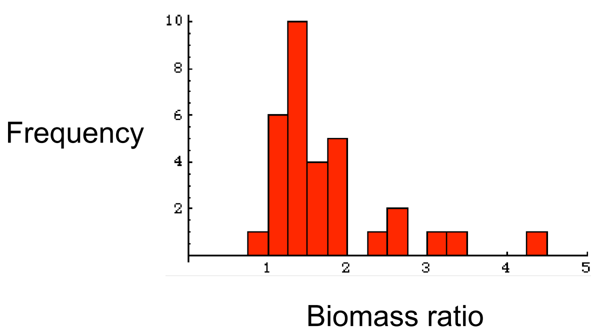
Assumptions of *t*-tests

- Random sample(s)
- Populations are normally distributed
- (for 2-sample *t*) Populations have equal variances

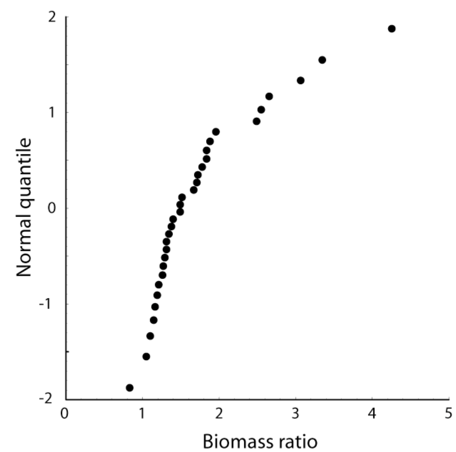
Detecting deviations from normality

- Previous data/ theory
- Histograms
- Quantile plots
- Shapiro-Wilk test

Detecting deviations from normality: by histogram

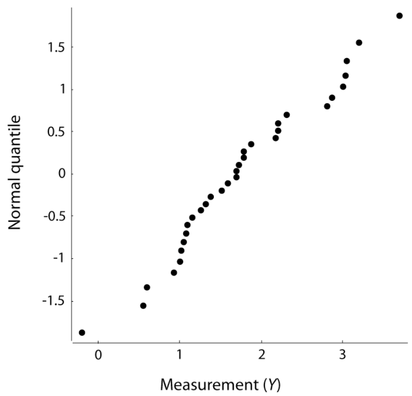


Detecting deviations from normality: by quantile plot



Detecting deviations from normality: by quantile plot

Normal data



Detecting differences from normality: Shapiro-Wilk test

A *Shapiro-Wilk test* is used to test statistically whether a set of data comes from a normal distribution.

What to do when the assumptions are not true

- If the sample sizes are large, sometimes the parametric tests work OK anyway
- Transformations
- Non-parametric tests
- Randomization and resampling

The normal approximation

- Means of large samples are normally distributed
- So, the parametric tests on large samples work relatively well, even for non-normal data.
- Rule of thumb- if $n >$ ~50, the normal approximations may work

Parametric tests - Unequal variance

- Welch's *t*-test would work

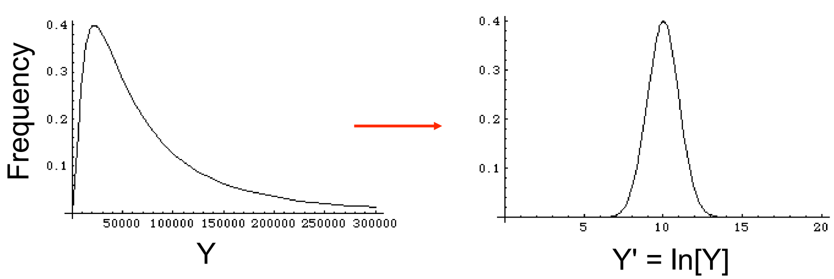
- If sample sizes are equal and large, then even a ten-fold difference in variance is *approximately* OK

Data transformations

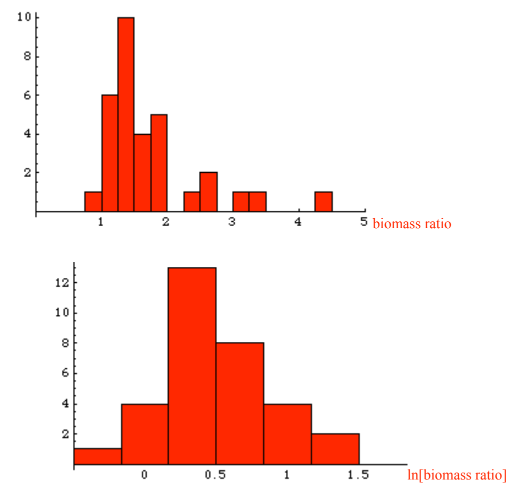
A *data transformation* changes each data point by some simple mathematical formula.

Log-transformation

$$Y' = \ln[Y]$$



Biomass ratio	ln[Biomass Ratio]
1.34	0.30
1.96	0.67
2.49	0.91
1.27	0.24
1.19	0.18
1.15	0.14
1.29	0.26

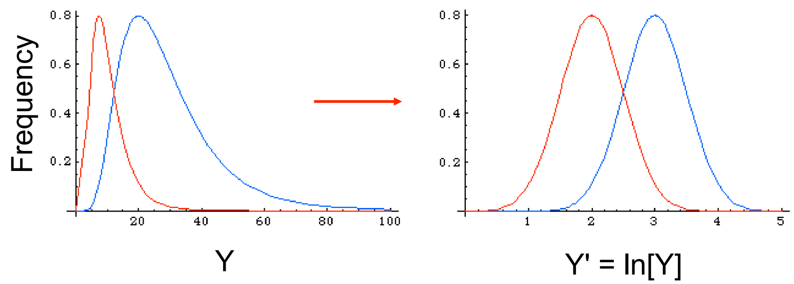


Carry out the test on the transformed data!

The log transformation is often useful when:

- the variable is likely to be the result of multiplication of various components.
- the frequency distribution of the data is skewed to the right
- the variance seems to increase as the mean gets larger (in comparisons across groups).

Variance and mean increase together --> try the log-transform



Other transformations

Arcsine	$p' = \arcsin\left[\sqrt{p}\right]$
Square-root	$Y' = \sqrt{Y + 1/2}$
Square	$Y' = Y^2$
Reciprocal	$Y' = \frac{1}{Y}$
Antilog	$Y' = e^Y$

Example: Confidence interval with log-transformed data

Data:	5	12	1024	12398
Log data:	1.61	2.48	6.93	9.43

$\bar{Y}' = 5.11$ $\quad s_{\ln[Y]} = 3.70$

$$\bar{Y}' \pm t_{0.05(2),3} \frac{s_{\ln[Y]}}{\sqrt{n}} = 5.11 \pm 3.18 \frac{3.70}{\sqrt{4}} = 5.11 \pm 5.88$$

$$-0.773 < \mu_{\ln[Y]} < 10.99$$

$$e^{-0.773} < e^{\mu_{\ln[Y]}} < e^{10.99}$$

$$0.46 < \mu_G < 59278$$

Valid transformations...

- Require the same transformation be applied to each individual
- Have one-to-one correspondence to original values
- Have a monotonic relationship with the original values (e.g., larger values stay larger)

Choosing transformations

- Must transform each individual in the same way
- You CAN try different transformations until you find one that makes the data fit the assumptions
- You CANNOT keep trying transformations until P <0.05!!!

Non-parametric methods

- Assume less about the underlying distributions
- Also called "distribution-free"
- "Parametric" methods assume a distribution or a parameter

Most non-parametric methods use RANKS

- Rank each data point in all samples from lowest to highest
- Lowest data point gets rank 1, next lowest gets rank 2, ...

Sign test

- Non-parametric test
- Compares data from one sample to a constant
- Simple: for each data point, record whether individual is above (+) or below (-) the hypothesized constant.
- Use a binomial test to compare result to 1/2.

Example: Polygamy and the origin of species

- Is polygamy associated with higher or lower speciation rates?

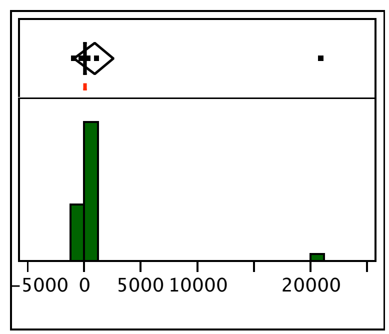
Arnqvist *et al*. (2000) Sexual conflict promotes speciation in insects. *PNAS* 97:10460-10464.

Order	Family	Multiple mating group	Number of species	Single mating group	Number of species
Beetles	Anobiidae	Ernobius	53	Xestobium	10
	Dermestidae	*Dermestes*	73	*Trogoderma*	120
	Elateridae	*Agriotes*	228	*Selatosomus*	74
Flies	Muscidae	*Coenosia*	353	*Delia*	289
	Cecidomyiidae	*Rhopalomyia*	157	*Mayetiola*	30
	Chironomidae	*Chironomus*	300	*Pontomyia*	4
	Chironomidae	*Stictochironomus*	34	*Clunio*	18
	Drosophilidae and Culicidae	Drosophilidae	3,400	Culicidae	3,500
	Dryomyzidae and Calliphoridae	Dryomyzidae	20	Calliphoridae	1,000
	Tephritidae	*Anastrepha*	196	*Bactrocera*	486
	Sciaridae and Bibionidae	Sciaridae	1,750	Bibionidae	660
	Scatophagidae	*Scatophaga*	55	*Musca*	63
Mayflies	Siphlonuridae	*Siphlonurus*	37	*Caenis*	115
Homoptera	Psyllidae	*Cacopsylla*	100	*Aonidiella*	30
Butterflies and moths	Noctuidae and Psychidae	Noctuidae	21,000	Psychidae	600
	Tortricidae	*Choristoneura*	37	*Epiphyas*	40
	Nymphalidae	*Eueides (aliphera* clade*)*	7	*Eueides (vibilia* clade*)*	5
	Nymphalidae	*Heliconius (silvaniform* clade*)*	15	*Heliconius (sarasapho* clade*)*	7
	Nymphalidae	*Polygonia /*	18	*Nymphalis*	6

Etc....

The differences are not normal

43	-47	154	64	127	296	16
-100	-980	-290	1090	-8	-78	70
20940	-3	2	8	12	227	1
61	1	79	78			



Hypotheses

H_0: The median difference in number of species between singly-mating and multiply-mating insect groups is 0.

H_A: The median difference in number of species between these groups is not 0.

7 out of 25 comparisons are negative

43	-47	154	64	127	296	16
-100	-980	-290	1090	-8	-78	70
20940	-3	2	8	12	227	1
61	1	79	78			

$$\Pr[X \leq 7] = \sum_{i=0}^{7} \binom{25}{i} (0.5)^{i} (0.5)^{25-i} = 0.02164$$

P = 2 (0.02164) = 0.043

The sign test has very low power

So it is quite likely to *not* reject a *false* null hypothesis.

Non-parametric test to compare 2 groups

The *Mann-Whitney* U *test* compares the central tendencies of two groups using ranks.

Performing a Mann-Whitney U test

- First, rank all individuals from both groups together in order (for example, smallest to largest)
- Sum the ranks for all individuals in each group --> R_1 and R_2

Calculating the test statistic, *U*

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1$$

$$U_2 = n_1 n_2 - U_1$$

U_1 is the number of times an individual from pop. 1 has a lower rank than an individual from pop. 2, out of all pairwise comparisons.

Example: Garter snake resistance to newt toxin

Rough-skinned newt

Comparing snake resistance to TTX (tetrodotoxin)

Locality	Resistance
Benton	0.29
Benton	0.77
Benton	0.96
Benton	0.64
Benton	0.70
Benton	0.99
Benton	0.34
Warrenton	0.17
Warrenton	0.28
Warrenton	0.20
Warrenton	0.20
Warrenton	0.37

This variable is known to be not normally distributed within populations.

Hypotheses

H_0: The TTX resistance for snakes from Benton is the same as for snakes from Warrenton.

H_A: The TTX resistance for snakes from Benton is different from snakes from Warrenton.

Calculating the ranks

Locality	Resistance	Rank
Benton	0.29	5
Benton	0.77	10
Benton	0.96	11
Benton	0.64	8
Benton	0.70	9
Benton	0.99	12
Benton	0.34	6
Warrenton	0.17	1
Warrenton	0.28	4
Warrenton	0.20	2.5
Warrenton	0.20	2.5
Warrenton	0.37	7

Rank sum for Warrenton: R=1+4+2.5+2.5+7=17

Calculating U_1 and U_2

$$U_1 = n_1 n_2 + \frac{n_1(n_1+1)}{2} - R_1 = 5(7) + \frac{5(6)}{2} - 17 = 33$$

$$U_2 = n_1 n_2 - U_1 = 5(7) - 33 = 2$$

For a two-tailed test, we pick the larger of U_1 or U_2:

$$U = U_1 = 33$$

Compare U to the U table

n_1

n_2	3	4	5	6	7	8	9	10	11	12	13	14	15
3	–	–	15	17	20	22	25	27	30	32	35	37	40
4	–	16	19	22	25	28	32	35	38	41	44	47	50
5	15	19	23	27	$\boxed{30}$	34	38	42	46	49	53	57	61
6	17	22	27	31	36	40	44	49	53	58	62	67	71
7	20	25	30	36	41	46	51	56	61	66	71	76	81
8	22	28	34	40	46	51	57	63	69	74	80	86	91
9	25	32	38	44	51	57	64	70	76	82	89	95	101
10	27	35	42	49	56	63	70	77	84	91	97	104	111
11	30	38	46	53	61	69	76	84	91	99	106	114	121
12	32	41	49	58	66	74	82	91	99	107	115	123	131
13	35	44	53	62	71	80	89	97	106	115	124	132	141
14	37	47	57	67	76	86	95	104	114	123	132	141	151
15	40	50	61	71	81	91	101	111	121	131	141	151	161

Compare U to the U table

- Critical value for U for n_1 =5 and n_2=7 is 30
- 33 ≥ 30, so we can reject the null hypothesis
- Snakes from Benton have a different distribution of resistance to TTX than the Warrenton snakes.

How to deal with ties

- Determine the ranks that the values would have got if they were slightly different.
- Average these ranks, and assign that average to each tied individual
- Count all those individuals when deciding the rank of the next largest individual

Ties

Group	*Y*	*Rank*
2	12	1
2	14	2
1	17	3
1	19	4.5
2	19	4.5
1	24	6
2	27	7
1	28	8

Mann-Whitney: Large sample approximation

For n_1 and n_2 both greater than 10, use

$$Z = \frac{2U - n_1 n_2}{\sqrt{n_1 n_2 (n_1 + n_2 + 1)/3}}$$

Compare this *Z* to the standard normal distribution

Example:

U_1=245 U_2=80
n_1=13 n_2=25

$$Z = \frac{2U - n_1 n_2}{\sqrt{n_1 n_2 (n_1 + n_2 + 1)/3}}$$
$$= \frac{2(245) - 13(25)}{\sqrt{13(25)(13 + 25 + 1)/3}}$$
$$= 2.54$$

$Z_{0.05(2)}$=1.96, Z>1.96, so we could reject the null hypothesis

Assumptions of Mann-Whitney U test

Both samples are random samples.

Both populations have the same shape of distribution.