Kurs Bio144:
Datenanalyse in der Biologie

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Week 8: Interpretation, causality, cautionary notes

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Overview (todo: check)

- *P*-values: Interpretation and (mis-)use
- Statistical significance vs biological relevance
- Relative importance of regression terms
- Causality vs correlation
- Bradford-Hill criteria for causal inference
- Experimental vs observational studies

Course material covered today

- todo

Optional reading:

- todo

Recap of Last week

- todo

P-values

Recap:

P-values are commonly used for *statistical testing*, e.g. by checking if $p < 0.05$.

Examples:

- T-test for a difference between two samples.
- χ^2-test for independence of two discrete distributions.
- Test if a regression coefficient $\beta_x \neq 0$ in a regression model.

P-values in regression models

In regression modelling, the *p*-value is often used as an indicator of covariate importance. Remember the mercury example:

	Coefficent	95%-confidence interval	*p*-value
Intercept	-0.68	from -0.88 to -0.47	< 0.0001
log10(Hg_soil)	0.033	from -0.05 to 0.11	0.42
vegetables	0.07	from -0.03 to 0.17	0.18
migration	-0.036	from -0.19 to 0.12	0.65
smoking	0.27	from 0.06 to 0.48	0.012
sqrt(amalgam)	0.33	from 0.24 to 0.42	< 0.0001
age	-0.042	from -0.06 to -0.02	0.0004
mother	-1.03	from -1.70 to -0.35	0.003
sqrt(fish)	0.079	from 0.03 to 0.13	0.004
last_fish	0.30	from 0.15 to 0.45	< 0.0001
age:mother	0.055	from 0.03 to 0.08	0.0002

A common practice is to look only at the *p*-value and use $p < 0.05$ to decide whether a variable has an influence or not.

P-values criticism

P-value **criticism is** as **old** as statistical significance testing (1920s!). Issues:

- The sharp line $p < 0.05$ is arbitrary and significance testing according to it may lead to *mindless statistics* (Gigerenzer, 2004).
- Model selection using *p*-values may lead to a model selection bias (see last week).
- *P*-hacking / dada dredging: Search until you find a result with $p < 0.05$.
- Publication bias: Studies with $p < 0.05$ are more likely to be published than "non-significant" results.
- Recent articles in *Science*, *Nature* or a statement by the *American Statistical Associaton (ASA)* in March 2016 show that the debate still continues (Claridge-Change and Assam, 2016; Goodman, 2016; Wasserstein and Lazar, 2016).

P-values even made it into NZZ (April 2016)

Überschätzte Statistiken

Daten-Analysen entscheiden heute darüber, ob ein Medikament als wirksam gilt. Bloss verstehen viele Forscher die Bedeutung dieser Berechnungen gar nicht. **Von Patrick Imhasly**

GETTY IMAGES

Daten werden meist von Leuten analysiert, die nicht dafür ausgebildet sind.

Karriere machen können nicht nur Menschen, sondern auch statistische Grössen. Das gilt besonders für den sogenannten p-Wert, mit dem jeder Mittelschüler und jede Studentin in Kontakt kommt, vor allem aber jeder, der im weitesten Sinn etwas mit Statistik zu tun hat. Inzwischen ist der p-Wert indes auf die schiefe Bahn geraten. Denn was der Vater der modernen Statistik, der britische Genetiker Ronald Fisher, 1920 als eine Art informelles Sensorium für die Aussagekraft von Daten entwickelte, ist in der Praxis oftmals zu einem simplen Lackmustest verkommen.

Ergibt die statistische Analyse von Daten einen p-Wert < 0,05 (5 Prozent) oder noch besser < 0,01 (1 Prozent), gelten diese als *signifikant* – den Daten wird dann automatisch Beweiskraft zugestanden. Das entscheidet etwa darüber, ob ein neues Medikament als wirksam eingestuft wird oder ob ein Forscher seine Studie in einem angesehenen Fachblatt publizieren kann. «Der p-Wert war aber nie dazu gedacht, wissenschaftliches Denken ausser Kraft zu setzen», hat sich Ron Wasserstein, der Direktor der Amerikanischen Statistischen Vereinigung (ASA), jüngst öffentlich beklagt.

Wissenschafter verwenden den p-Wert immer häufiger, ohne zu verstehen, was er bedeutet – das fördert schlechte Forschung und untergräbt die Glaubwürdigkeit der Wissenschaft. Der Mediziner und Epidemiologe John Ioannidis von der Universität Stanford sprach in einem Kommentar von «Drogenabhängigen»: «Der falsche Gebrauch des p-Wertes ist dermassen einfach und erfolgt so automatisiert, dass manche süchtig werden danach – vor allem wenn sie mit Forschungsgeldern und Publikationen belohnt werden.» Angesichts der Missstände sah sich die ASA jetzt veranlasst, zum ersten Mal in ihrer fast 180-jährigen Geschichte Empfehlungen zu veröffentlichen, wie man mit einer statistischen Grösse vernünftig umgeht.

Wider die Null-Hypothese

«Der p-Wert sagt nicht das aus, was man gemeinhin von ihm erwartet», erklärt der Berner Epidemiologe Peter Jüni, der seit kurzem am Applied Health Research Centre der Universität Toronto tätig ist. Das bedeutet: Der p-Wert misst nicht die Wahrscheinlichkeit, ob eine bestimmte Hypothese zutrifft, und auch nicht, ob ein bestimmtes Resultat zufällig zustande gekommen ist, wie die ASA festhält. Vielmehr dienen p-Werte dazu, die sogenannte Null-Hypothese zu testen und möglichst zu verwerfen.

Die Hypothese in einer Patientenstudie könnte zum Beispiel lauten, dass ein Medikament A gegen Nierenprobleme besser wirkt als ein Medikament B. Die Null-Hypothese besagt dann genau das Gegenteil davon, nämlich dass das Medikament A nicht besser wirkt als das Medikament B. Beim Test berechnet der Forscher im Prinzip, wie gross die Wahrscheinlichkeit für das Auftreten eines tatsächlich festgestellten oder noch grösseren Unterschieds zwischen den beiden untersuchten Medikamenten ist – unter der Annahme, dass die Null-Hypothese stimmt. Diese Wahrscheinlichkeit ist der p-Wert, und je geringer er ist, desto weniger spricht für die Null-Hypothese. Ein p-Wert von 0,05 bedeutet, dass das festgestellte (oder ein noch extremeres) Resultat unter den Bedingungen der Null-Hypothese mit einer Wahrscheinlichkeit von lediglich 5 Prozent zustande kommen kann – und nicht, dass eine bestimmte Hypothese mit einer Sicherheit von 95 Prozent wahr ist.

«Studien führen heute zu dermassen vielen Daten, dass man allen Unfug testen kann und so zu Hunderten von p-Werten kommt.»

Über die eigentlich untersuchte Hypothese kann der p-Wert nur indirekt etwas aussagen, weil er über zwei Ecken gedacht ist. Der ominöse Wert liefert also keine ultimativen Beweise für einen postulierten Unterschied oder Zusammenhang. «Der p-Wert ist eine bedingte und nicht eine absolute Wahrscheinlichkeit», erklärt Peter Jüni. «Doch genau das verstehen viele Forscher nicht, und es interessiert sie auch nicht.»

Hinzu kommt, dass die Signifikanzgrenzen von 5 Prozent bzw. 1 Prozent historisch entstanden und keineswegs klar definierte Werte sind. Ronald Fisher, der Erfinder des p-Werts, überliess es jedenfalls ausdrücklich der Interpretation des einzelnen Forschers, ab welcher Grösse ein p-Wert in einer Untersuchung Aussagekraft haben soll. «Trotzdem haben sich die willkürlich gewählten Signifikanzgrenzen ins Gehirn von Generationen von Forschern gebrannt», sagt Leonhard Held vom Institut für Epidemiologie, Biostatistik und Prävention der Universität Zürich.

Die britischen Statistiker Jonathan Sterne und George Davey Smith haben schon vor 15 Jahren im «British Medical Journal» dazu aufgerufen, die Resultate von medizinischen Studien nicht mehr als «signifikant» oder «nichtsignifikant» darzustellen, sondern im Kontext der gesamten Untersuchung und anhand anderer Ergebnisse zu interpretieren. Genützt hat das herzlich wenig, wie das Team von John Ioannidis in einer soeben erschienenen Studie festgestellt hat. Demnach sind in den vergangenen 25 Jahren in der biomedizinischen Forschung immer mehr Studien erschienen, die p-Werte angeben, die zudem immer klarer signifikant ausfallen. Gleichzeitig werden Zusatzinformationen zu den festgestellten Effekten immer seltener («JAMA», Bd. 315, S. 1141).

5%

Kleiner als dieser Wert muss der sogenannte p-Wert in einem statistischen Test sein, dann gelten die Daten aus einer Studie als aussagekräftig. Doch die Grenze ist willkürlich gewählt. (*pim.*)

Es gibt Alternativen

Das Problem ist dabei nicht nur, dass der p-Wert ein trügerisch einfaches statistisches Instrument ist. «Studien führen heute zu dermassen vielen Daten, dass man allen Unfug testen kann und so zu Hunderten von p-Werten kommt», erklärt Leonhard Held. «Der eine oder andere fällt dann bestimmt signifikant aus, auch wenn kein Effekt vorhanden ist.» Leonhard Held und Peter Jüni verlassen sich deshalb bei der Planung und Auswertung von Studien schon lange nicht mehr nur auf die p-Werte.

Jüni empfiehlt, die Resultate von Studien mindestens mit Vertrauensintervallen zu versehen, die spezifische Aussagen über die Unsicherheit einer Schätzung machen. Und Held erforscht alternative Masse für statistische Evidenz – zum Beispiel Bayes-Faktoren. Mit ihrer Hilfe lässt sich die Wahrscheinlichkeit einer Hypothese anhand der Daten anpassen, statt dass diese wie beim p-Wert nach einem Schwarz-Weiss-Schema angenommen oder abgelehnt wird.

Note: R.A. Fisher, the "inventor" of the p-value (1920s) didn't mean the p-value to be used in the way it is used today (which is: doing a single experiment and use $p < 0.05$ for a conclusion)!

From Goodman (2016):

> *Fisher used "significance" merely* **to indicate that an observation was worth following up, with refutation of the null hypothesis justified only if further experiments "rarely failed" to achieve significance.** *This is in stark contrast to the modern practice of making claims based on a single demonstration of statistical significance.*

The misuse of p-values has led to a reproducibility crisis in science!

Essay

Why Most Published Research Findings Are False

John P. A. Ioannidis

Summary

There is increasing concern that most current published research findings are false. The probability that a research claim is true may depend on study power and bias, the number of other studies on the same question, and, importantly, the ratio of true to no relationships among the relationships probed in each scientific field. In this framework, a research finding is less likely to be true when the studies conducted in a field are smaller; when effect sizes are smaller; when there is a greater number and lesser preselection of tested relationships; where there is greater flexibility in designs, definitions, outcomes, and analytical modes; when there is greater financial and other interest and prejudice; and when more teams are involved in a scientific field in chase of statistical significance. Simulations show that for most study designs and settings, it is more likely for a research claim to be false than true. Moreover, for many current scientific fields, claimed research findings may often be simply accurate measures of the prevailing bias. In this essay, I discuss the implications of these problems for the conduct and interpretation of research.

factors that influence this problem and some corollaries thereof.

Modeling the Framework for False Positive Findings

Several methodologists have pointed out [9–11] that the high rate of nonreplication (lack of confirmation) of research discoveries is a consequence of the convenient, yet ill-founded strategy of claiming conclusive research findings solely on the basis of a single study assessed by formal statistical significance, typically for a *p*-value less than 0.05. Research is not most appropriately represented and summarized by *p*-values, but, unfortunately, there is a widespread notion that medical research articles

It can be proven that most claimed research findings are false.

should be interpreted based only on *p*-values. Research findings are defined here as any relationship reaching formal statistical significance, e.g., effective interventions, informative predictors, risk factors, or associations.

is characteristic of the field and can vary a lot depending on whether the field targets highly likely relationships or searches for only one or a few true relationships among thousands and millions of hypotheses that may be postulated. Let us also consider, for computational simplicity, circumscribed fields where either there is only one true relationship (among many that can be hypothesized) or the power is similar to find any of the several existing true relationships. The pre-study probability of a relationship being true is $R/(R+1)$. The probability of a study finding a true relationship reflects the power $1-\beta$ (one minus the Type II error rate). The probability of claiming a relationship when none truly exists reflects the Type I error rate, α. Assuming that c relationships are being probed in the field, the expected values of the 2×2 table are given in Table 1. After a research finding has been claimed based on achieving formal statistical significance, the post-study probability that it is true is the positive predictive value, PPV. The PPV is also the complementary probability of what Wacholder et al. have called the false positive report probability [10]. According to the 2

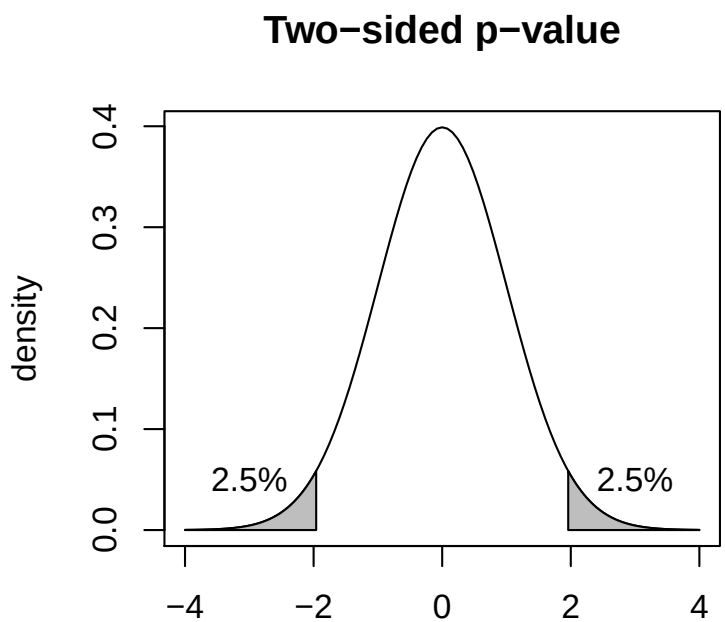
(Ioannidis, 2005)

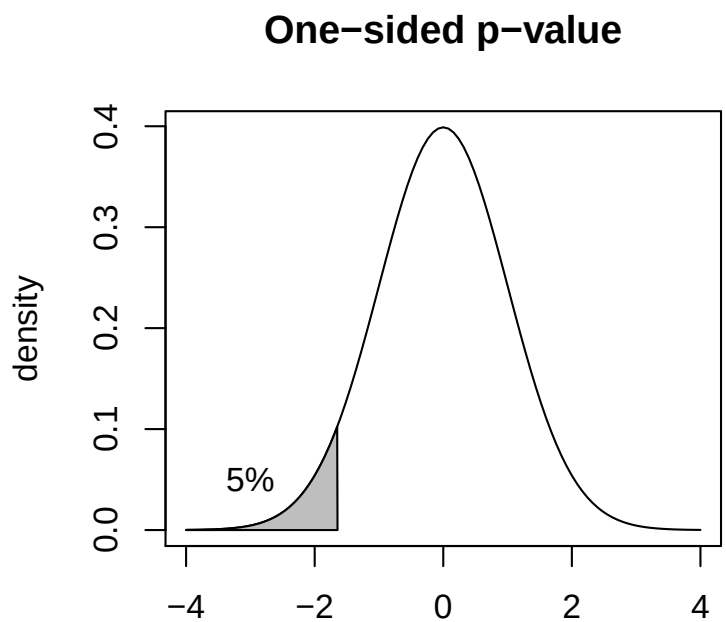
What is the problem with the *p*-value?

Many applied researchers do not really understand what the *p*-value actually is.

The **formal definition of *p*-value** is the probability of an observed data summary (e.g., an average) and its more extreme values, given a specified mathematical model and hypothesis (usually the "Null").

(Goodman, 2016)





Klicker-Exercise

▸ Klicker-Exercise

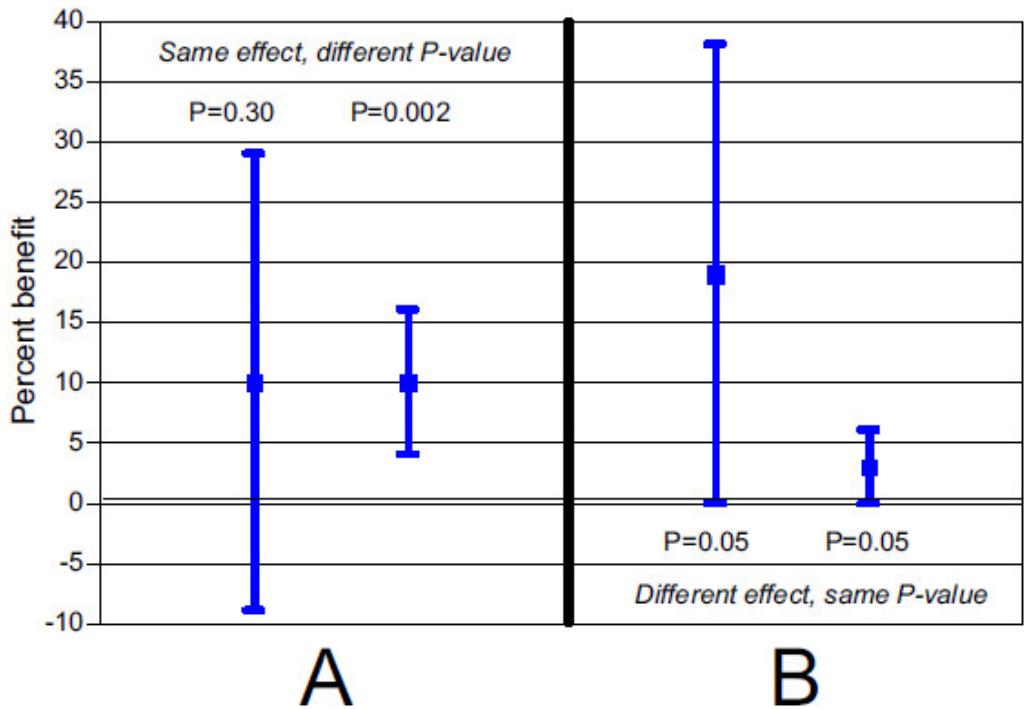
http://www.klicker.uzh.ch/bkx

+ Discussion of the results!

Significance vs relevance

In regression models:

- A low p-value does not automatically imply that a variable is "important".
- "Is there an effect?" v.s. "How much of an effect is there?".



(from Goodman, 2008)

Shall we abolish p-values?

No: p-values are not "good" or "bad". They contain important information, and they have have **strengths** and **weaknesses**.

Suggestions:

- Use p-values, but don't over-interpret them, use them properly.
- Look at effect sizes and confidence intervals.
- Look at relative importances of covariates.
- **Don't use p-values for model selection.**

Suggestion 1: Proper interpretation of p-values

Rather than a black-and-white decision ($p < 0.05$), Martin Bland suggests to regard p-values as continuous measures for statistical evidence (Introduction to Medical Statistics, 4th edition, Oxford University Press):

$p > 0.1$	little or no evidence against the null hypothesis
$0.1 > p > 0.05$	weak evidence
$0.05 > p > 0.01$	evidence
$0.01 > p > 0.001$	strong evidence
$p < 0.001$	very strong evidence

But: The level of significance must also depend on the context!

In the Hg example:

	Coefficent	95%-confidence interval	p-value
Intercept	-0.68	from -0.88 to -0.47	< 0.0001
log10(Hg_soil)	0.033	from -0.05 to 0.11	0.42
vegetables	0.07	from -0.03 to 0.17	0.18
migration	-0.036	from -0.19 to 0.12	0.65
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sqrt(fish)	0.079	from 0.03 to 0.13	0.004
last_fish	0.30	from 0.15 to 0.45	< 0.0001
age:mother	0.055	from 0.03 to 0.08	0.0002

- **Little or no evidence:** Hg soil, vegetables from garden, migration background
- **Weak evidence:** Smoking
- **Strong evidence:** Mother, monthly fish consumption
- **Very strong evidence:** Amalgam, age, last fish ($>$ or $<$ 3 days), interaction of age and mother

Suggestion 2: Report effect sizes....

Ask: **Is the effect size relevant?**

Example

WHO recommendation concerning smoking and the consumption of processed meat. Both, smoking and meat consumption, appear to be carcinogenic.

- 50g processed meat per day increases the risk for colon cancer by a factor of 1.18 (+18%).
- Smoking increases the risk for cancer by a factor of 3.6 (+260%).

Thus: Although both, meat consumption and smoking, are carcinogenic ("significant"), their **effect sizes are vastly different**!

...and 95% CIs

Ask: **Which range of true effects is statistically consistent with the observed data?**

Example

Body fat example, slide 39 of week 1.

The effect estimate for the effect of BMI on body fat is given as $\hat{\beta}_{BMI} = 1.82$, 95% CI from 1.61 to 2.03.

Interpretation: for an increase in the bmi by one index point, roughly 1.82% percentage points more bodyfat are expected, and all true values for β_{BMI} between 1.61 and 2.03 are **compatible with the observed data**.

However...

- The choice of the 95% is again somewhat arbitrary. We could also go for 90% or 99% or any other interval, but 95% has established as a commonly accepted range.

- The 95% CI should **not be misused for simple hypothesis testing** in the sense of

  "Is 0 in the confidence interval or not?"

  Because this boils down to checking whether $p < 0.05$ ...

Suggestion 3: Look at relative importances of covariates

- Ultimately, the popularity of p-values is based on the wish to judge which covariates are **relevant** in a model, particularly in observational studies.
- The problem with this: Low p-values do not automatically imply high relevance (Cox, 1982).
- Alternative: **relative importances** of explanatory variables that measure the proportion (%) of the responses' variability explained by each variable.

Relative importance: Decomposing R^2

Remember: R^2 indicates the proportion of variance explained by **all** covariates in a model

$$y_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + \ldots + \beta_2 x_i^{(m)} + e_i \ .$$

The aim or relative importance is to decompose R^2 such that

- each variable $x^{(j)}$ is attributed a fair share r_j.
- the sum of all importances sums up to R, that is, $\sum_{j=1}^{p} r_j = R^2$.

Further, it is required that

- all shares are ≥ 0.

Question: How would you define/calculate relative importance?

- **Idea 1:** Fit simple models including only one covariate at the time, *i.e.*:

$$y_i = \beta_0 + \beta_j x_i^{(j)} + e_i$$

  for each variable $x^{(j)}$ and use the respective R^2 as r_j.

- **Idea 2:** Fit the linear model twice, once with and once without the covariate of interest, and then take the increase of R^2 as r_j.

Problem: In practice, regressors $x^{(j)}$ are *always correlated*, thus both ideas lead to $\sum_j r_j \neq R^2$!

To understand the problem of ideas 1 and 2, let us fit three models for $\log(Hg_{\text{urine}})$ with

- $x^{(1)} = \sqrt{\text{Number of monthly fish meals}}$
- $x^{(2)} =$ binary indicator if last fish meal was less than 3 days ago.

These two variables are correlated (people who consume a lot of fish are more likely to have it consumed within the last 3 days).

$$y_i = \beta_0 + \beta_1 x_i^{(1)} + + e_i \qquad R^2 = 0.12 \qquad (1)$$

$$y_i = \beta_0 + \beta_2 x_i^{(2)} + + e_i \qquad R^2 = 0.08 \qquad (2)$$

$$y_i = \beta_0 + \beta_1 x_i^{(1)} + \beta_2 x_i^{(2)} + e_i \qquad R^2 = 0.14 \qquad (3)$$

Note: The R^2 of the model with both covariates is much less than the sum of the R^2 from models (1) and (2)!

$\Rightarrow$ The increase of R^2 upon inclusion of a covariate depends on the covariates that are already in the model!

A better way to calculate relative importance?

Various proposals to calculate relative importance (R^2 decomposition) have been proposed. The (currently) most useful is given by the following idea, called **LMG** (**L**indemann, **M**erenda and **G**old 1980):

- Fit the model for all possible orderings of the covariates.
- Record the incrase in R^2 each time a variable is included.
- Average over all orderings of the covariates.

Luckily, the `R`-package `relaimpo` (Groemping 2006) contains the function `calc.relimp()` that does this for us!

Hg results

Which proportion (%) of variance in $\log(Hg_{\text{urine}})$ is explained by each covariate?

```
> library(relaimpo)
> lmg.hg <- calc.relimp(r.lm.hg)$lmg
```

Variable	Rel. imp. (%)	p-value
$\log(Hg_{\text{soil}})$	0.10	0.42
Vegetable	0.46	0.18
Migration	0.43	0.65
Smoking	1.21	0.012
Amalgam	19.69	$<$0.0001
Age	1.25	0.0004
Mother	1.08	0.0031
Fish	7.26	0.0042
Last fish	7.34	$<$0.0001
Age:mother	6.56	0.0002

Several variables have very low p-values, but their relative importance differs clearly.

$\Rightarrow$ Relative importance gives intuitive complementary information to p-values, effect sizes and confidence intervals!

Does relative importance solve all the problems?

Unfortunately not...

Relative importance should be understood as a complement to standard statistical output.

There are several limitations to it:

- Rel.imp. of a variable may heavily depend on the other variables included in the model, especially when there are strongly correlated variables (see next slide).
- Hard to generalize to other, non-linear regression models.

Groemping 2007:

"...a request for a decomposition of R^2 is often driven by a desire to prioritize intervention actions with the intention to influence the response. It is important to notice that any intervention bears the risk [...] of not only influencing the targeted regressor but also the correlation structure among regressors. Thus, unexpected results may occur regarding changes of the response's variance. In this way, the benefit of the concept of decomposing R^2 is more limited than the typical user might realize."

Example

Compare the estimated relative importance for the variable `fish` (monthly fish meals) for two cases:

Model 1
Original Hg model.

Model 2
Model without the indicator variable `last_fish`.

- **Case 1:** Relative importance of `fish`: 7.26% (see slide 25).
- **Case 2:** Relative importance of `fish`: 10.75% .

Interpretation: If one of two correlated variables is removed, the other absorbs some of the importance from it.

Ev give another example where rel. imp is calculated, e.g. from previous weeks.

Causality vs correlation

In explanatory models the ultimate goal usually is to reveal causal relationships between the covariates and the response.

Examples:

- Does Hg in the soil influence Hg-levels in humans?
- Does inbreeding negatively affect population growth of Swiss Alpine ibex (Steinbock)?
- Does exposure to Asbest lead to illness or death?
- ...

However: Regression models actually only reveal associations, that is, correlations between **x** and **y**!

Bradford-Hill-Criteria for causal inference I

In 1965 the Epidemiologist Bradford Hill presented a list of criteria to assess whether there is some causality or not. However, he wrote "None of my nine viewpoints can bring indisputable evidence for or against the cause-and-effect hypothesis and none can be required sina qua non."

Bradford-Hill Criteria:

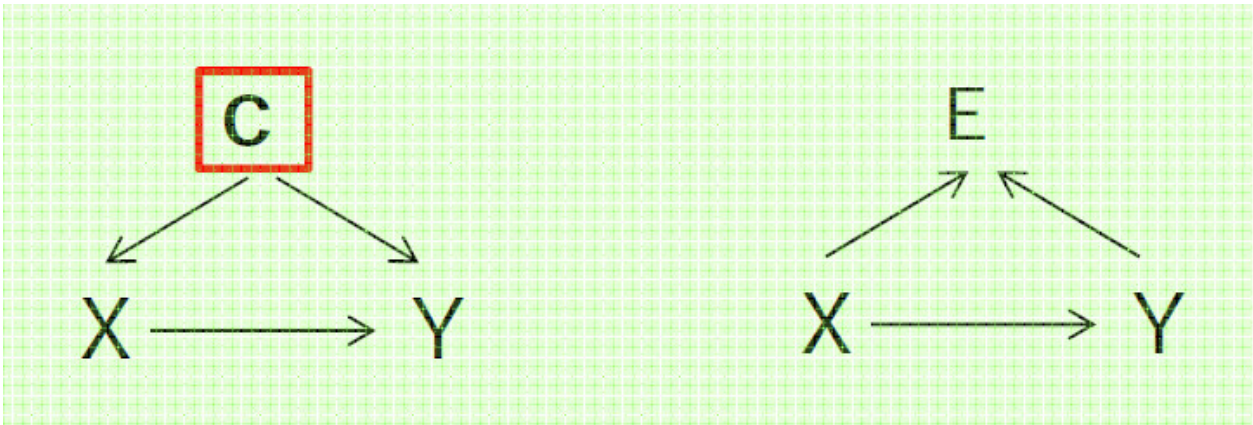
1. **Strength:** A causal relationship is likely when the observed association is strong.
2. **Consistency:** A causal relationship is likely if mutiple independent studies show similar associations.
3. **Specificity:** A causal relationship is likely when a covariate x is associated only with one potential outcome y and not with other outcomes.
4. **Temporality:** The effect has to occur after the cause.
5. **Biological gradient:**

Bradford-Hill-Criteria for causal inference II

6. **Plausibility:**
7. **Coherence:**
8. **Analogy:**
9. **Experiment:**

Causality considerations for model selection

It is **widely unknown** that a model can be broken by the inclusion of a "wrong" covariate, which is causally associated in the wrong direction:



Remember: Avoid to include covariates in your model that are caused by the outcome!

Example: ...

Experimental vs observational studies

Summary

References:

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