

# Lecture 4 – Review of Probability and Statistics

STAT/BIOF/GSAT 540: Statistical Methods for High Dimensional Biology

Keegan Korthauer

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Slides by: Sara Mostafavi and Keegan Korthauer

# Preview of next 6 lectures

- **2020/01/15 - Lecture 4: Stats Philosophy, Math/stat background & review**
- 2020/01/20 - Lecture 5: Statistical Inference - two group comparisons
- 2020/01/22 - Lecture 6: Statistical Inference - linear regression and ANOVA
- 2020/01/27 - Lecture 7: Statistical Inference - linear models (more than two groups, and interaction testing)
- 2020/01/29 - Lecture 8: Statistical Inference - continuous model + limma
- 2020/02/03 - Lecture 9: Statistical Inference - multiple testing

# Outline for today

- Intro: Philosophy, goals, and central concepts
- Review: Random Variables, Probability Distributions, Sampling Distribution, Estimation, Inference, CLT, Hypothesis Testing

Your goals:

1. be familiar with the terminology
2. have a clear understanding of the concepts

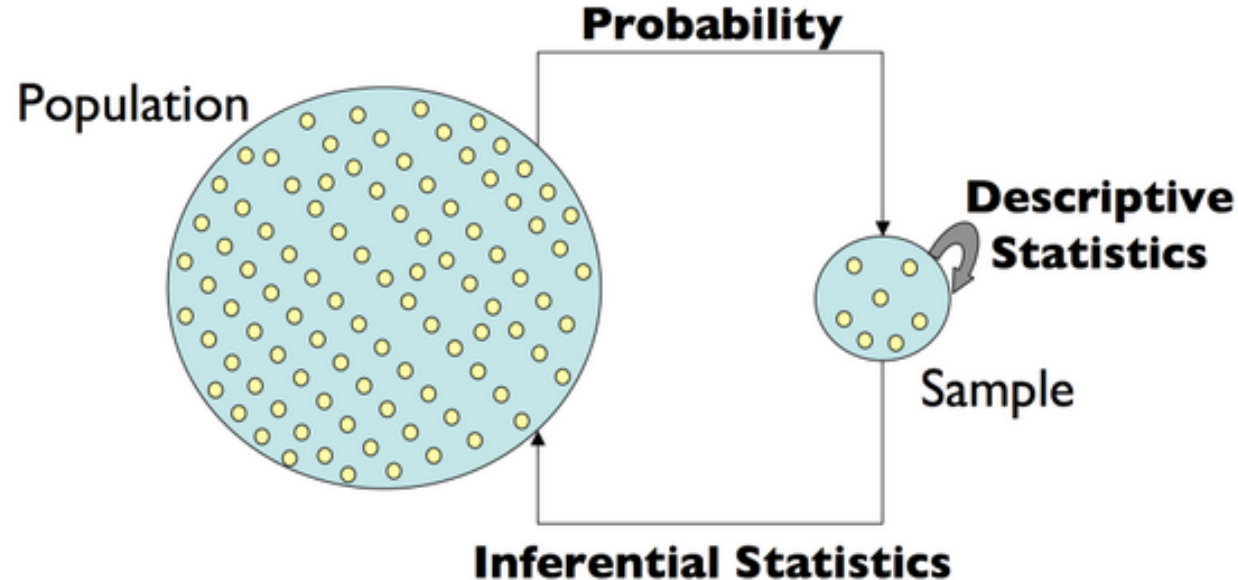
# What is Statistics?

# Statistics

- The field of statistics concerns the science of **collecting, analyzing/modeling, interpreting** data and **communicating uncertainty** about the results
  - Data science and machine learning have enabled application to 'big data'
- Statistical and computational methods should not be used as generic "recipes" to follow → non-robust science
- We aim for:
  - rigorous understanding to perform routine statistical analysis
  - solid foundation to follow up on specific topics

# Statistical Inference

A framework for generating conclusions about a population from noisy data from a sample



- Language of **probability** enables us to make *predictions* and discuss *uncertainty*
- **Statistical inference** enables us to *understand* the data and make *conclusions*
- We need both to learn from data

# Review: terminology & basic concepts

- Random variables and their distributions
- Models, parameters, and their estimators
- Central Limit Theorem (CLT)
- Hypothesis Testing

# Variables

**Variable (*noun*):** an element, feature, or factor that is liable to vary or change

- In statistical terminology, a **variable** is an unknown quantity that we'd like to study
- Most research questions can be formulated as
  - | What's the *relationship* between two or more variables?



# Random variables

**Random Variable (RV):** A variable whose value results from the measurement of a quantity that is subject to variation (e.g. the *outcome* an experiment)

- Examples: a coin flip, a dice throw, the expression level of gene X
- An RV has a *probability distribution*

# Distributions of RVs




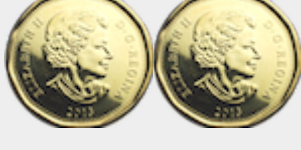
**Probability:** A number assigned to an outcome/event that describes the extent to which it is likely to occur

- Must satisfy certain rules (e.g. be between 0 and 1)
- Represents the (long-term) *frequency* of an event

**Probability distribution:** A mathematical function that maps outcomes/events to probabilities





# Example experiment: Two coin tosses

- **Experiment:** Toss two coins
- **Sample space:** set of all possible outcomes  $S = \{TT, TH, HT, HH\}$
- **Random Variable of interest:** number of heads

	Outcome	Number of Heads
TT		0
HT		1
TH		1
HH		2




# Assigning probability to outcomes

- Let:
  - $\omega$  = an outcome
  - $X(\omega)$  = number of heads in  $\omega$
- Each possible outcome is associated with a probability
- **Event:** A set of outcomes that satisfy some condition
- Each realization of the RV corresponds to an **event** (e.g.  $X(\omega) = 1$  corresponds to the outcomes  $TH$  and  $HT$  )

	$\omega$	$X(\omega)$	Probability
TT		0	0.25
HT		1	0.25
TH		1	0.25
HH		2	0.25

# Assigning probability to events

The probability distribution of the Random Variable  $X$  tells us how likely each event (number of heads) is to occur in the experiment

Event	$x$	$P(X = x)$
	0	0.25
	1	0.50
	2	0.25

Note on notation:  $P(X = x)$  can also be written as  $P_X(x)$

# Two types of random variables

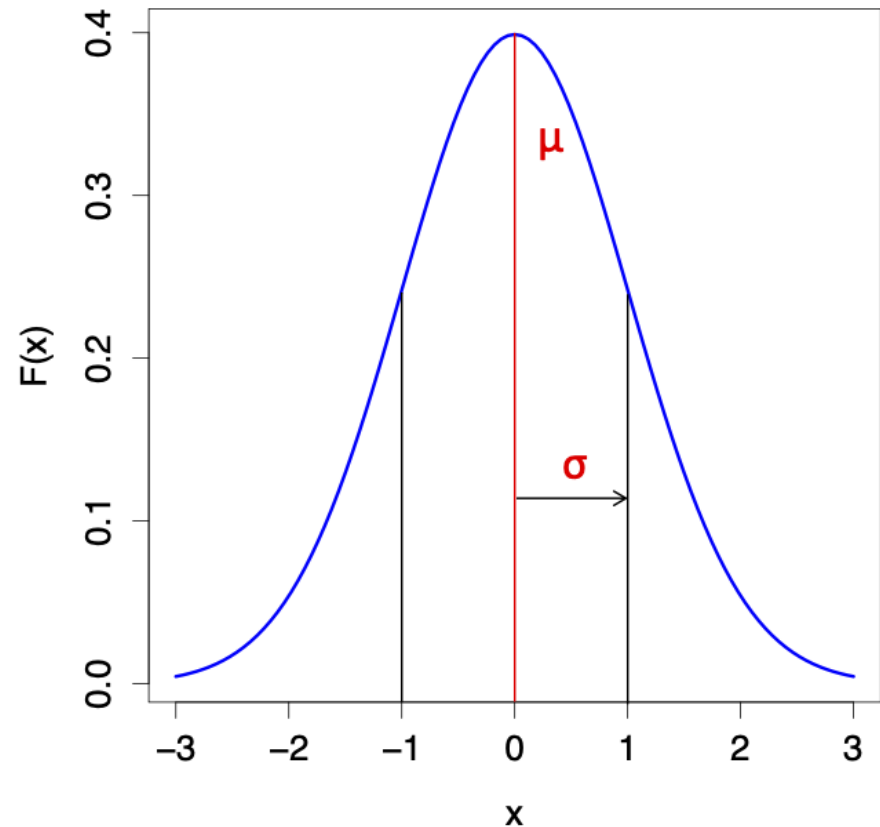
- A **discrete** RV has a countable number of possible values
  - e.g. throwing dice, genotype measured on a SNP chip
- A **continuous** RV takes on values in an interval of numbers
  - e.g. expression level of a gene, blood glucose level, height of individuals

# Standard Gaussian (Normal) distribution

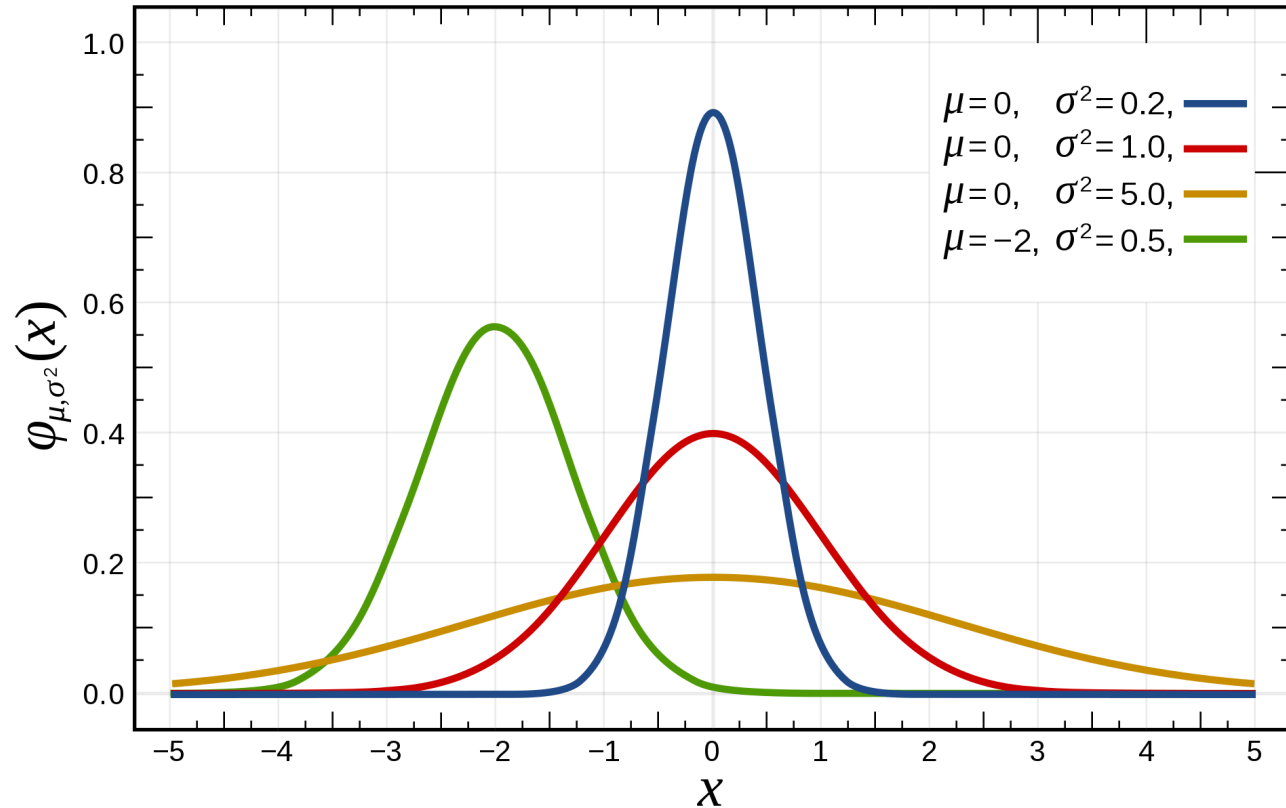
- probability density function:

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

- Mean =  $\mu$
- Standard Deviation =  $\sigma$
- For convenience, we write  $N(\mu, \sigma^2)$
- When  $\mu = 0$  and  $\sigma = 1$ , this is the *Standard Normal* distribution  $N(0, 1)$



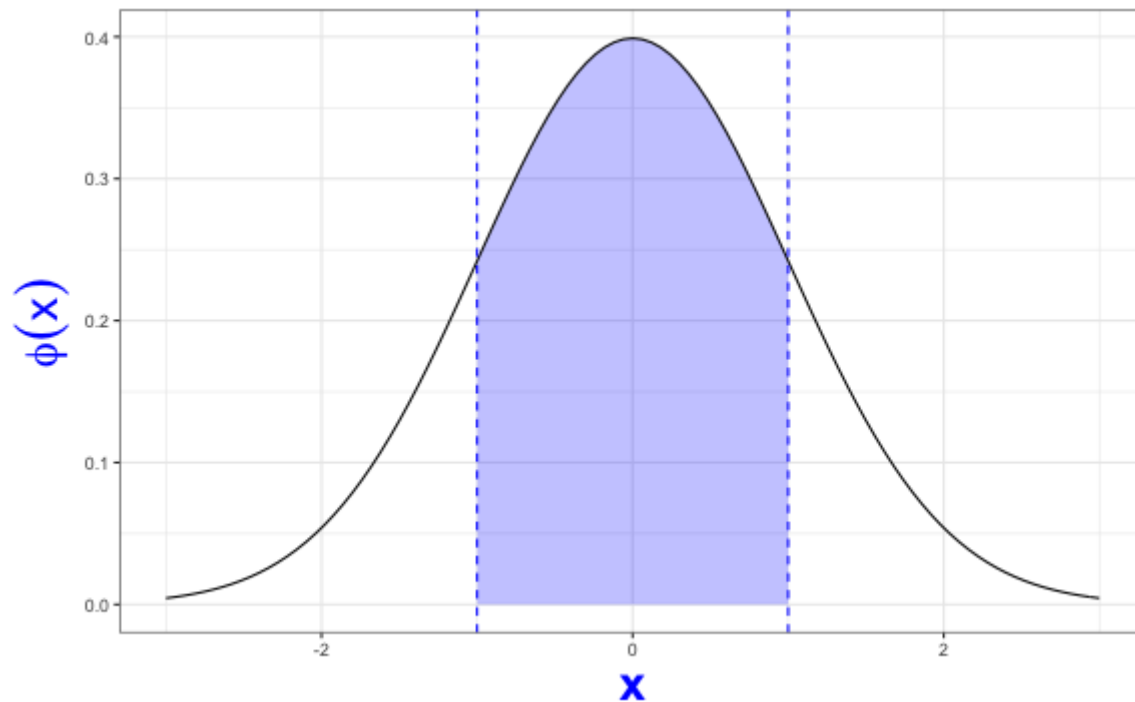
# Gaussian (Normal) distribution



Probability density function:  $f(x|\mu, \sigma^2) = \phi(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-\mu)^2}{2\sigma^2}}$



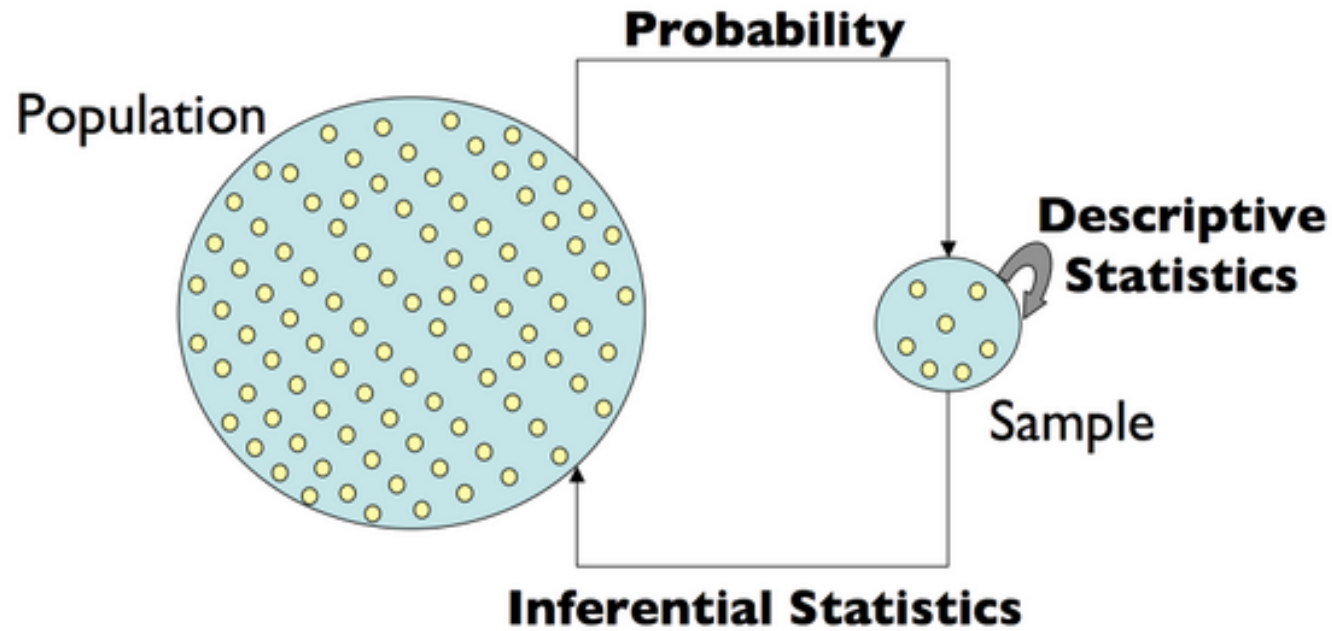
Density  $\rightarrow$  probability requires integration



# Statistical Inference

- The **parameter space** is the set of all possible values for the parameter
- One major goal: to "figure out" (i.e. estimate) the **parameter values**
  - i.e. *"fit the model to the data"*
- The model is a representation that (we hope) approximates the data and (more importantly) the population that the data were sampled from
- We can then use this model for:
  - hypothesis testing
  - prediction
  - simulation

# Statistical Inference



# IID

- A requirement (assumption) in many settings is that the data are IID: **I**ndependent and **I**dentically **D**istributed
- **Identically Distributed**: a set of observations (events) are from the same population (i.e. they have the same underlying probability distribution)
  - e.g. a t-test assumes that under the null, all observations come from the same normal distribution
- **Independent**: all samples satisfy the condition

$$P(A, B) = P(A)P(B)$$

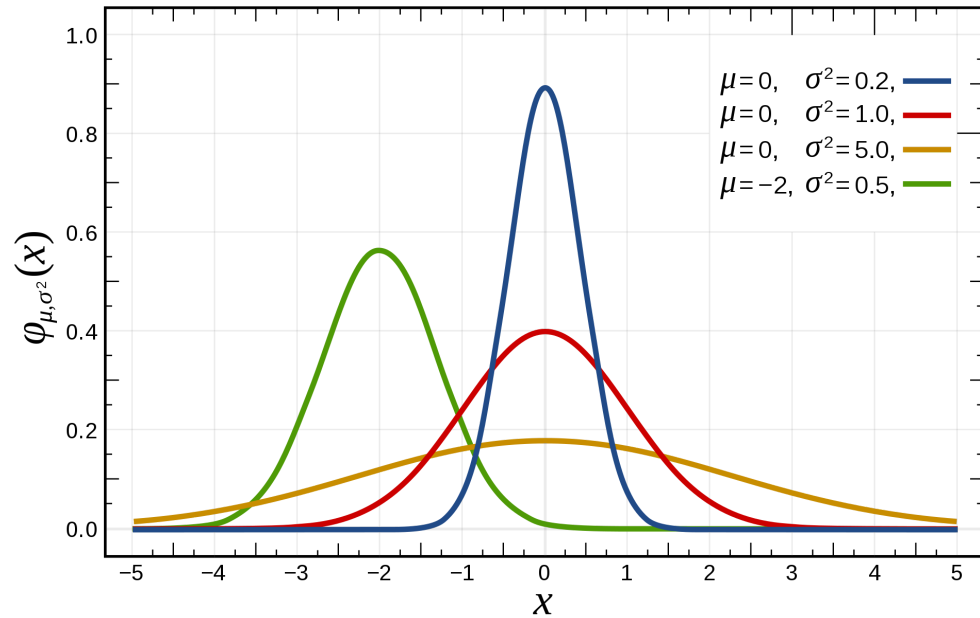
where  $A$  and  $B$  are events

- i.e. the joint probability is the product of the individual event probabilities
- The above statement is for two events, but the same definition applies for any number of events (without loss of generality for any number of events)

# Violations of independence

- Experimental design is in part about trying to avoid unwanted dependence
- Example of design with violation of independence assumption:
  - | Height measurements of individuals sampled from *related* females in a tall family

# Parameters of the normal distribution



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

- Mean =  $\mu$
- Standard Deviation =  $\sigma$
- For convenience, we write  $N(\mu, \sigma^2)$
- Population parameters are unknown

# Parameter estimation

- **Estimator:** A function (or rule) used to estimate a parameter of interest
- **Estimate:** A particular realization (value) of an estimator

# Estimators for normally distributed data

- If we are given a sample of  $n$  observations from a normally distributed population, how do we estimate the parameter values  $\mu$  and  $\sigma$ ?
- Recall  $\mu$  is the mean and  $\sigma$  the standard deviation of the distribution

$$\hat{\mu} = \bar{x} = \frac{x_1 + x_2 + \dots + x_n}{n} = \frac{1}{n} \sum_{i=1}^n x_i$$

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



# Estimators vs Parameters

	Estimators	Parameters
Summarize	Sample	Population (ground truth)
Value	Computed from data	Unknown
Notation	$\hat{\theta}$	$\theta$

# Normal Mean: Estimator vs Parameter

	Estimator	Parameter
Summarizes	Sample/data	Population (ground truth)
Value	$\bar{x} = \frac{1}{n} \sum_{i=1}^n x_i$	Unknown
Notation	$\hat{\mu}$	$\mu$

# Normal Standard Deviation: Estimator vs Parameter

	Estimator	Parameter
Summarizes	Sample/data	Population (ground truth)
Value	$s = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$	Unknown
Notation	$\hat{\sigma}$	$\sigma$

# Estimator for normally distributed data

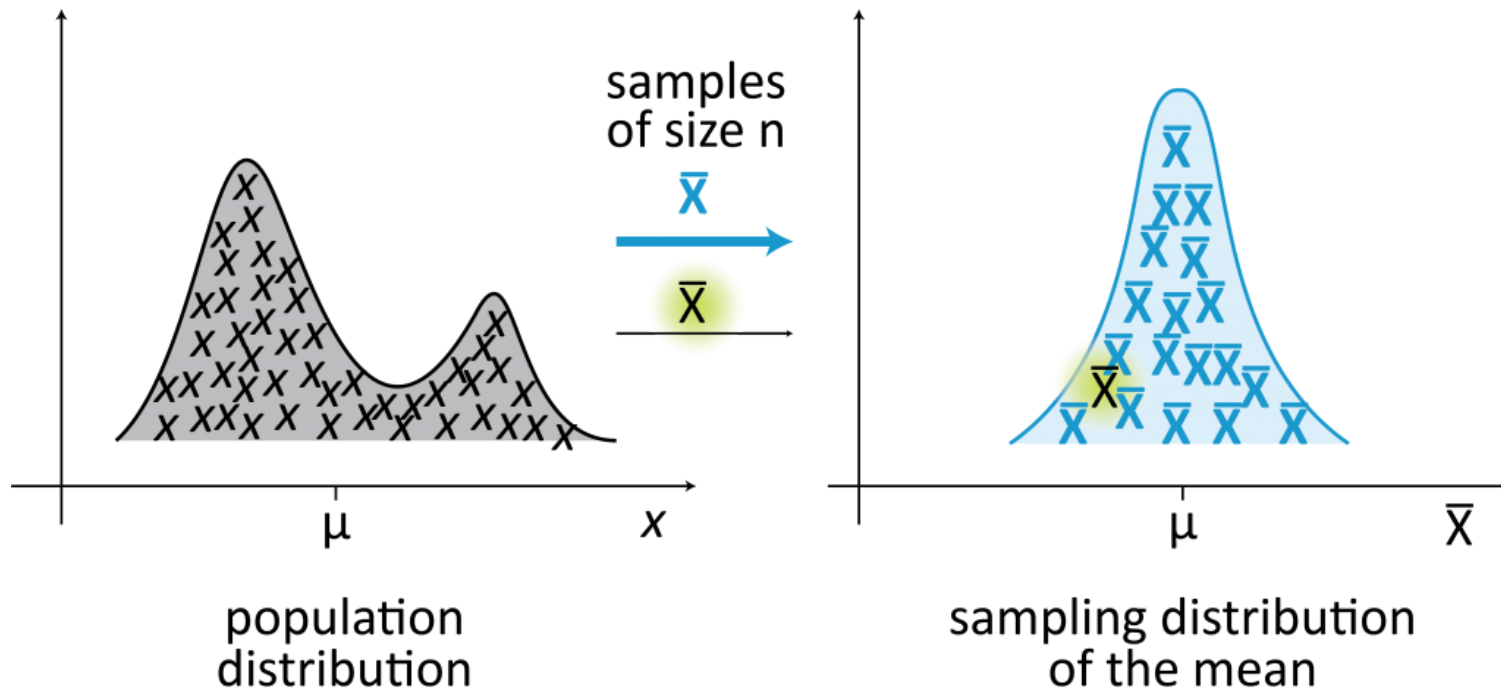
- Let's say we collected a **sample** from a population we assume to be normal
- We estimate the mean  $\hat{\mu} = \bar{x}$
- How good is the estimate?
- The answer depends on:
  - sample size
  - variability of the population

# Sampling distribution

- **Statistic:** any quantity computed from values in a sample
- Any function (or statistic) of a sample (data) is a random variable
- Thus, any statistic (because it is random) has a probability distribution function → specifically, we call this the *sampling distribution*
- Example: the sampling distribution of the mean

# Sampling distribution of the mean

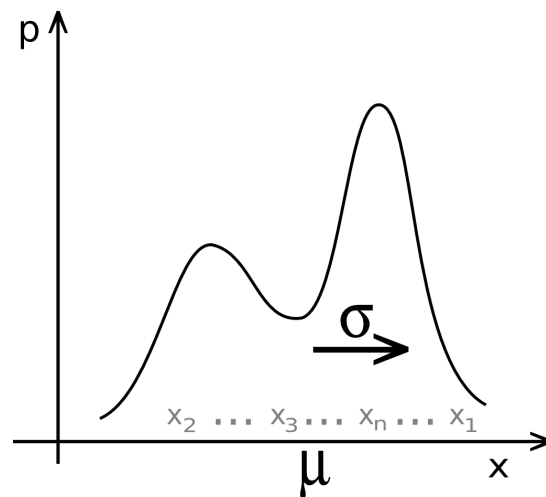
The sample mean  $\bar{x}$  is an RV, so it has associated probability or sampling distribution



# Central Limit Theorem (CLT)

By the *Central Limit Theorem (CLT)*, we know that the sampling distribution of the mean is Normal:

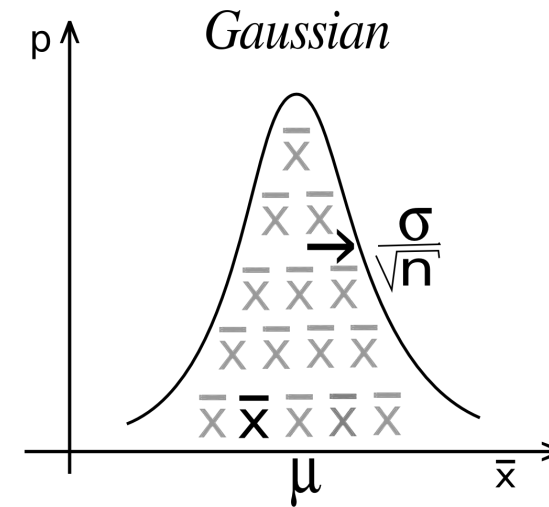
- with mean  $\mu_{\bar{X}} = \mu$  and standard deviation  $\sigma_{\bar{X}} = \frac{\sigma}{\sqrt{n}}$



population  
distribution

samples  
of size  $n$

Two horizontal arrows pointing right. The top arrow is thin and labeled  $\bar{x}$ . The bottom arrow is thick and labeled  $\bar{X}$ .



sampling distribution  
of the mean

## ⚠ Standard deviation vs Standard error ⚠

- The sampling distribution of the mean (by CLT):

$$\bar{X} \sim N\left(\mu, \frac{\sigma^2}{n}\right)$$

- The *standard error* of the mean is  $\frac{\sigma}{\sqrt{n}}$
- The *standard deviation* of  $X$  is  $\sigma$

## Estimation of parameters of the sampling distribution of the mean

Just as we estimated  $\mu$  and  $\sigma$  before, we can estimate  $\mu_{\bar{X}}$  and  $\sigma_{\bar{X}}$

- $\hat{\mu}_{\bar{X}} = \hat{\mu} = \bar{x}$
- $\hat{\sigma}_{\bar{X}} = \frac{\hat{\sigma}}{\sqrt{n}} = \frac{s}{\sqrt{n}}$



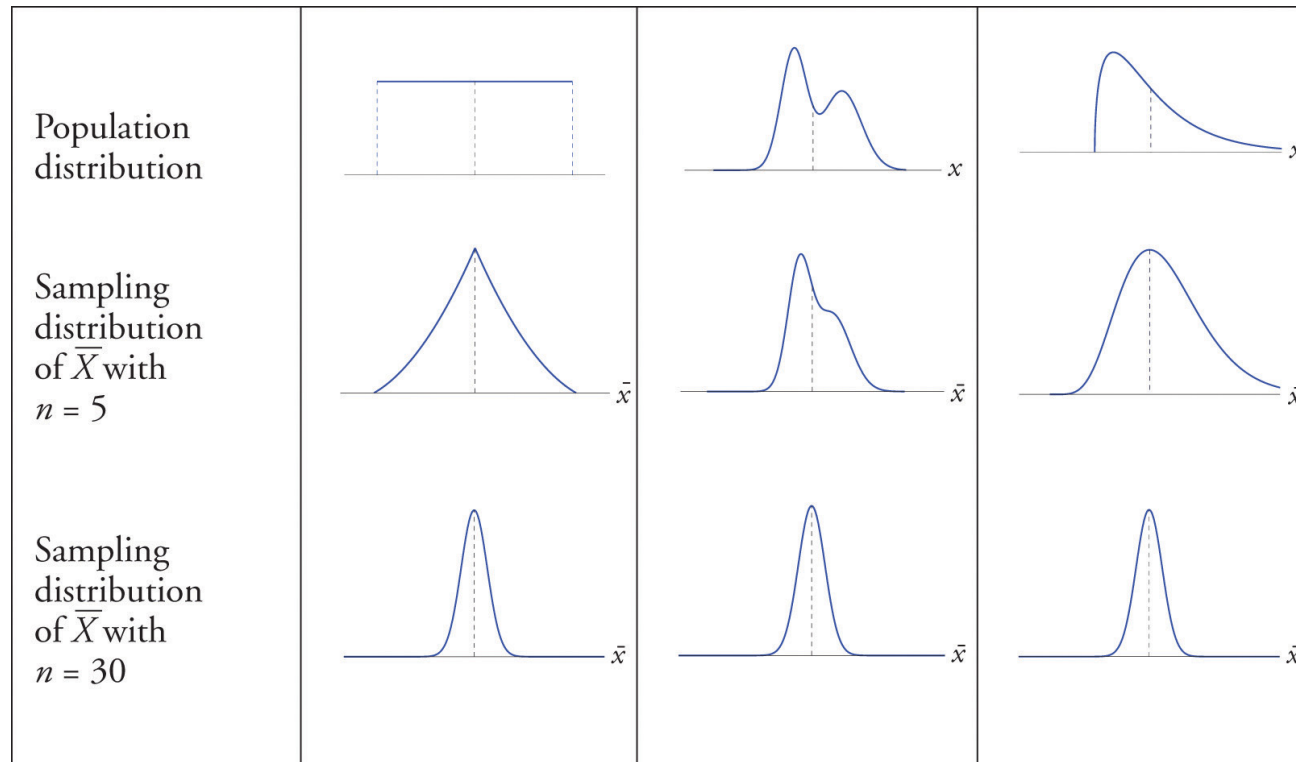
# Standard error of the mean

$$\hat{\sigma}_{\bar{X}} = \frac{\hat{\sigma}}{\sqrt{n}} = \frac{s}{\sqrt{n}}$$

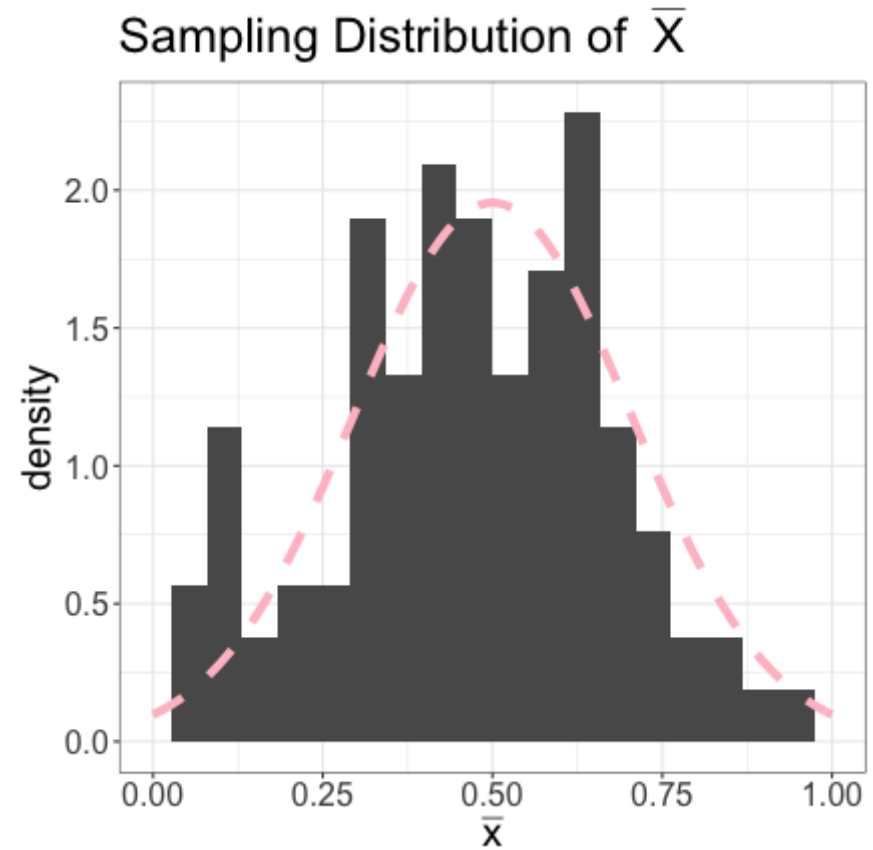
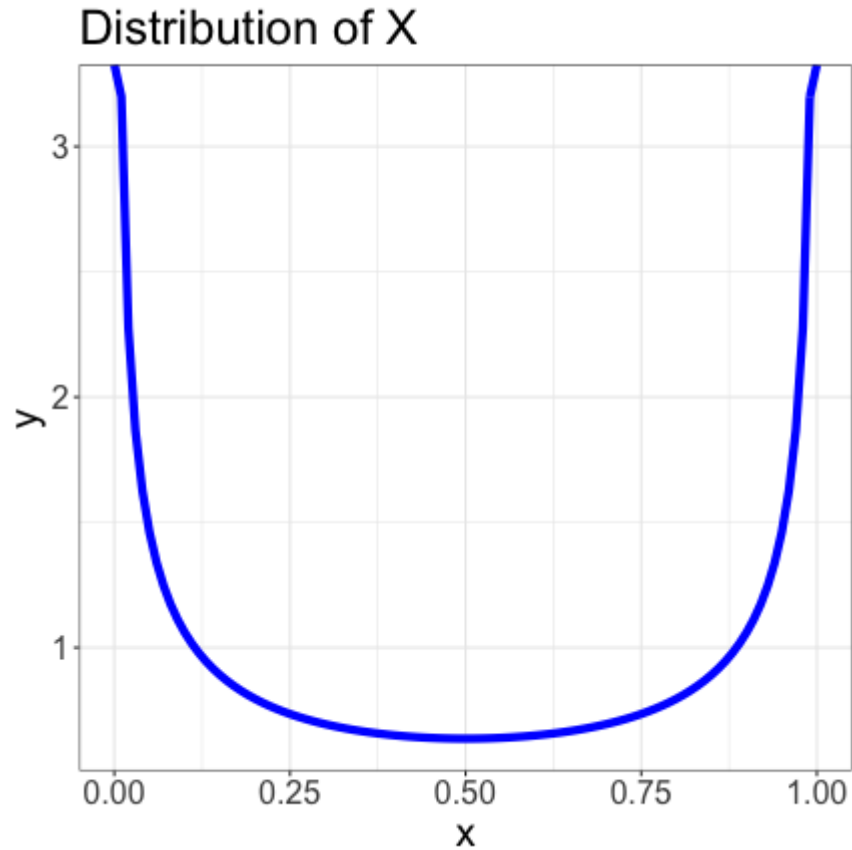
- The standard error (SE) of the mean reflects uncertainty about the value of the population mean  $\mu$
- The CLT assumes a 'large enough' sample:
  - when the sample size is  $\sim 30$  or more, the normal distribution is a good approximation for the sampling distribution of the mean
  - for smaller samples, the SE  $\frac{s}{\sqrt{n}}$  is an underestimate

# CLT applies to any population (regardless of distribution)

Let  $X_1, X_2, \dots, X_n$  be a random sample from a population with a non-normal distribution. If the sample size  $n$  is sufficiently large, then the sampling distribution of the mean will be approximately normal:  $\bar{X} \sim N(\mu, \frac{\sigma^2}{n})$

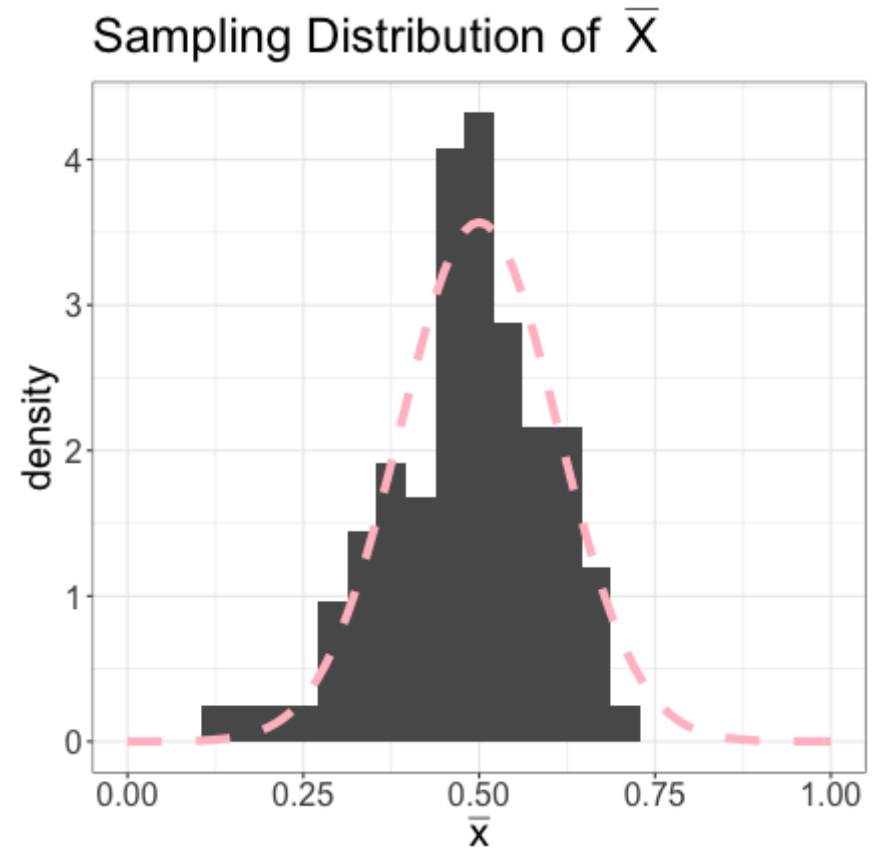
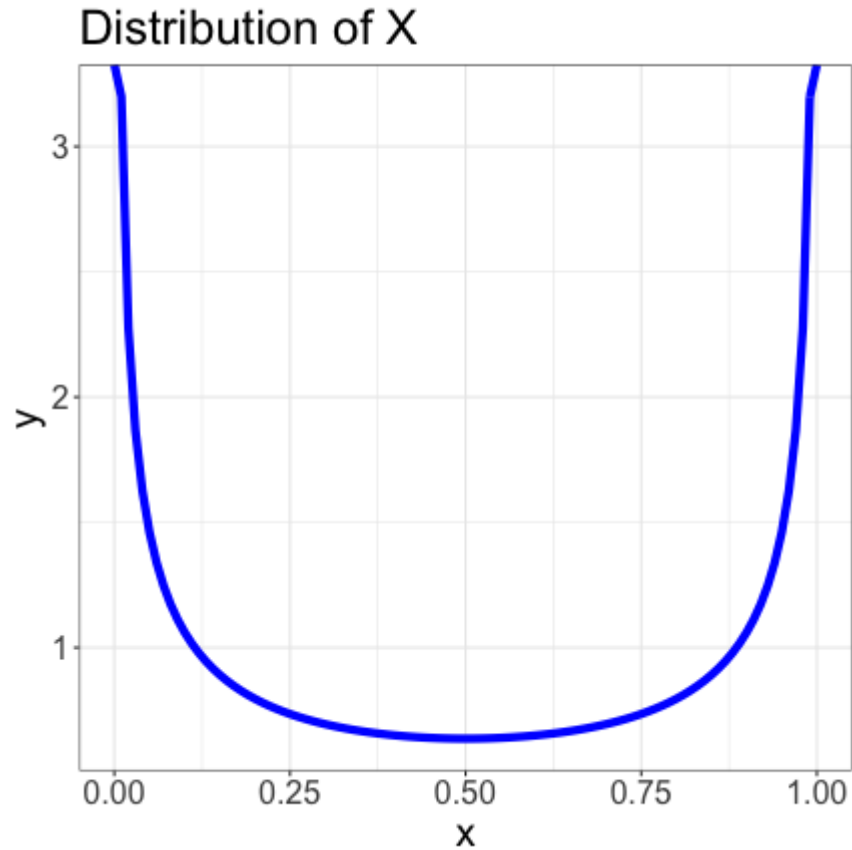


# Illustration ( $n = 3$ )



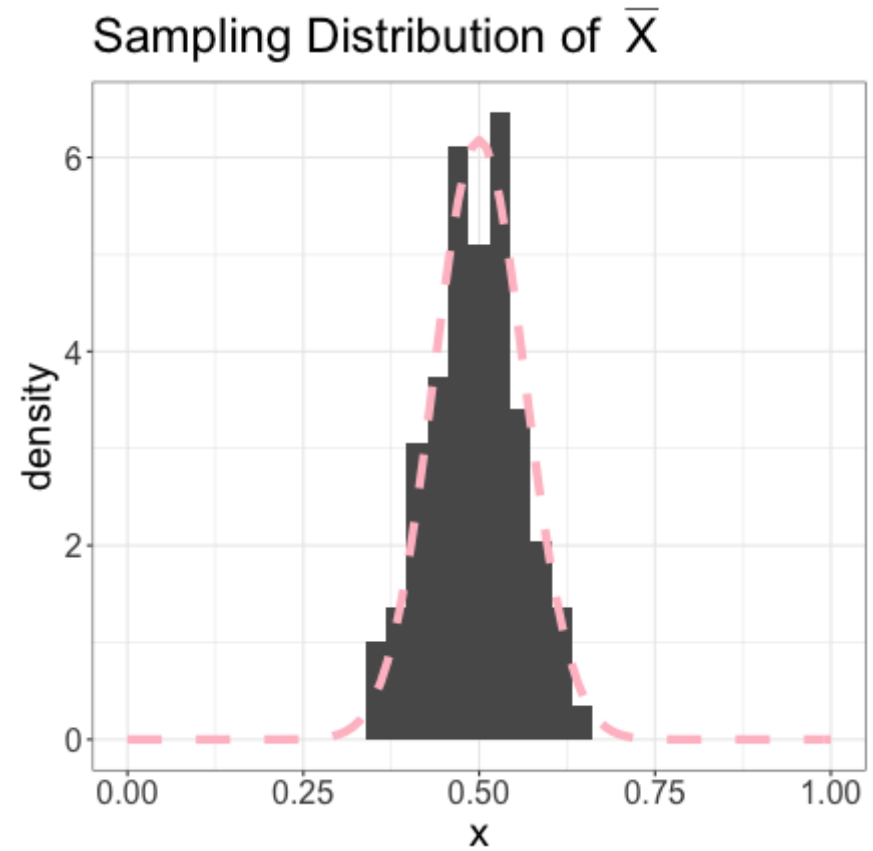
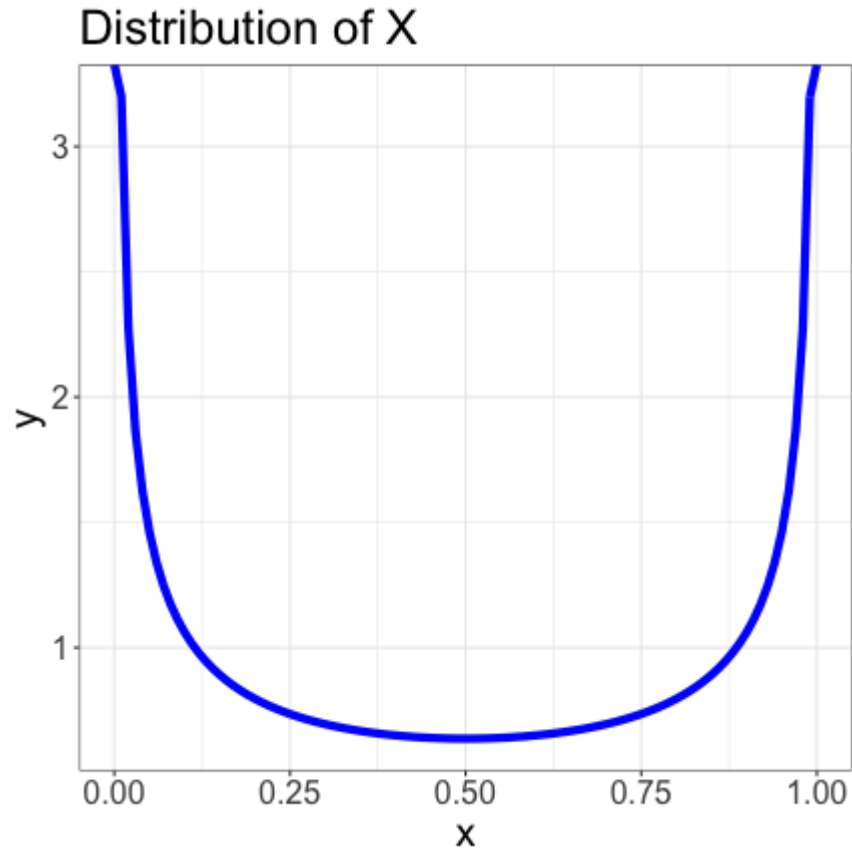
On right: dashed pink line is  $N(\mu, \sigma^2/n)$

# Illustration ( $n = 10$ )



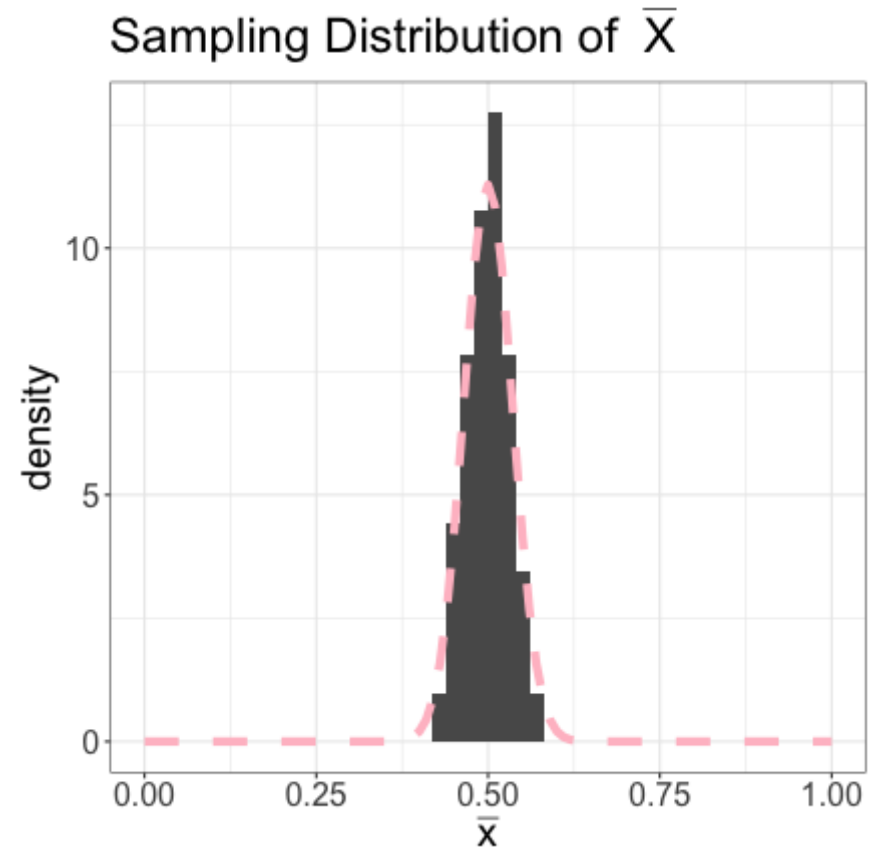
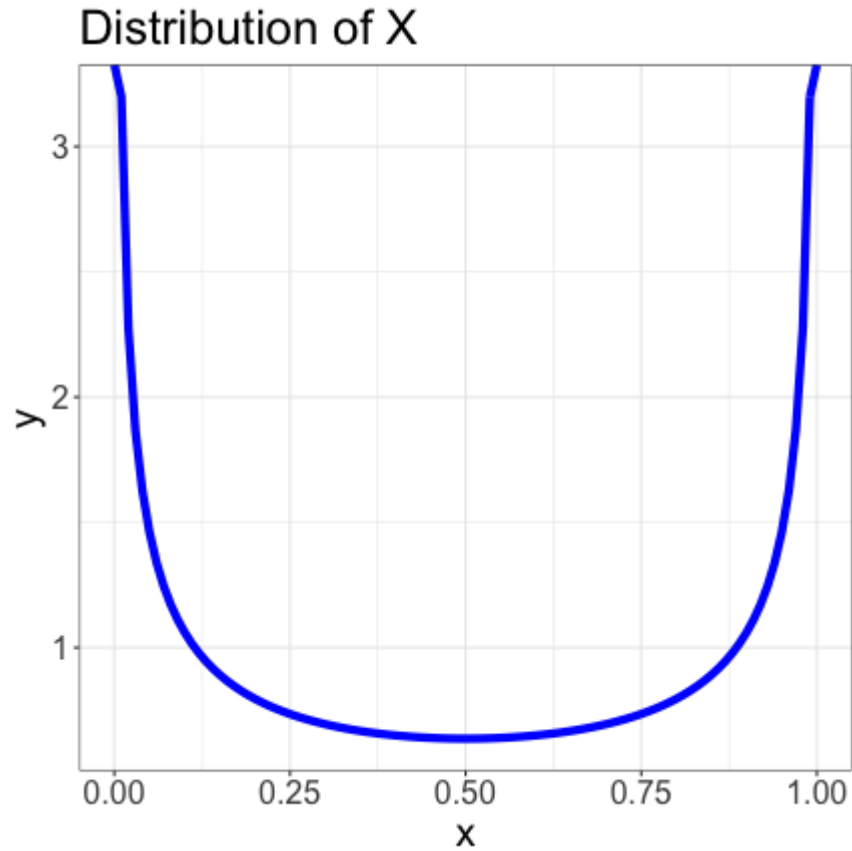
On right: dashed pink line is  $N(\mu, \sigma^2/n)$

# Illustration ( $n = 30$ )



On right: dashed pink line is  $N(\mu, \sigma^2/n)$

# Illustration ( $n = 100$ )



On right: dashed pink line is  $N(\mu, \sigma^2/n)$

# Hypothesis Testing

- **Hypothesis:** A *testable (falsifiable)* idea for explaining a phenomenon
- **Statistical hypothesis:** A hypothesis that is testable on the basis of observing a process that is modeled via a set of random variables
- **Hypothesis Testing:** A formal procedure for determining whether to *accept* or *reject* a statistical hypothesis
- Requires comparing two hypotheses:
  - $H_0$ : null hypothesis
  - $H_A$  or  $H_1$ : alternative hypothesis

# Hypothesis Testing: motivating example

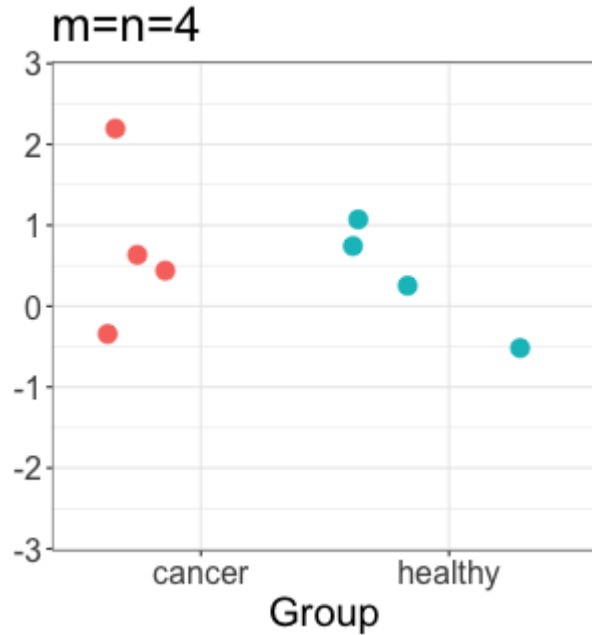
- The expression level of gene  $g$  is measured in  $n$  patients with disease (e.g. cancer), and  $m$  healthy (control) individuals:
  - $z_1, z_2, \dots, z_n$  and  $y_1, y_2, \dots, y_m$
- Is gene  $g$  is differentially expressed in cancer vs healthy samples?
  - $H_0 : \mu_Z = \mu_Y$
  - $H_A : \mu_Z \neq \mu_Y$
- In this setting, hypothesis testing allows us to determine whether observed differences between groups in our data are *significant*



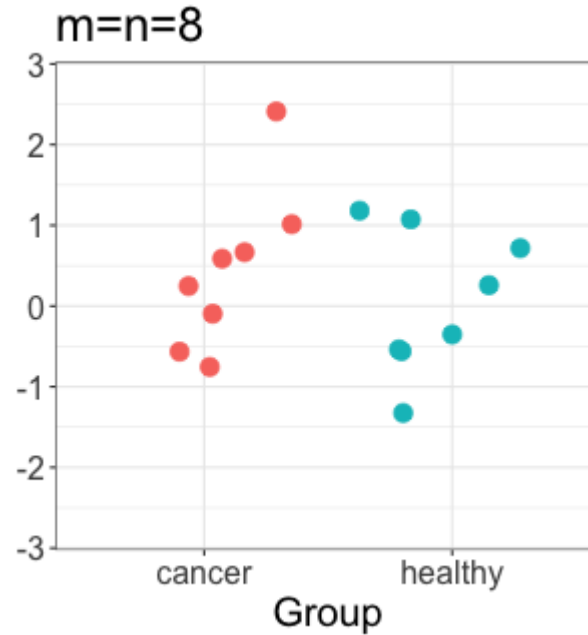
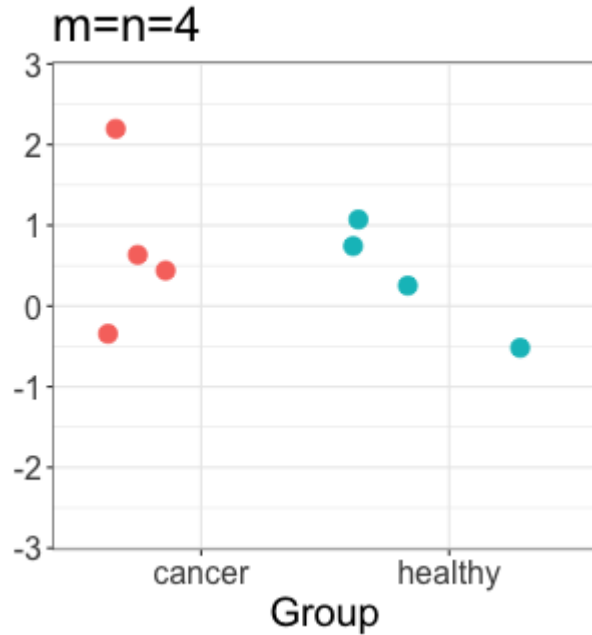
# Steps in Hypothesis Testing

1. Formulate your hypothesis as a statistical hypothesis
2. Define a test statistic (RV) that corresponds to the question.  
You typically know the expected distribution of the test statistic *under the null*
3. Compute the p-value associated with the observed test statistic under the null distribution  $p(t|H_0)$

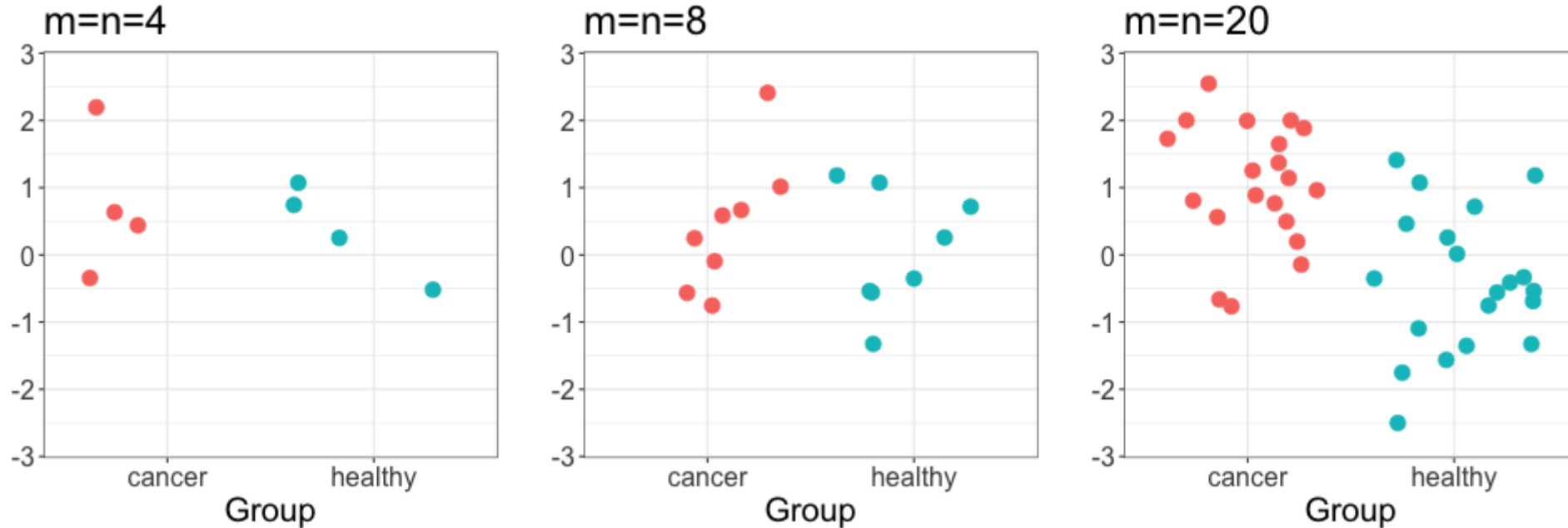
# Motivating example (cancer vs healthy gene expression)



# Motivating example (cancer vs healthy gene expression)

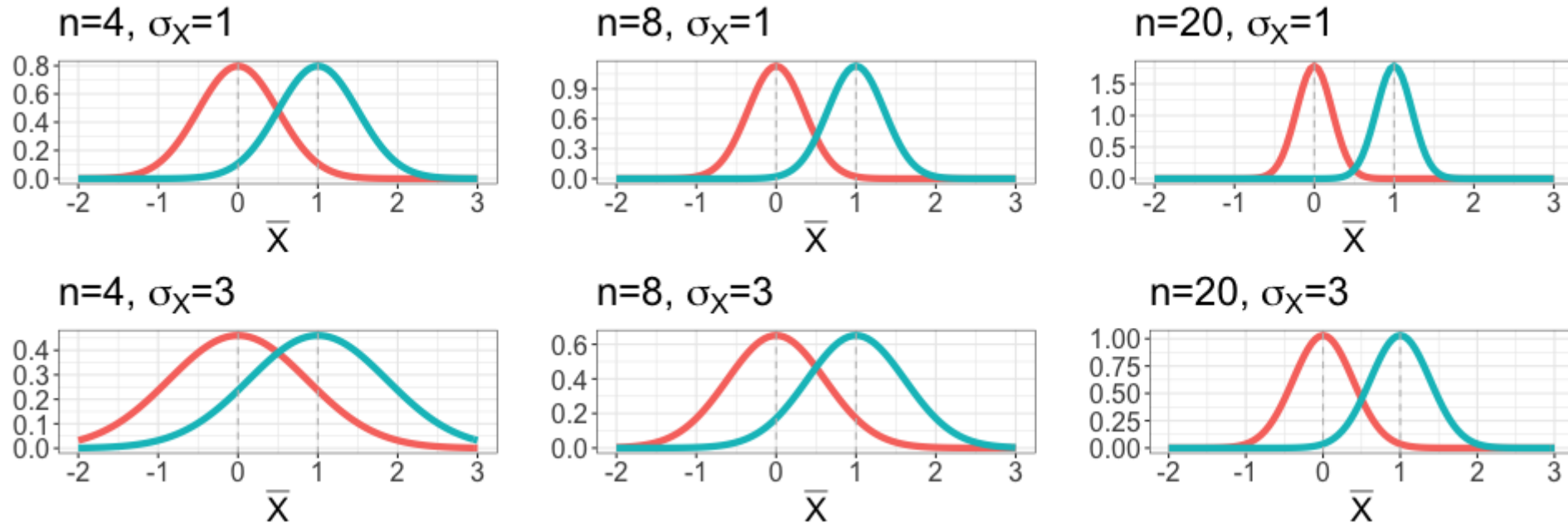


# Motivating example (cancer vs healthy gene expression)



All three samples drawn from iid Normal distributions with equal variance and  $\mu_Z - \mu_Y = 1$

# Is there a significant difference between the two means?



- Mean difference needs to be put into context of the *spread (standard deviation)*
- Also depends on the sample size

# t-statistic / t-test

- Measures difference in means, adjusted for spread/standard deviation:

$$t = \frac{\bar{z} - \bar{y}}{SE_{\bar{z} - \bar{y}}}$$

for  $z_1, z_2, \dots, z_n$  expression measurements in healthy samples and  $y_1, y_2, \dots, y_m$  cancer samples

- Standard error estimate for the difference in means:

$$SE_{\bar{z} - \bar{y}} = s_p \sqrt{\frac{1}{n} + \frac{1}{m}}, \text{ where } s_p^2 = \frac{s_z^2 + s_y^2}{(n - 1) + (m - 1)}$$

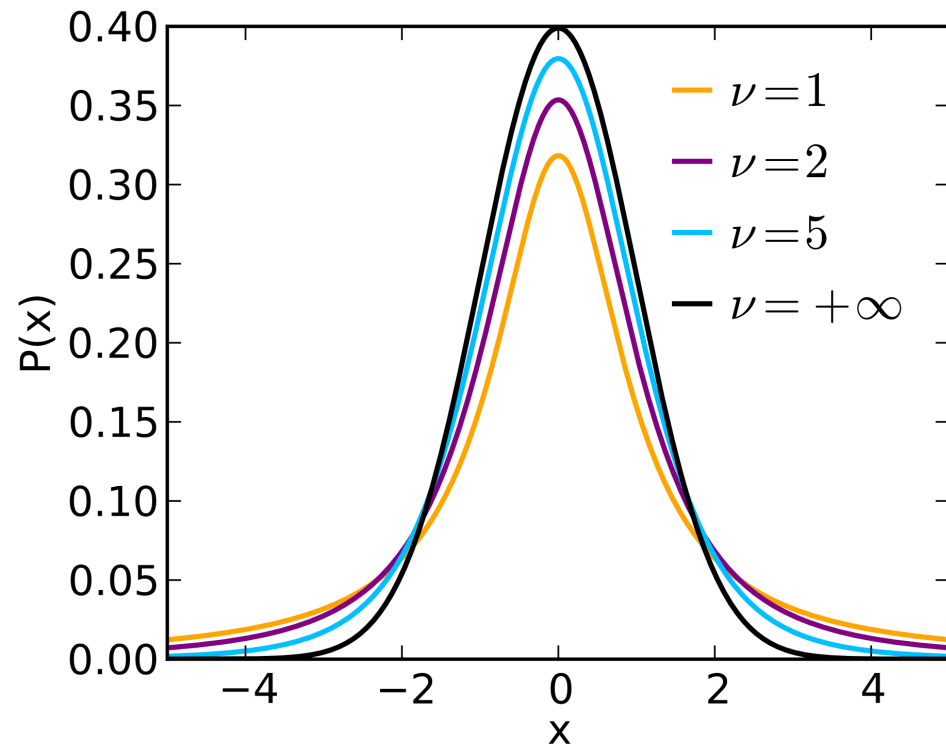
# t-test

- From the theory, we know the distribution of our test statistic, if we are willing to make some assumptions
- If we assume:
  - Z and Y are normally distributed
  - Z and Y have equal variance

Then our t-statistic follows a t distribution with  $m+n-2$  degrees of freedom

$$t \sim t_{n+m-2}$$

# t distribution



- t statistic value tells us how extreme our observed data is relative to the null
- obtain p-value by computing area to the left and/or right of the t statistic (one-sided vs two-sided)



# Summary

- Random variables are variables that have an associated probability distribution
- Any statistic of sampled data is an RV, and hence has an associated probability distribution
- The CLT gives us the sampling distribution of the mean
- Hypothesis testing gives us a framework to assess a statistical hypothesis under the null