

Tutorials are not just exercises or applications.

They are intentionally designed to complement and strengthen the material covered in lectures, helping you develop a deeper understanding of key concepts.



### Tutorial 2: Statistical Hypothesis Testing

The principles of statistical hypothesis testing: generating evidence-based conclusions without complete biological knowledge!

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Statistics does not remove **uncertainty** - it formalizes it and communicates **confidence**.

In statistics we quantify **uncertainty** to justify **confidence**.

Understanding these two ideas is essential for developing lasting statistical reasoning.

They form the foundation of statistical inference and hypothesis testing.



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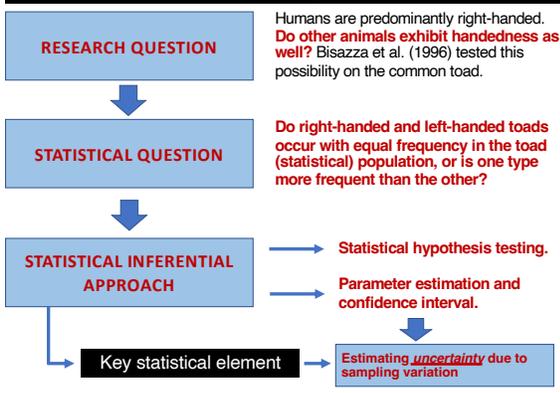
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#### Schematic summary of lecture 2



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**Statistical inference:** the process of quantifying *uncertainty* due to sampling variation in order to draw conclusions from samples (despite incomplete knowledge) about entire populations.

**Sampling variation drives statistical uncertainty.**  
Observed proportions differ from the true population value simply because samples are random. Some samples will be closer to the true value than others, meaning the level of uncertainty varies across samples.

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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.

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**Statistical inference:** We use sampling theory - either computationally based or calculus based (more common) - to derive the sampling distribution needed to quantify uncertainty around a sample estimate of interest (e.g., the proportion of right- and left-legged toads).

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**Estimation [ & associated confidence intervals] and statistical hypothesis testing agree but have different interpretations**

45% right-handed      65% right-handed  
 $H_0$  55%      Don't reject  $H_0$ ;  $p > 0.05$   
 "Quantitative" statement      "Qualitative" statement

30% RH      48% RH  
 $H_0$  39%      Reject  $H_0$ ;  $p < 0.05$   
 "Quantitative" statement      "Qualitative" statement

*Remember*

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**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)

we can state that we have some confidence (say 95%) that the true parameter of interest (say number of right-legged frogs is between two values)

95% confidence interval for the proportion of right-legged toads  
 52.4% right-legged      93.6% right-legged

77.8%

(14 right- and 4 left-legged toads, i.e., 14/18 = 77.8%)

A large confidence interval (e.g., 95% or 99%) provides a most plausible range for a parameter of interest (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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**Statistical inference**

Operationally simple, but conceptually essential.  
 Understanding the underlying theory is what makes statistics intuitive rather than mechanical.

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> binom.test(14, 18, 0.5, alternative="two.sided")
Exact binomial test

data: 14 and 18
number of successes = 14, number of trials = 18, p-value = 0.03888
alternative hypothesis: true probability of success is not equal to 0.5
95 percent confidence interval:
 0.5242722  0.9359888
sample estimates:
probability of success
 0.7777778
    
```

95% confidence interval for the proportion of right-legged toads  
 52.4% right-legged      93.6% right-legged

77.8%

(14 right- and 4 left-legged toads, i.e., 14/18 = 77.8%)

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**Statistical inference:** The role of sampling theory

If sampling is random and the model assumptions (e.g., normal distribution) are satisfied, two key statistical results follow:

[1] Across a very large number of repeated samples from the same population, 95% of the confidence intervals constructed in this way will contain the true population value.

[2] Because of this long-run (repeated sample) property, we can say that a confidence interval built from a single random sample is associated with 95% confidence of containing the true population value.

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**Statistical inference:** The role of sampling theory

*Why not a 100% confidence interval?*

Because 100% certainty requires intervals so wide that they contain all possible values - and therefore tell us nothing useful.

In many cases, this would mean an interval from  $-\infty$  to  $+\infty$ ; for proportions, from 0% to 100%.

*Statistics trades certainty for information:* Narrow intervals (e.g., 95%) allow learning; 100% intervals guarantee coverage but eliminate inference.

As we accept slightly less certainty (e.g., 95% instead of 100%), the interval becomes narrower, allowing us to make more precise statements about where the true value is likely to lie. However, this precision comes with a small risk of being wrong.

In statistics, we deliberately accept a limited risk of error in order to gain informative, precise estimates. This balance—less certainty for more precision—is what makes statistical inference possible.

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**Statistical inference:** The role of sampling theory

*Why not a 100% confidence interval?*

Absolute certainty requires intervals so wide that they convey no information.

Greater certainty requires wider intervals; greater precision requires accepting some uncertainty.

Precision (confidence interval width) can be increased by increasing sample size (among other features).

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Imagine a weather forecast saying: "Tomorrow's temperature will be between  $-50^{\circ}\text{C}$  and  $+50^{\circ}\text{C}$ ."

That forecast is certain, but completely useless.

A forecast like "between  $14^{\circ}\text{C}$  and  $18^{\circ}\text{C}$ " is far more informative, but it accepts that the true value might occasionally fall outside that range.

*Inference is not about being certain—it is about managing uncertainty. Narrow intervals support decisions, provided we plan for the possibility of being wrong.*

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

More traditionally we use the proportion instead of the numbers  
 (easier to generalize mathematically)

It is expected that 1 sample confidence interval over 20 does not include the true population proportion (i.e., 95%). This interval is in red.

There are different ways to estimate the confidence interval for proportions (here we used the *exact* method which can produce non-symmetric intervals and tend to be wider than the asymptotic estimation).

For now, the estimation method is not important; what's important is the rationale. We will see some more details later in this lecture.

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

Now 1000 estimates for the confidence intervals. The point here is that we can produce a really large number of intervals & 95% of them will contain the true population value

intervals not containing the true parameter are plotted in red (i.e., 5% of the intervals).

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**The role of sample size in increasing confidence and decreasing uncertainty**

One way to improve estimation & reduce uncertainty is to increase sample size.

Remember though that increasing sample may be extremely timely and financially consuming, and even ethically irresponsible (e.g., collecting and manipulating too many individuals that can put biological populations and species at risk).

Sample size = 18

Sample size = 1000

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**Very important & often confusing!**

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".

BUT, we cannot say that "there is a 95% probability that the true population mean lies within the confidence interval".

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**Very important & often confusing!**

*We cannot say that "there is a 95% probability that the true population mean lies within the confidence interval".*

The population parameter (e.g., the true proportion) is a fixed but unknown value; but it does not change from sample to sample.

What does vary is the confidence interval, because it is built from a random sample. Across many repeated samples, some intervals will capture the true value, and some will not.

Once a specific interval is calculated from a single sample, the interval is fixed. At that point, the true parameter is either inside it or outside it, i.e., there is no randomness left.

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**Confidence intervals are not well grasped by a large number of users of statistics!**

CANADIAN JOURNAL OF SCIENCE, MATHEMATICS AND TECHNOLOGY EDUCATION, 44(1), 23-34, 2014  
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DOI: 10.1080/14926156.2014.874615

**Routledge**  
Taylor & Francis Group

**Confidence Trick: The Interpretation of Confidence Intervals**

Colin Foster  
*School of Education, University of Nottingham, Nottingham, United Kingdom*

**Often stated by students and practitioners of statistics:** "there is a 95% probability that the true population mean lies within the confidence interval". We can't state that; either the parameter is within the interval or not! So, no probability attached to this condition.

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Let's take a break - 1 minute



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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 confident that one single sample confidence interval (i.e., we usually only have one sample) will most *likely* (but without a precise probability) 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!

*Confidence is about trust in the method, not a probability attached to the parameter. Once the interval is computed, the true value is either inside it or not.*

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

Assume that 77.7% (14/18 individuals) of toad population is right-legged and 22.2%(4/18 individuals) left-handed. Assume this population to be mathematically infinite.

20 random samples

number of right-legged toads

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

Assume that 77.7% (14/18 individuals) of toad population is right-legged and 22.2%(4/18 individuals) left-handed. Assume this population to be mathematically infinite.

100000 random samples

proportion of right-legged toads in each of the 100000 samples

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

Assume that 77.7% (14/18 individuals) of toad population is right-legged and 22.2%(4/18 individuals) left-handed. Assume this population to be mathematically infinite.

100000 random samples

Calculate the 2.5% percentile and 97.5% percentile as the confidence interval.

proportion of right-legged toads

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**How are confidence intervals computationally derived?**  
 Building knowledge about sampling theory using the "bag approach"

Assume that 77.7% (14/18 individuals) of toad population is right-legged and 22.2%(4/18 individuals) left-handed. Assume this population to be mathematically infinite.

In this way, 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!

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**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.

```

> binconf(14,18,method = "all")
      PointEst Lower Upper
Exact    0.7777778 0.5236272 0.9359088
Wilson   0.7777778 0.5470542 0.9699987
Asymptotic 0.7777778 0.5857194 0.9698362

> binconf(778,1000,method = "all")
      PointEst Lower Upper
Exact    0.778 0.7589431 0.8034691
Wilson   0.778 0.7512054 0.8026670
Asymptotic 0.778 0.7522419 0.8037581
    
```

This may happen because different methods are used to approximate the distribution of a variable.

Some confidence intervals for some distributions can be modelled exactly (e.g., normal) whereas others only approximated (e.g., binomial).

The exact method is better but can be computationally intense (impractical for large sample sizes) compared to the asymptotic (approximation via a normal approximation); the Wilson is better for small sample sizes than the asymptotic.

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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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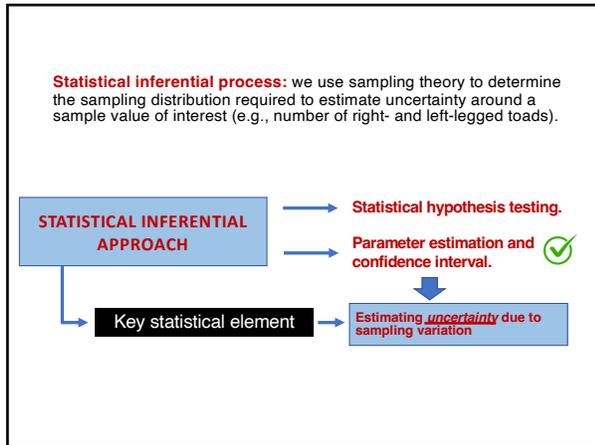
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*Frequentist Statistical Inference: An Intimate Stranger - Routine in use, mysterious in meaning!*

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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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**The intuition behind the framework of statistical hypothesis testing**

You can generate evidence for or against a hypothesis (handedness) using a computational approach (the "bag approach"). All you need is to assume a particular hypothesis as true (**null hypothesis**) and then reject it (or not) in support of an **alternative hypothesis!**

**Null hypothesis ( $H_0$ ):** the proportion of right- and left-handed toads in the population ARE equal.

**Alternative hypothesis ( $H_A$ ):** the proportion of right- and left-handed toads in the population ARE NOT equal.

It estimates a p-value (seen often as more "quantitative") than when contrasted to a threshold (alpha, i.e., significance level) it "forces" us into a yes (reject  $H_0$ ) or no (don't reject  $H_0$ ) answer.

R <sub>ight</sub>	L <sub>eft</sub>
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Statistical hypothesis testing can be also understood as building the confidence interval for samples that come from a population of "no interest"

↑

➔

20 random samples

➔

proportion of right-legged toads

Assume that 50% of toad population is right-legged and 50% left-handed. In other words, assume that the null hypothesis is true.

Assume this population to be mathematically infinite.

It is expected that 1 sample confidence interval over 20 does not include the true population proportion (i.e., .95% of intervals). This interval is in red.

$H_0$  true

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Statistical hypothesis testing can be also understood as building the confidence interval for samples that come from a population of "no interest"

It is expected that 1 sample confidence interval over 20 does not include the true population proportion (i.e., 95% of intervals). This interval is in red.

The principle of statistical hypothesis testing is that if a sample confidence interval covers the value underlying the null hypothesis (here 50%), then we should reject the null hypothesis.

$H_0$  true

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Statistical hypothesis testing can be also understood as building the confidence interval for samples that come from a population of "no interest"

While only 5% of the confidence intervals based on the 50% right-legged population rejects  $H_0$  when it should not (type I error); many intervals also do not reject when they should reject (type II error). Therefore, we can't state that a  $H_0$  is true; all we can say is that we have evidence to reject it.

Type II errors (probability of not rejecting a  $H_0$  that is false) will depend here on the sample size and the value of the true population.

$H_0$  is false

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Errors underlying statistical hypothesis testing – using confidence intervals to increase the ability to understand this notion

Statistical decision	Reality (unknown)	
	$H_0$ true	$H_0$ false
Reject $H_0$	Type I error	Correct
Do not reject $H_0$	Correct	Type II error

Inference is not about being certain—it is about managing uncertainty. Narrow intervals support decisions, provided we plan for the possibility of being wrong

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**Errors underlying statistical hypothesis testing – using confidence intervals to increase the ability to understand this notion**

Reality (unknown)		
Statistical decision	$H_0$ true	$H_0$ false
Reject $H_0$	Type I error	Correct
Do not reject $H_0$	Correct	Type II error

Inference is not about being certain – it is about managing uncertainty. Narrow intervals support decisions, provided we plan for the possibility of being wrong

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Frequentist statistical hypothesis testing uses p-values to support statistical conclusions (e.g., do not reject vs reject the null hypothesis). And confidence intervals “always” (rarely not) agree with p-value-based decisions.

⇒
1000000000 random samples (~infinite)

Number of right-handed toads	Probability of those samples
0	0.000004
1	0.00007
2	0.0006
3	0.0031
4	0.0117
5	0.0327
6	0.0708
7	0.1214
8	0.1669
9	0.1855
10	0.1669
11	0.1214
12	0.0708
13	0.0327
14	0.0117
15	0.0031
16	0.0006
17	0.00007
18	0.000004
Total	1.0

Assume that 50% of toad population is right-handed and 50% are left-handed. Assume this population to be mathematically infinite.

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**Contrasting the confidence intervals from samples from a population where  $H_0$  is true against the null distribution assuming  $H_0$  is true. Any wrong assessment is then obviously an error (wrong assessment).**

In this example, we know which assessments are correct or incorrect because the samples were generated from a population in which the null hypothesis ( $H_0$ ) is known to be true.

In real applications, we do not know whether our samples truly come from the population assumed under  $H_0$ . This setup was used purely for pedagogical purposes—to illustrate the fundamental properties of confidence intervals and statistical hypothesis testing.

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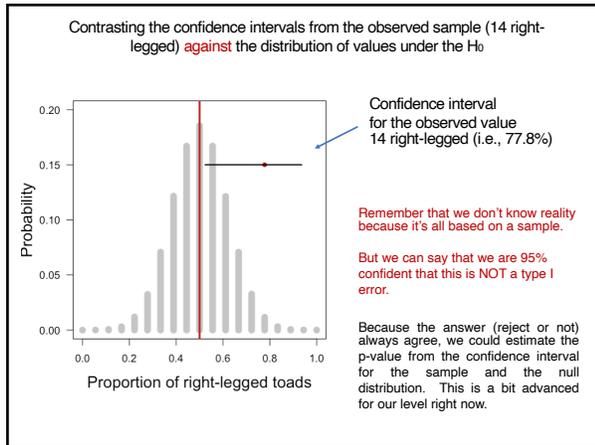
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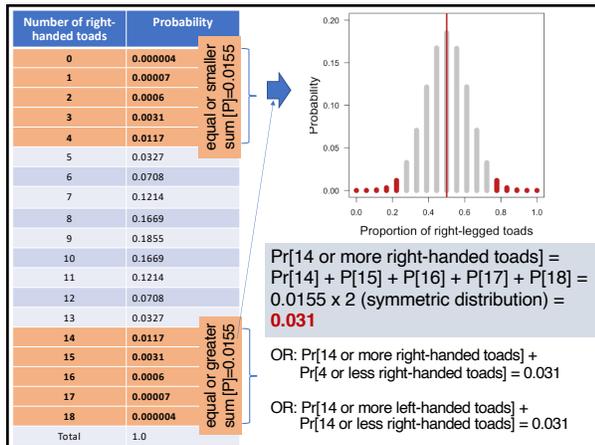
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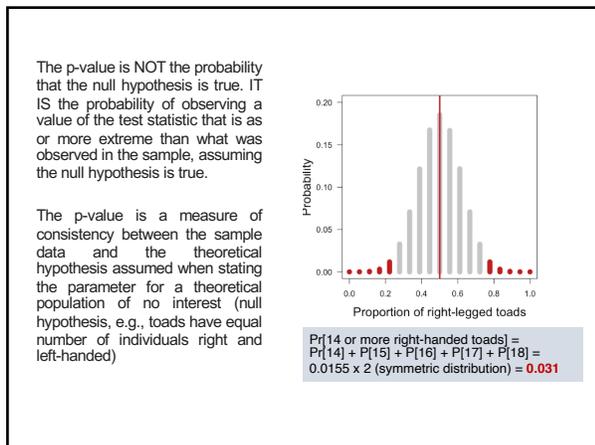
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Let's take a break - 1 minute



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

The **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":

We can say that we have evidence to reject (can't say *not accept*) the null statistical hypothesis, but we cannot say that we have evidence to accept a specific alternative hypothesis.

This is because our decision is based entirely on the sampling distribution expected under chance alone, assuming the null hypothesis ( $H_0$ ) is true (e.g., 50% right-legged and 50% left-legged toads).

By rejecting  $H_0$ , we conclude that the observed data are inconsistent with what would be expected under the null model (i.e., probability distribution assuming  $H_0$  as true). This provides support for the research hypothesis (that something other than chance is at work), but not confirmation of any particular alternative, since infinitely many alternative hypotheses could also explain the deviation from  $H_0$ .

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**The process of statistical hypothesis testing:  
SUMMARY OF critical details**

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.

*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.*

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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  $H_0$ ) or a p-value is significant (reject  $H_0$  in favour of  $H_A$ ).

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



The American Statistician

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**Moving to a World Beyond "p < 0.05"**

Ronald L. Wasserstein, Allen L. Schirm & Nicole A. Lazar

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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  $H_0$ ).
2. P-values do not measure the probability that the studied research hypothesis is true.
3. 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.



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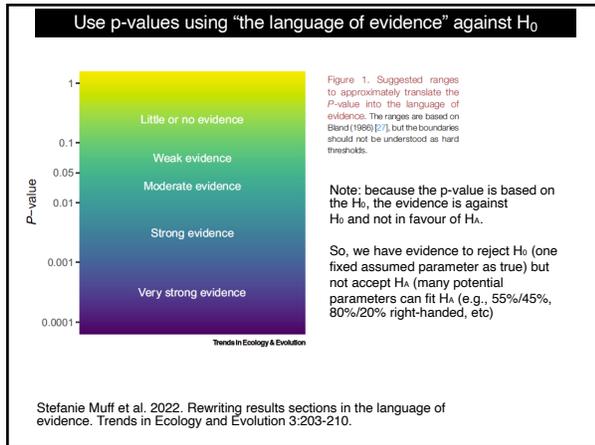
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**The don'ts about P values and hypothesis testing (Wasserstein et al. 2019)**

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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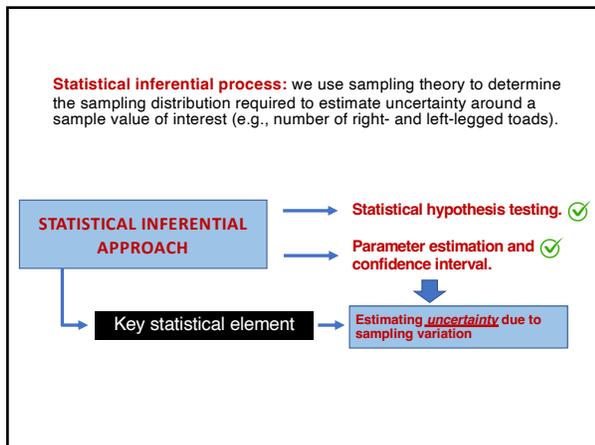
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