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# Remember: random sampling minimizes sampling error, uncertainty & inferential bias (i.e., how close or far sample values for the statistic of interest are from the true population value for that statistic)

The common requirement for statistical inference is that data come from a **random sample**. A random sample is one that fulfills two criteria:

**1)** Every observational unit in the population (e.g., individual tree) have an **equal chance** of being included in the sample.

2) The selection of observational units in the population (e.g., individual tree) must be **independent**, i.e., the selection of any unit (e.g., individual tree) of the population must not influence the selection of any other unit.

Samples are biased when some observational units of the intended population have lower or higher probabilities to be sampled.









Statistical inferential process: The role of sampling theory in parameter estimate and estimating confidence intervals

"The purpose of statistical inference is to develop theory and methods to make inference on the unknown parameters based on observed data" (Hong, 2017)





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If sampling is random and distributional properties of the population are met (e.g., observations are dichotomous), then two critical statistical features will happen:

[1] 95% of the "infinite" (really large value) number of confidence intervals that could be built based on each possible sample from a given population will contain the true population value.

[2] Because of statement 1, we can be then 95% confident that the interval estimated based on a single sample contains the true population value of the population from where that sample was taken!

Why not a 100% confidence interval? There are some important reasons for that, but one is because intervals (smaller than 100%) become narrower with increased sample sizes (100% intervals would cover the entire possible range for the parameter which is not very useful for inference in general; and in many cases would go from  $-\infty$  and  $+\infty$ ; and here would go from 0% to 100%).

























For any given sample confidence interval, we can state that "we are 95% confident that the true population mean lies between the lower and upper limits of the interval".

We cannot say that "there is a 95% probability that the true population mean lies within the confidence interval". Either the parameter is within the interval or not!





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# Confidence intervals are not well grasped by many (probably most) users of statistics! RECAP

Confidence intervals is a concept based on sampling theory.

Here, sampling theory relates to repeated sampling making certain assumptions about the statistical population.

We use the principle of repeated sampling to model the expectations of sampling variation. Under repeated sampling, if we were to estimate a confidence interval for each sample, 95% of them would contain the true population parameter.

As such, we can be confidence that one single sample confidence interval (i.e., we usually only have one sample) will most likely contain the true population value.

A large confidence interval (e.g., 95% or 99%) provides a most plausible range for a parameter (true population value). Values lying within the interval are most plausible, whereas values outside are less plausible, based on the sample data alone.

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#### How are confidence intervals computationally derived?

Building knowledge about sampling theory using the "bag approach"

Let's go back to the original results of Bisazza et al. (1996) in which 14 toads were 14 right-legged and 4 were left-legged (i.e., 77.8% right- and 22.2% left-legged). Let's estimate its confidence interval based on the "bag approach"

#### BUT FIRST REMEMBER:

[1] 95% of the "infinite" (really large value) confidence intervals that could be built based on each possible sample from a given population will contain the true population value.

[2] Because of statement 1, we can be then 95% confident that the interval estimated based on a single sample contains the true population value of the population from where that sample was taken!















## How are confidence intervals computationally derived?

Building knowledge about sampling theory using the "bag approach"

For binomial distributions, i.e., distributions that have two possible outcomes (here right- and left-legged individuals), there are a few different ways to estimate confidence intervals – and they differ somewhat, particularly when sample sizes are smaller.



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#### PREVIOUS SLIDE INTO "SOLID" WORDS

#### Statistical inferential process: The role of sampling theory?

Estimating uncertainty from sample-based values (information) that allows generalization to entire populations

The basic idea of statistical inference is to assume that the observed data (e.g., 77.8% of frogs were right-legged) is generated from a probability distribution (all possible sample values for the population of interest; e.g., frogs) which is modelled by a function in the form of a probability distribution (e.g., Exact, Wilson, Asymptotic).

Sampling theory is applied to predict sampling uncertainty from sample estimates that is then used to estimate uncertainty. The prediction is made by making assumptions about certain aspects of the sample or populations to estimate the sampling distribution for the value of interest (e.g., number of right- and left-legged toads).

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# statistical hypothesis testing is an intimate stranger!!

Most users know how to implement and interpret it, but they don't really understand its philosophy and how it really works.

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#### Tackling research hypotheses using the framework of statistical hypothesis testing

The **statistical hypothesis framework** (most often involving statistical tests) is a quantitative method of statistical inference that allows to generate evidence for or against a research hypothesis.

CONFUSING: BUT ONLY GENERATES SUPPORT AGAINST THE STATISTICAL NULL HYPOTHESIS (NOT FOR). It also doesn't generate support for (or against) the alternative hypothesis.

But by building support AGAINST a statistical null hypothesis, one builds support FOR research hypothesis.

A small p-value makes us reject the null hypothesis of equal proportion of limb usage and therefore provides support to the research hypothesis of handedness.



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# $R_{\rm i}$



















Statistical hypothesis testing also estimates p	Number of right- handed toads	Probability of those samples
reject versus reject). In this case, one	0	0.000004
estimates the confidence interval for a sample value that is truly	1	0.00007
	2	0.0006
	3	0.0031
	4	0.0117
R R R L, R	5	0.0327
I RR R I I I I I I I I I I I I I I I I	6	0.0708
	7	0.1214
	8	0.1669
	9	0.1855
	10	0.1669
	11	0.1214
Assume that 50% of toad population is right-legged and 50% arts left- handed. Assume this population to be mathematically infinite.	12	0.0708
	13	0.0327
	14	0.0117
	15	0.0031
	16	0.0006
	17	0.00007
	18	0.000004

1.0

Total

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The p-value is NOT the probability that the null hypothesis is true. IT IS the probability of observing a value of the test statistic that is as or more extreme than what was observed in the sample, assuming the null hypothesis is true.

The p-value is a measure of consistency between the sample data and the theoretical hypothesis assumed when stating the parameter for a theoretical population of no interest (null hypothesis, e.g., toads have equal number of individuals right and left-handed)







## Decision in statistical hypothesis testing - what do P-values represent?

The  $\ensuremath{\textbf{p-value}}$  is the probability of the observed sample data assuming that the null hypothesis is true.

The smallest the P-value, the stronger the evidence against the initial assumption (model) based on the parameter assumed for the theoretical population (i.e., null hypothesis).

That's not to say that handedness is true OR false but rather that we have strong evidence to say that lack of handedness (i.e., 50%/%50) is unlikely.

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 Decision in statistical hypothesis testing – what do P-values represent?

 P = 0.031

 AGAIN, and VERY IMPORTANT, and also "confusing":

 So, we can say that we have evidence to reject the null statistical hypothesis BUT we cannot say that we have evidence to accept the alternative statistical hypothesis.

 That's because we made our decision based on the sampling distribution of values expected under chance alone from a population where the null hypothesis H0 is true (i.e., 50% right-legged and 50% left-legged).

 BUT, by rejecting the statistical null hypothesis, we build evidence towards the research hypothesis and not towards accepting the alternative hypothesis (remember that the null distribution is built based

on the null as there are infinite possible alternative hypothesis).

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## Decision in statistical hypothesis testing - what do P-values represent?

Research hypotheses cannot be proven right or wrong from the data. Hypotheses can be said to be either refuted (evidence is against the research hypothesis) or supported (evidence is in favour of the research hypothesis) by the data generated.

The p-value is NOT the probability that the null hypothesis is true. IT IS the probability of observing a value of the test statistic that is as or more extreme than what was observed in the sample, assuming the null hypothesis is true.

The p-value is a **measure** of **consistency** between the sample data and the theoretical hypothesis assumed when stating the parameter for a theoretical population of no interest (null hypothesis, e.g., toads have equal number of individuals right and left-handed)

### The process of statistical hypothesis testing:

Statistical hypothesis testing asks how unusual it is to get the observed value for the sample data within the distribution built assuming the null hypothesis as true.

Statistical hypotheses are about populations but are tested with data from samples.

Statistical hypothesis (usually) assumes that sampling is random.

The null hypothesis is usually the simplest statement, whereas the alternative hypothesis is usually the statement of greatest interest.

A null hypothesis is often specific (specific parameter for the theoretical population); an alternative hypothesis often is not.

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#### What does the significance level ( $\alpha$ level) represent?

There is disagreement among statisticians and users about whether to reject or not reject (referred as to thresholding) statistical hypotheses based on p-values.

i.e., whether to use  $\alpha$  as a threshold for making a decision to state whether a p-value is non-significant (do not reject H<sub>0</sub>) or a p-value is significant (reject H<sub>0</sub> in favour of H<sub>A</sub>).

Although I agree with these arguments it is unlikely that radical changes will arrive in research behaviour any time soon!

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#### The don'ts about P values and hypothesis testing (Wasserstein et al. 2019)

1. P-values indicate how incompatible the observed data are with a specified statistical model (e.g., the one assumed under  ${\sf H}_0).$ 

 $\ensuremath{\mathbf{2}}$  . P-values do not measure the probability that the studied research hypothesis is true.

 Scientific conclusions and business or policy decisions should not be based only on whether a p-value passes a specific threshold (alpha) – (even though they currently are)

4. A p-value, or statistical significance, does not measure the biological importance of a result.

- There are other important don'ts that we will see later in the course.

The Adversaria Statisticals



# The don'ts about P values and hypothesis testing (Wasserstein et al. 2019)

Despite the limitations of p-values, we are not recommending that the calculation and use of p-values be discontinued. Where p-values are used, they should be reported as continuous quantities (e.g., p = 0.08) and not yes/no reject the null hypothesis.

The biggest push today is to abandon the idea of statistical significance. In other words, to abandon the almost universal and routine practice to state that if the probability is smaller than or equal to alpha, than we should state that the results are significant.

Abandoning significance is easily said than done. The majority of researchers do report results as significant or non-significant. We will try to guide you in a more nuanced ways in our course but it's hard to get away from this common culture in the statistical applications in biology and in most other fields.

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