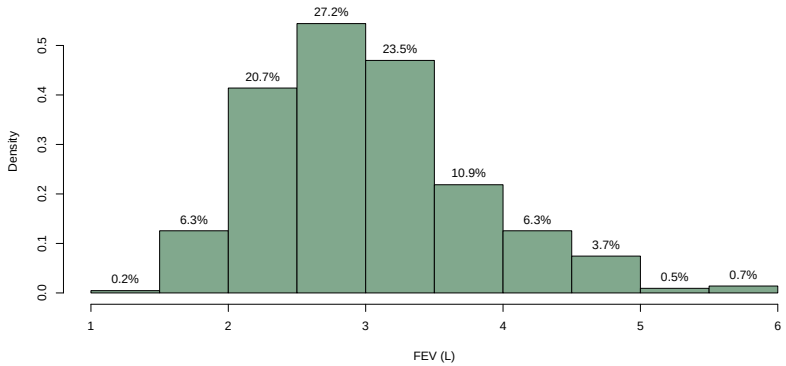


"Density" is more abstract but

- gives right scale for density function estimate (easy to correctly plot candidate model on top of histogram)
- allows for varying "bins"
- allows for comparison between very different sample sizes

Histogram (with density) of FEV

The histogram (with density on the y-axis) estimates the density function.

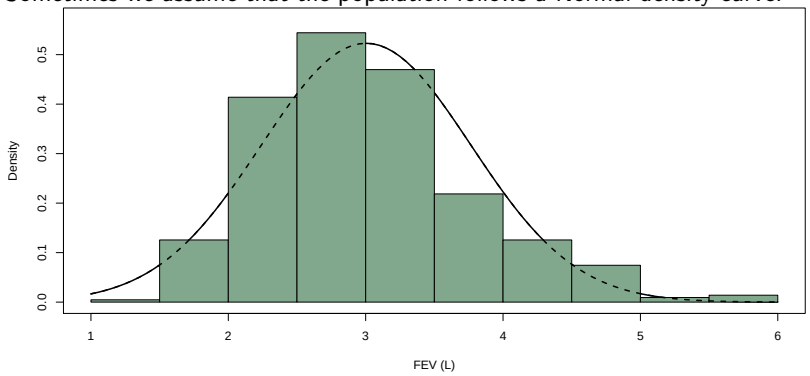


Summary

Graph	Summary Measure	Theory
CF	percentiles	(Cdf)
Boxplot	min, Quartiles, max	
Bar charts		Probability functions (discrete/categorical RV)
Histograms		Density functions (continuous RV)
	median, IQR	any distribution
	mean, s.d.	symmetrical distribution ($\approx$ Normal distribution)

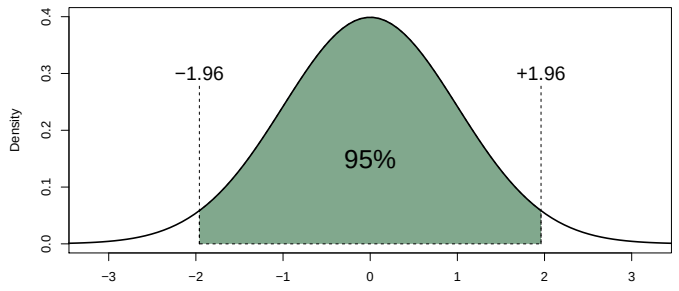
The Normal Distribution

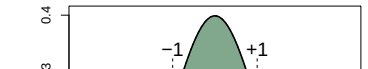
Sometimes we assume that the population follows a Normal density curve.



Properties of the standard Normal distribution (and why you need to know the number 1.96)

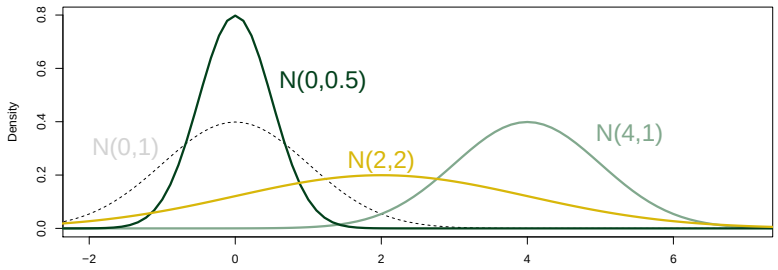
The standard Normal distribution has a standard deviation of 1.
95% of the 'probability mass' lies within ± 1.96.





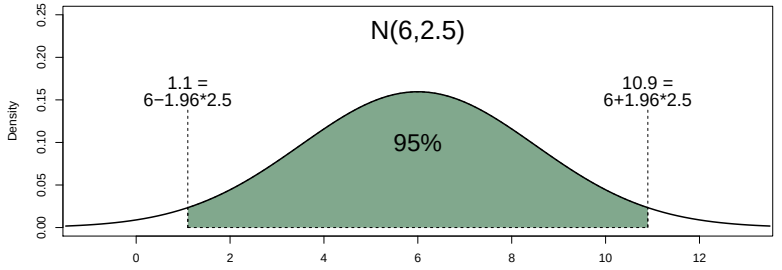
The Normal distribution $N(\mu, \sigma)$

is determined by its mean (μ) and standard deviation (σ).
If X is N(0,1) then $\mu + \sigma X$ is N(μ, σ).



Properties of the Normal distribution

If X is N(μ, σ), then 95% of observations will be between $\mu - 1.96\sigma$ and $\mu + 1.96\sigma$.



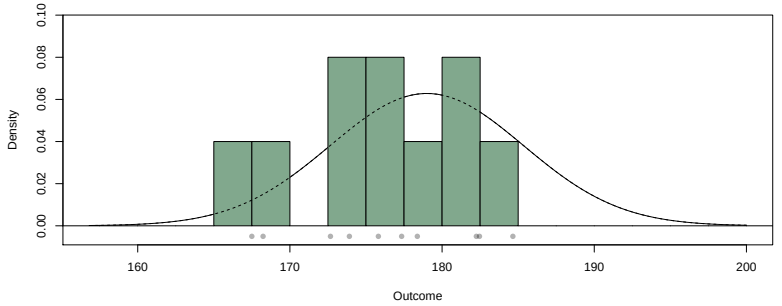
Attempt to visualize sampling from a given model

Assume that height of individuals in some population

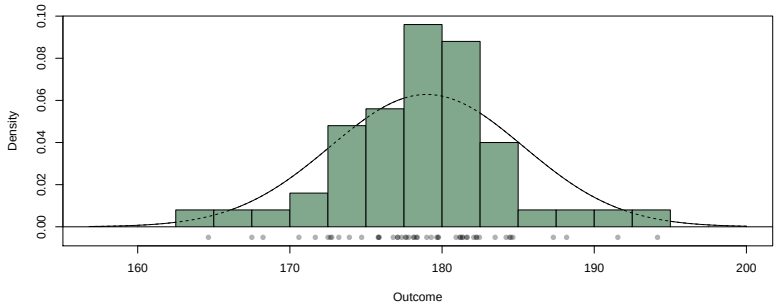
- is Normally distributed,
- has mean (μ) 179 (cm), and,
- has s.d. (σ) 6.35 (cm).

The following three slides show histogram of samples of sizes 10, 50 and 500, respectively.

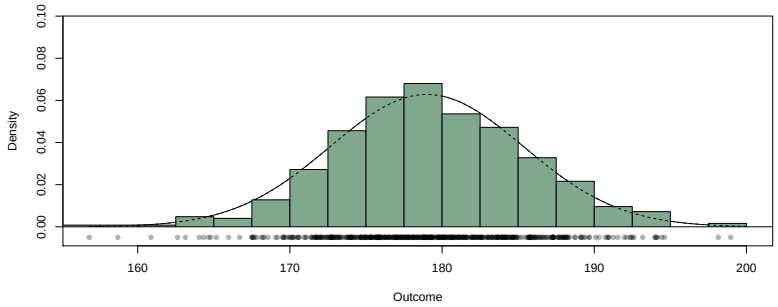
$n = 10$



$n = 50$



$n = 500$



Properties of samples and sample means

Suppose we have a sample of size 10 from the population.

We want to know the *population mean* μ.

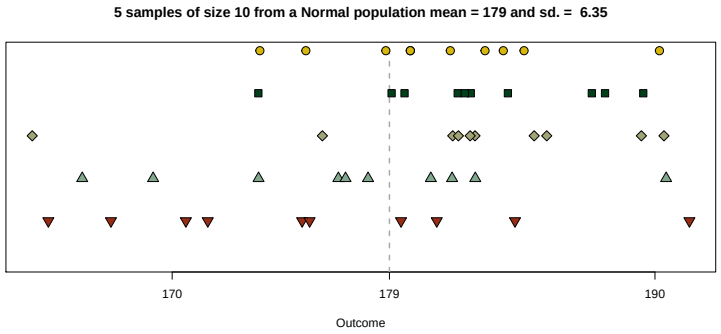
We can estimate μ with the *sample mean* μ^*.

If the sample is drawn *at random* then μ^* is *unbiased* (on average correct)

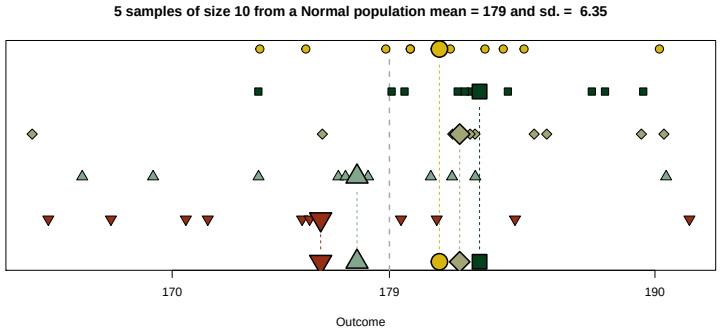
But we also want to know its *precision* - often by calculating its standard deviation.

Consider several iterations of the procedure of drawing a sample of size 10.

It is clear that there is variation within a sample and thus a standard deviation can be calculated (directly from the sample itself) . . .



. . . *but* there is also a variation associated with a sample mean (but we do not tend to see it). *Anything* calculated from a (random) sample (e.g. a mean value or a regression coefficient) is a random number and thus has a standard deviation (but we typically need theory to calculate this!)



Properties of sample means

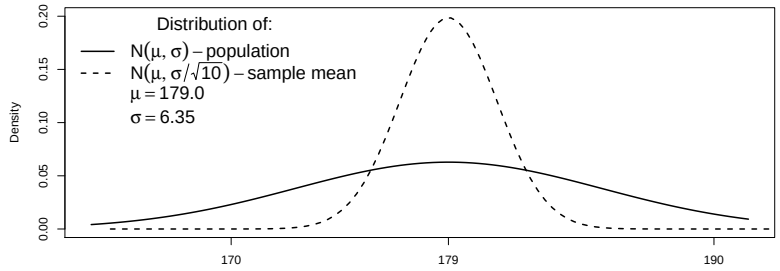
Standard Error of the Mean (SEM)
The standard deviation of the sample mean is referred to as SEM and a measure of (approx.) the average distance the population mean. Thus a measure of the precision the sample mean is as an estimator of the population mean.

In a Normal model the SEM is the population s.d. dived by $\sqrt{n}$.

Standard Errors (SE)
More generally, the standard deviation of something calculated from a sample is called its standard error.

Later we will use these to create *confidence intervalls*.

In this example we can exactly specify the distribution of the sample mean.



Normal population ⇒ Normal mean

On the previous slide you saw

- a Normal population curve, and
- the Normal curve for the sample mean (for sample size 10).

In this case this is the true (derived from theory) density curve for the sample mean.

Properties of the Normal distribution will aid further analysis using the mean of the sample.

What happens if the population follows some non-Normal curve?

The Central Limit Theorem (CLT)

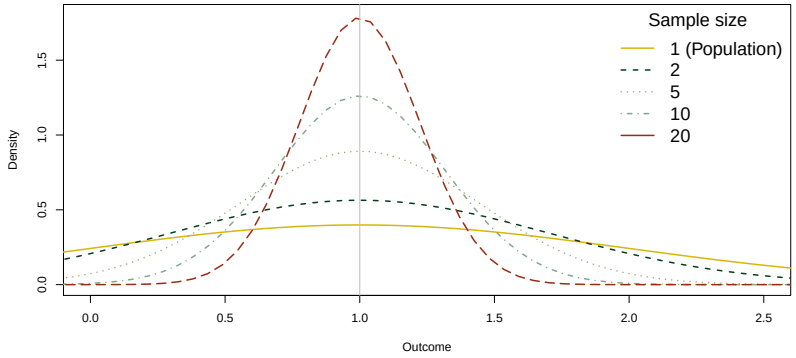
CLT: Regardless of the true population density curve, the sample mean density can be made (with arbitrarily good approximation) Normal by choosing n large enough.

- How large does n have to be?
  Depends on how skew the population density is.
  - In general $n = 20$ will suffice.
  - If population is Normal then $n = 1$ is enough.
- CLT applies to many 'statistics' (= functions of samples).

The next 2 slides shows theoretical distributions for a population and sample means thereof, for varying sample sizes.
Check out Seeing Theory for an interactive version.

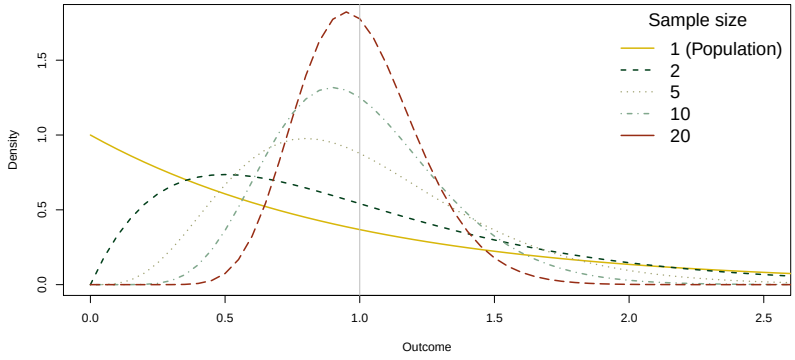
Visualization of sample mean from normal population

The distribution of sample means from a *normal* distribution.



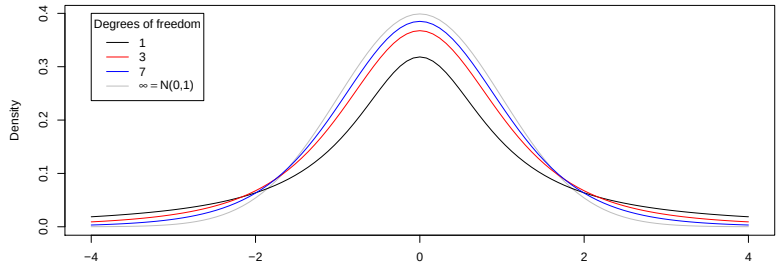
Attempt to visualize CLT for the sample mean

The distribution of sample means from a skewed distribution.



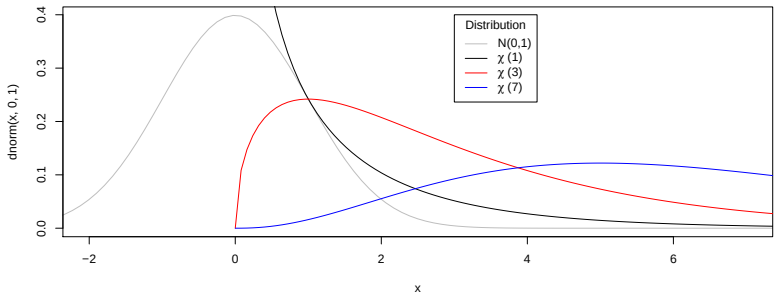
The t-distribution

Calculating standard test statistics from a normal distribution requires estimating the standard deviation. For **small samples** the change in distribution motivates making exact calculations. The t-distribution is "increasingly normal" as its parameter (degrees of freedom) increases.



The χ^2-distribution

If you add k standard normal random numbers, each squared, then the resulting distribution is $\chi^2(k)$. As we will see this is useful in the context of categorical variables.



Visual tests of normality

Perhaps surprisingly, quite often we rely on *visual* rather than *formal* tests of model assumptions.

A common formal test of normality is the Shapiro-Wilks test.

Many plots can provide a visual test of normality, but a common one is the Q-Q plot. 'Q' is for *quantile*.

Quantile? Quantiles divides your data into (roughly) equal piles.

- the median is the 2-quantile
- the tertiles are the 3-quantiles (the $33\frac{1}{3}$ percentile and the $66\frac{2}{3}$ percentile)
- the quartiles (Q1, Q2 and Q3) are the 4-quantiles.
- . . . and so on.

Cross-over data

13 patients had their peak expiratory flow (PEF, l/min) recorded once after inhaling each of two different asthma drugs (the order of which were random).

In *paired* data one usually look at the 13 differences as a measurement of effect size.

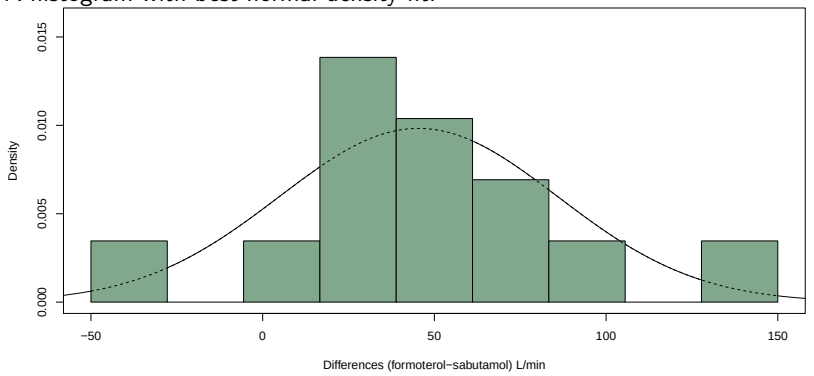
Data:

40, 50, 70, 20, 40, 30, -35, 15, 90, 30, 30, 80, 130

Is the normal distribution a good model for these 13 numbers?

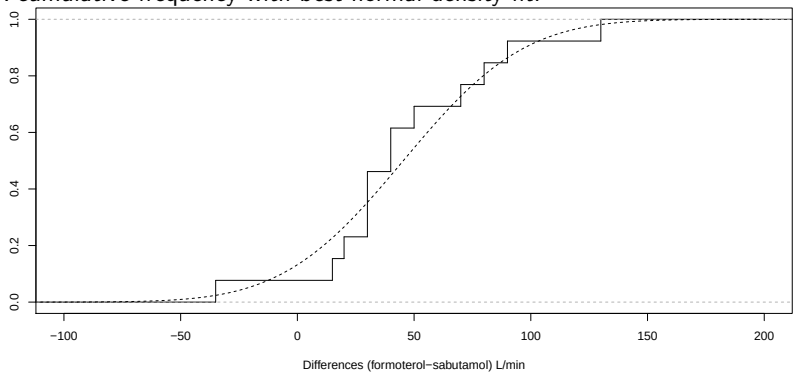
PEV

A histogram with best normal density fit.



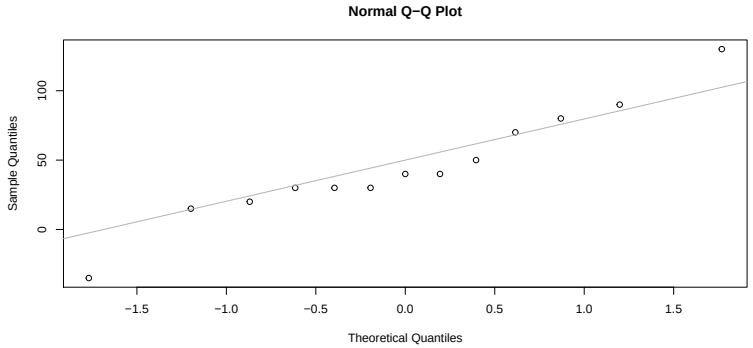
PEV

A cumulative frequency with best normal density fit.

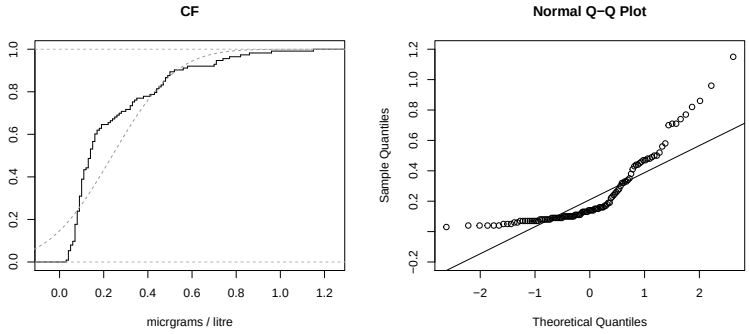


The Quantile-Quantile plot

If the effect size is Normally distributed its QQ-plot should be a straight line (approximately).
A QQ-plot plots the sample (of size n) against the n-quantiles of the (standard) Normal distribution.



The S100β measurements is certainly not normally distributed.



A reminder ahead of time

It is very rarely the actual data that is tested for normality!

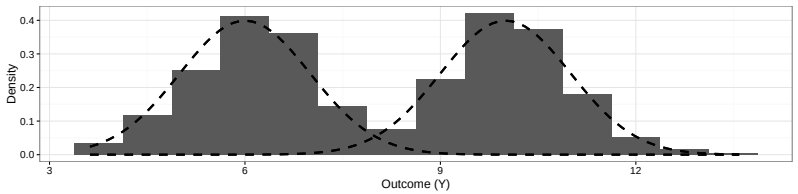
Most of the time the models that assume normality does so for the *error terms*, i.e. there is a model, depending on the covariates x, for the outcome Y such that

$$Y = \text{some deterministic function of x} + \text{noise.}$$

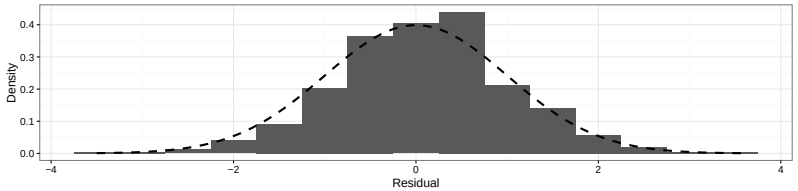
E.g. a 2-sample t-test assumes that an outcome is normally distributed around a group-specific mean. Data for such a test might look like this

outcome (Y)	5.1	6.2	7.9	9.2	4.7	...
group (x)	A	A	B	B	A	...

We cannot test the entire Y data for normality. This is evident if imagine the group effect to be very large...



It is the deviations (noise/error term) around each group-specific mean that is supposed to be normal, an estimation of which is called *residuals*. Subtract the (estimated) group effect from each datapoint to get:



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Second Summary

95% of observations from a Normal population lies within 1.96 multiples of the (population) s.d. from the (population) mean.

Means and, in particular sd's, must be distinguished on three levels

- population,
- sample, and
- estimate.

The s.d. of the latter is called the Standard Error

The CLT explains why many estimates ('statistics') are (approx.) Normally distributed even though the population may not be.