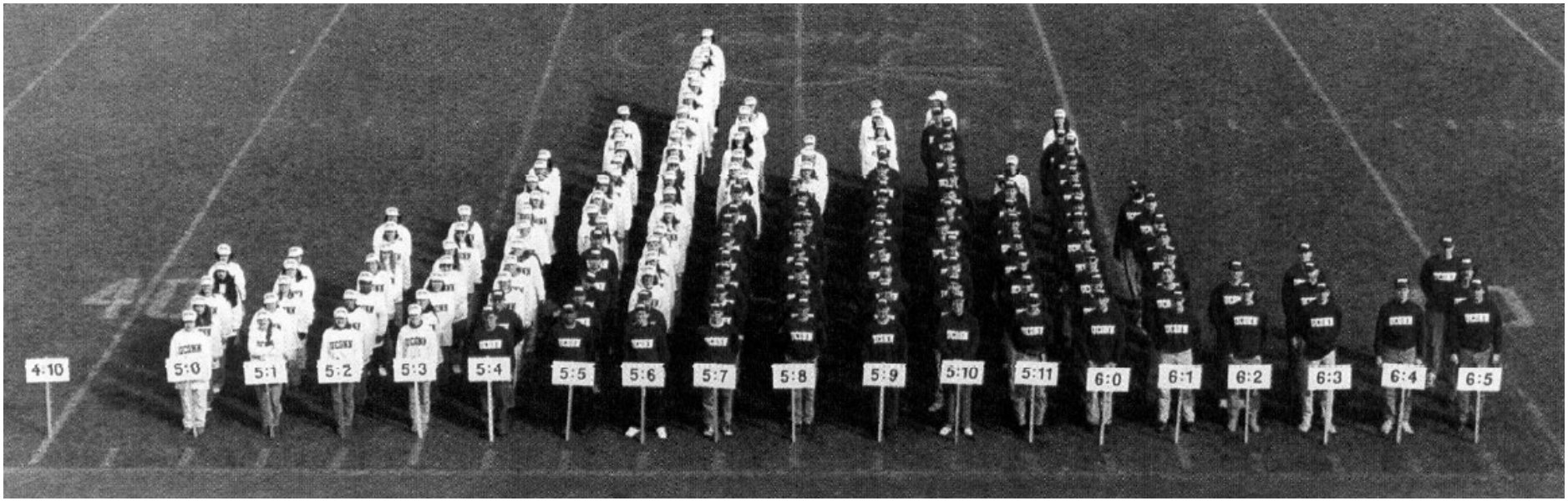
Introduction to Biostatistics



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

Introduction to Biostatistics
Lecture 1B and 2

Henrik Renlund

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 *variable* for each unit of study

ID	Sex	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. `Sex`, `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 of the *outcome variable* determines what analyses are available.

Data management

Make sure you and your software agree on variable formats.

This is especially important if data has been transferred, e.g. between formats or operating systems.

Common problems:

- date- and categorical data stored as integers
- numerical values stored as text (due to ',' vs. '.')
- how are missing values represented? "" (blank), ".", "-99"?

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 sex, age, BMI (some missing) and smoking status (some missing). Worry: the reason for being missing depends on the value.

The statistical software default is to include only those individuals with complete 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).

Imputation

Suppose you want to model some outcome against 10 covariates. If an individual has 1 value missing, they will be excluded from the complete case analysis *event though they could contribute with 9 other covariate values*.

Imputation methods tries to fill in the blanks; e.g. with typical values (e.g. mean or sampled values) or predicted values (from regression models, possibly using more covariates).

It might even to this several times to create multiple data sets over which the analysis will be averaged.

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

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):

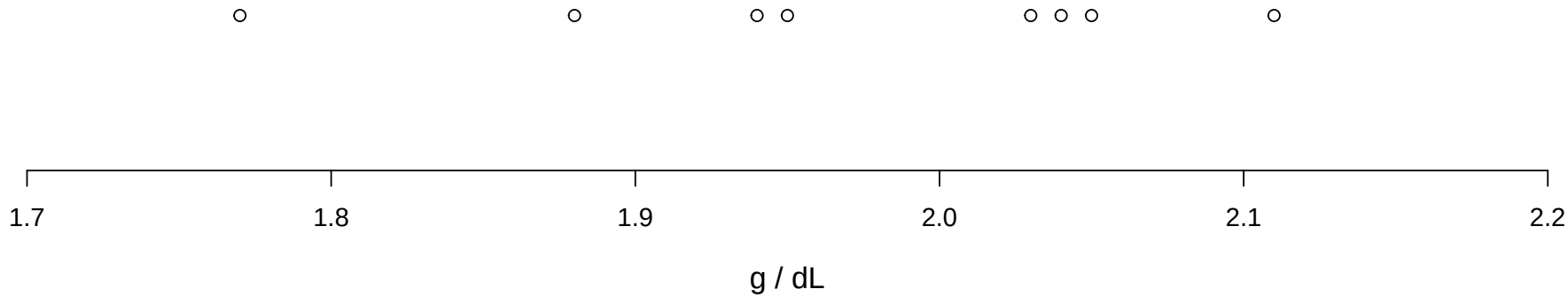
1.88 2.03 2.11 1.77 2.04 2.05 1.94 1.95

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.



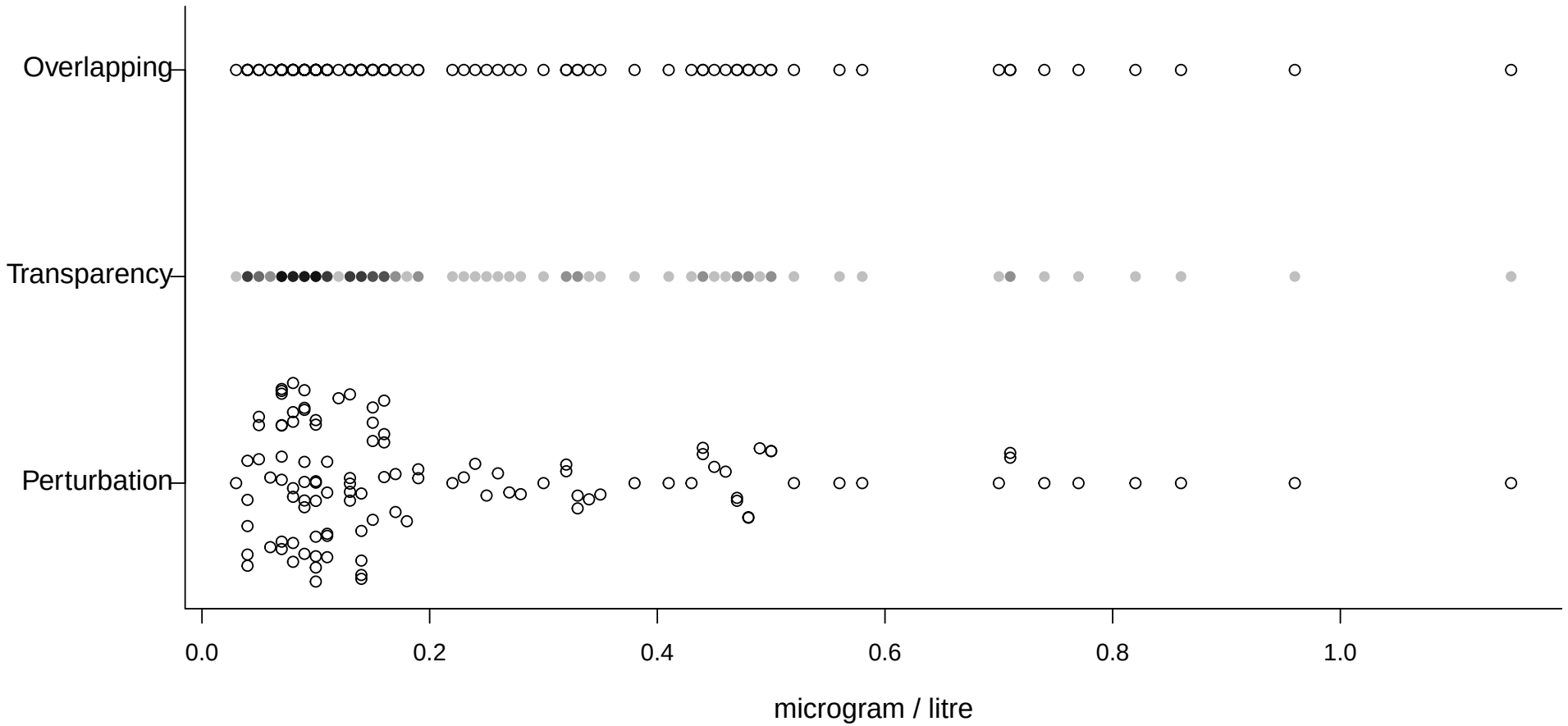
If there are non-unique (or close) points, the data set may appear smaller than it really is.

This can be alleviated by

- perturbation, or,
- (alpha) transparency.

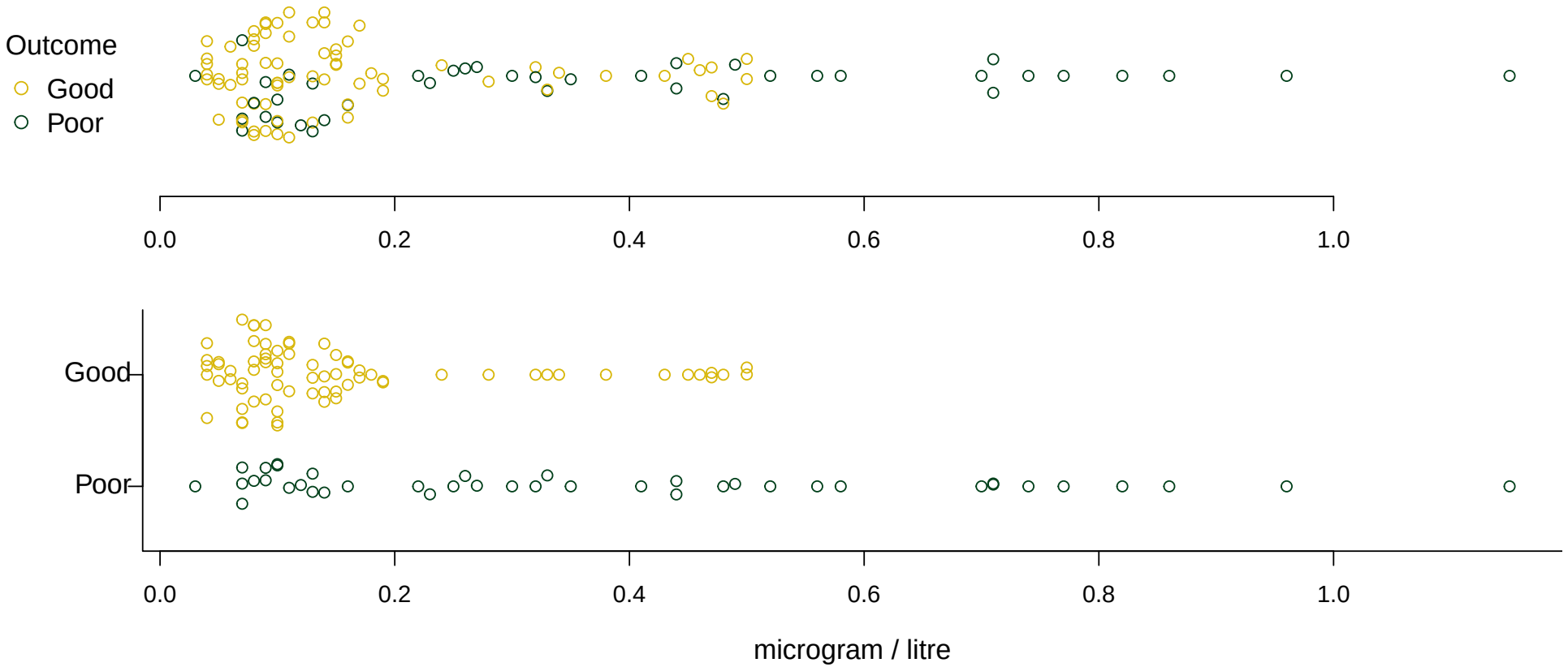
Subarachnoidal bleeding

A biomarker - the protein S100B - was measured for 113 individuals with aneurysmal subarachnoid hemorrhage.



Dotplot and groups

Dotplots can display groups.



"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 / *value*	Diabetes: No		Diabetes: Yes	
	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*
Sex				
M	64%	27	52%	22
F	36%	15	48%	20
Happiness				
⌒	61%	19	36%	15
—	23%	7	36%	15
⌣	16%	5	28%	12

Percentiles (Measure of location)

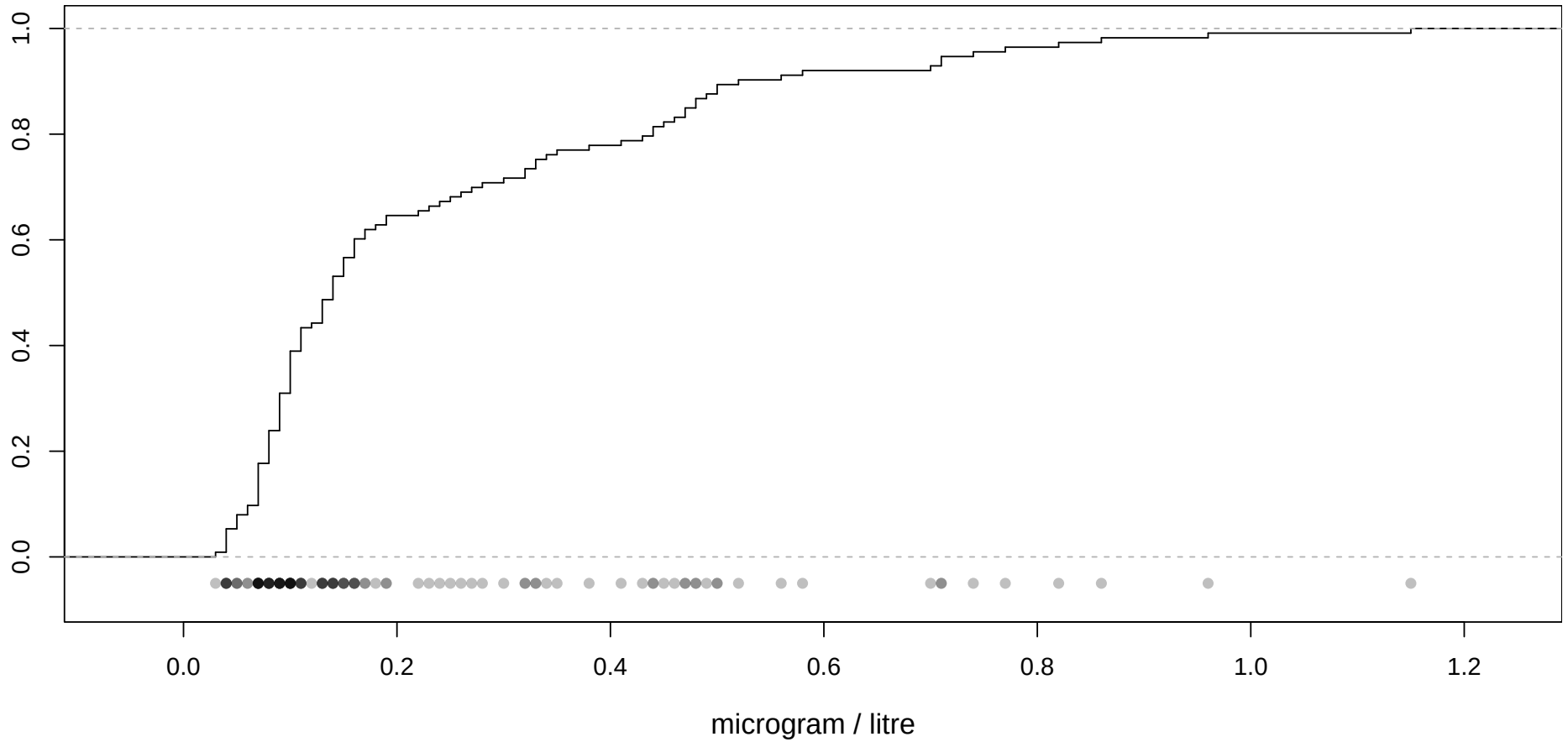
- The kth percentile is a value v such that k percent of your data lies below (or at) v. (Usually not uniquely defined.)
- The 50th percentile (the *median*) is the point which divides your ordered sample equally. (Only 'unique' if sample is odd, else use mean of the two midpoints.)
- *The Quartiles:* 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) also called the *empirical cumulative distribution function* (ECDF)

Creating a CF

A CF shows the cumulative frequency (or cumulative proportion) and thus starts at 0 for points smaller than the smallest point of the data set. Then it is a step-wise function with jumps according to:

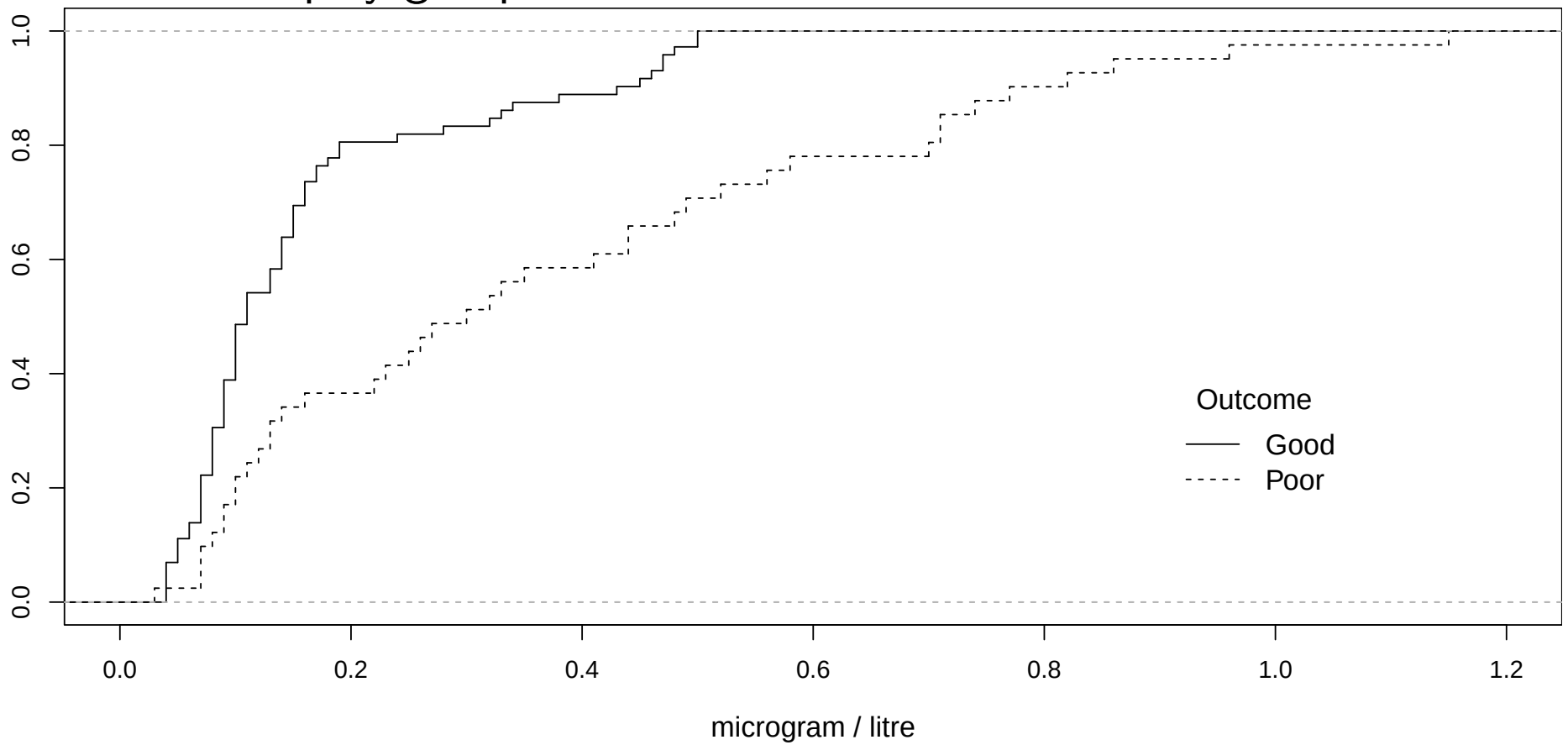
Values	Count	Cumulative count	Cumulative proportion
0.03	1	1	$\frac{1}{113} \approx 0.009$
0.04	5	6	$\frac{6}{113} \approx 0.053$
0.05	3	9	$\frac{9}{113} \approx 0.080$
$\vdots$	$\vdots$	$\vdots$	$\vdots$
1.15	1	113	1.000

Cumulative frequency for S100B



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.

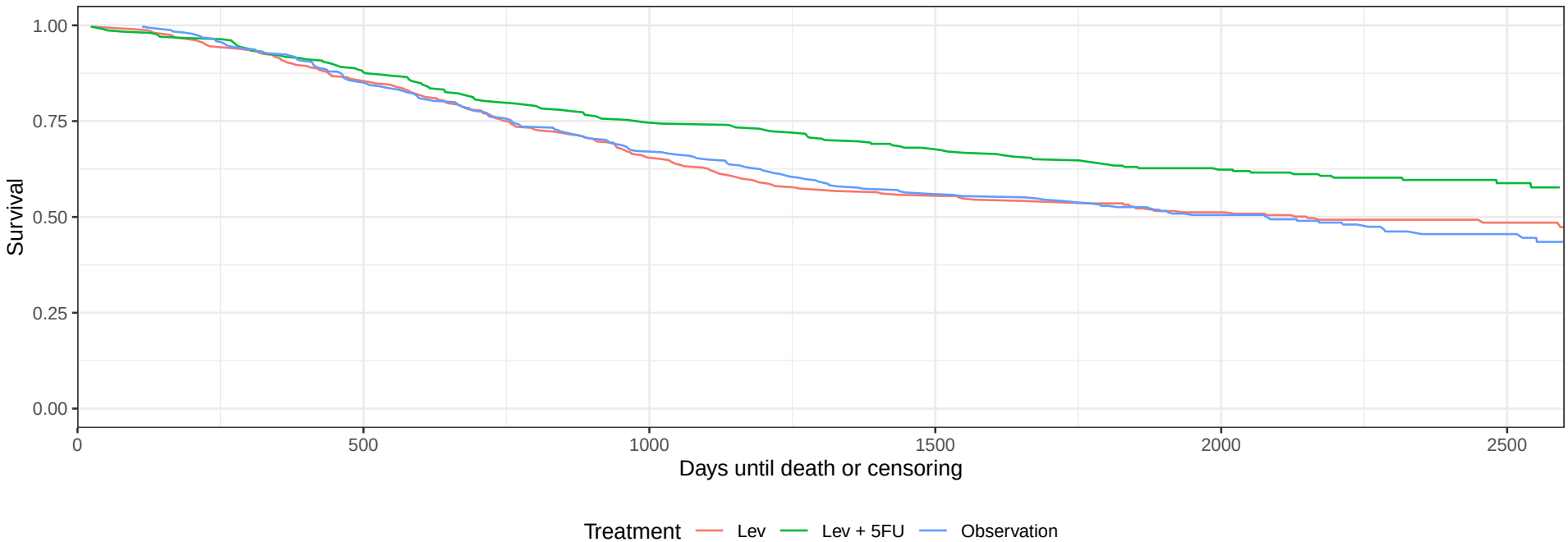
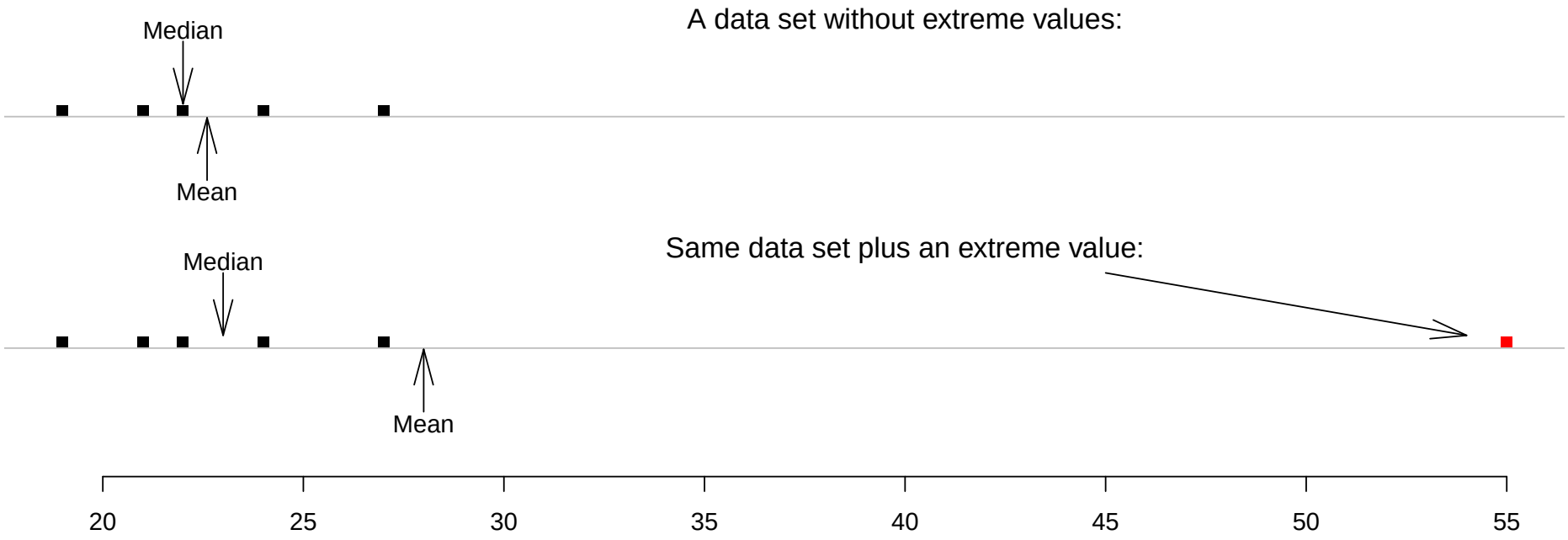


Figure: Survival curves for 3 different treatments of colon cancer; observation only, Levasimole, or Levasimole and 5-FU. (Moertel 1991)

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

Ex: The number of hospitalization days per individual in Uppsala is likely to be very skewed. The median might be of most interest on an individual level, wheras the mean (which is essentially the sum) is of more interest to whoever is in charge of the hospital budget (as well as other things).

In fact: the median is probably 0! One could e.g. describe this distribution by the median among those with non-zero hospitalization days.

Measures 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,

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

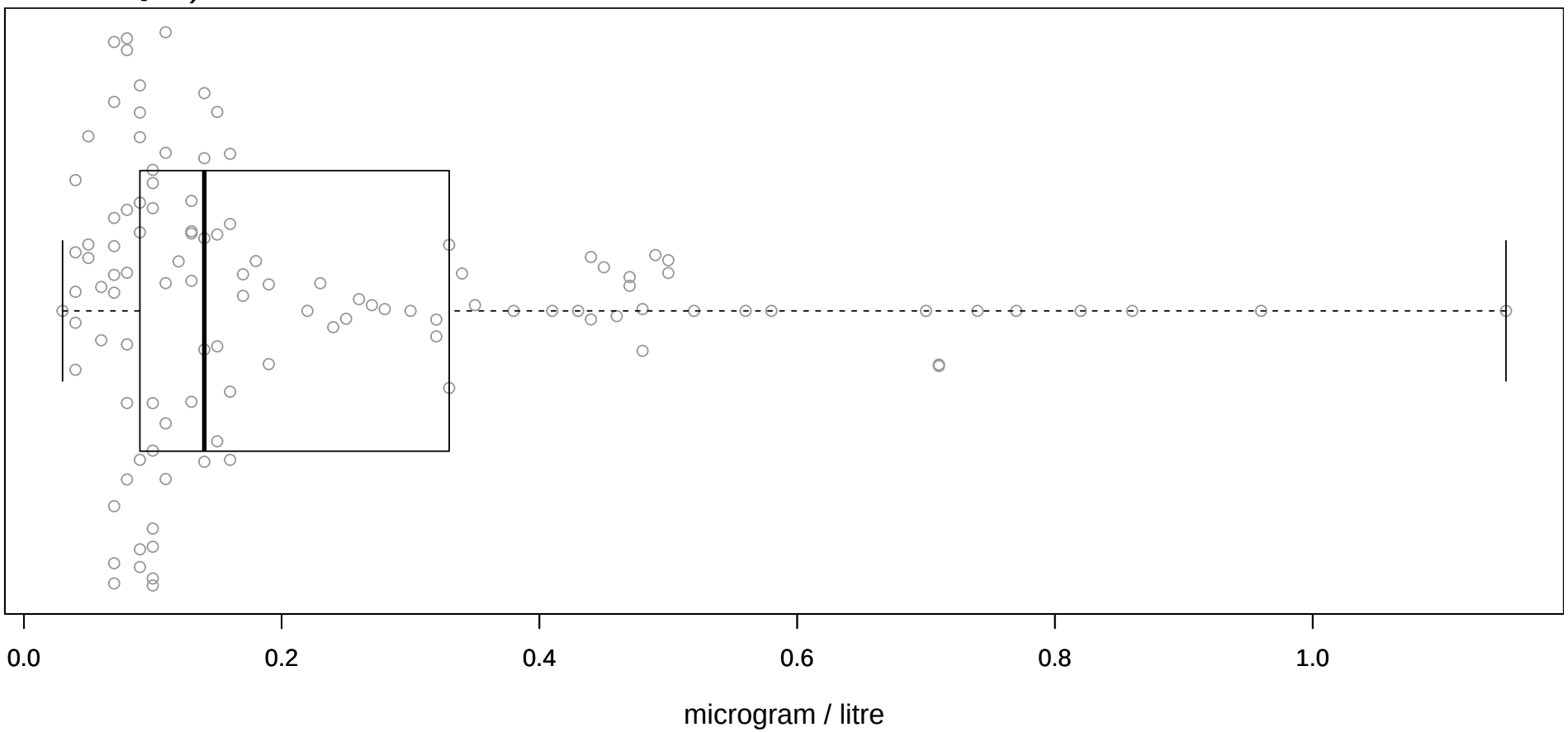
Where $x_1, x_2, \ldots, x_n$ is the sample and $\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$ is the mean.

It is the typical distance between a value and the mean value.

Note: the sd in the previous salary example is 3.0 and 13.5 if the boss is excluded or included, respectively.

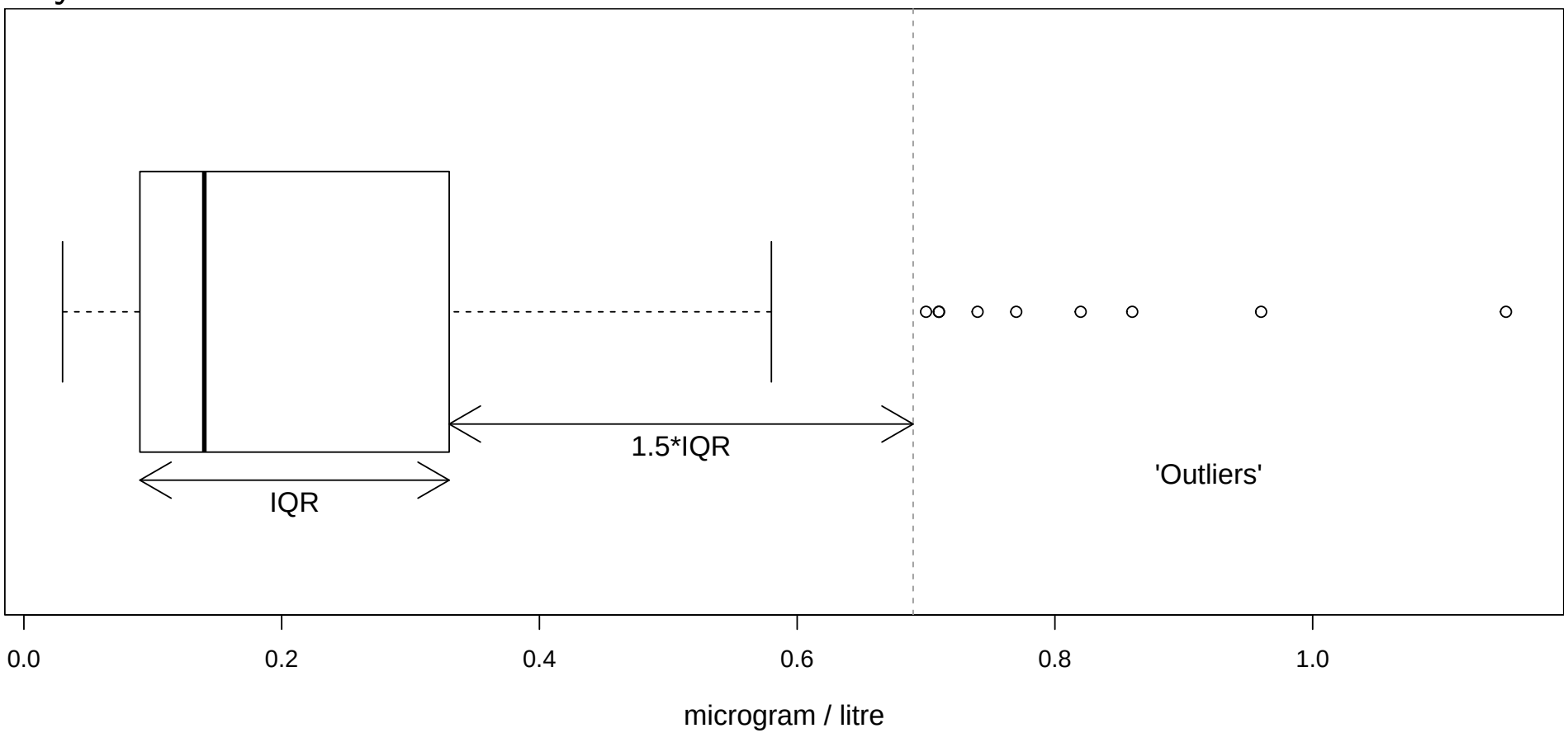
Boxplot of S100B

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



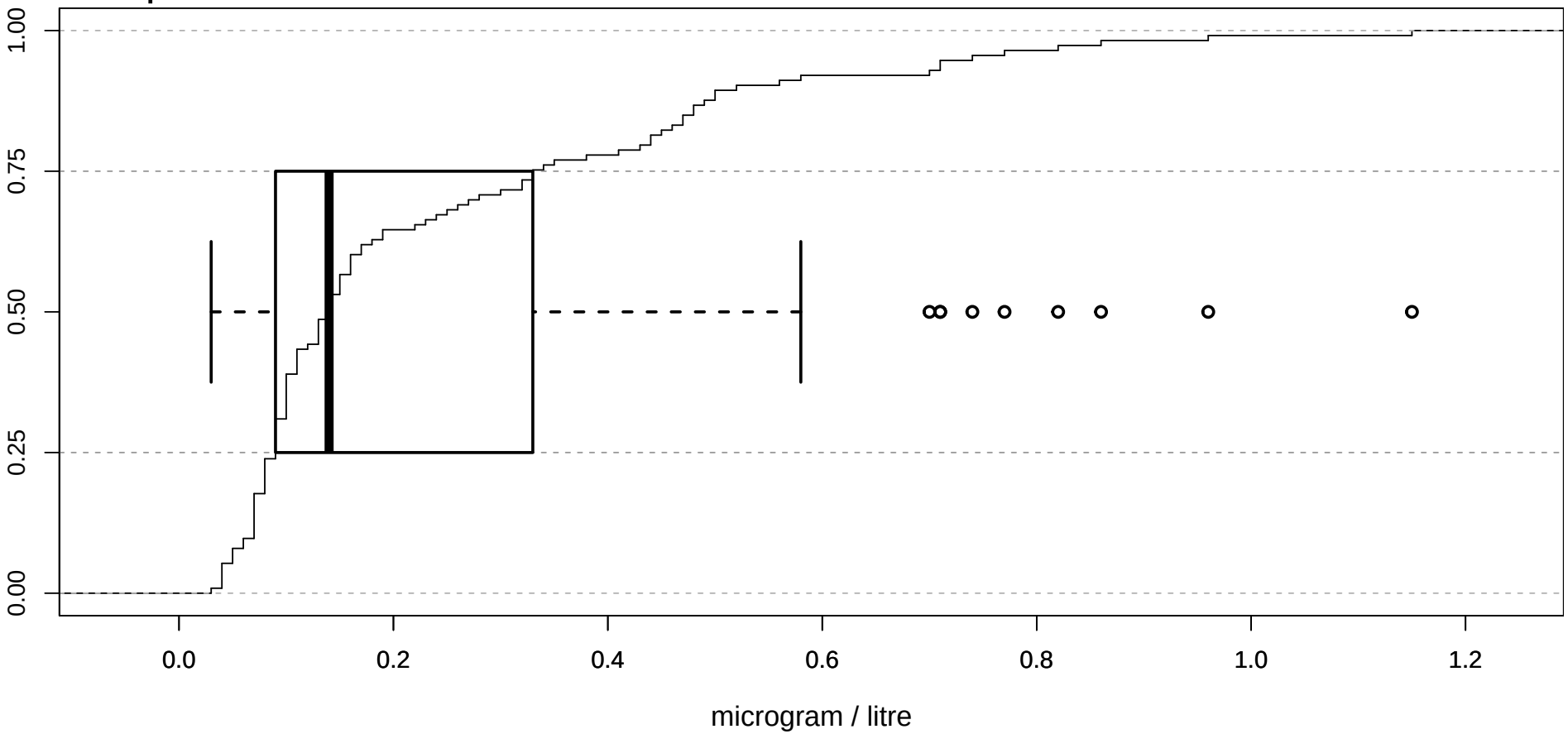
Boxplot

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



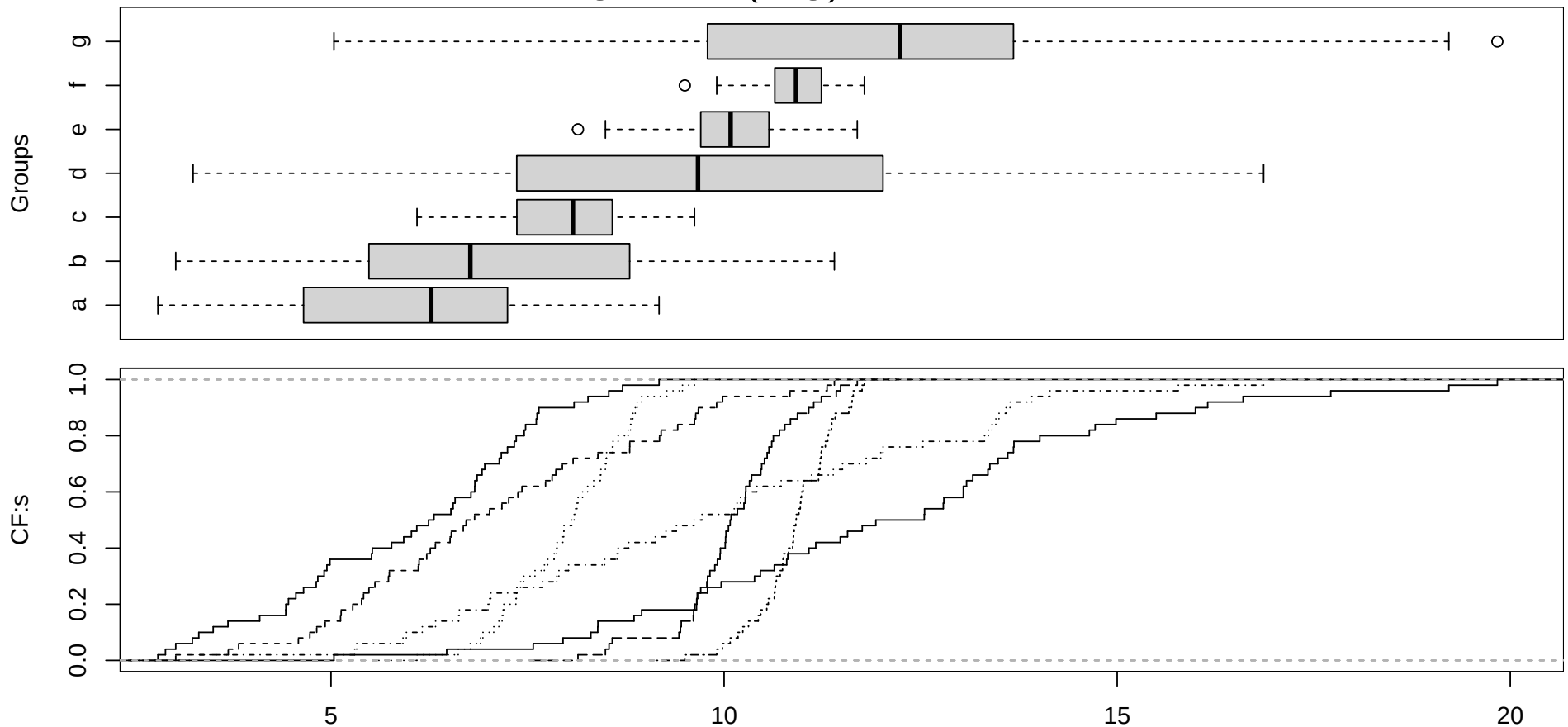
Connection between boxplot and cumulative frequency

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.
- Colors are tricky (colorblindness, black/white-printing, etc.) - a website like Colorbrewer (https://colorbrewer2.org) might guide color choice.

Probability theory and models

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*.

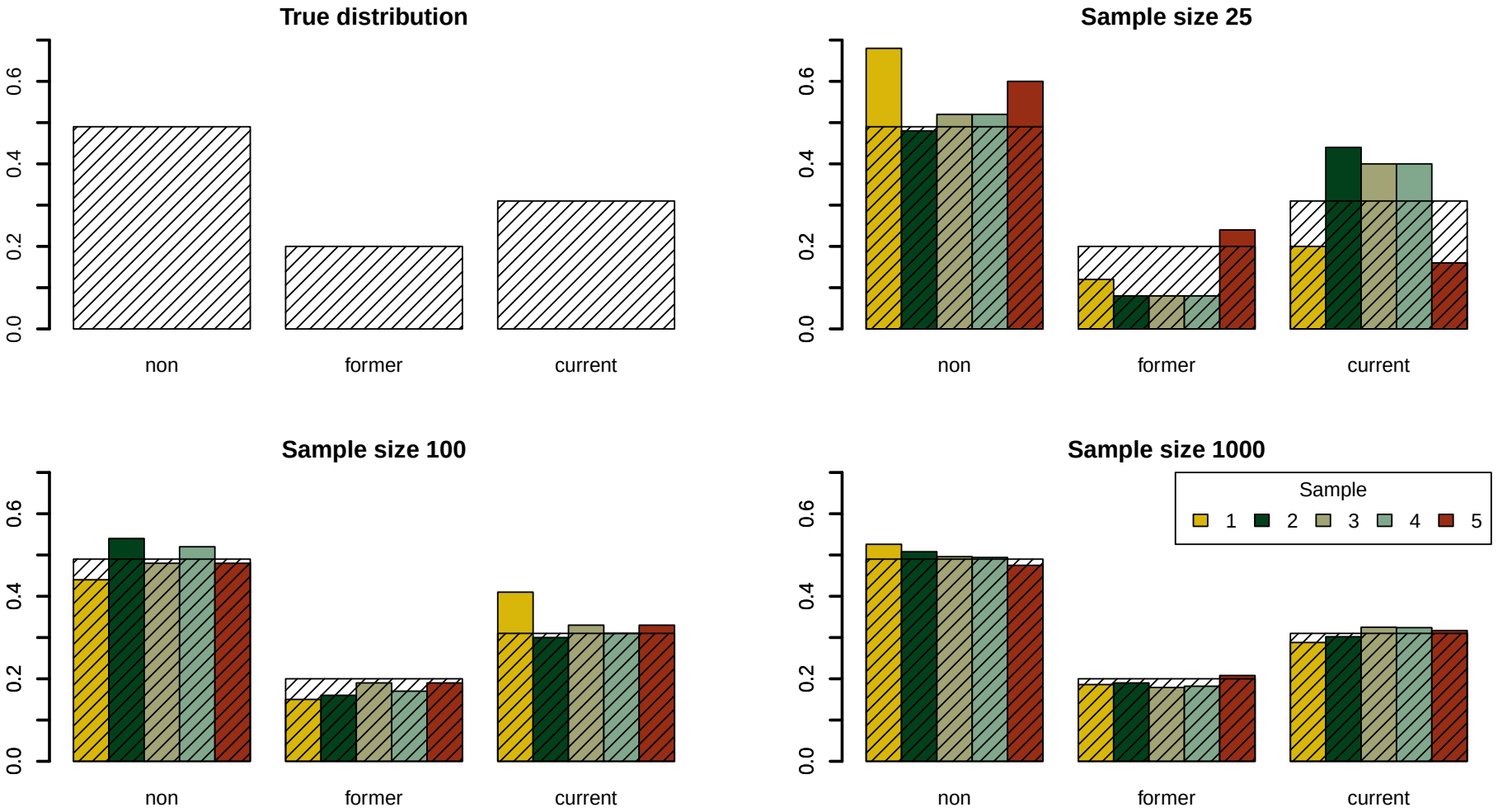
Let $Z =$'the outcome of the throw of a die'. Then $\text{Prob}(Z = k) = 1/6$ for all $k = 1, 2, \ldots, 6$, or, equivalently

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 random variable 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



The Law of Large Numbers tells us that the sample distribution will increasingly look like the population 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.

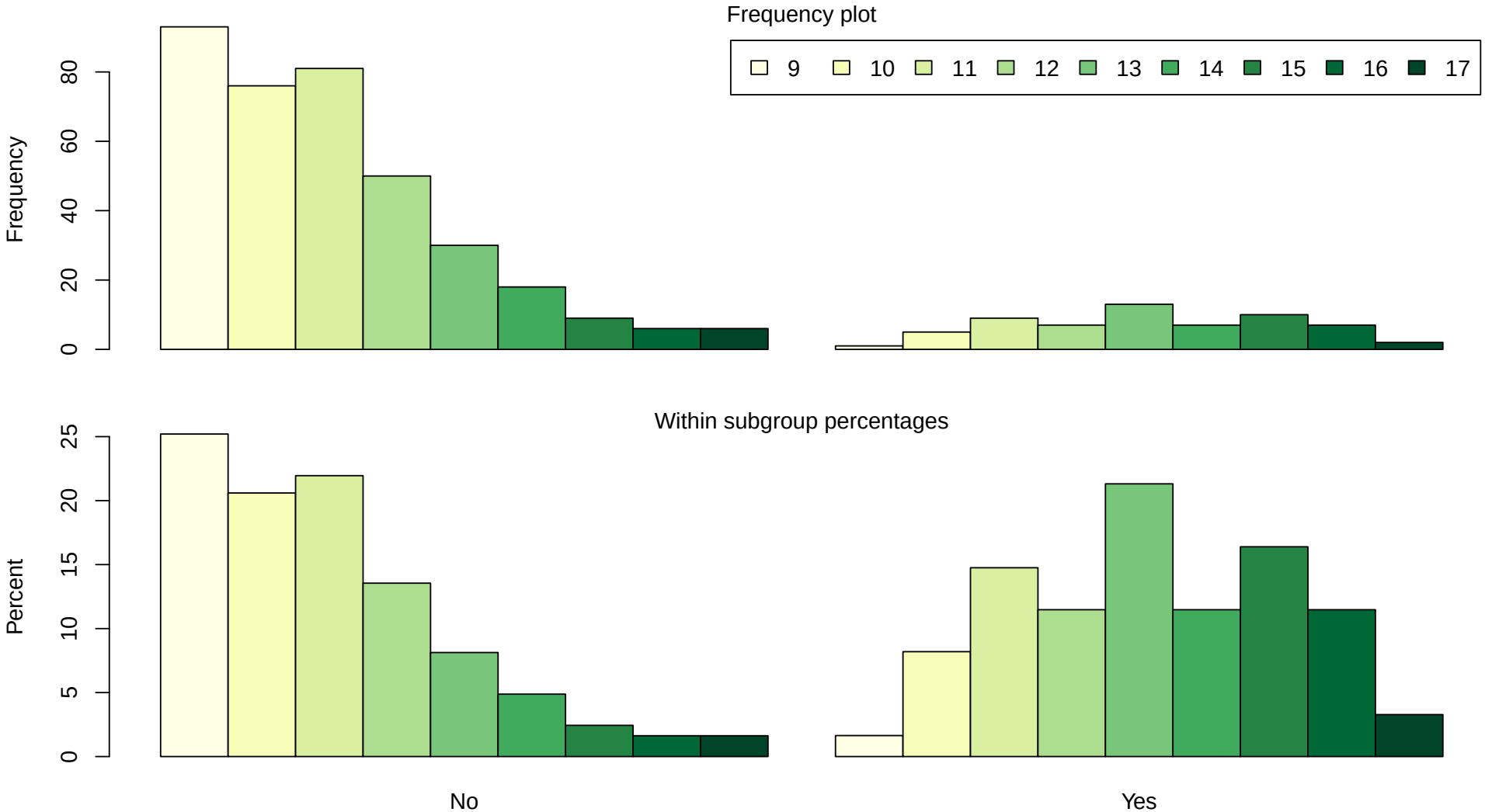
A barchart is a way to visualize a variable with a small number of unique values (often categorical). They are visual analogous of tables.

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

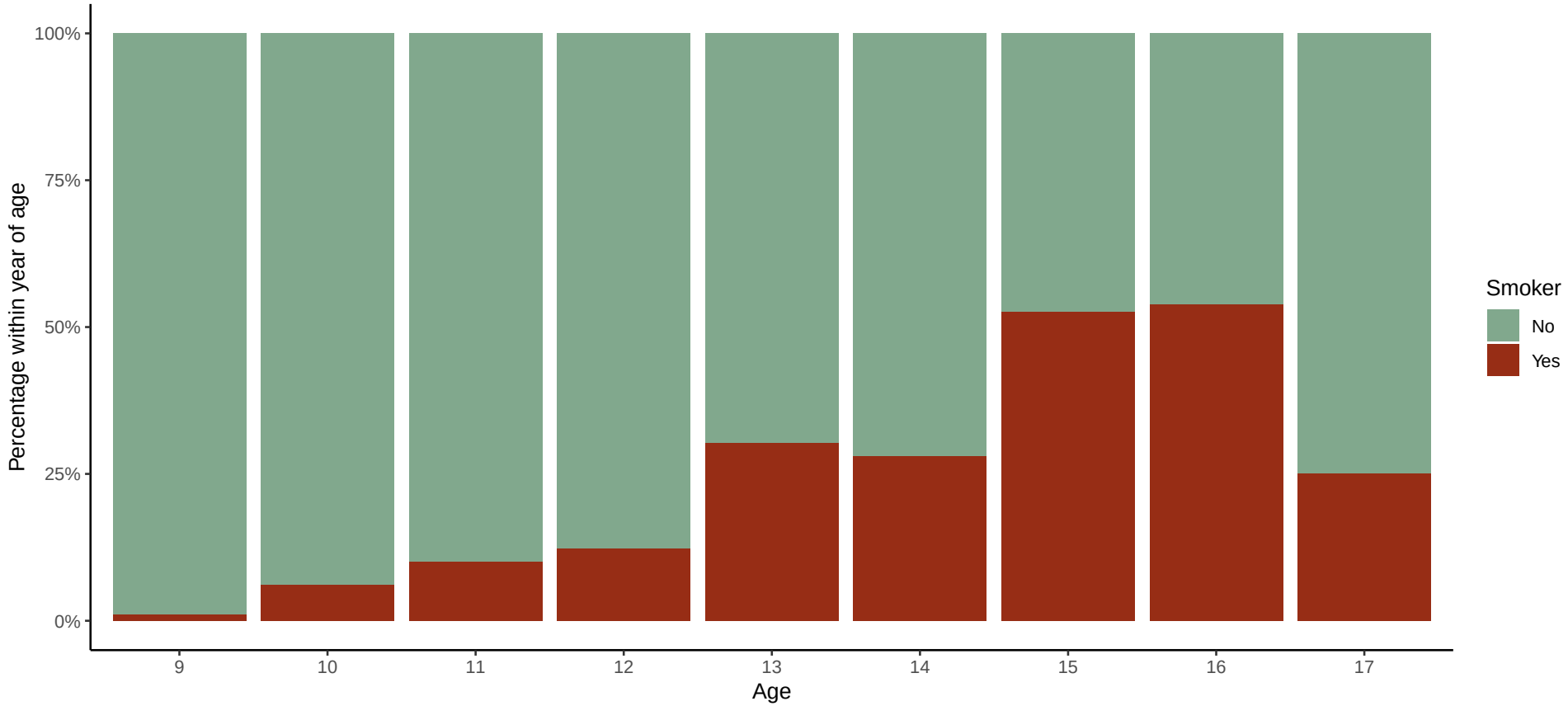
Visualizing 'age' versus 'smoking'

Absolute frequencies can be hard to compare if groups are unbalanced - use subgroup percentages instead.



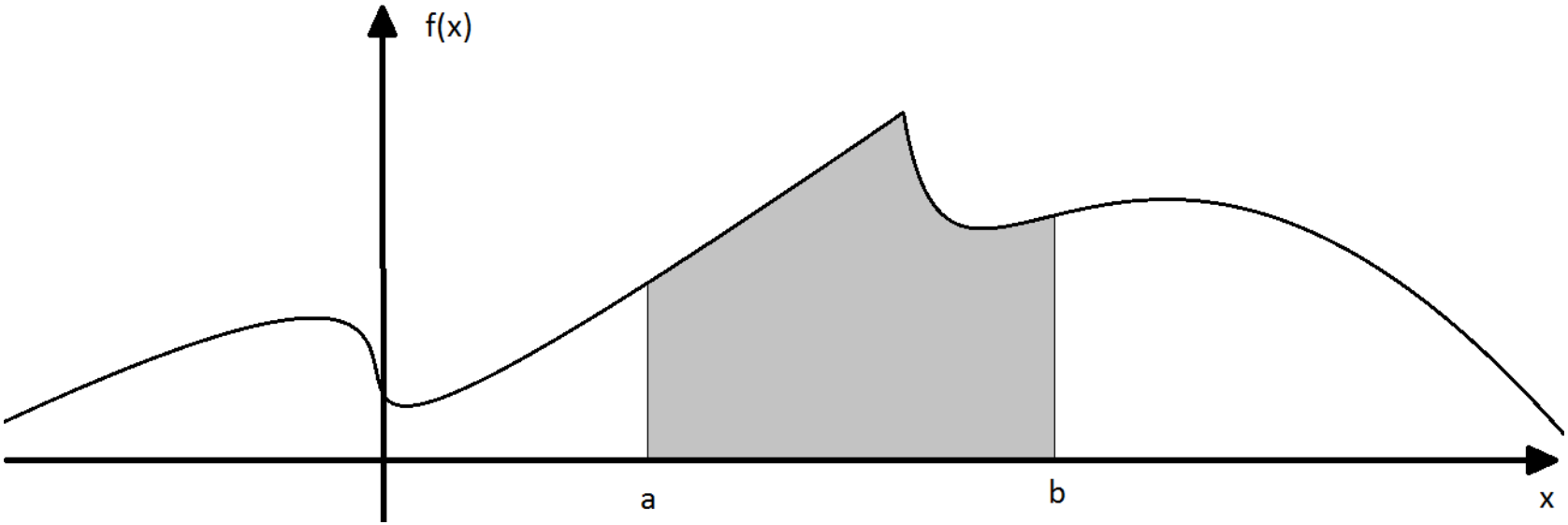
Flipping the axes

The "right" visualization might not be obvious. A binary categorical variable can be suitable to *stack* in a bar chart:



Probability model for continuous data

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

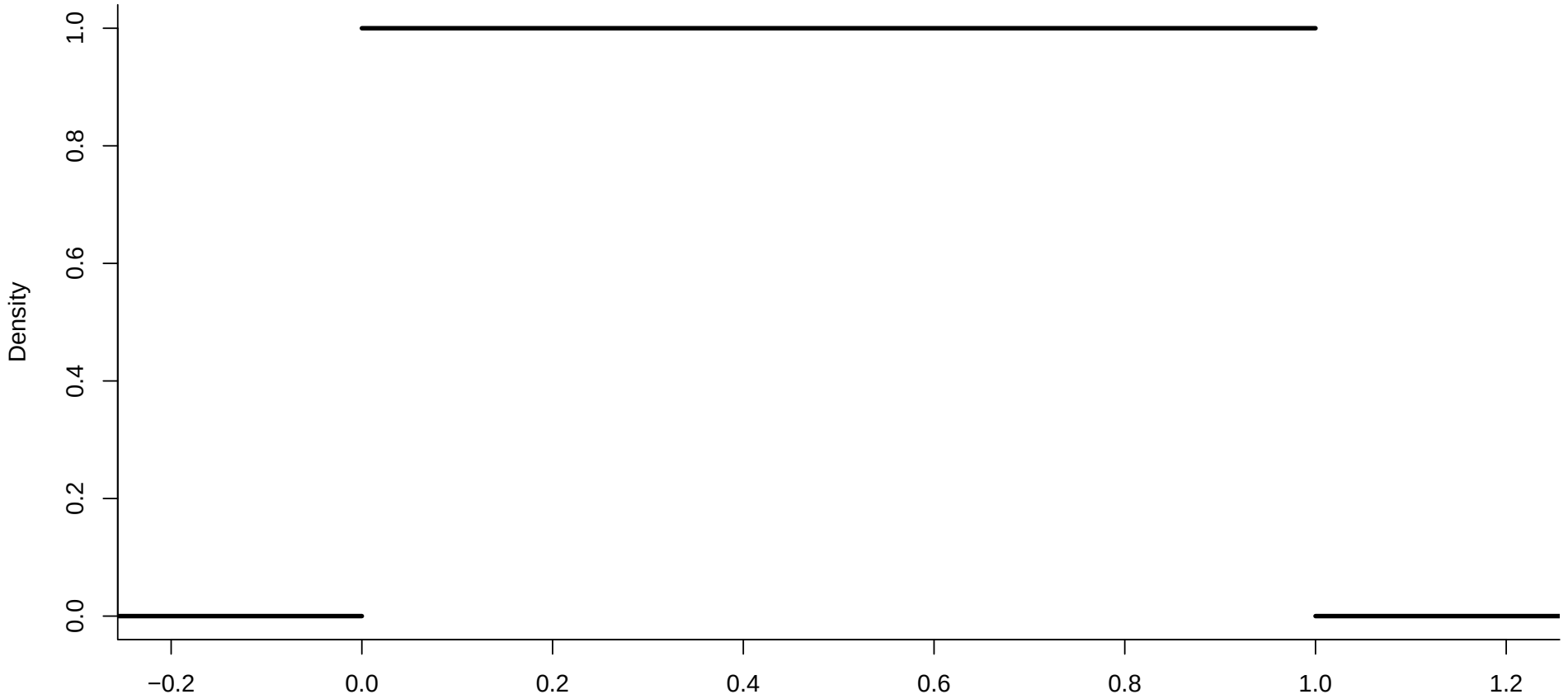


If X has density function f as above, then we compute probabilities as

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

Example: The Uniform distribution

A computer generated random number typically tries to mimic the *uniform distribution* (on the $[0, 1]$ interval).



Any number (or interval) within $[0, 1]$ is as likely as any other (of the same length).

Making a histogram

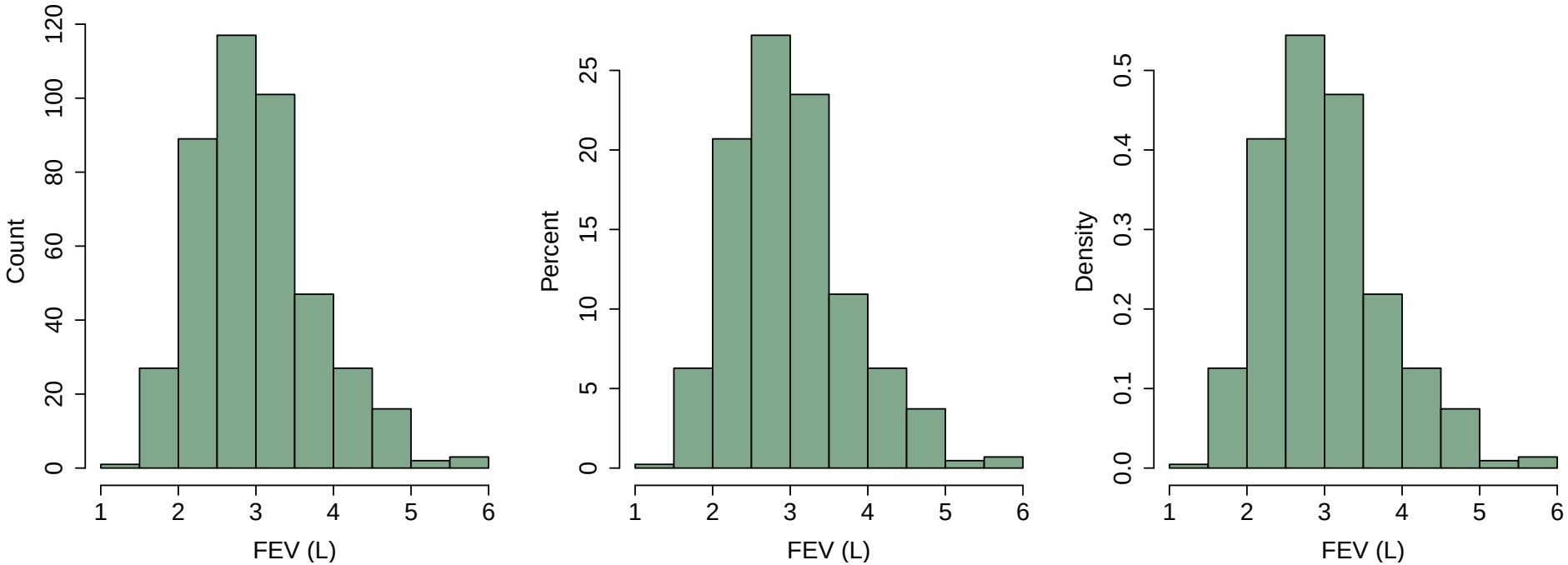
A histogram is a categorization of the x-axis into "bins", typically as intervals of the same range, and a statistic associated with each.

The FEV dataset has 430 (numercial) FEV-measurements between 1 and 6.

Data underlying a histogram:

Interval	Count	Proportion	Density
$1.0 - 1.5$	1	$\frac{1}{430} \approx 0.0023$	$\frac{1/430}{1.5-1.0} \approx 0.0047$
$1.5 - 2.0$	27	0.063	0.13
$2.0 - 2.5$	89	0.21	0.41
$\vdots$	$\vdots$	$\vdots$	$\vdots$
$5.5 - 6.0$	3	0.0070	0.014

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



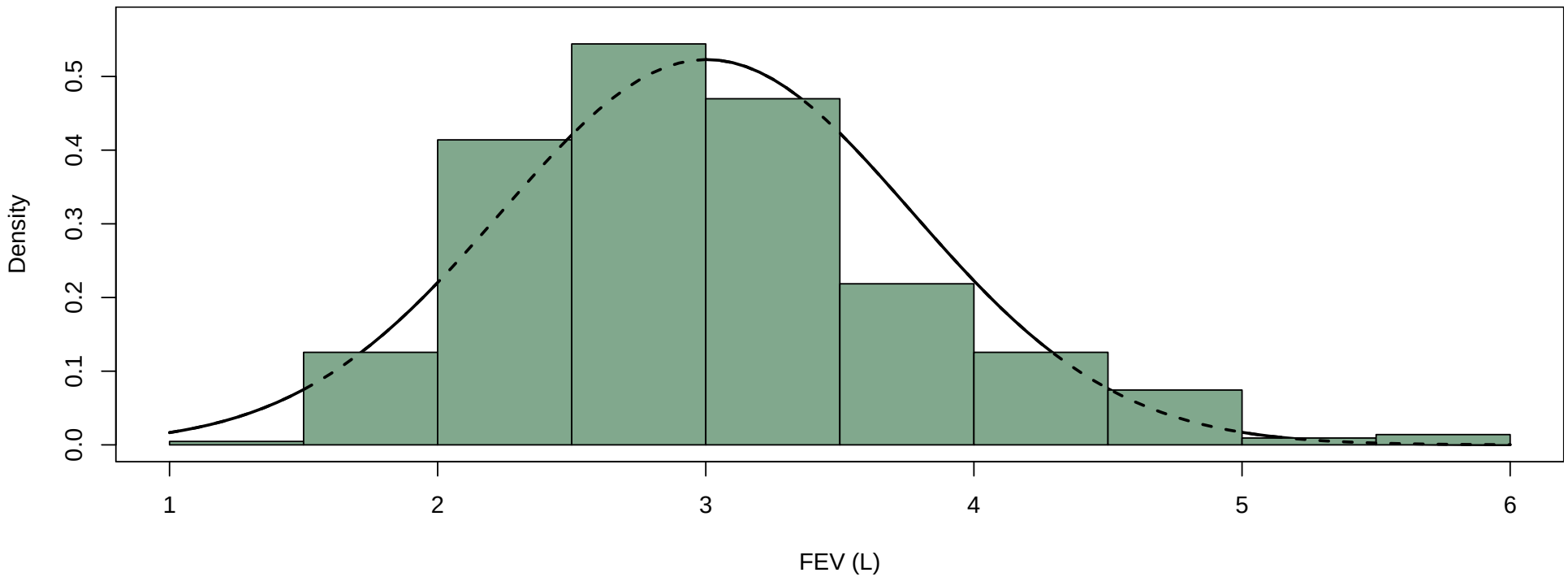
"Density" is more abstract but

- 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

The Normal Distribution

Sometimes we assume that data follows a Normal density curve.

(**Beware!** In many models/tests it is *not* the data in front of you which is assumed normal. More on that later in the course.)



The Normal Distribution

The Normal, Gauss, or Bell, curve is centered at (the mean) μ with a standard devation of σ, according to the equation:

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\mu)^2}{2\sigma}}.$$

The special case of $\mu = 0$ and $\sigma = 1$ is called a *standard normal distribution*.

Note: Any sample can be "standardized" by subtracting the mean and dividing by the standard deviation.

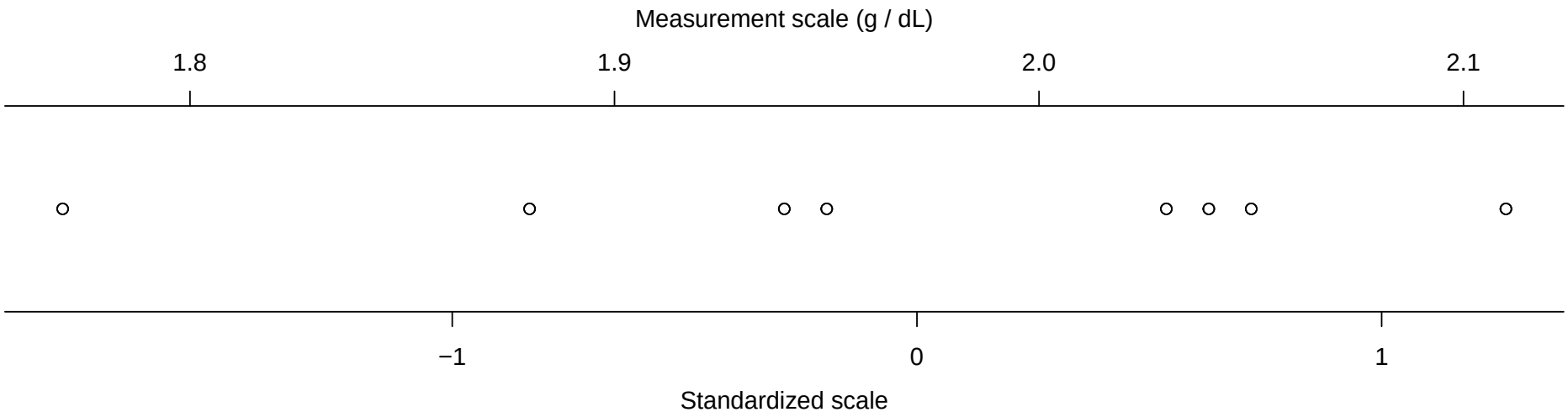
Digression on standardization:

If $x_1, x_2, \ldots, x_n$ is a sample (of size n) with mean $\bar{x} = \frac{1}{n}\sum_1^n x_i$ and $sd = \sqrt{\frac{1}{n-1}\sum_1^n (x_i - \bar{x})^2}$, then the transformation

$$\frac{x_1 - \bar{x}}{sd}, \frac{x_2 - \bar{x}}{sd}, \ldots, \frac{x_n - \bar{x}}{sd},$$

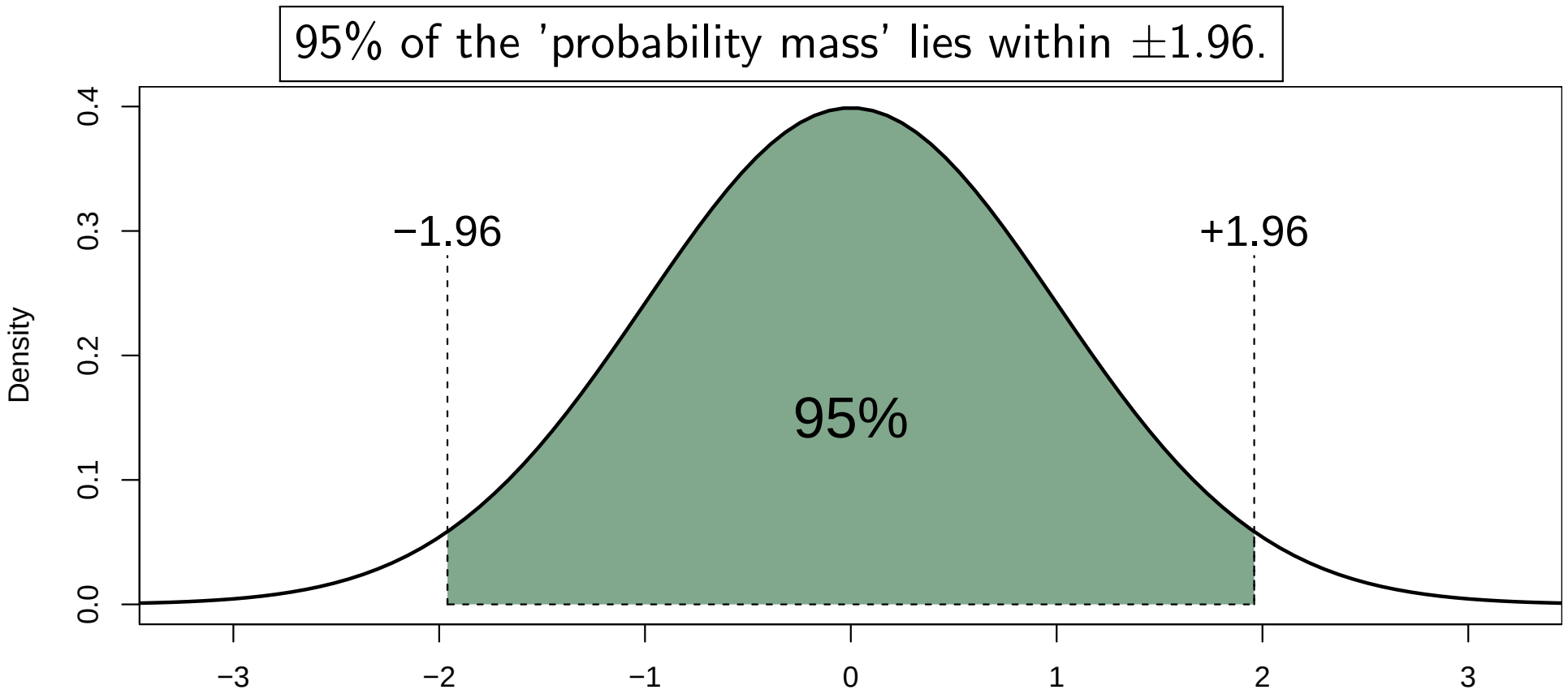
is *standardized* (it has mean 0 and sd 1).

Here is the albumin measurements again; orginal and standardized:



Properties of the standard Normal distribution

The standard Normal distribution is centered at 0 and has a standard deviation of 1.



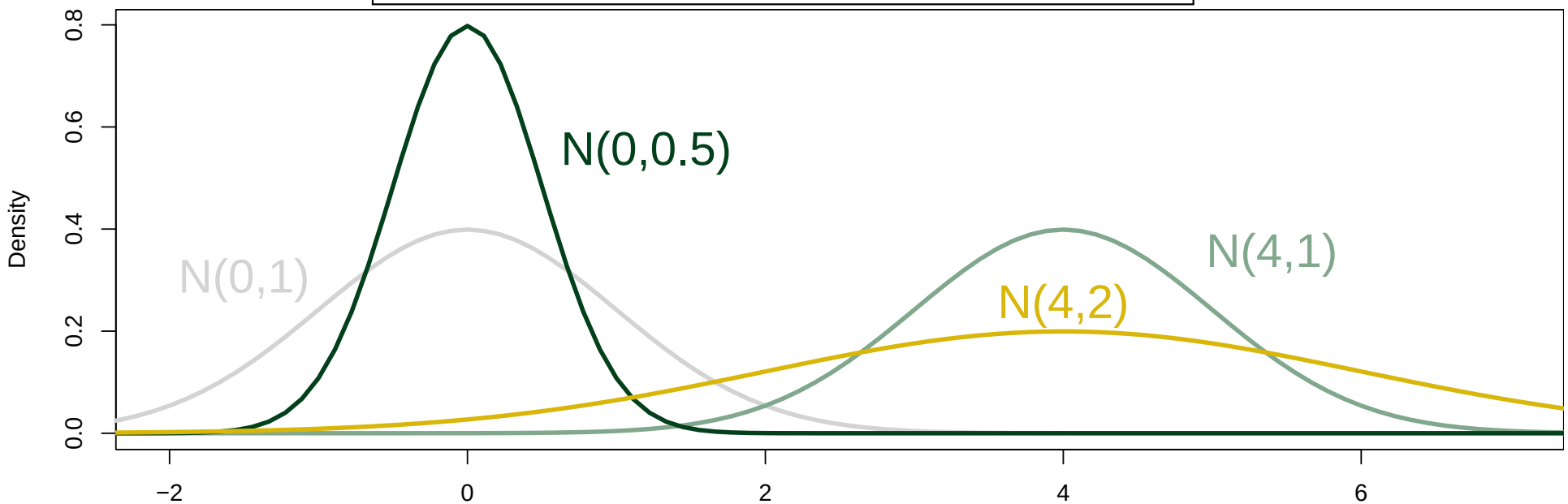
This will be useful when creating *confidence intervals*.

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

is determined by its mean (μ) and standard deviation (σ).

If Y is N(μ, σ) then $(Y - \mu)/\sigma$ is N$(0, 1)$.

If X is N(0,1) then $\mu + \sigma X$ is N(μ, σ).



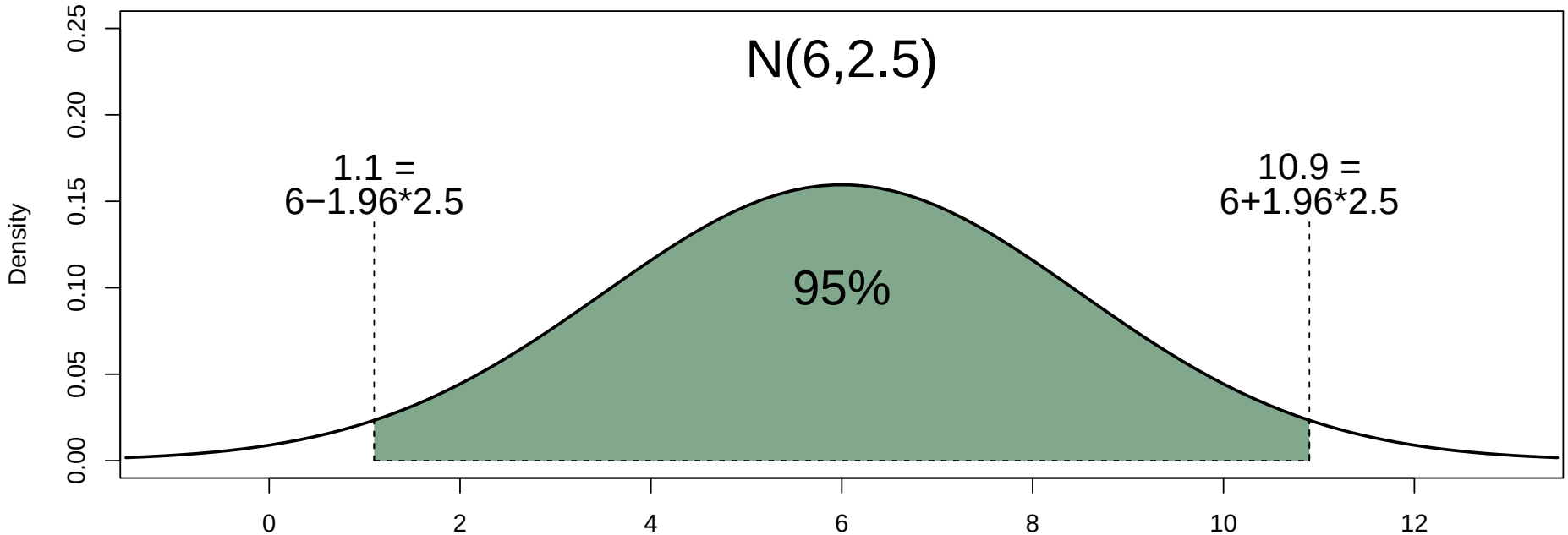
In fact, if X_1 and X_2 are Normal (and *independent*), then so is

$$a + bX_1 + cX_2.$$

Properties of the Normal distribution

If X is N(μ, σ), then 95% of observations will be between

$$\mu - 1.96\sigma \text{ and } \mu + 1.96\sigma.$$



Estimators

Suppose you're trying to estimate μ the average height of a population and you have a random sample of the heights of 10 individuals available; $x_1, x_2, \ldots, x_{10}$.

Consider the following strategies for estimating μ:

- A: By the largest value; $\max x_i$
- B: By the first value; x_1
- C: By the mean value; $\bar{x} = \frac{1}{10} \sum x_i$

Two leading questions:

- What's bad about A?
- Why is C better than B?

Unbiased estimators

An estimator is unbiased if it is right on average (as a strategy / as a random variable), i.e. there is no systematic error. Strategy A is biased.

Strategies B and C are both unbiased, but C has a smaller standard deviation (varies less). Why? Intuitively, some of the x_i's are going to be "large" and some are going to be "small", and the average value will cancel some of these.

For an unbiased estimator, the standard deviation (called the *standard error*) is the "typical" error, i.e. a measure of *precision*.

If we also knew the distribution of the estimator, we could calculate things like confidence intervals.

Next up: *How does the distribution of the mean value depend on the distribution of the population?*

Estimator distribution: 2 special cases

Sometimes our model assumptions gives us the answer.

1. If the population is normally distributed $N(\mu, \sigma)$
The mean value is a linear combination and so *by theory* (details skipped) the mean value has the distribution:

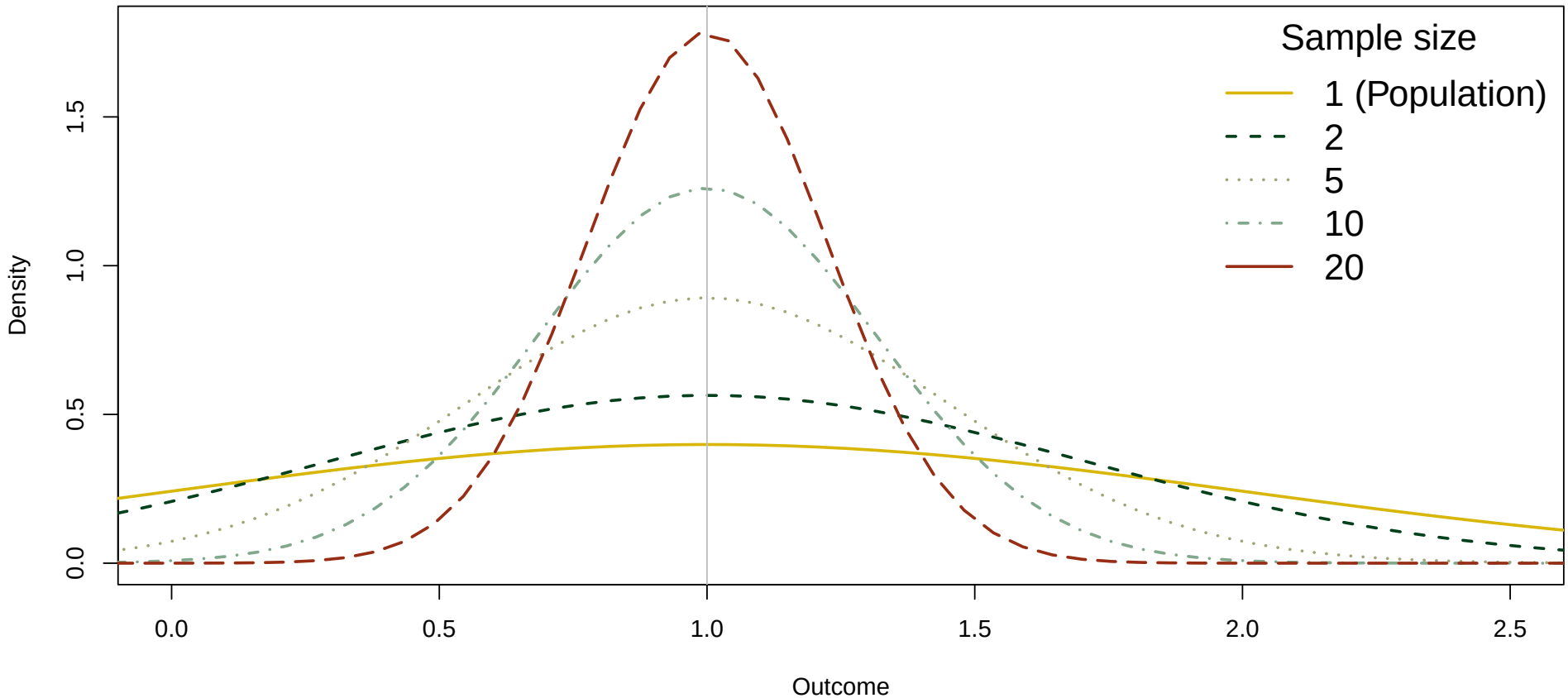
$$N\left(\mu, \frac{\sigma}{\sqrt{n}}\right),$$

where n is the sample size.

2. If the population has a gamma-distribution (skewed).
Details are unimportant, the point is that the mean value distribution *can* be calculated (look 2 slides ahead).

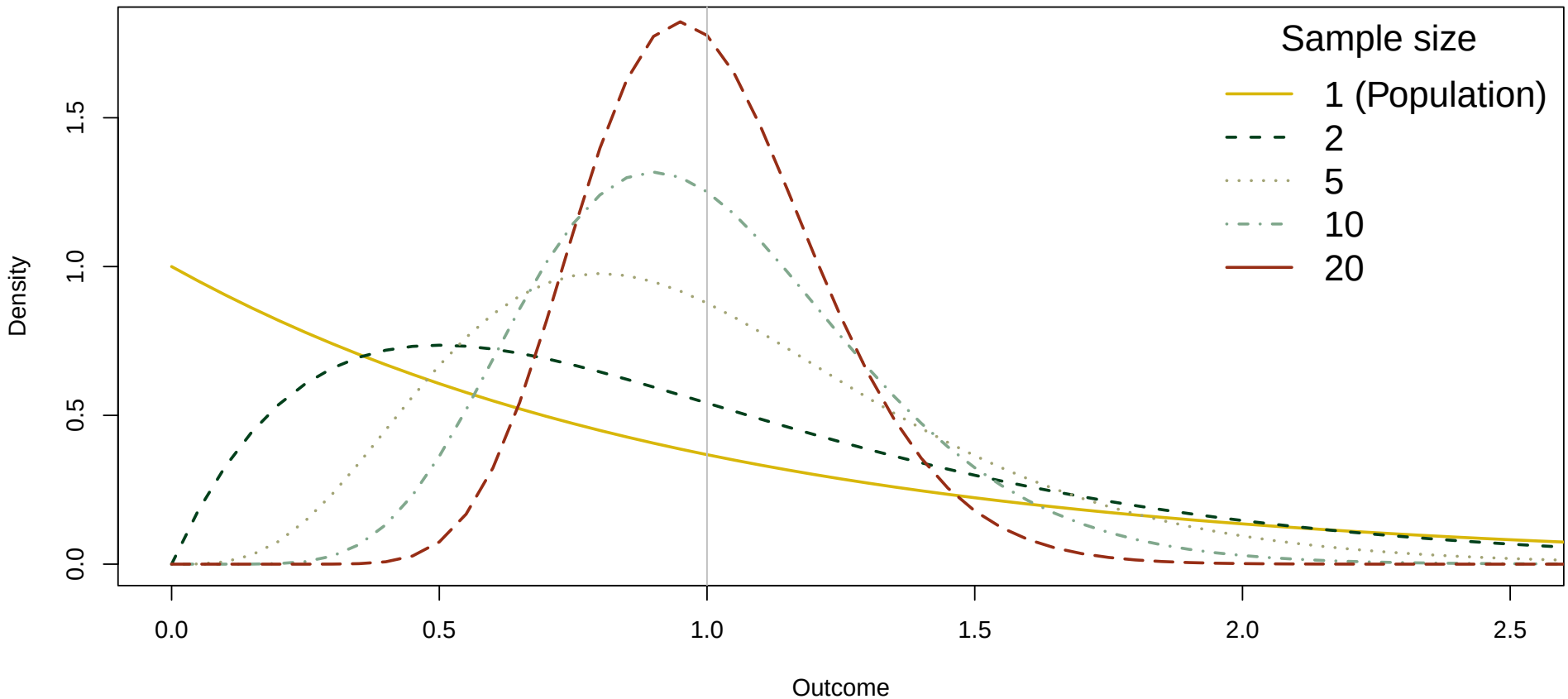
Sample mean distribution (normal population)

The distribution of sample means from a *normal* distribution. In this case the sample mean *is normally distributed* (but with a smaller sd).



Sample mean distribution (gamma population)

The distribution of sample means from a skewed gamma distribution. The sample mean distribution becomes increasingly symmetric.



The Central Limit Theorem (CLT)

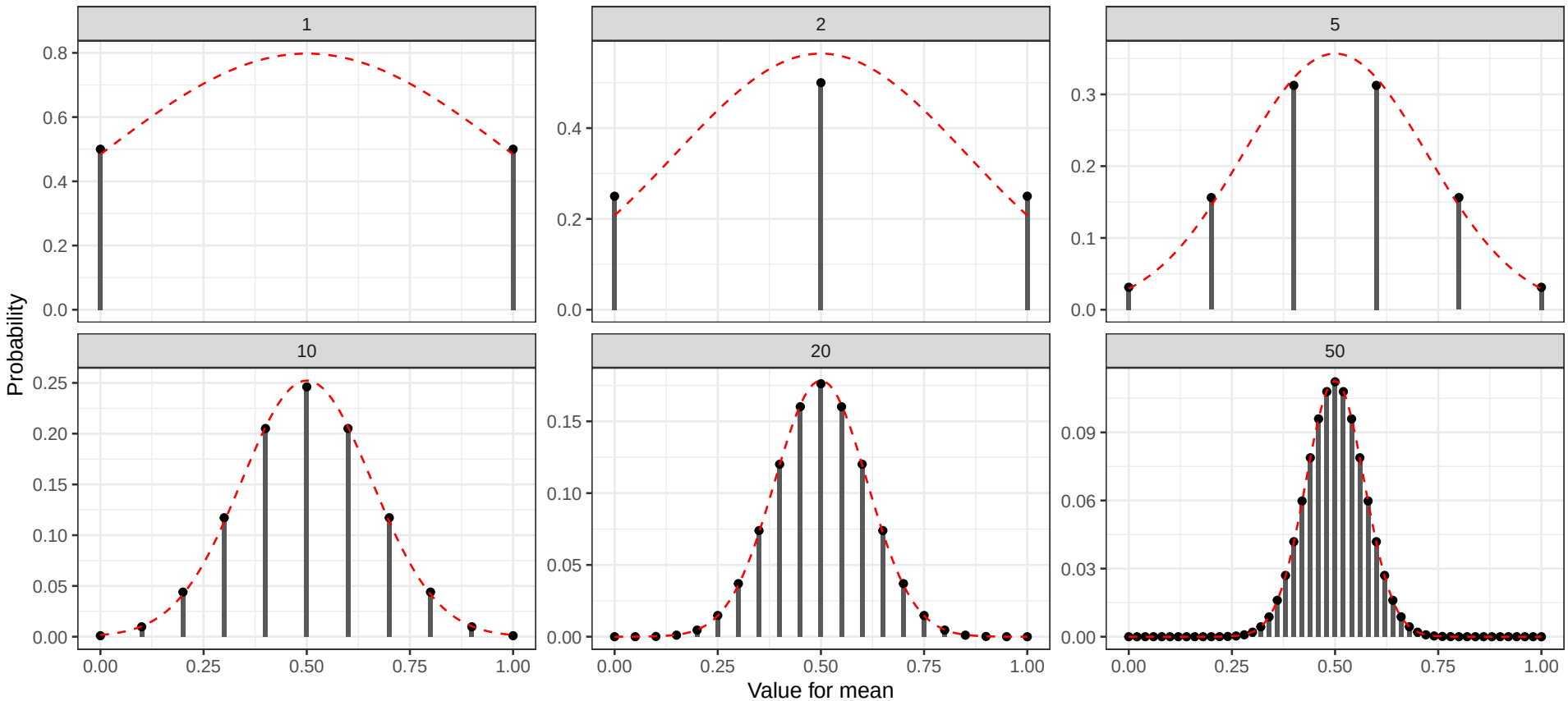
CLT: Regardless of the population density curve, the sample mean density can be made (with arbitrarily good approximation) Normal by choosing n large enough.

- 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).

This is great news! This means we (often) only need to calculate the standard error.

Sample mean distribution of coin flips

Even non-continuous (discrete) distributions can be approximated with the normal distribution. (Normal density curve rescaled by $1/n$.)



Visual tests of normality: Cross-over data

13 patients had their peak expiratory flow (PEF, l/min) recorded 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.

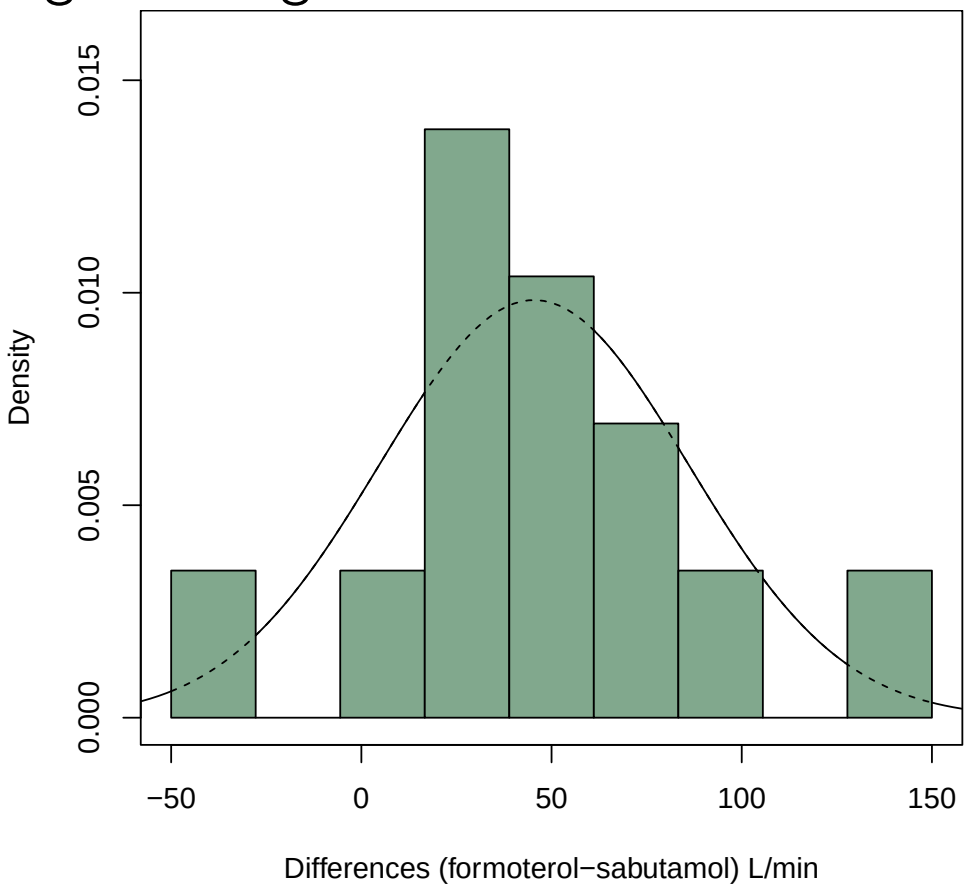
Data:

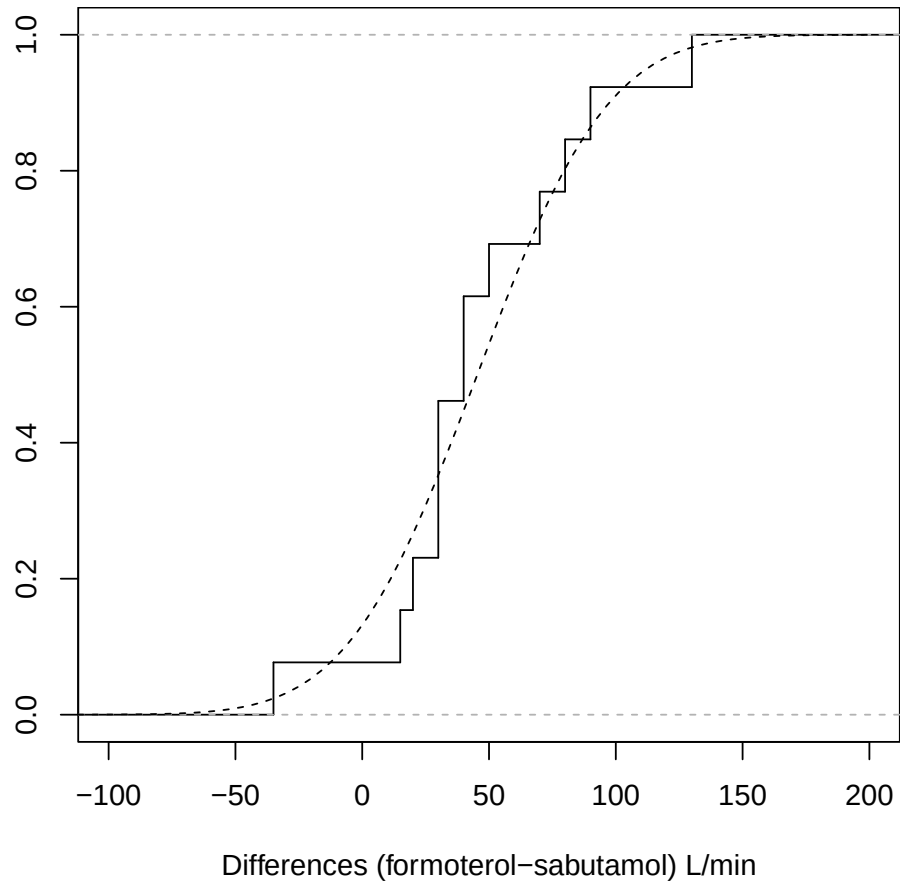
40, 50, 70, 20, 40, 30, -35, 15, 90, 30, 30, 80, 130

Is the normal distribution a good model for these 13 numbers?
Perhaps surprisingly, quite often we rely on *visual* rather than *formal* tests of model assumptions.

PEV

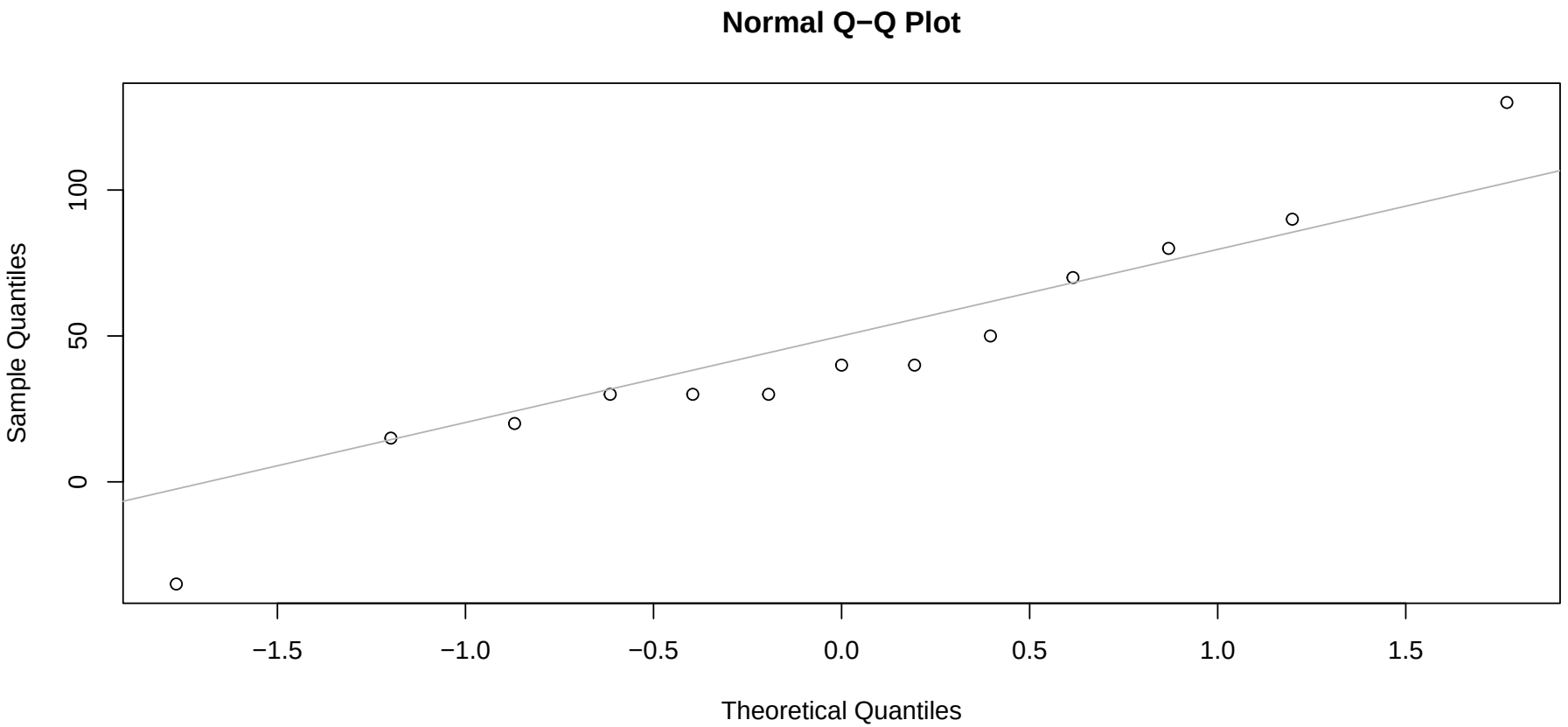
One could ascertain the plausibility of an underlying normal distribution via e.g. a histogram or a CF.



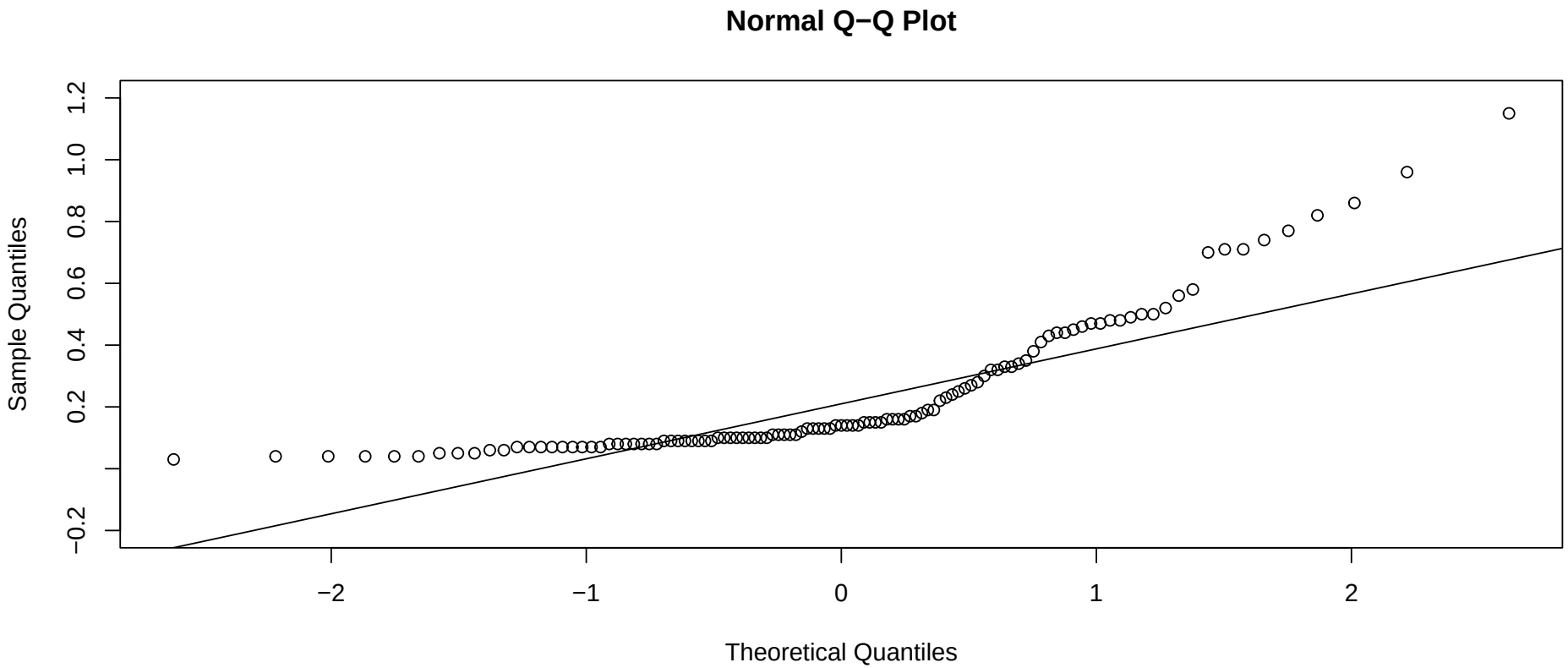


The Quantile-Quantile plot

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 S100B measurements is certainly not normally distributed.



Caution

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

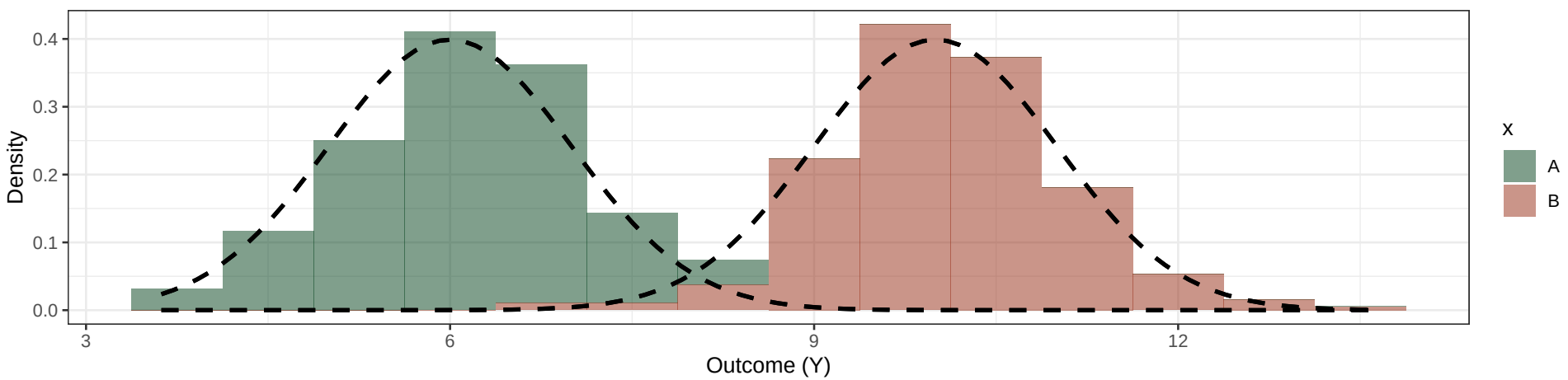
Most of the time the models that assume normality does so for the *error terms*, i.e. there is a model, depending on the covariates x, for the outcome Y such that

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

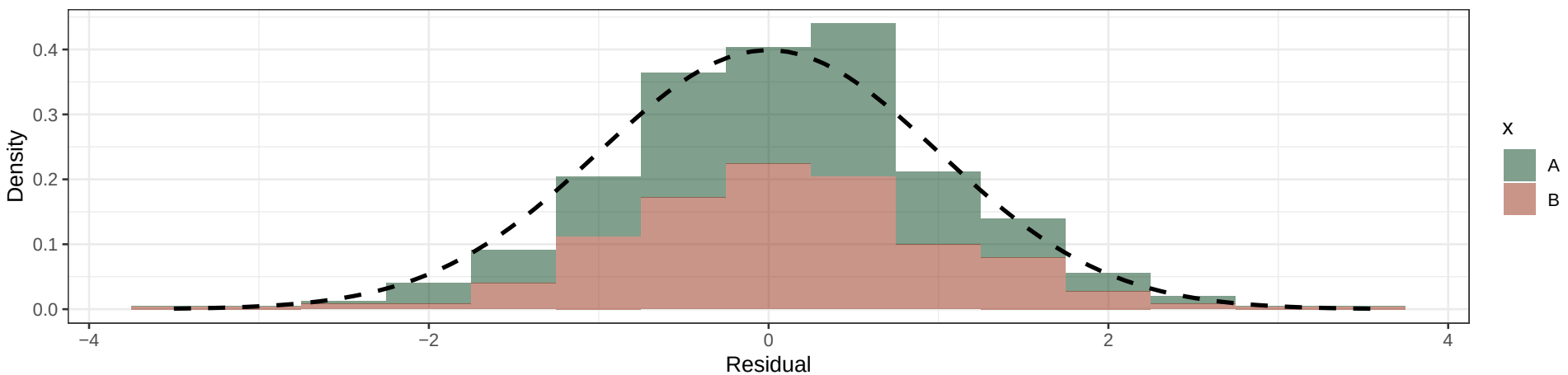
E.g. a 2-sample t-test assumes that an outcome is normally distributed around a group-specific mean. Data for such a test might look like this

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...

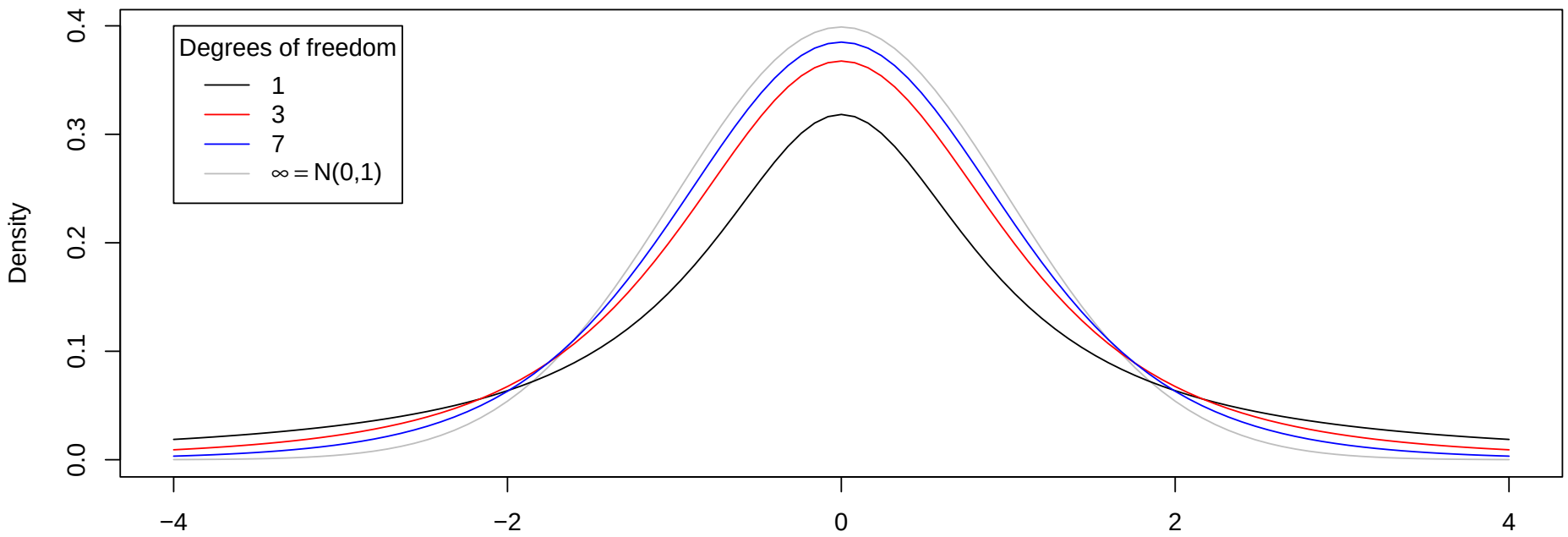


It is the deviations (noise/error term) around each group-specific mean that is supposed to be normal, an estimation of which is called *residuals*.
Subtract the (estimated) group effect from each datapoint to get:



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 standard 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.