Grundpraktikum Bioinfo - Week 1

Biological Data Analysis

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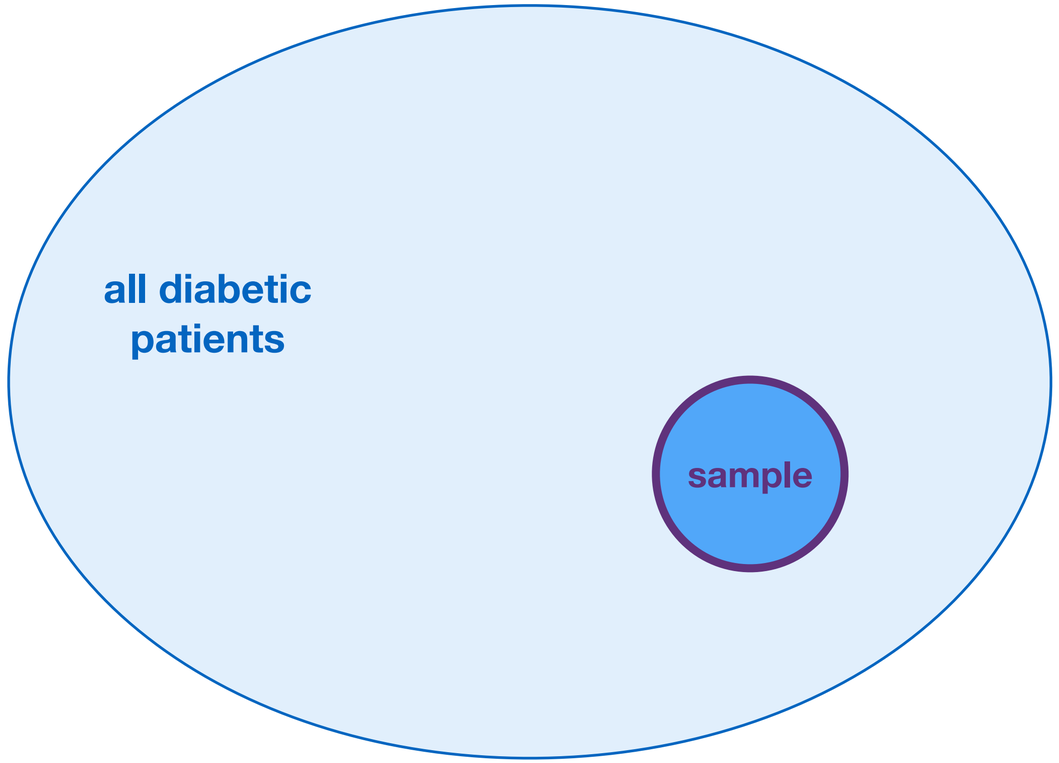
5. Distributions

Principles of statistical inference

- can we learn general principles from a small sample?
- Example: opinion polls / clinical study / ...



Is the sample representative of the general population?

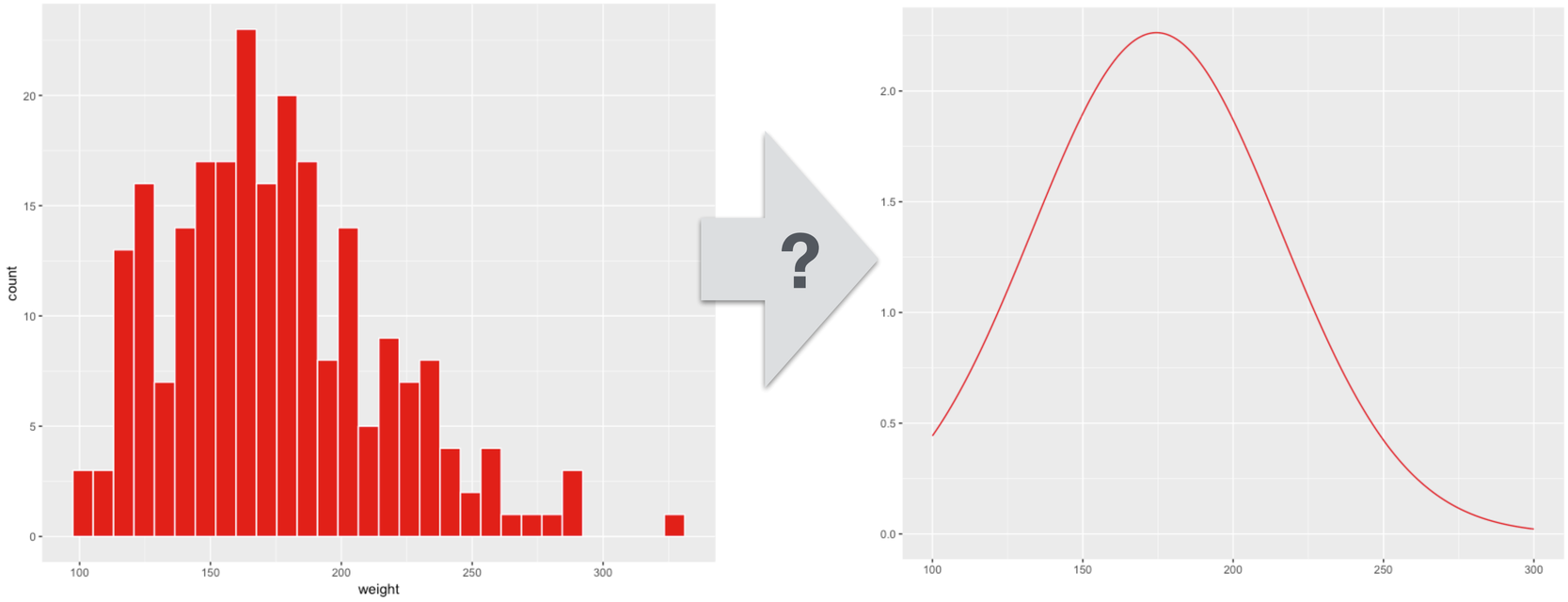
<u>Assumption</u>:

sample is drawn from the same distribution as the general population!

Diabetic patients

sample

population?



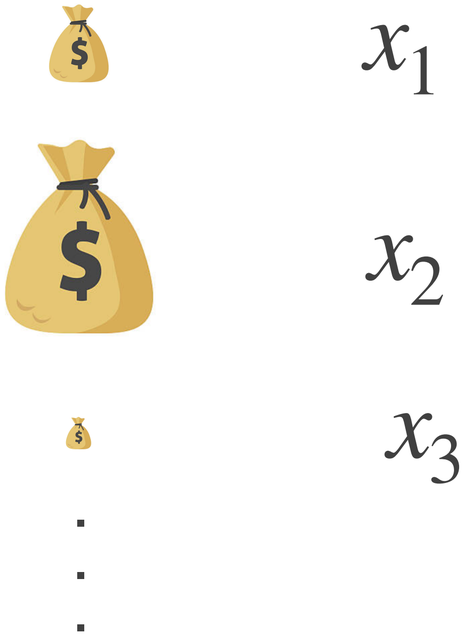
inference: How can we estimate the parameters (mean, spread,…) of the population based on the sample?

Random variables

- a **random variable** *X* describes a **stochastic process**

Random variable X

Realizations x_i



x_1

x_2

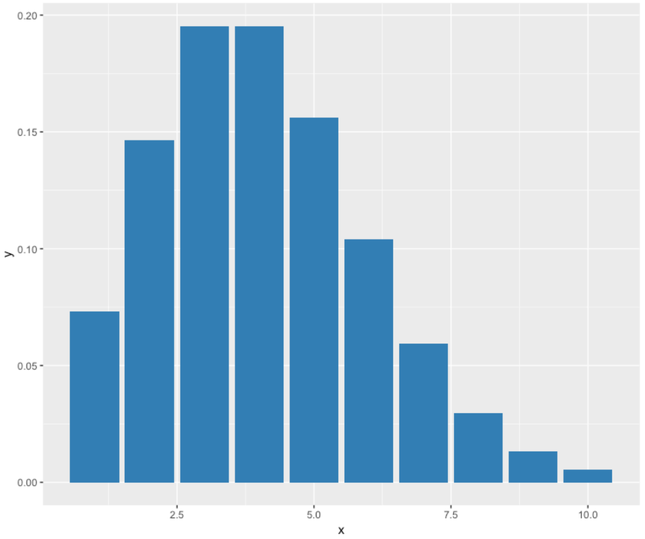
x_3

Random variables

- Random variable X can be described using
  - **expectation *E(X)***: “theoretical" mean of the entire population
  - **Variance *Var(X)***: “theoretical" variance of the entire population
  - **Density distribution** (continuous RV) *f(X)*
  - **Probability distribution** (discrete RV) *p(X)*

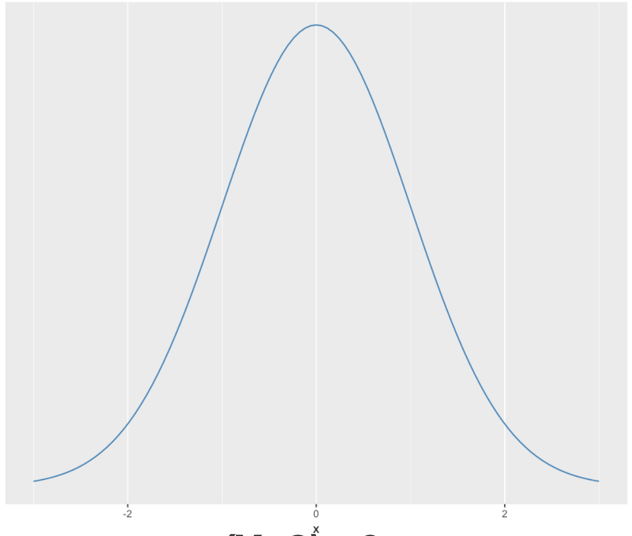
Discrete probability distribution

$$\sum_{i \in A} p(X_i) = 1$$



p(X=2) : probability that X takes value 2

Continuous density distribution



$$\int_{x \in A} f(x)dx = 1$$

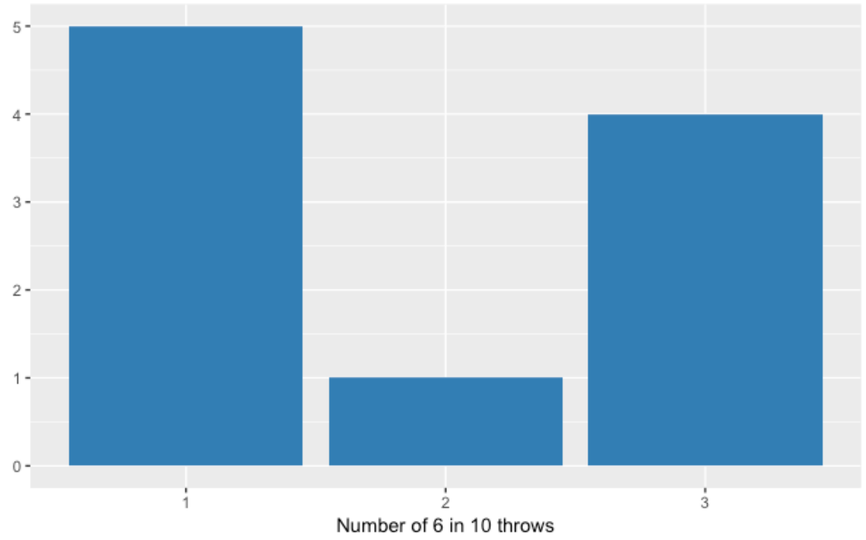
p(X=2) =0
p(2 < X < 3) >0

Example of a discrete RV

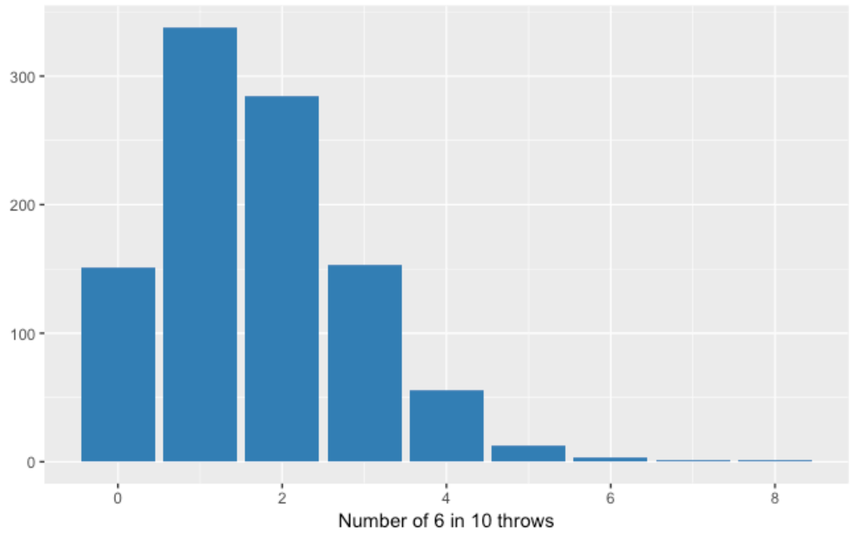
- Random variable X : number of 6 obtained in 10 dice throws

$$X \in \{0,\ldots,10\}$$

10 games

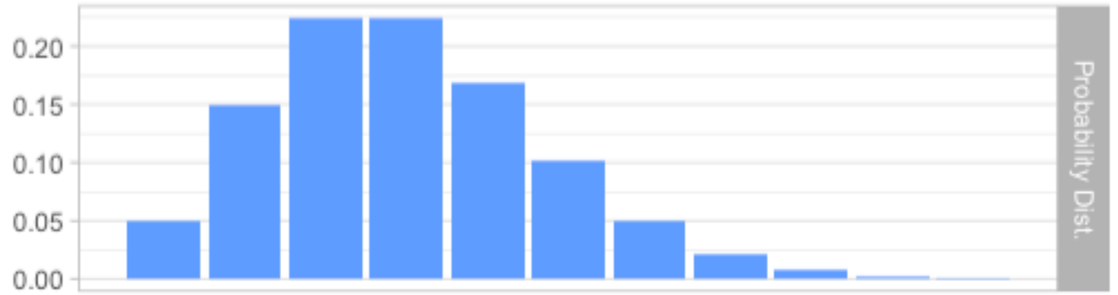


1000 games



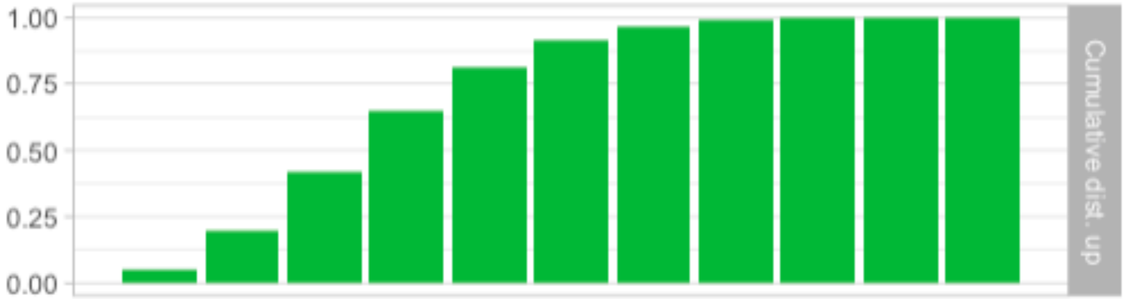
Cumulative distribution function (CDF)

- Example: **discrete RV**
- Probability distribution *P(k)*



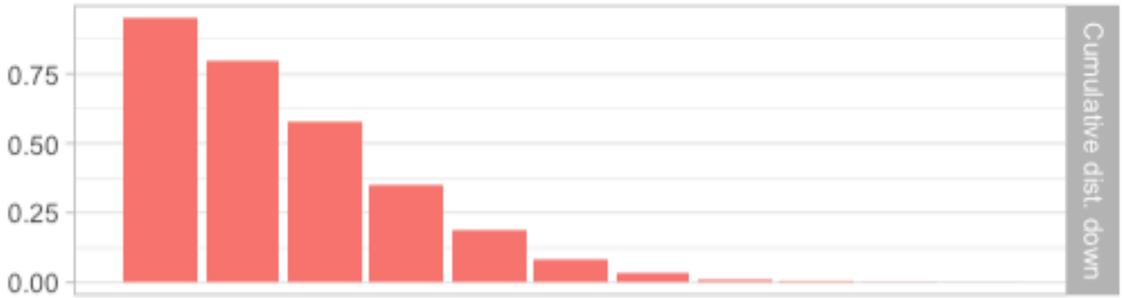
  - Cumulative distribution

$$F_X(k) = P(x \leq k)$$



  - Complementary cumulative distribution

$$\bar{F}_X(k) = p(x > k) = 1 - F_X(k)$$

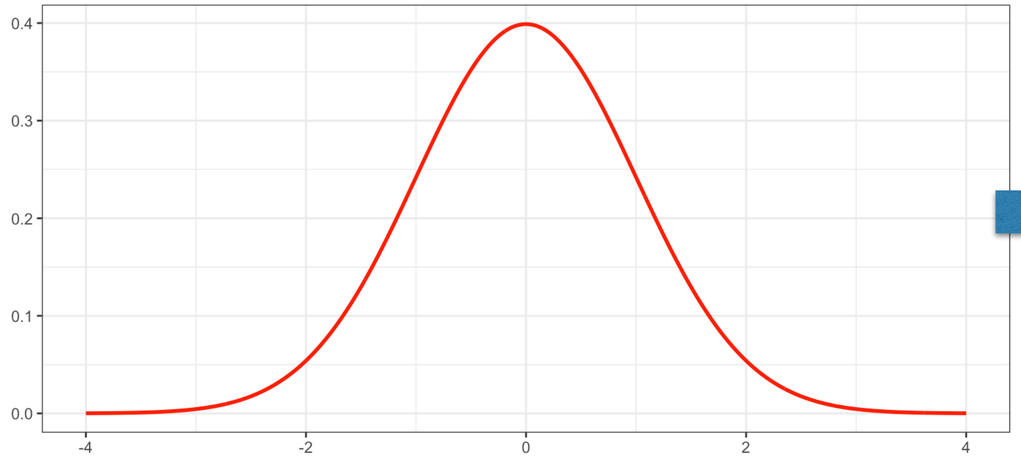


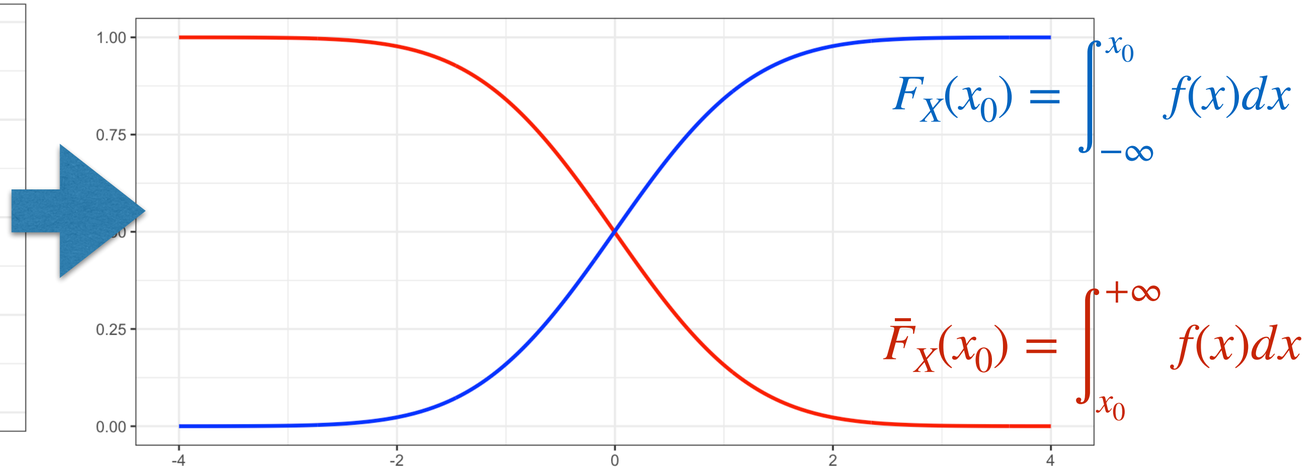
Cumulative distribution (CDF)

- **Continuous** random variable X
- density distribution *f(X)*
  - cumulative distribution
  - complementary cumulative distribution

$$F_X(x_0) = P(x < x_0) = \int_{-\infty}^{x_0} f(x)\,dx$$

$$\bar{F}_X(x_0) = P(x > x_0) = \int_{x_0}^{+\infty} f(x)\,dx$$





Cumulative distribution of the uniform distribution?

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Discrete distributions

Binomial distribution

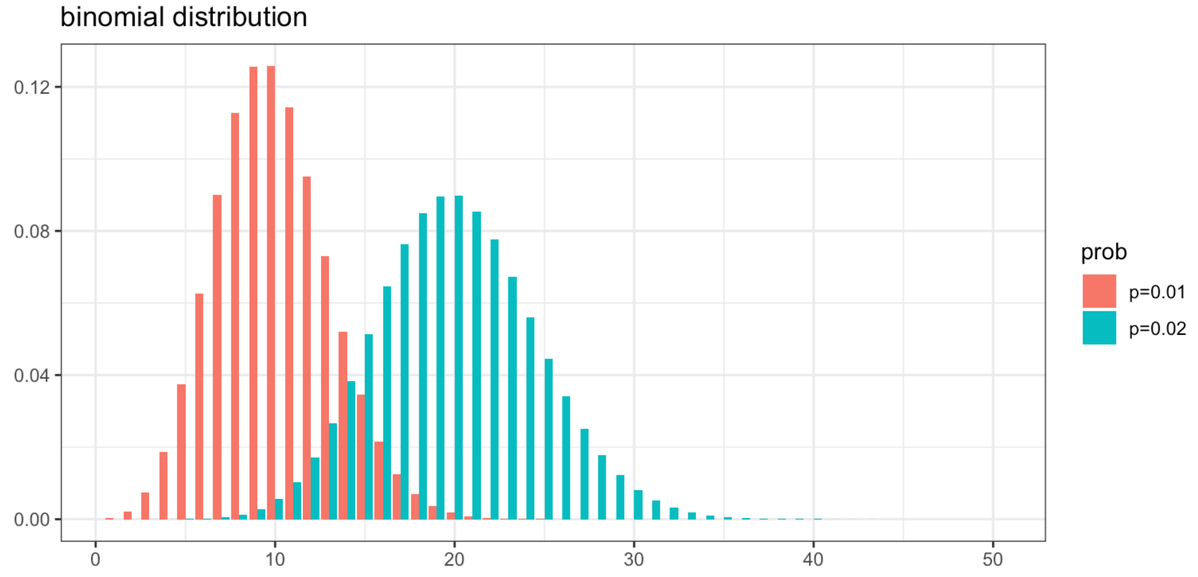
- Number of successes in ***L*** independent trials, each with probability of success ***p***
- Example of DNA polymerase
  - error rate at each position ***p*** (constant, independent of the previous position)
  - sequence of length ***L*** → number ***k*** of errors (= “successes”) ?

$$p(k) = \binom{L}{k} p^k (1-p)^{L-k}$$

$$E(X) = Lp$$

$$Var(X) = E(X)(1-p)$$

(how to compute the cumulative distribution?)



(what is L here?)

Poisson distribution

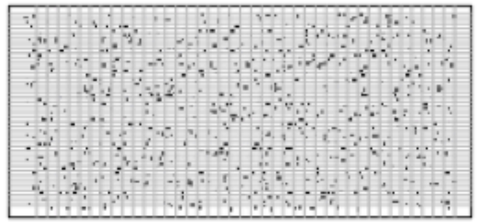
- Measures the number of events in a time period for a given **rate** of events
- Example: number of drops per tile during a rainshower
- Rate needs to be constant and independent of previous period!

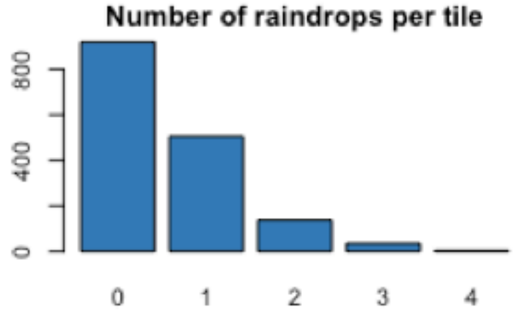
$$p(k) = \frac{\lambda^k}{k!} e^{-\lambda}$$

$$E(X) = \lambda$$

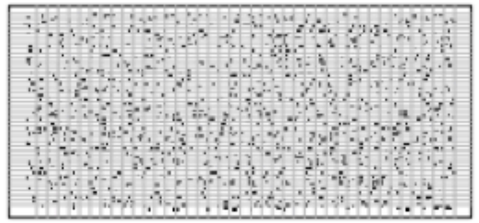
$$Var(X) = \lambda$$

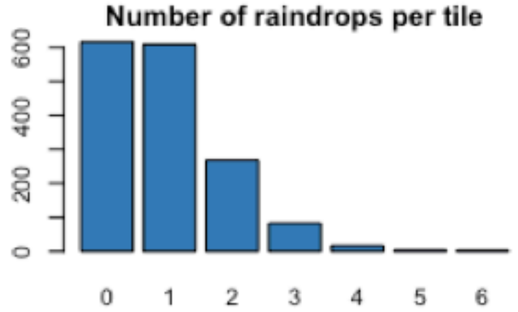
$\lambda = 0.56$



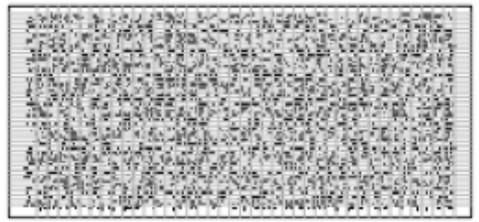


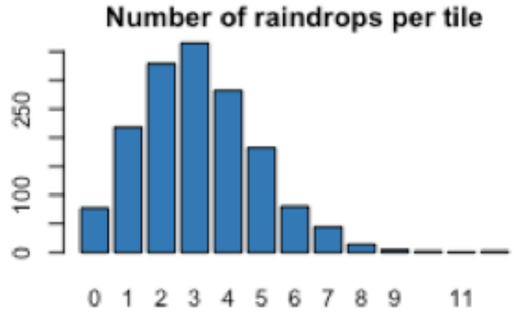
$\lambda = 0.94$





$\lambda = 3.125$





λ = drop per tile per unit time

Negative binomial

- Independent trials, constant probability p
- Different questions can be asked
  - ***Number of successes in L trials?***
    → **Binomial distribution** with 2 parameters *(L, p)*
  - ***Waiting time until r successes reached?***
    → **Negative binomial distribution** with 2 parameters *(r, p)*
  - Example: Taq-polymerase has accuracy (1-p) with p ~ 1/900 (one error every 900 bases)
    → length distribution of sequences with r = 3 errors?

Negative binomial

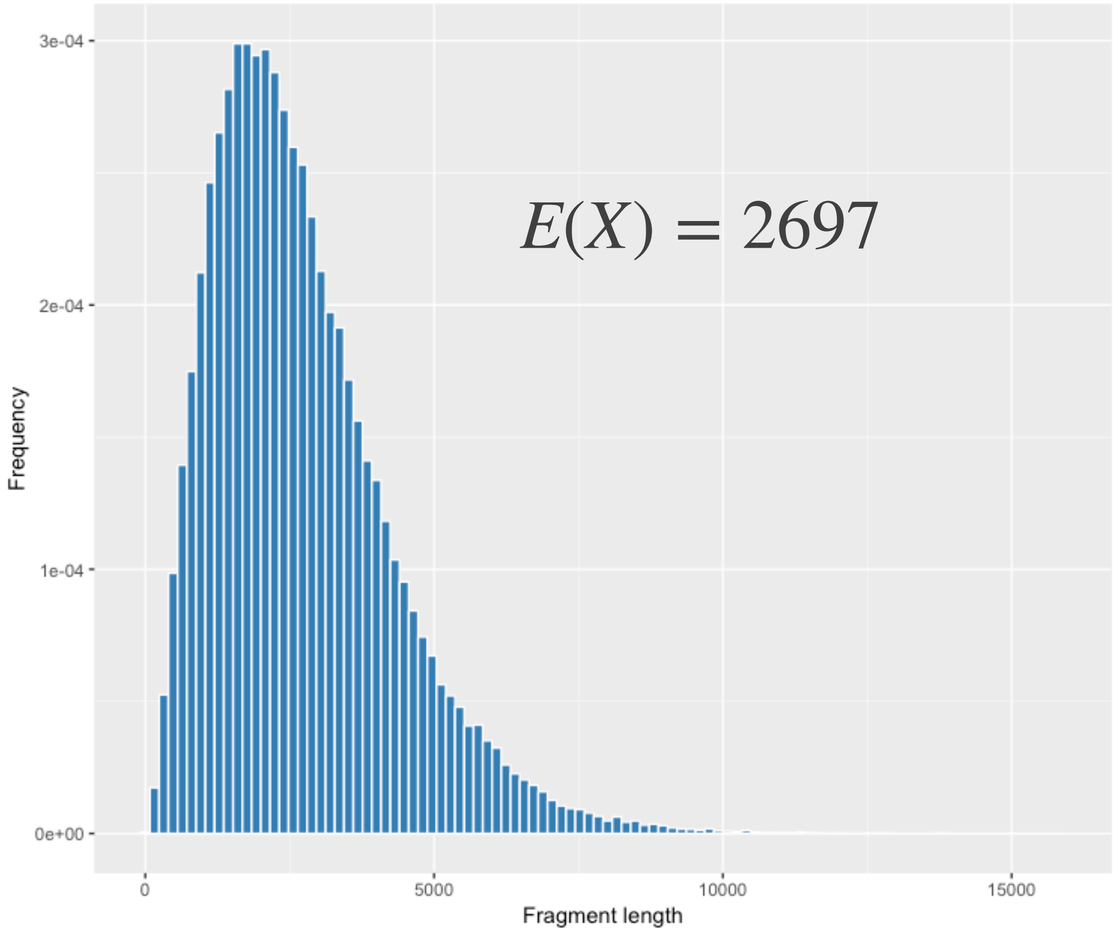
$$p(k) = \binom{k+r-1}{r-1} p^r (1-p)^k$$

$$E(X) = \frac{(1-p)r}{p}$$

$$Var(X) = \frac{E(X)}{p} = \frac{(1-p)r}{p^2}$$

k = number of error-free bases

p = 1/900, r = 3 errors

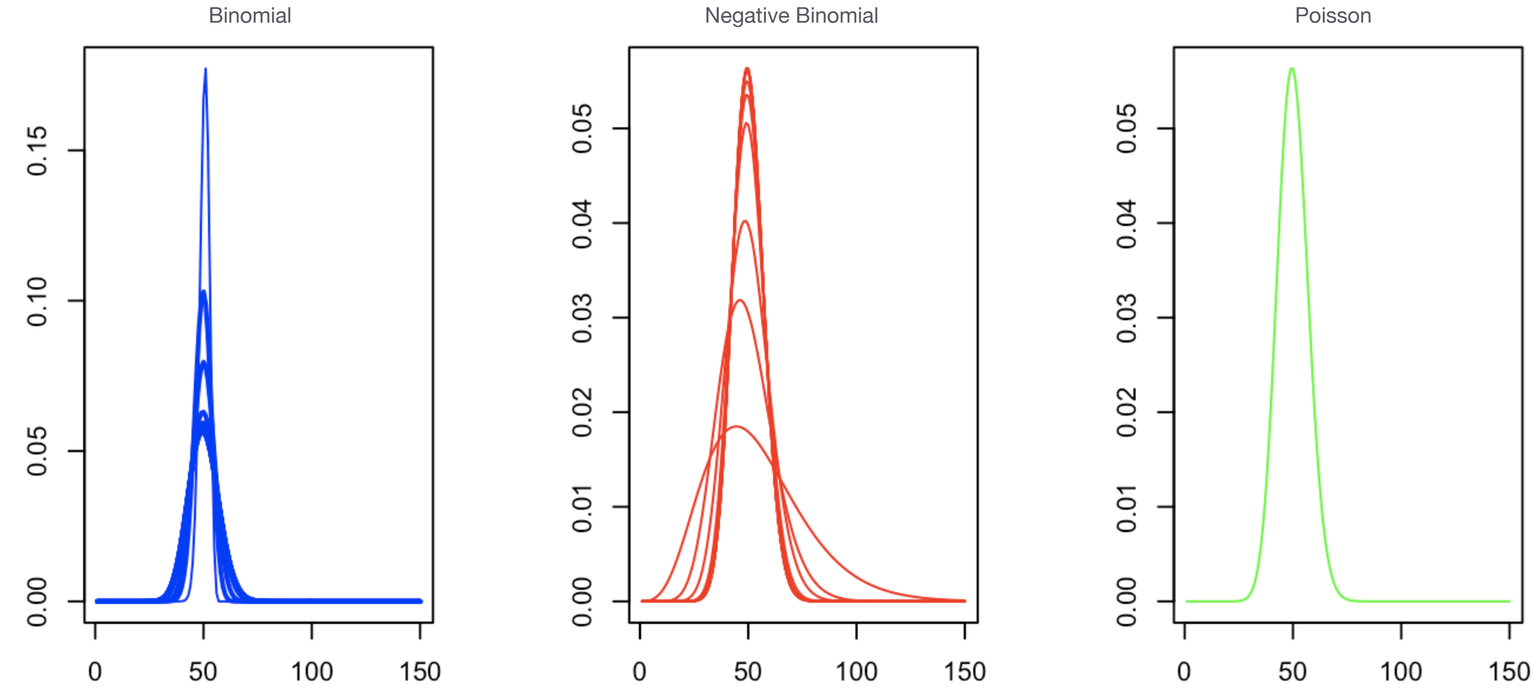


Negative binomial

- The variance of the NB distribution can be made **arbitrarily wide**, by making p smaller

$$Var(X) = \frac{E(X)}{p} = \frac{(1-p)r}{p^2}$$

- This can be usefull in modelling processes showing an **over-dispersion**



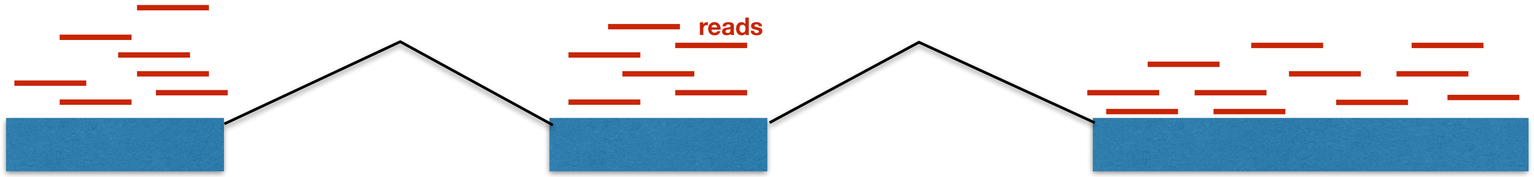
$$Var(X_B) = (1-p)E(X_B)$$

$$Var(X_{NB}) = \frac{E(X_{NB})}{p}$$

$$Var(X_P) = E(X_P)$$

Over-dispersion

- RNA-seq: mRNA molecules are fragmented and sequenced as short “reads” (e.g. 75-150 bp)
- reads are mapped onto the genome
- for each transcript, the number of reads is recorded



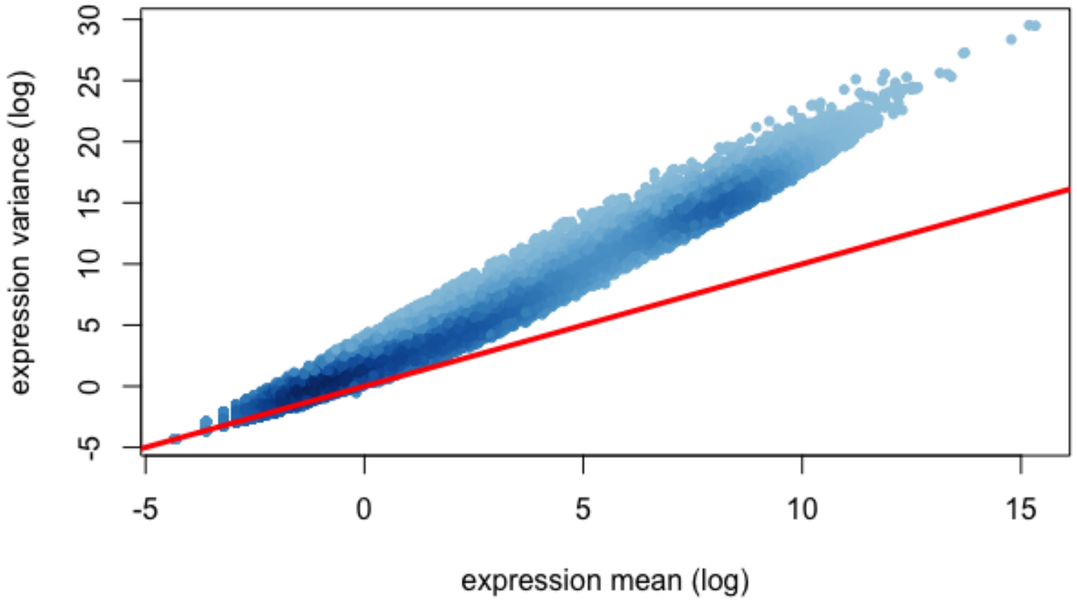
samples / replicates

transcripts

Description	GTEX-1117F-0226-SM-5GZZ7				GTEX-111CU-1826-SM-5GZYN				GTEX-111FC-0226-SM-5N9B8				GTEX-111VG-2326-SM-5N9I		
DDX11L1 3	4	1	1	0	2	1	3	5	1	0	1	3	1	1	3
WASH7P 616	395	826	364	301	419	340	451	424	331	304	430	508	414	911	505
MIR1302-11	2	1	1	0	1	0	2	3	1	1	1	0	0	1	0
FAM138A 1	0	1	1	0	0	2	3	2	0	1	2	0	2	1	2
OR4G4P 0	0	0	0	0	0	2	1	0	0	0	0	0	1	1	0
OR4G11P 0	2	2	0	0	1	0	0	0	1	0	0	1	1	1	2
OR4F5 0	0	0	0	2	0	0	0	1	1	0	0	2	1	1	0
RP11-34P13.7	8	3	12	12	2	4	10	9	3	14	9	6	4	1	12
CICP27 11	29	9	18	5	5	7	4	11	18	11	12	9	9	18	14
AL627309.1	264	300	114	1364	227	276	518	52	91	105	558	354	91	195	587
RP11-34P13.15	0	2	1	1	1	1	2	0	0	1	0	0	1	3	3
RP11-34P13.14	5	0	0	16	4	9	9	0	0	2	16	10	1	8	12
RP11-34P13.13	57	31	24	104	31	69	104	18	65	18	116	34	23	82	146
RNU6-1100P	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
RP11-34P13.9	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0
AP006222.2	52	113	168	7	100	46	25	29	44	256	35	152	79	159	511
RP4-669L17.10	18	15	47	42	54	42	23	33	20	26	20	38	15	17	128
RP4-669L17.8	1	1	2	2	6	0	3	0	3	0	0	0	1	1	2
CICP7 5	6	5	2	2	1	2	4	4	4	2	7	11	8	3	13
RP4-669L17.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
OR4F29 0	1	0	0	0	3	0	1	0	0	0	0	0	2	1	2
WBP1LP7 0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	2
AL732372.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RP4-669L17.2	0	0	0	1	1	0	0	2	1	1	0	1	1	0	0
RP5-857K21.15	3	0	4	2	2	1	2	2	2	2	0	1	2	2	2
RP4-669L17.1	3	3	1	0	2	2	1	0	5	2	2	6	1	2	5

RNA-seq data

- each dot is a transcript; several replicates (~patients) available
- x-axis : mean number of reads per transcript (over all replicates)
- y-axis : variance of number of reads per transcript (over all replicates)
- **variance is larger than mean**: cannot be described by Poisson or binomial process!



if number of reads per transcript would be a Poisson distribution, dots should lie on the y = x line

Continuous distributions

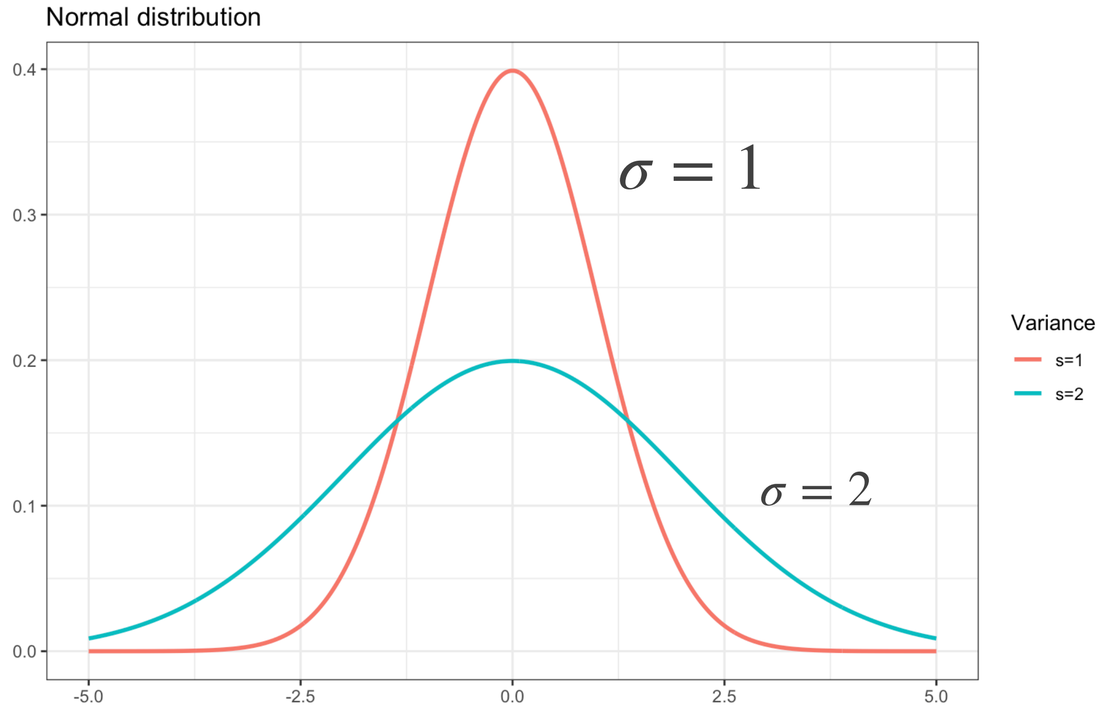
Normal distribution

- Normal distribution plays a central role in statistics and data analysis due to *Central Limit Theorem*
- 2 parameters: expectation $\mu$and standard deviation σ

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

$$E(X) = \mu$$

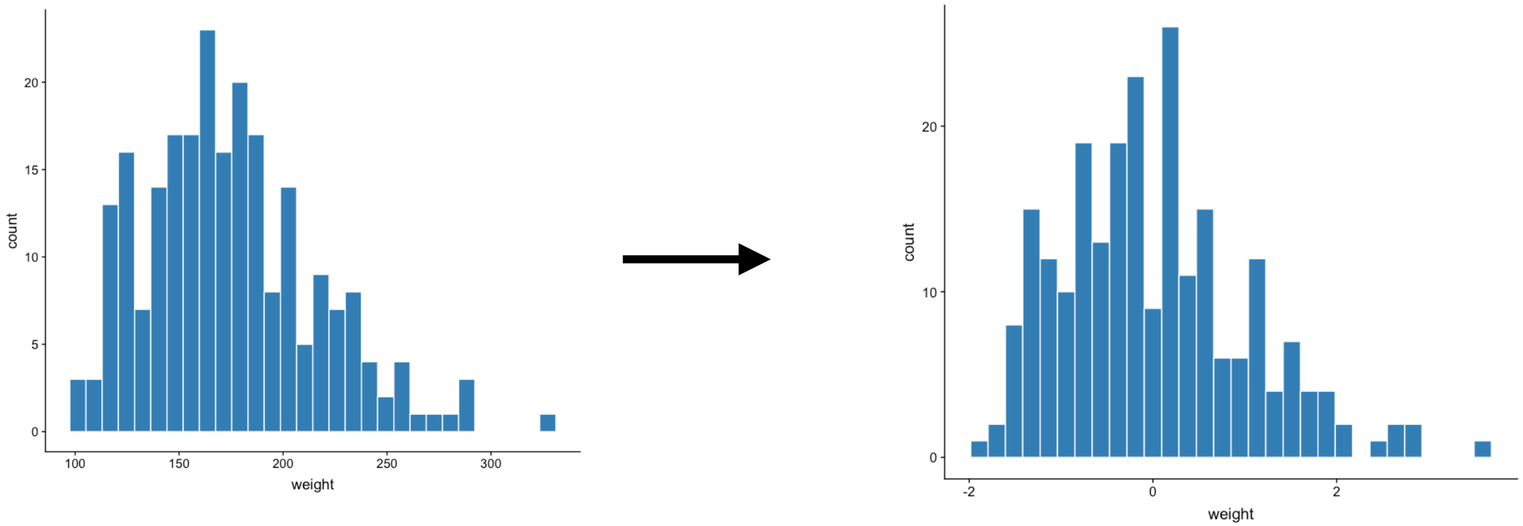
$$Var(X) = \sigma^2$$



Normal distribution

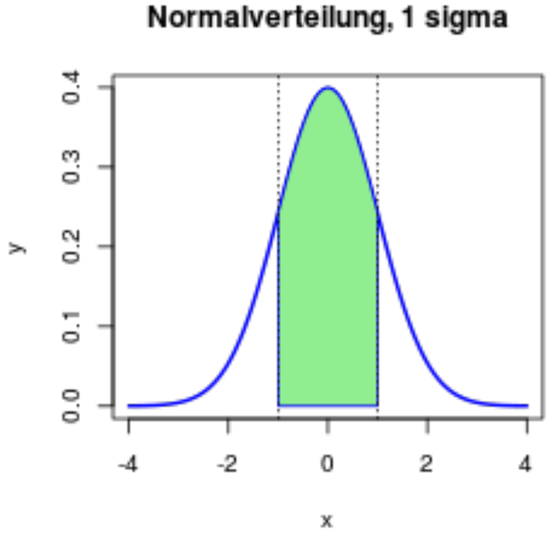
- The normal distribution with $\mu = 0$ and $\sigma = 1$ is called the **Standard Normal distribution** (SND) $\mathcal{N}(0,1)$
- Every normal distribution $\mathcal{N}(\mu, \sigma)$ can be transformed into the SND through a Z-transformation
  By definition, the new RV Z has expectation 0 and variance 1
- The Z-transformation can be applied to any distribution!

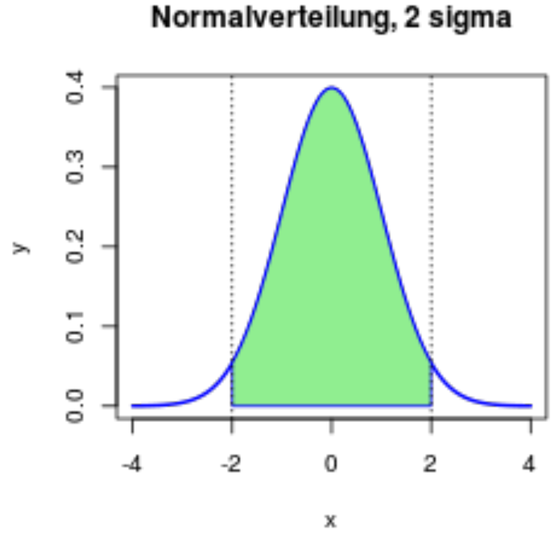
$$X \longrightarrow Z = \frac{X - \mu}{\sigma}$$



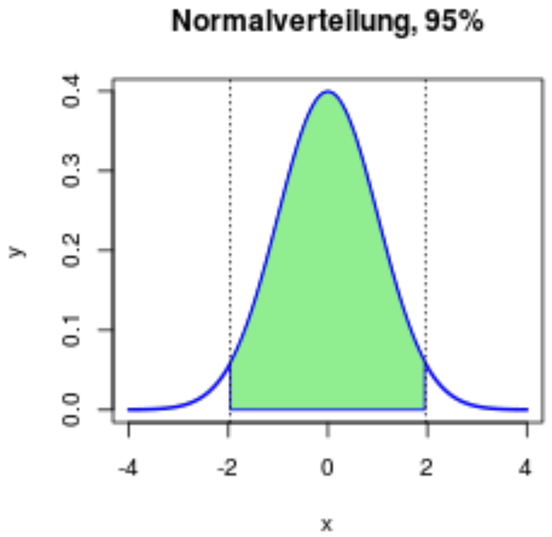
Properties of SND

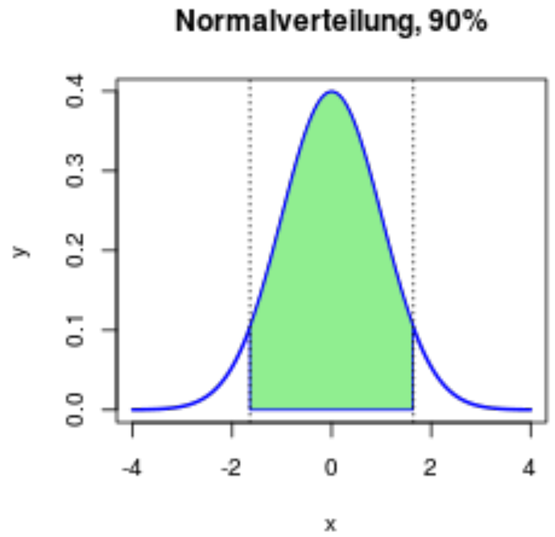
- the area represents…
  - [-1 , +1] = 68%
  - [-2 , +2] = 95.4%
  - [-1.96 , +1.96] = 95%
  - [-1.64 , +1.64] = 90%
- Critical values
  - t_{95} = 1.96
  - t_{90} = 1.64





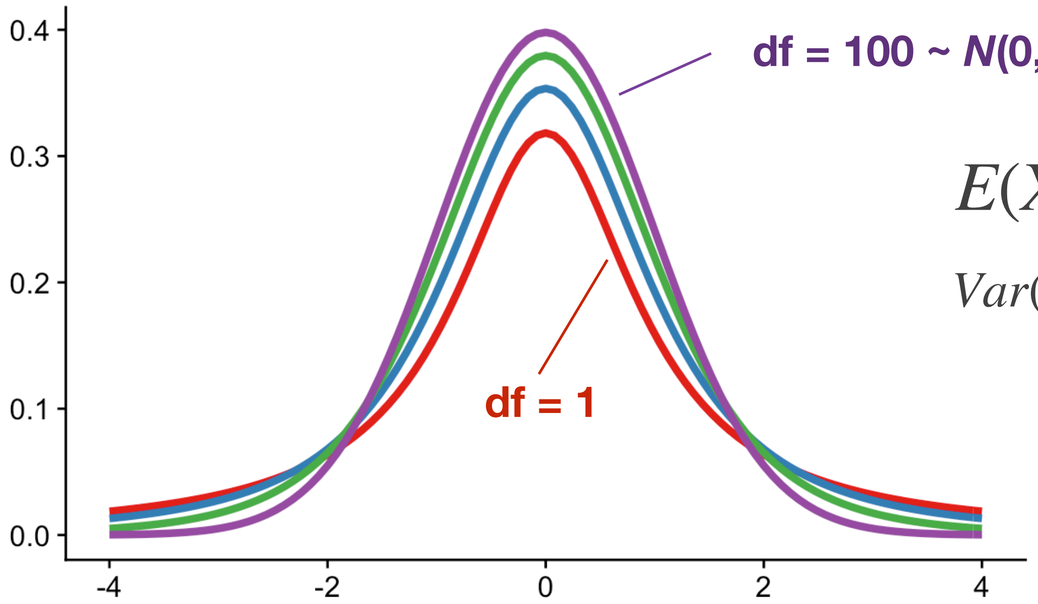
$$\int_{-\infty}^{+\infty} f(X)dX = 1$$





t-distribution

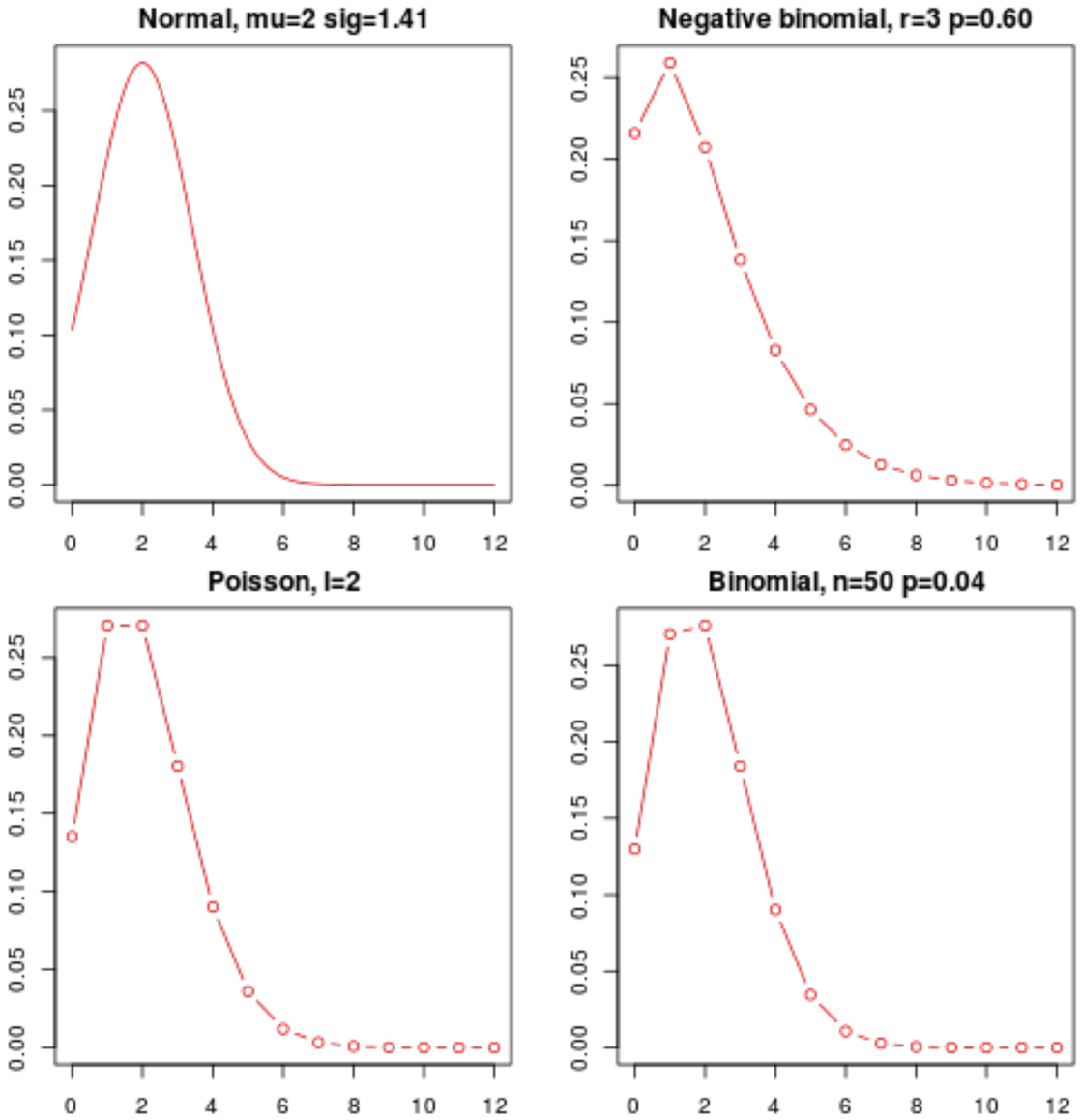
- Student's t-distribution is a continuous distribution describes the **distribution of mean values over small samples**
- Parameter: *degree of freedom (df)*
- Tends to the SND for large number of degrees of freedom



$$E(X) = 0$$

$$Var(X) = \frac{df}{df - 2} \quad \text{for} \quad df > 2$$

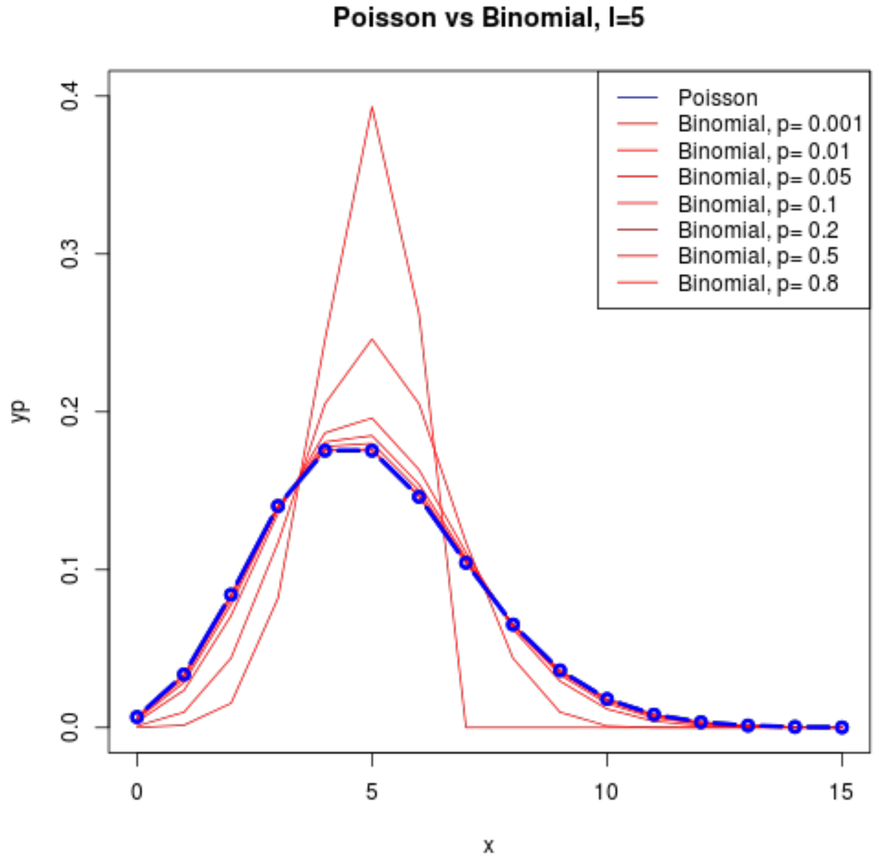
Related distributions



Poisson vs. binomial

$$Binom(n, p) \longrightarrow Pois(\lambda = np)$$

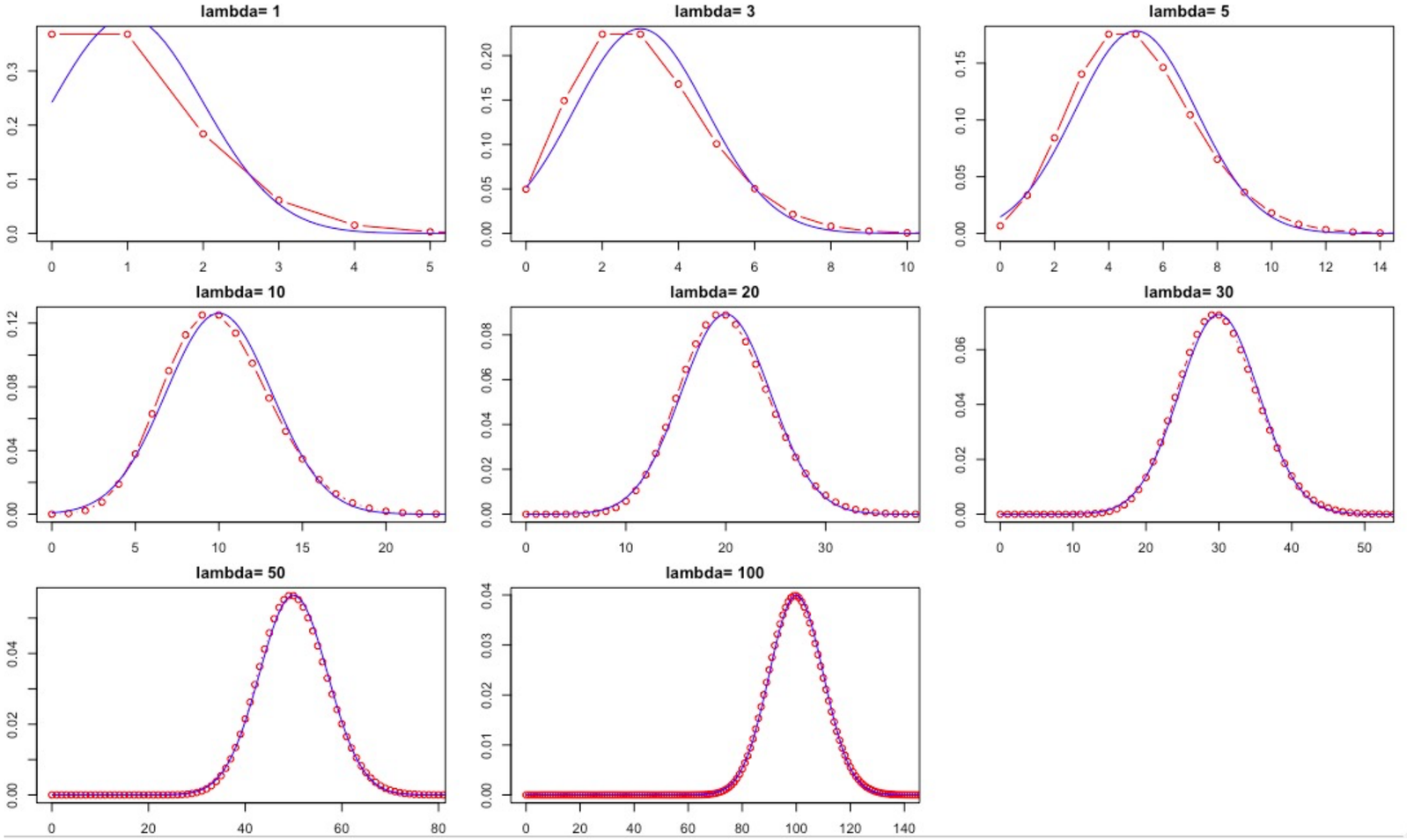
$$n \to \infty; p \to 0; np = \lambda$$



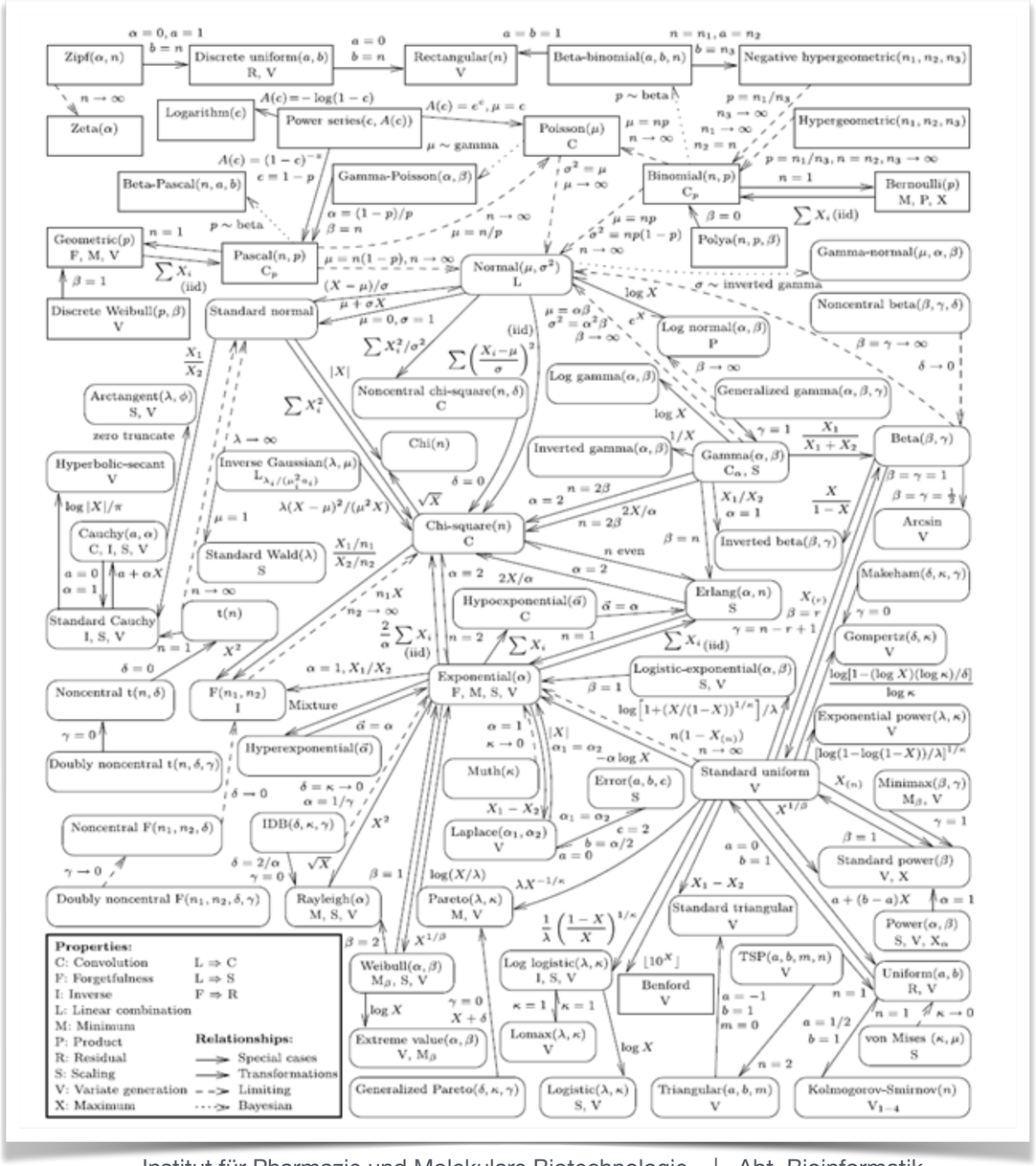
Poisson vs. normal

$$Pois(\lambda) \longrightarrow \mathcal{N}(\lambda, \sqrt{\lambda})$$

$$\lambda \gg 1$$



Distribution = italian wedding party!



IPMB
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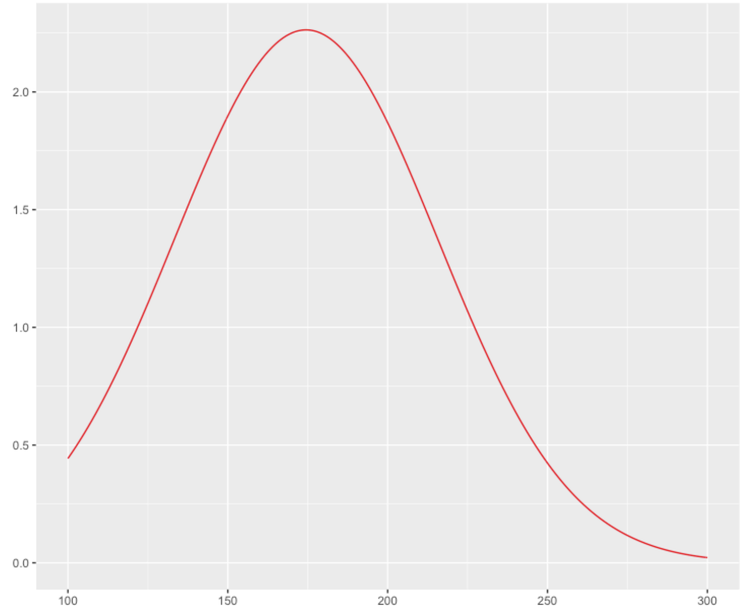
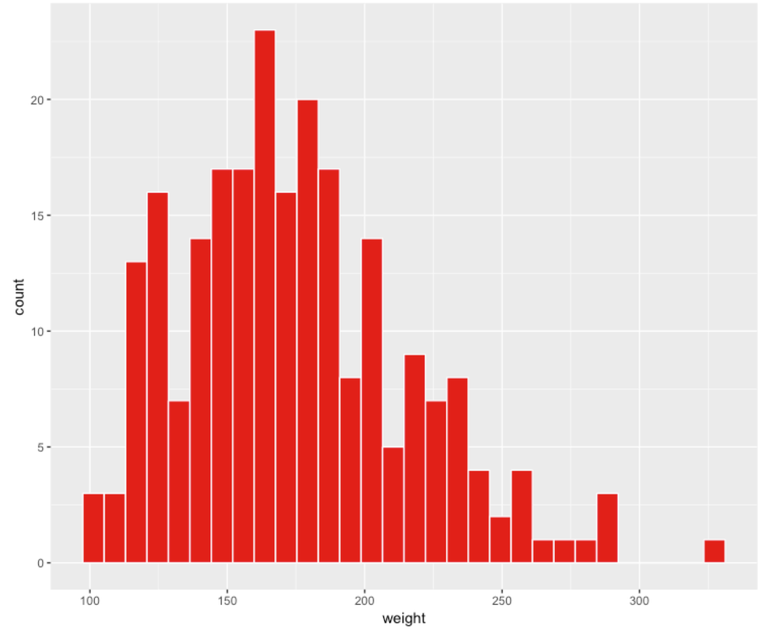
ZUKUNFT SEIT 1386

Statistical inference

Statistical inference





How can we estimate the parameters of the population (expectation, variance, ..) …

… using the sample distribution?

Random variables and samples

- Parameters associated to the **random variable** are indicated in **greek letters**
- Parameters associated to **sample** are in **roman letter**

Random variable	Sample
random variable X	realizations $x_1, x_2, \ldots, x_n$
probability distribution p(k) density distribution f(X)	histogram
Expectation $E(X) = \mu$	Mean / median $\bar{x} = m$
Variance $Var(X) = \sigma^2$	Sample variance $Var(x_i) = s^2$

Can we use m and s to estimate μ and σ ?

Infering the expectation

- Example: **number of children per family**
  → we conduct a survey and interview every day *n* families
- Random variable : ***N_i* = {number of children in the i-th family}**
- The N_i are random variables, independent of each other, and identically distributed (“i.i.d. = independent, identically distributed")

$$M_n = \frac{1}{n} \sum_{i=1}^{n} N_i$$

- M_n is a **new random variable**
  → estimate its probability distribution p(k), expectation and variance

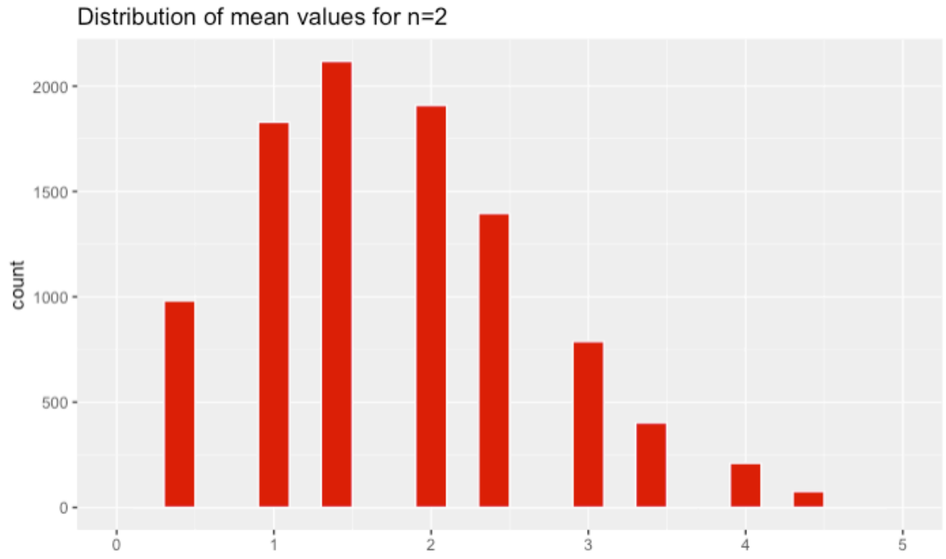
Example n=2

	Day_1	Day_2	Day_3	Day_4	Day_5	Day_6	Day_7	Day_8	Day_9	Day_10
Family_1	1	0	4	4	3	2	4	2	0	0
Family_2	3	2	1	1	2	2	4	3	1	1

...

	Day_1	Day_2	Day_3	Day_4	Day_5	Day_6	Day_7	Day_8	Day_9	Day_10
m_2	2	1	2.5	2.5	2.5	2	4	2.5	0.5	0.5

...



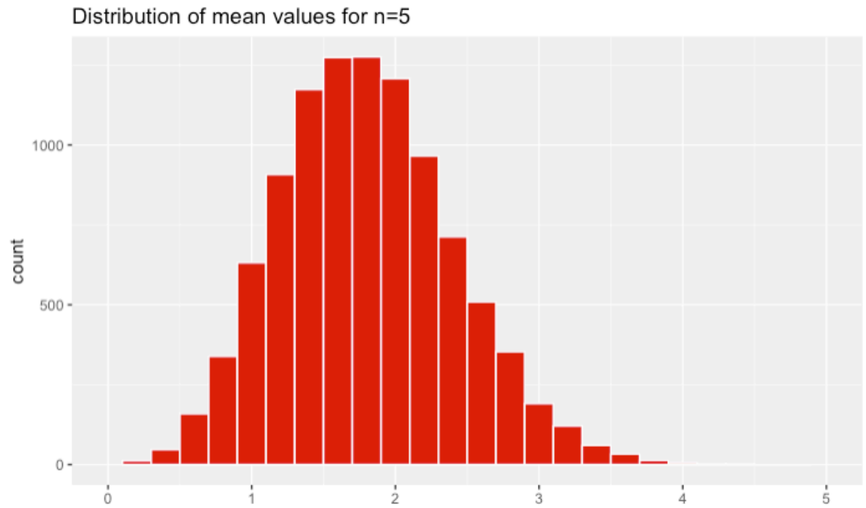
Example n=5

	Day_1	Day_2	Day_3	Day_4	Day_5	Day_6	Day_7	Day_8	Day_9	Day_10
Family_1	1	0	4	4	3	2	4	2	0	0
Family_2	3	2	1	1	2	2	4	3	1	1
Family_3	1	4	2	0	2	2	2	1	1	2
Family_4	3	2	2	1	6	1	3	1	1	1
Family_5	4	1	0	4	2	0	0	1	0	3

...

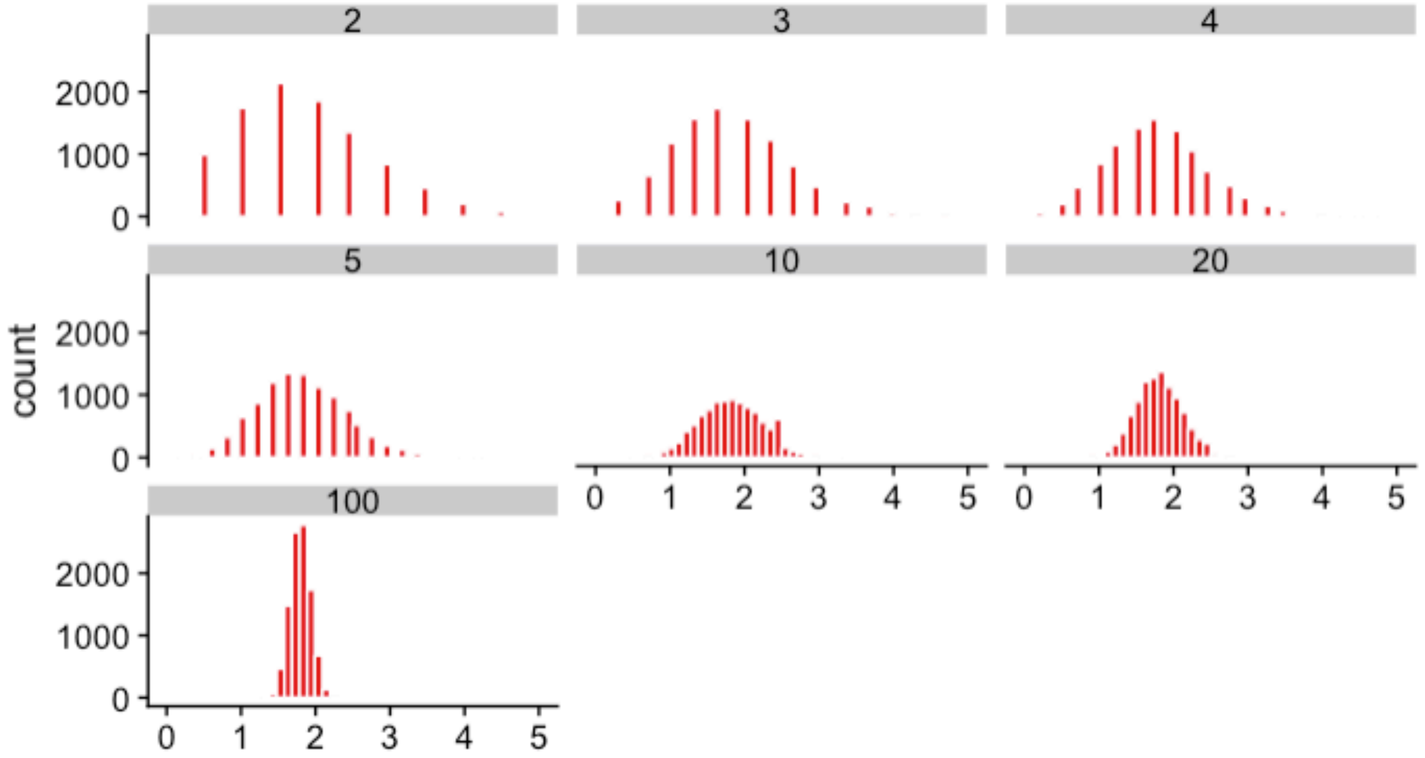
	Day_1	Day_2	Day_3	Day_4	Day_5	Day_6	Day_7	Day_8	Day_9	Day_10
m_5	2.4	1.8	1.8	2	3	1.4	2.6	1.6	0.6	1.4

...

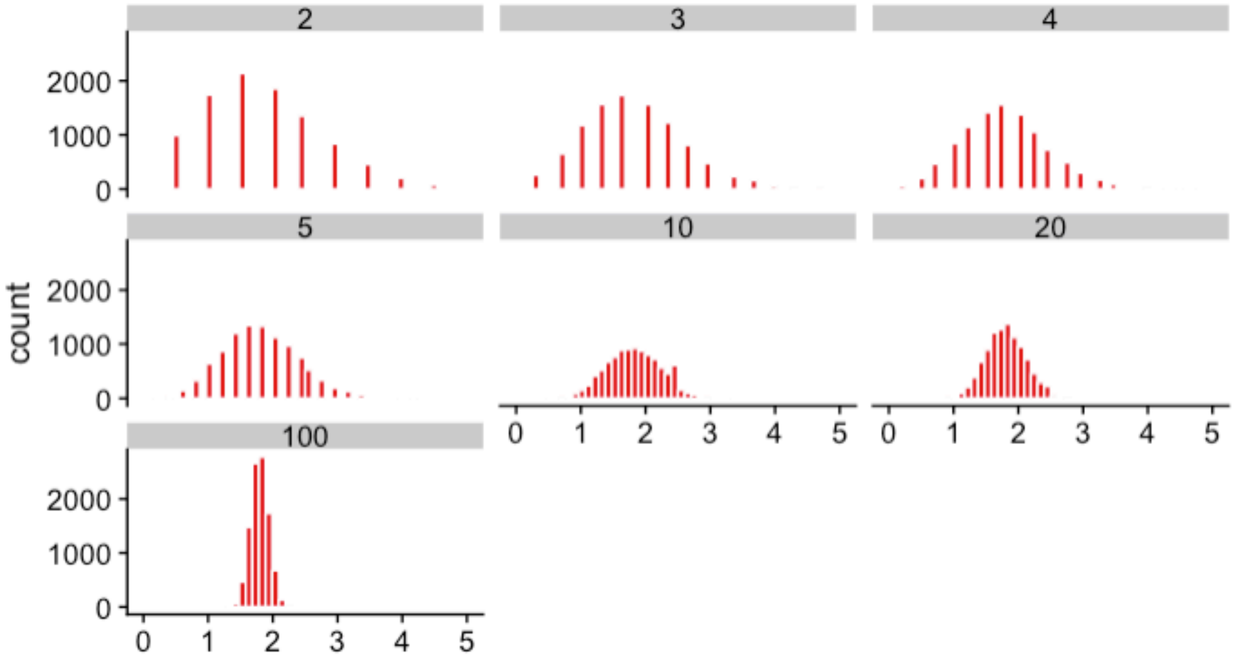


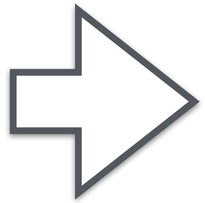
Distribution of means

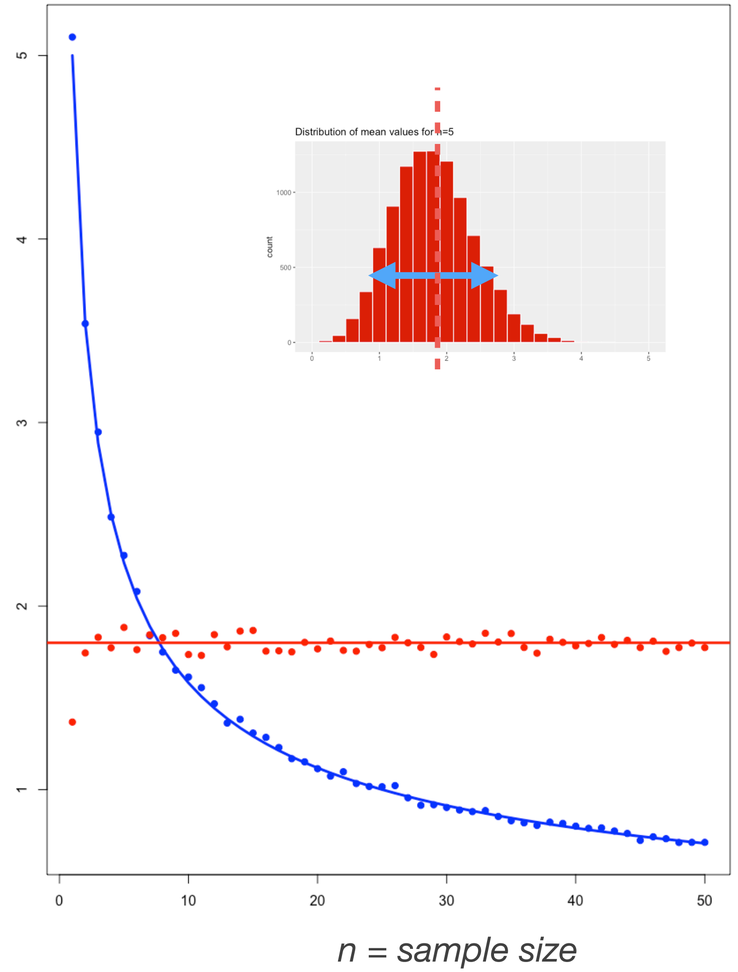
- For increasing sample size n:
  - distribution of mean values **tends to a normal distribution**
  - distribution of mean values **becomes narrower**
- **less fluctuations of the mean between samples**



Distribution of means







$$M_n \longrightarrow \mathcal{N}(\mu, \sigma/\sqrt{n})$$

mean of the sample is a good ("unbiased") estimator of the expectation

Standard error vs. Standard deviation

- **Standard deviation** describes the *spread in a distribution*

$$\text{Standard deviation} : \sigma$$

- **Standard error** measures the *spread in the estimation of the mean*

$$\text{Standard error} : \frac{\sigma}{\sqrt{n}}$$

Central Limit Theorem

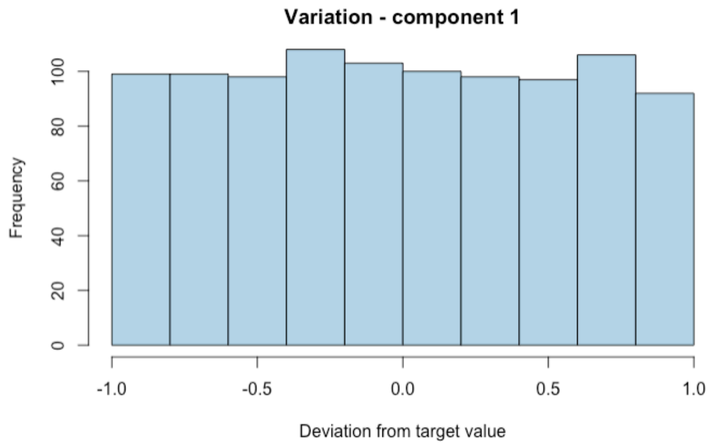
- $\{X_1, X_2, \ldots, X_n\}$ *n* random variables such that
  - independent
  - identically distributed
  - $E[X_i] = \mu$
  - $Var(X_i) = \sigma^2$

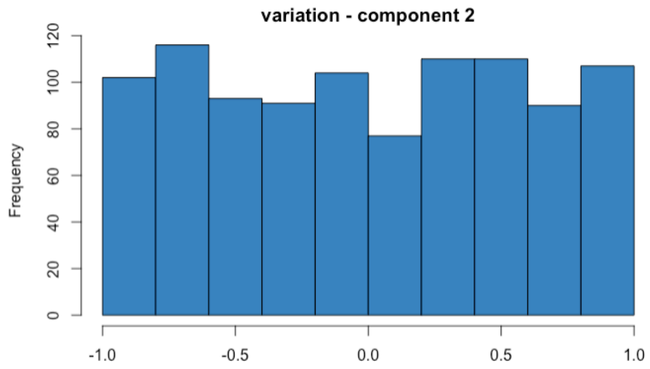
$$\bar{X} = \frac{1}{n}\sum_{i=1}^{n} X_i \quad \xrightarrow[n \to \infty]{} \quad \mathcal{N}(\mu, \frac{\sigma^2}{n})$$

$$\frac{\bar{X} - \mu}{\sigma/\sqrt{n}} \quad \xrightarrow[n \to \infty]{} \quad \mathcal{N}(0,1)$$

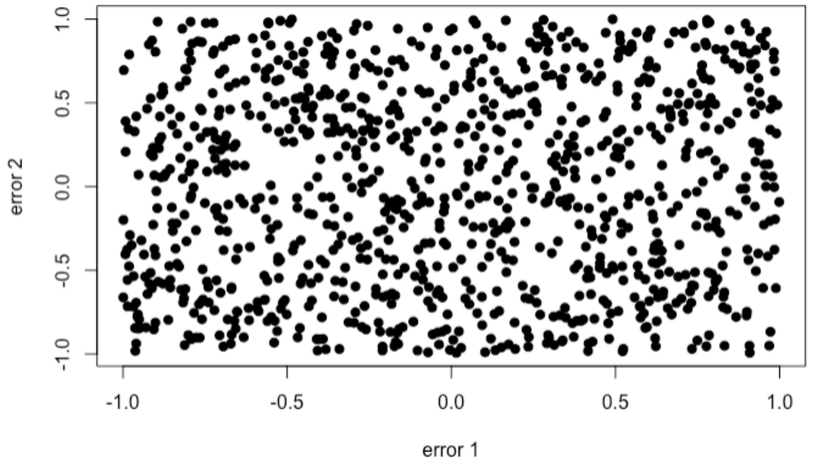
Central Limit Theorem

- **Example**: effect of multiple sources of errors
- Production process - multiple sources of error X_i on the final weight of the product
- Assume these errors are i.i.d (independent, identically distributed - for example uniformly distributed)





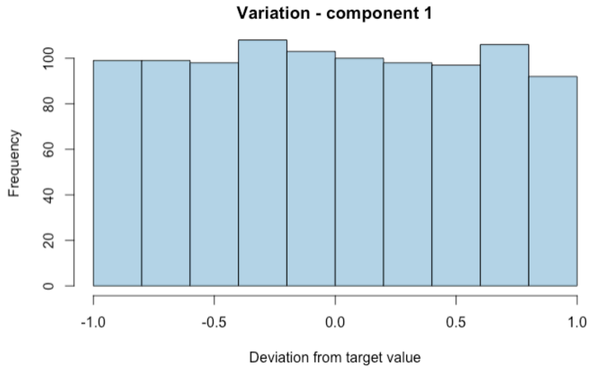
identically distributed (uniform)

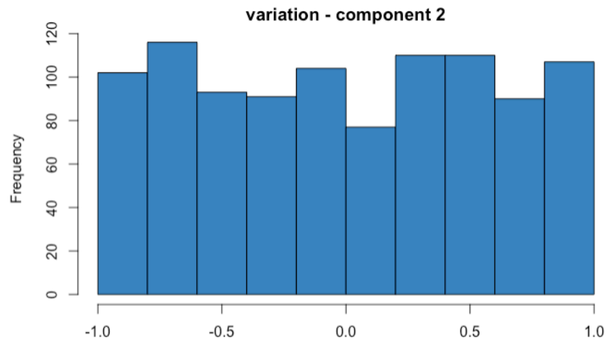


independent (no correlation)

Central Limit Theorem

- **Example**: effect of multiple sources of errors
- Total error on final product = mean of errors (for example on weights)



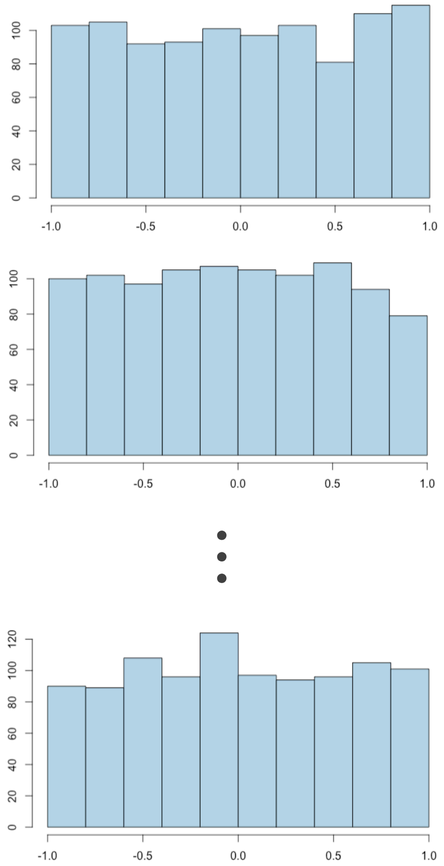


mean

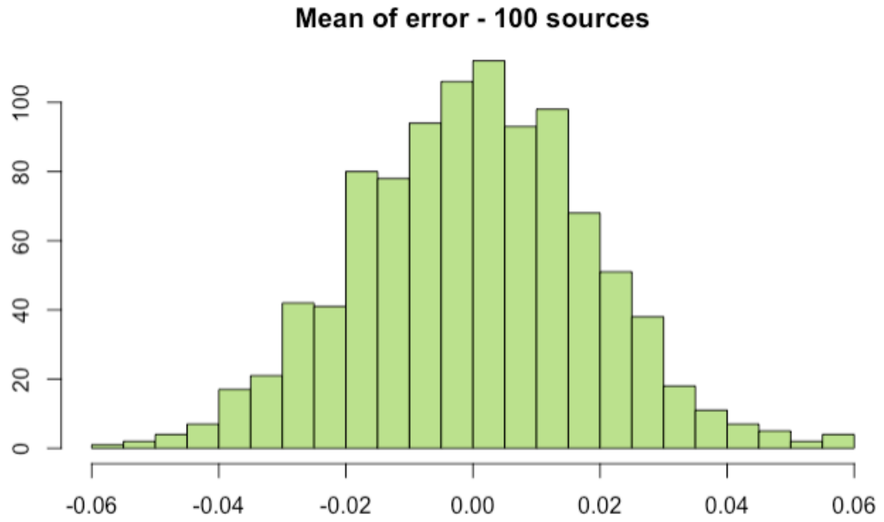
This is no longer a uniform distribution!

Central Limit Theorem

- **Example**: effect of multiple sources of errors
- Total error on final product = sum of errors (for example on weights)



mean



Converges to a normal distribution!

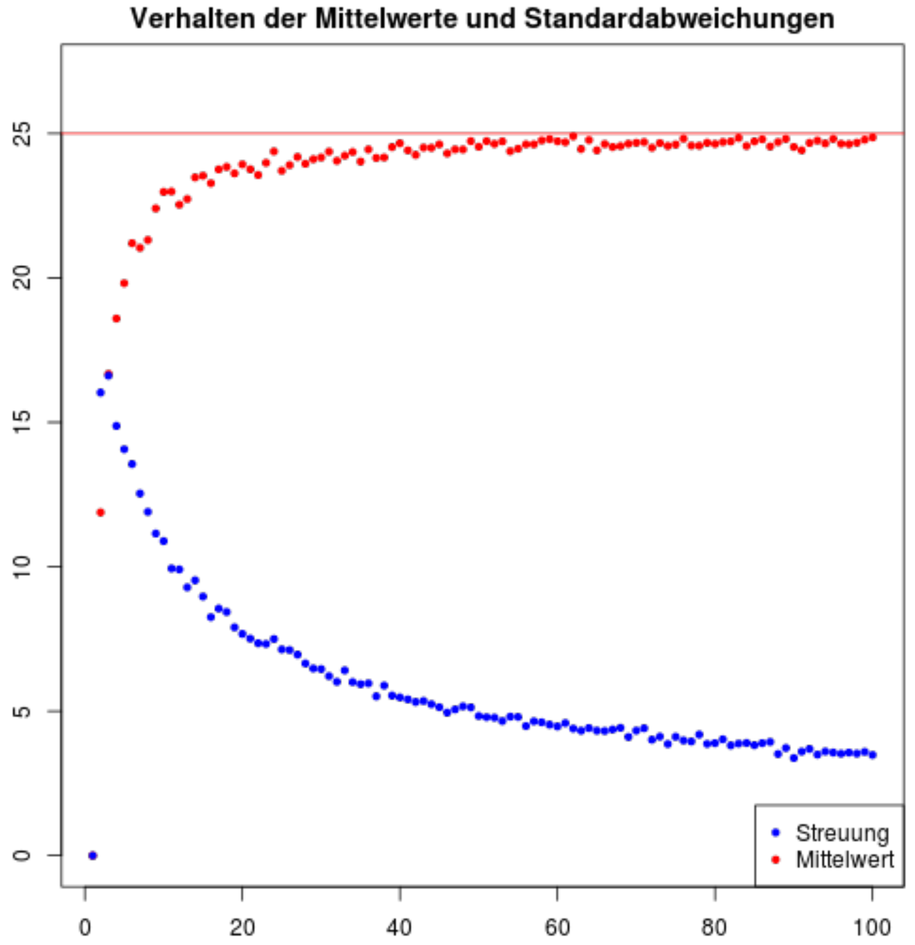
Estimation of variance

- Estimated sample variance

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

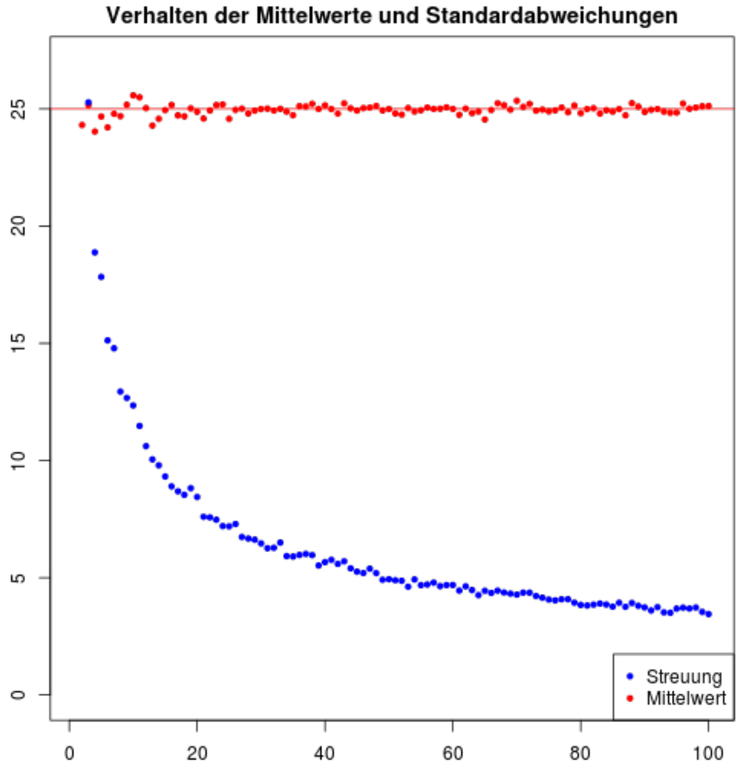
- converges **only asymptotically** to the true variance of the random variable

sample variance is a <u>biased</u> estimator of the population variance!



Estimating variance

$$Var_n = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2 \longrightarrow Var_n = \frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2$$



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

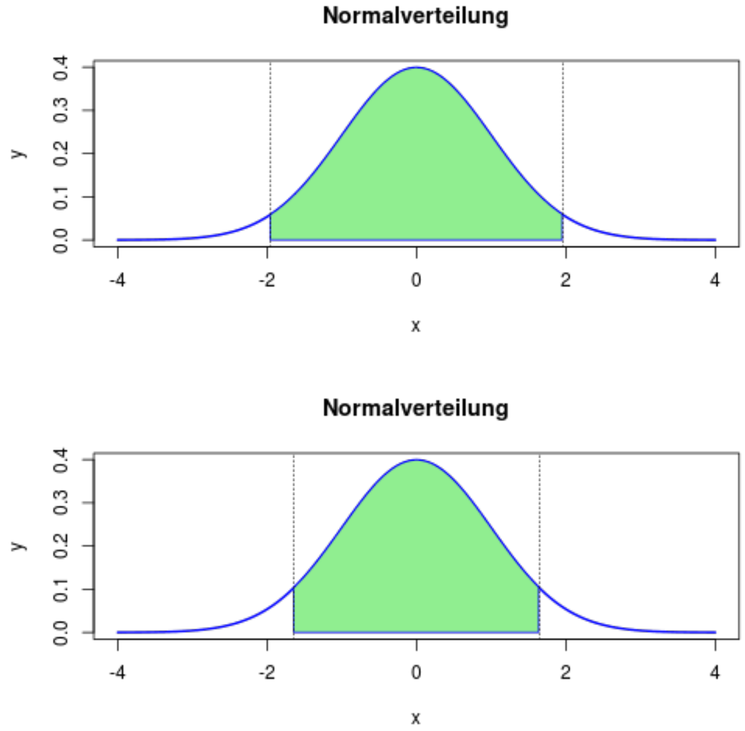
is a unbiased estimator of the population variance!

Confidence interval

$$M_n \xrightarrow{n \gg 1} \mathcal{N}(\mu, \frac{\sigma}{\sqrt{n}})$$

Central Limit Theorem

$$\mathrm{M}_n \sim \mathcal{N}(\mu, \frac{\sigma}{\sqrt{n}}); \frac{M_n - \mu}{\sigma/\sqrt{n}} \sim \mathcal{N}(0,1)$$



- in 95% of the cases, $\left|\frac{m_n - \mu}{\sigma/\sqrt{n}}\right|$ is smaller than t_{95}=1.96
- 95% confidence interval:

$$\mu \in [m_n - t_{95}\frac{\sigma}{\sqrt{n}} \; ; \; m_n + t_{95}\frac{\sigma}{\sqrt{n}}]$$

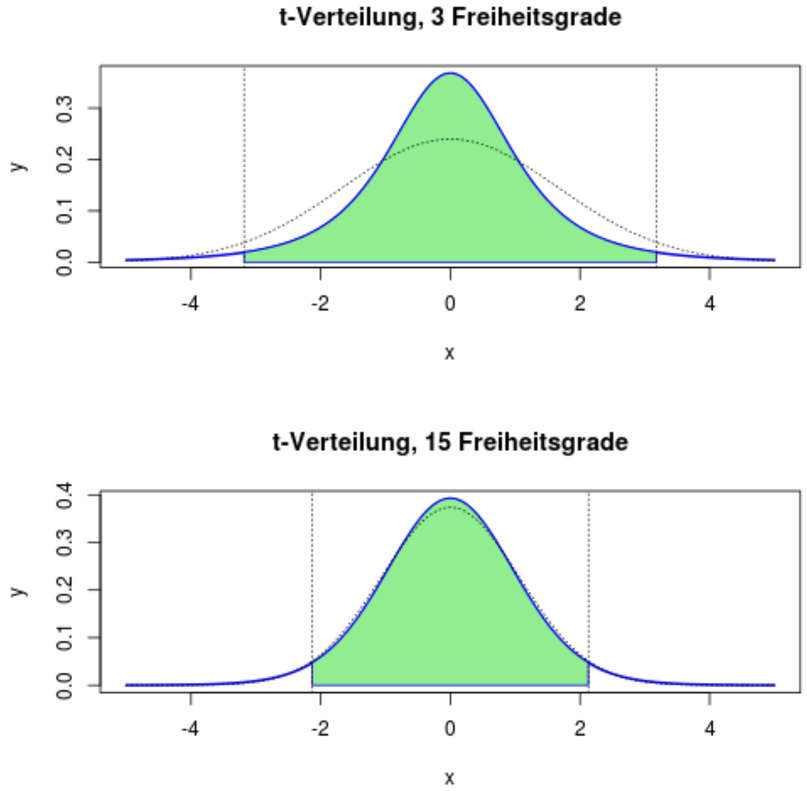
- **Problem**: we do not know the value of the population standard deviation σ
- Replace by its estimator

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

Confidence interval

for small samples:

$$\frac{M_n - \mu}{\sigma/\sqrt{n}} \sim t_{n-1}$$



- in 95% of the cases, $\left|\frac{m_n - \mu}{\sigma/\sqrt{n}}\right|$ is smaller than $t_{95,n-1}$
- *95% confidence interval:*

$$\mu \in [m_n - t_{95,n-1}\frac{\sigma}{\sqrt{n}} \;;\; m_n + t_{95,n-1}\frac{\sigma}{\sqrt{n}}]$$

- **Problem**: we do not know the value of the population standard deviation σ
- Replace by its estimator

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

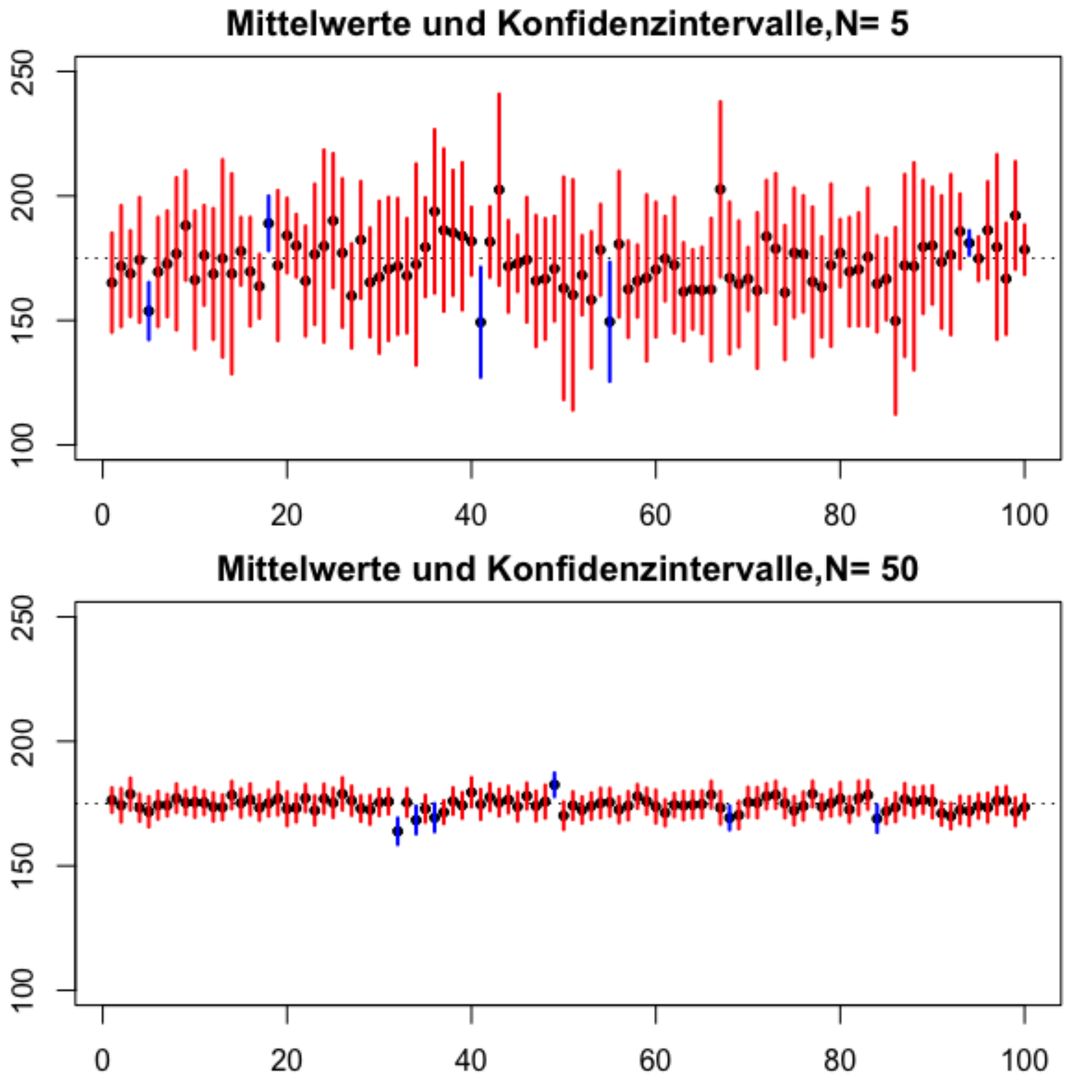
Critical values for t-distribution

df	t_95	t_90
2	4.302653	2.919986
3	3.182446	2.353363
4	2.776445	2.131847
5	2.570582	2.015048
8	2.306004	1.859548
10	2.228139	1.812461
15	2.131450	1.753050
20	2.085963	1.724718
50	2.008559	1.675905
100	1.983972	1.660234
200	1.971896	1.652508
Normal	1.959964	1.644854

Interpretation of confidence interval

- 95% confidence interval
  - 100 samples with n elements
  - sample distribution with expectation value μ
  - estimate mean and CI

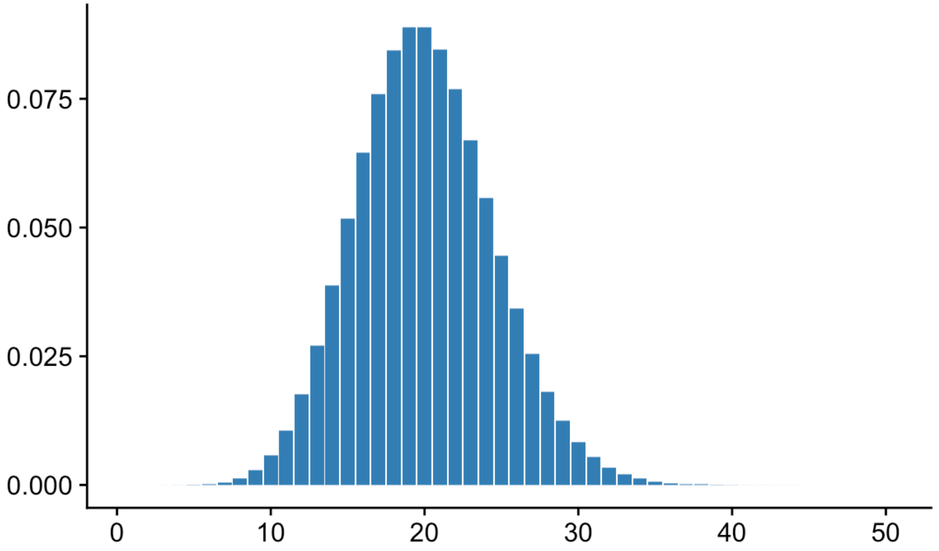
- True expectation value lies in 95% of the cases within the confidence interval



Confidence interval for Poisson distributions

- I find k=20 raisins in my raisin bun → what's in the recipe?
- Number of raisins *N* per bun is a **random variable**, described by a Poisson distribution

$$N \sim Pois(\lambda = k) \qquad Pois(k) \overset{k \gg 1}{\longrightarrow} \mathcal{N}(k, \sqrt{k})$$



$$\mu \in [m_n - t_{95}\frac{\sigma}{\sqrt{n}} \; ; \; m_n + t_{95}\frac{\sigma}{\sqrt{n}}]$$

(Sample size n=1 !)

$$\mu \in [k - t_{95}\sqrt{k} \; ; \; k + t_{95}\sqrt{k}]$$

$$11.2 \leq \mu \leq 28.8$$

Confidence interval Poisson distribution

How to reduce the confidence interval?

- **eat more buns!** n=10 samples with 1 bun
- Compute the mean over the sample $\bar{k} = 20.2$

28 17 22 19 18 ...

$$\mu \in [m_n - t_{95}\frac{\sigma}{\sqrt{n}} \; ; \; m_n + t_{95}\frac{\sigma}{\sqrt{n}}]$$

$$\mu \in [\bar{k} - t_{95}\frac{\sqrt{\bar{k}}}{\sqrt{10}} \; ; \; \bar{k} + t_{95}\frac{\sqrt{\bar{k}}}{\sqrt{10}}]$$

$$17.4 \leq \mu \leq 22.9$$