



Swiss Institute of
Bioinformatics



Best Practices for Investigating Gene Expression Differences by Biological Sex

Julien Roux, Vidhya Jagannathan
October 27th, 2025



 @julienroux.bsky.social

«Without careful methodology, the pursuit of sex difference research, despite a mandate from funding agencies, will result in a literature of contradiction»

Janet Rich-Edwards



Today's schedule

09:15 – 10:15 Introduction

10:15 – 10:30 Coffee break

10:30 – 12:00 Biological sex in experimental design (Talk+hands-on by Frédéric Schütz)

12:00 – 13:00 Lunch break

13:00 – 15:00 Hands-on session in subgroups

15:00 – 15:30 Coffee break

15:30 – 17:00 Finishing hands-on, wrap up and discussions

Exam?



Introducing ourselves

Vidhya Jagannathan (she/her)



- » MSc: Biology and Diploma in Bioinformatics, M.K.U, India
- » PhD: Applied EM Algorithm to TFBS, University of Lausanne, Swiss Institute of Bioinformatics
- » Postdoc: hormonal influence in breast cancer tissues using machine learning algorithms
- » Now: Senior scientist, Vetsuisse faculty, University of Bern
 - » Member, EU-SABV Consortium – exploring sex as a biological variable

Universität Bern | Universität Zürich

vetsuisse-fakultät

u^b

UNIVERSITÄT
BERN



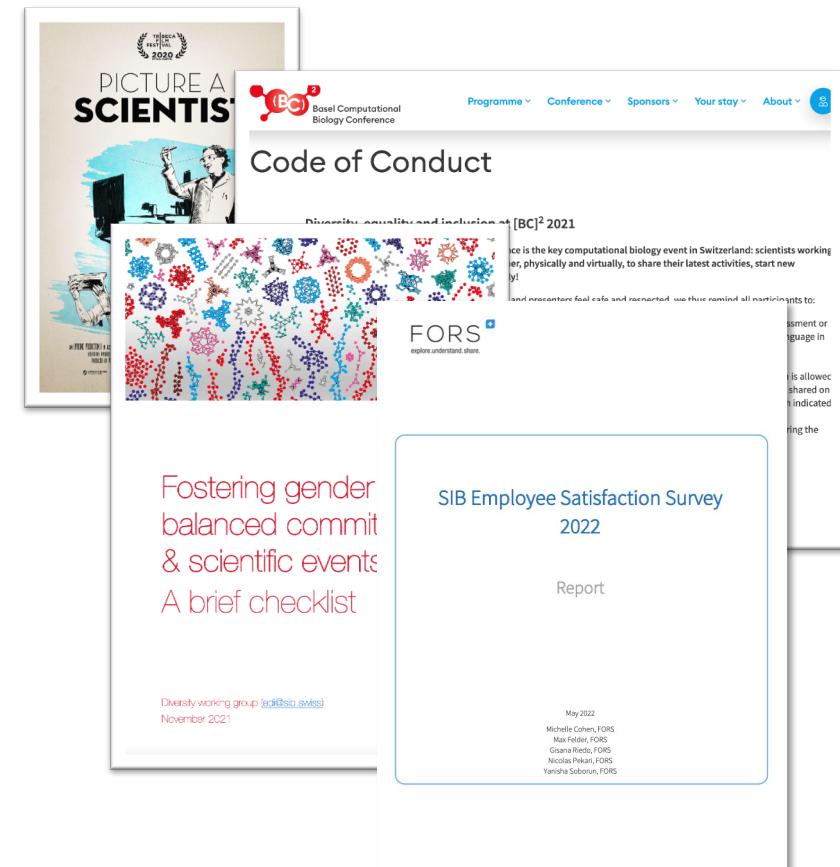
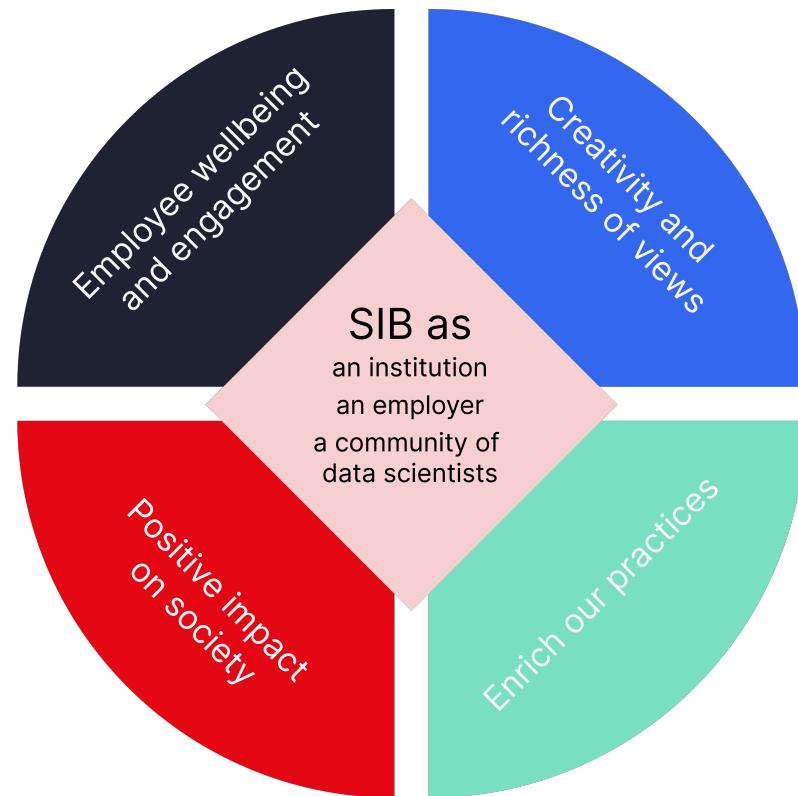
Introducing ourselves

Julien Roux (he/him)

- » MSc: Bioinformatics and Modeling, National Institute of Applied Sciences (INSA), Lyon, France
- » PhD: Evolutionary genomics, University of Lausanne, Swiss Institute of Bioinformatics
- » Postdoc: Evolutionary genomics, University of Chicago
- » Now: Senior scientist, Bioinformatics Core Facility, Department of Biomedicine, University of Basel



The SIB Diversity focus group



Want to contribute? Join us!



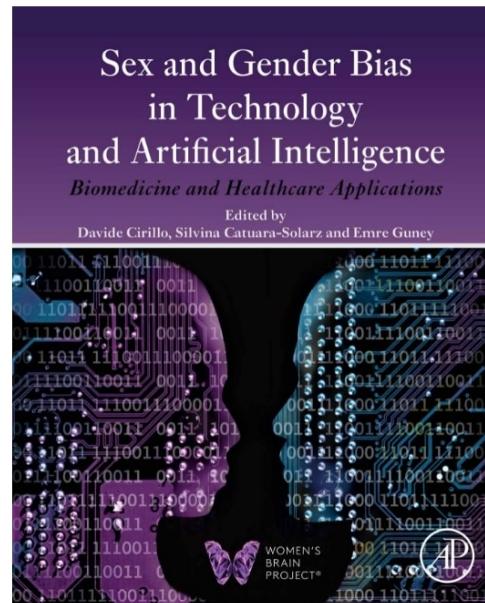


Swiss Institute of
Bioinformatics

SIB DAYS – 24 JUNE 2024 – BIEL

Incorporating biological variable in the design of biomedical research

Workshop from the SIB Diversity focus



Introducing ourselves

Frédéric Schütz

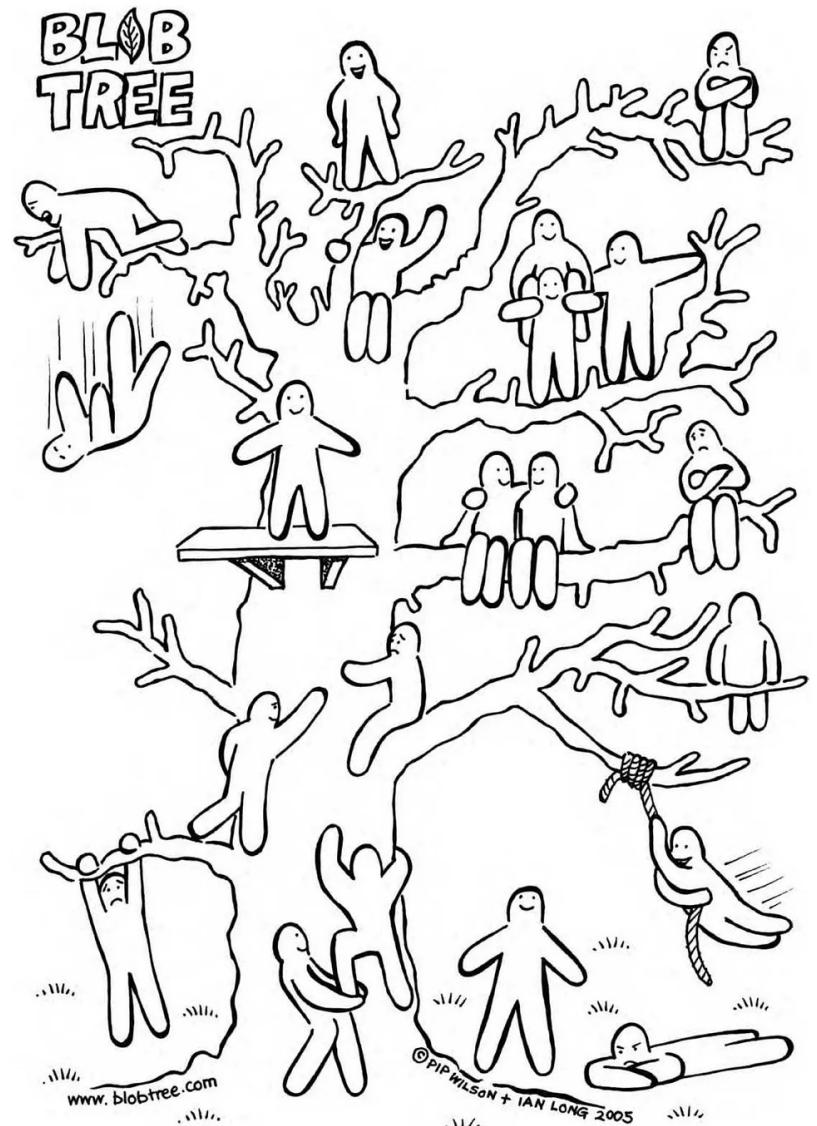
- » Biostatistics platform
- » Center for Integrative Genomics
- » University of Lausanne

- » SIB group leader
- » Chair of the Training Advisory Committee



Introducing yourselves

- » You
- » Your research project(s)
- » Expectations from the course?
- » How this course could be relevant?
- » Experience with bioinformatics?
Transcriptomics data analysis?
- » Involved in design of experiments?



Code of conduct

SIB abides by the ELIXIR Code of Conduct

- » Topic is complex, very oriented and cleaving
- » Disclaimer: we are not specialist, but willing to learn together
- » Be respectful, be inclusive, be open to discussion

<https://elixir-europe.org/events/code-of-conduct>

https://api.swiss-academies.ch/site/assets/files/25607/kodex_layout_en_web-1.pdf

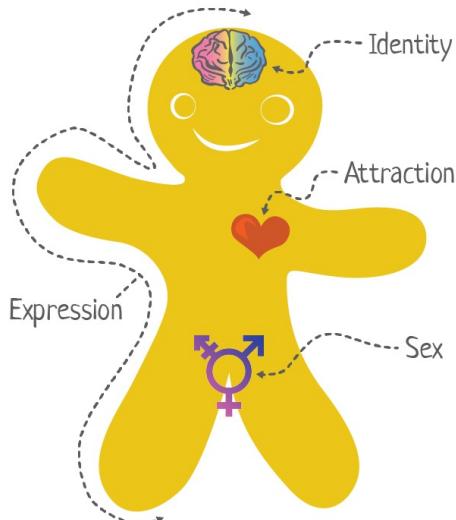


What is sex? What is gender?

Sex and gender are often used interchangeably, despite having different meanings



The Genderbread Person^{v4} by itspronouncedMETROsexual.com



∅ means a lack of what's on the right side



Gender Identity

∅ → Woman-ness
∅ → Man-ness



Gender Expression

∅ → Femininity
∅ → Masculinity



Anatomical Sex

∅ → Female-ness
∅ → Male-ness

Identity ≠ Expression ≠ Sex
Gender ≠ Sexual Orientation

Sex Assigned At Birth
Female Intersex Male

Heart icon: Sexually Attracted to... and/or (a/o)
∅ → Women a/o Feminine a/o Female People
∅ → Men a/o Masculine a/o Male People

Heart icon: Romantically Attracted to...
∅ → Women a/o Feminine a/o Female People
∅ → Men a/o Masculine a/o Male People

Genderbread Person Version 4 created and uncopyrighted 2017 by Sam Killermann

For a bigger bite, read more at www.genderbread.org

<https://www.itspronouncedmetrosexual.com/2018/10/the-genderbread-person-v4/>



What is sex? What is gender?

Sex and gender are often used interchangeably, despite having different meanings

Gender is a multidimensional sociocultural construct



Gender



Traditional male dress (Baranya, Hungary, 1920s)



Amazons in battle. Greek relief (4th century BC.

Kunsthistorische Museum, Vienna)

Slide courtesy of Davide Cirillo



Gender

Two-spirit (Indigenous North Americans)



George Catlin, "*Dance to the Berdache*" (1861-1869)

Muxes (Mexico)



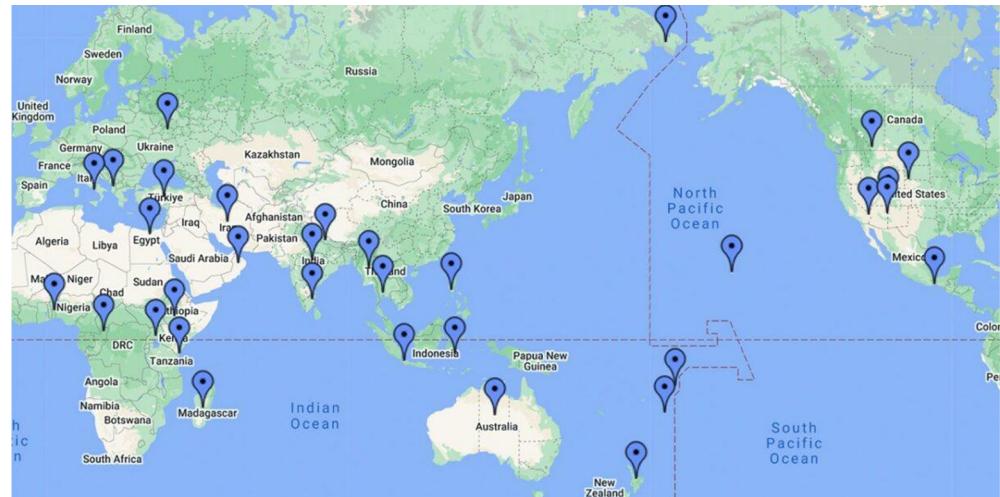
Vogue Mexico

Slide courtesy of Davide Cirillo



The Gender, Sex, and Sexual Orientation (GSSO) ontology

- gender identity (302)
 - exploring gender identity
 - female gender identity
 - male gender identity
 - no gender identity
 - nonbinary gender identity (294)
 - exclusive gender identity (278)
 - culturally-specific gender identity (274)
 - intersex-specific gender identity (1)
 - neurotype-specific gender identity
 - fluctuating gender identity (2)
 - indescribable gender identity (1)
 - multiple gender identity (3)
 - non-gendered identity (2)
 - overlapping gender identity (1)
 - partial gender identity (2)
 - other gender identity
 - unknown gender identity (1)



A map of gender-diverse cultures
https://www.pbs.org/independentlens/content/two-spirits_map-html/

<https://www.ebi.ac.uk/ols4/ontologies/qsoo>

Slide courtesy of Davide Cirillo



Today's focus on biological sex

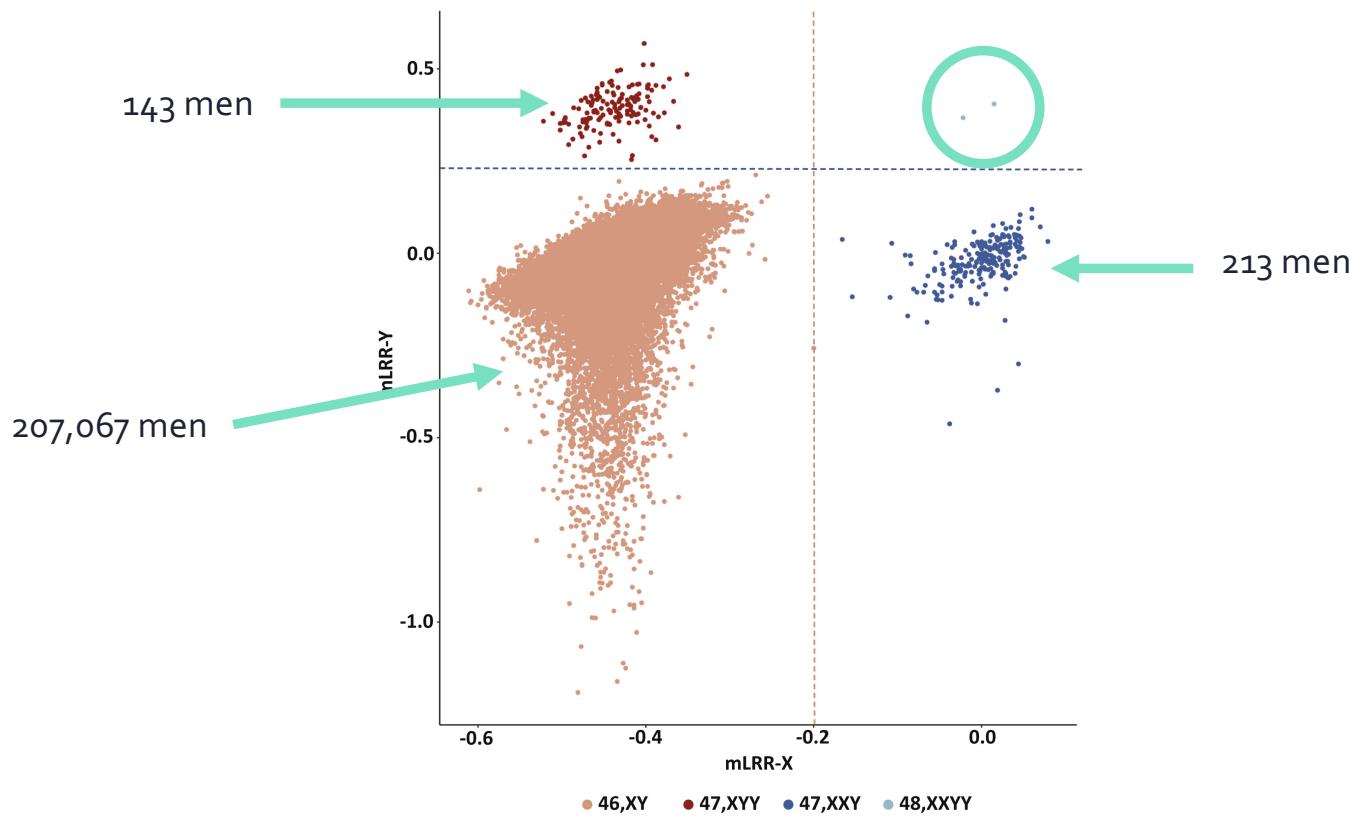
Why?

» Simpler?

» Quite topical



Sex chromosomes in males in UK Biobank



<https://www.sciencedirect.com/science/article/pii/S1098360022007778>



Biological sex: definition

Sex refers to a set of **biological attributes** in humans and animals.

It is primarily associated with physical and physiological features including **chromosomes, gene expression, hormone levels and function, and reproductive/sexual anatomy**.

Sex is usually categorised as female or male but **there is variation in the biological attributes** that comprise sex and how those attributes are expressed.

In the news...

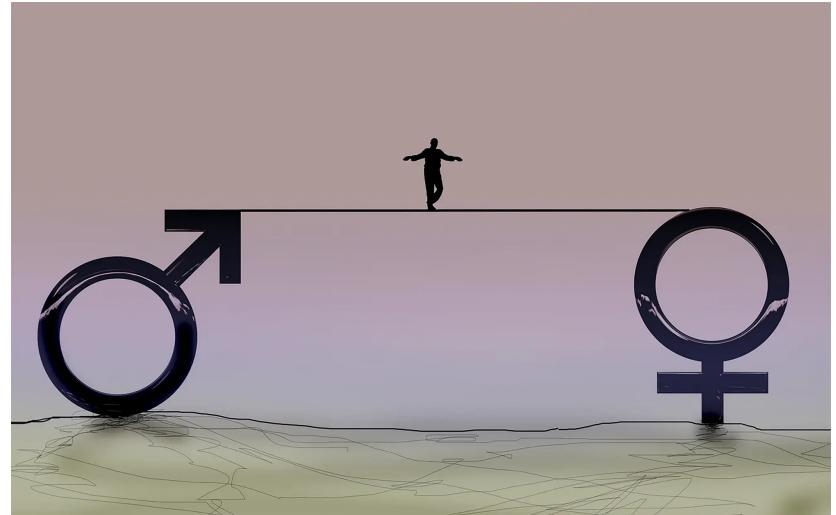
- »» Trump makes 'two sexes' official and scraps DEI policies (links 1 and 2)
 - » "Female" means a person belonging, at conception, to the sex that produces the large reproductive cell.
 - » "Male" means a person belonging, at conception, to the sex that produces the small reproductive cell.
 - »» Strong reaction from the scientific community
 - » Letter from presidents of Society for the Study of Evolution, American Society of Naturalists and Society of Systematic Biologists ("a revised version is in progress", but see link 3)
 - » Reaction to the reactions...
- <https://www.bbc.com/news/articles/czx84en1yp40>
 - <https://www.whitehouse.gov/presidential-actions/2025/01/defending-women-from-gender-ideology-extremism-and-restoring-biological-truth-to-the-federal-government/>
 - <https://medium.com/@alyision42/letter-to-the-us-president-and-congress-on-the-scientific-understanding-of-sex-and-gender-992051a60318>



Is it so simple?

- » How to assign sex at birth?
- » People with infertility?
- » Rare cases of people having both types of reproductive organs and producing both gametes

<https://academic.oup.com/bjs/article-abstract/69/5/279/6185759>



Gary Waters - Getty Images

In the news...



≡ MENU

World Boxing to introduce mandatory sex testing for all boxers that want to participate in its competitions*

May 30, 2025



PRESS RELEASE 30 JUL 2025

World Athletics introduces SRY gene test for athletes wishing to compete in the female category

Eligibility policy for men's and women's competitions approved

Following a period of thorough consultation with leading experts on the field, FIS presented to the Council a science-based eligibility policy for men's and women's competitions.

The eligibility conditions laid out in the policy are grounded on the presence or absence of the so-called SRY gene, the sex-determining gene present on humans' Y chromosome.

Essentially, only SRY-negative competitors may compete in women's competitions.



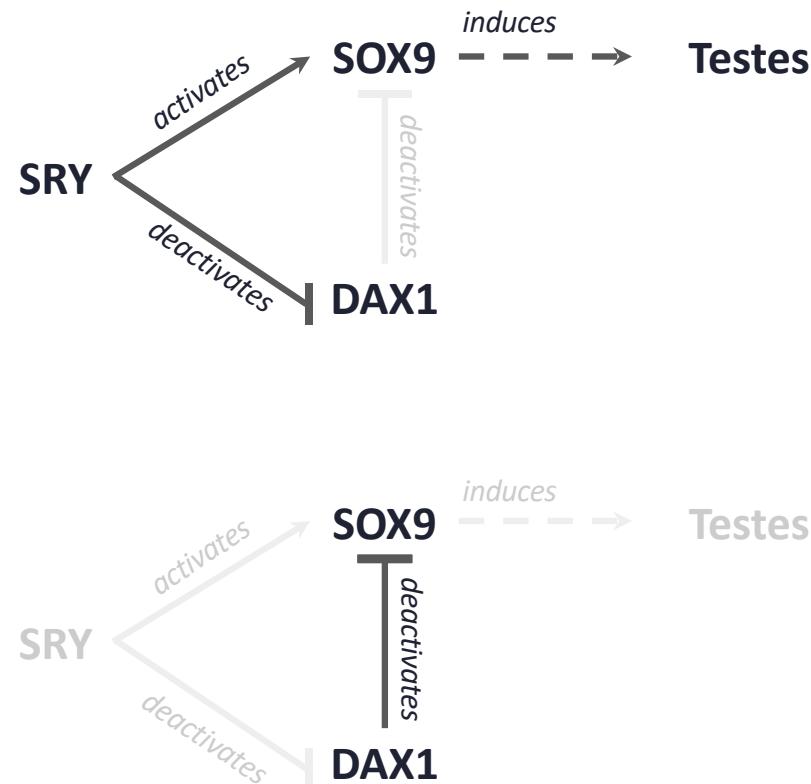
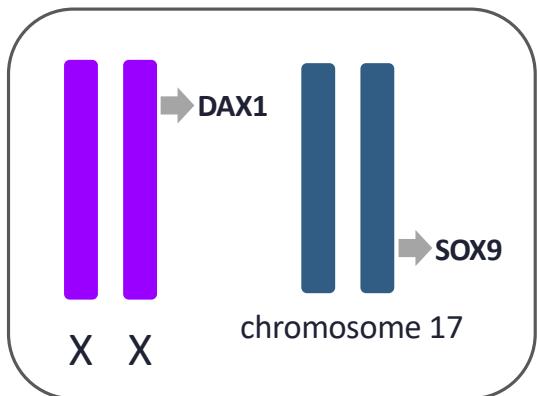
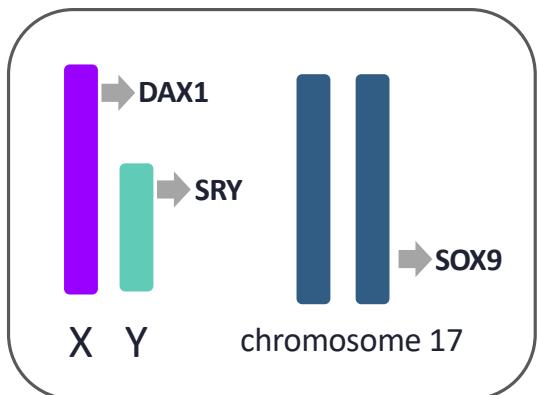
"This policy is the cornerstone of our commitment to protect women's sport, and we are convinced that there is only one fair and transparent way to do that: by relying on science and biological facts."

— Johan Eliasch, FIS President

<https://www.fis-ski.com/inside-fis/news/2025-26/fis-council-reinforces-commitment-to-athlete-safety>

<https://worldathletics.org/news/press-releases/sry-gene-test-athletes-female-category>





Slide courtesy of Davide Cirillo



Sex screening was used in the past

The IOC used **visual inspections** in the 1960s, but there were concerns they were degrading and invasive, before mandatory **chromosome-based** cheek swab tests were introduced.

There were still issues, however. Spanish hurdler Maria Jose Martinez Patino was barred from competition in 1986 after failing the test, but later examinations showed that while she had XY chromosomes she also had CAIS - meaning her body never gained benefits from the increased testosterone created by her internal testes.

She was reinstated, and the IOC switched to a **SRY gene test**, but amid a number of 'false positives', and fears that female athletes were being **punished for natural variations**, sex verification tests were abolished in the 1990s.

World Athletics insists the new tests are now "extremely accurate and the risk of false negative or positive is extremely unlikely".

<https://www.bbc.com/sport/athletics/articles/c9d07jq6vpeo>



Is it so simple? (2)

- » A male lab technician can contaminate swab samples
- » SRY is not *per se* providing an advantage in competition
- » Testosterone *is* linked to performance
- » Link 2:
 - » Between 2000 and 2023, 50-60 athletes *with DSD* competed in finals in athletics
 - » "The numbers, which were derived by anti-doping tests that revealed high testosterone levels – and arguably therefore may not capture every case – are significantly higher than many in the sport had expected."
- » The test does not tell if SRY is functioning, whether a testis has formed, whether testosterone is produced and, if so, whether it can be used by the body (link 1)
- » Genetic testing without medical prescription illegal in some countries
- » No genetic counselling to the athletes
- » No psychological support if a test is positive
 - <https://www.mcri.edu.au/news/insights-and-opinions/world-athletics-sry-gene-conversation>
 - <https://www.theguardian.com/sport/2025/sep/19/sex-tests-brought-in-after-data-showed-50-60-dsd-athletes-in-finals-world-athletics-says>



Today's focus on biological sex

Why?

- » Simpler?
- » Quite topical
- » Relevant as biologist/bioinformatician
- » Relevant as researcher aiming at conducting ethical and responsible research



Relevant as biologist

- » In animal studies, we usually only talk about biological sex
- » Large proportion of mammalian traits influenced by sex
 - » <https://www.nature.com/articles/ncomms15475>
- » Sex-differences in tissues
 - » <https://elifesciences.org/articles/99602>
 - » <https://faseb.onlinelibrary.wiley.com/doi/full/10.1096/fj.14-255000>
- » Reported sex differences in multiple diseases, e.g.:
 - » Sex-specific genetic architecture of human disease
<https://www.nature.com/articles/nrg2415>
 - » Neuromuscular disorders <https://www.sciencedirect.com/science/article/pii/S0047637423000192>
 - » Lung disease (COPD) <https://www.atsjournals.org/doi/abs/10.1165/rcmb.2024-0226OC>
- » Age-related somatic mutations, e.g., loss of Y chromosome in red blood cells
 - » <https://www.nature.com/articles/s43856-025-00966-9>
 - » <https://academic.oup.com/eurheartj/article/46/17/1603/8009275>

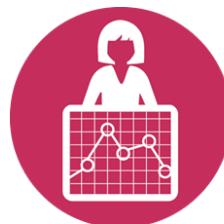
Funding agencies requirements

NIH's 2014 policy: "NIH expects that **sex as a biological variable** will be factored into research designs, analyses, and reporting in vertebrate animal and human studies"



Consider

Design studies that take sex into account, or explain why it isn't incorporated



Collect

Tabulate sex-based data



Characterize

Analyze sex-based data



Communicate

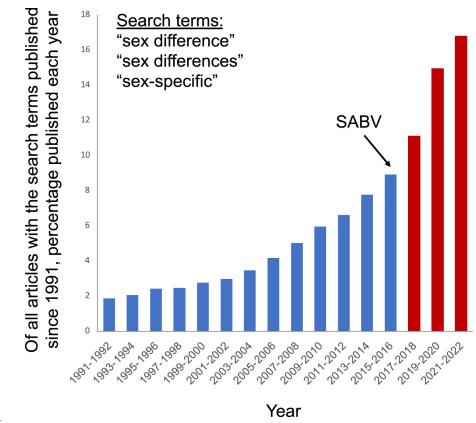
Report and publish sex-based data

<https://orwh.od.nih.gov/sex-as-biological-variable>



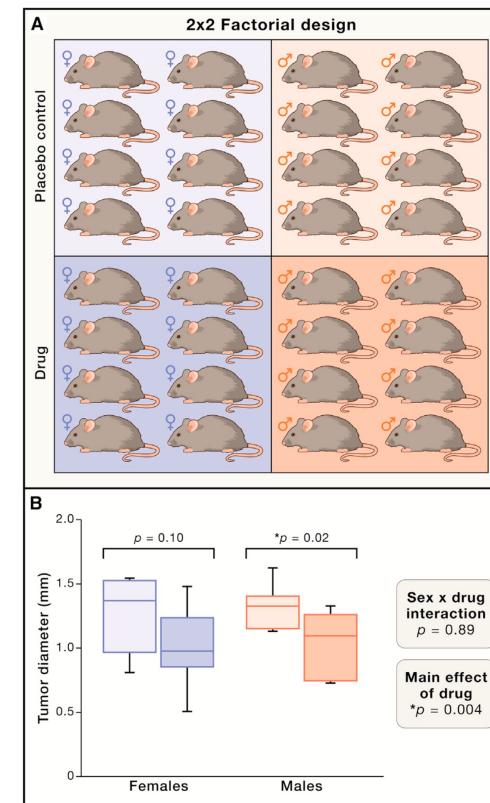
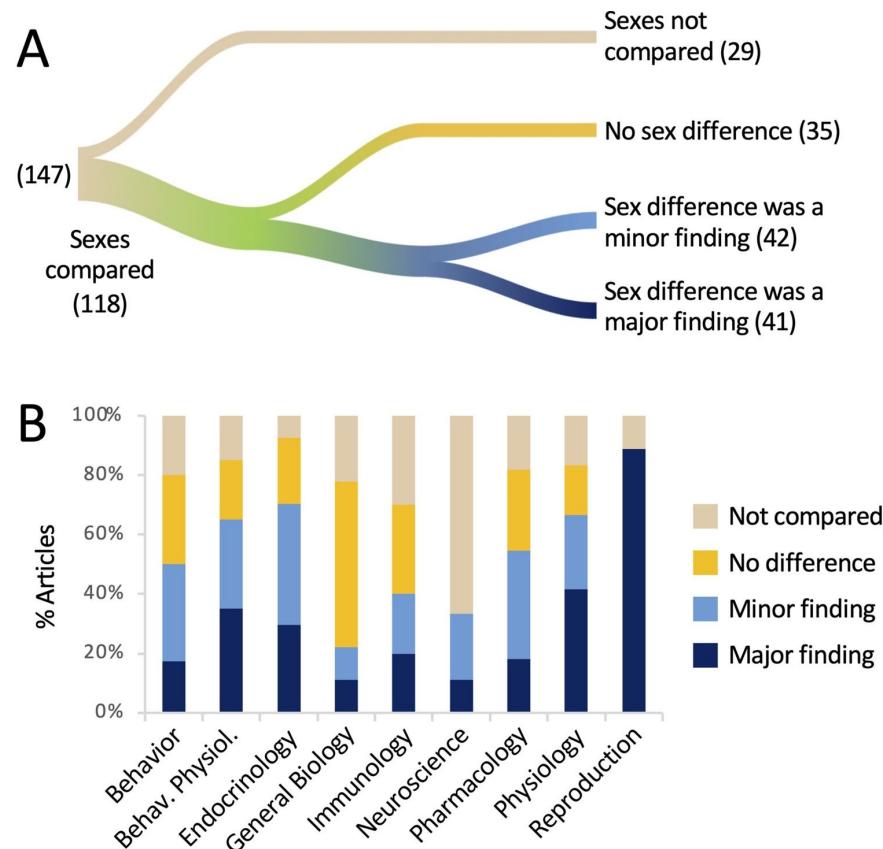
But...

- » Increase in mentions of sex-differences in published articles
- » «*Without careful methodology, the pursuit of sex difference research, despite a mandate from funding agencies, will result in a literature of contradiction*»
Janet Rich-Edwards et al. (2018) Endocrine Reviews



[https://www.whijournal.com/article/S1049-3867\(23\)00072-5/fulltext](https://www.whijournal.com/article/S1049-3867(23)00072-5/fulltext)
<https://academic.oup.com/edrv/article/39/4/424/4967741>

The «DISS» error: difference in sex-specific significance



<https://elifesciences.org/articles/70817>
<https://www.sciencedirect.com/science/article/pii/S00928674240017>

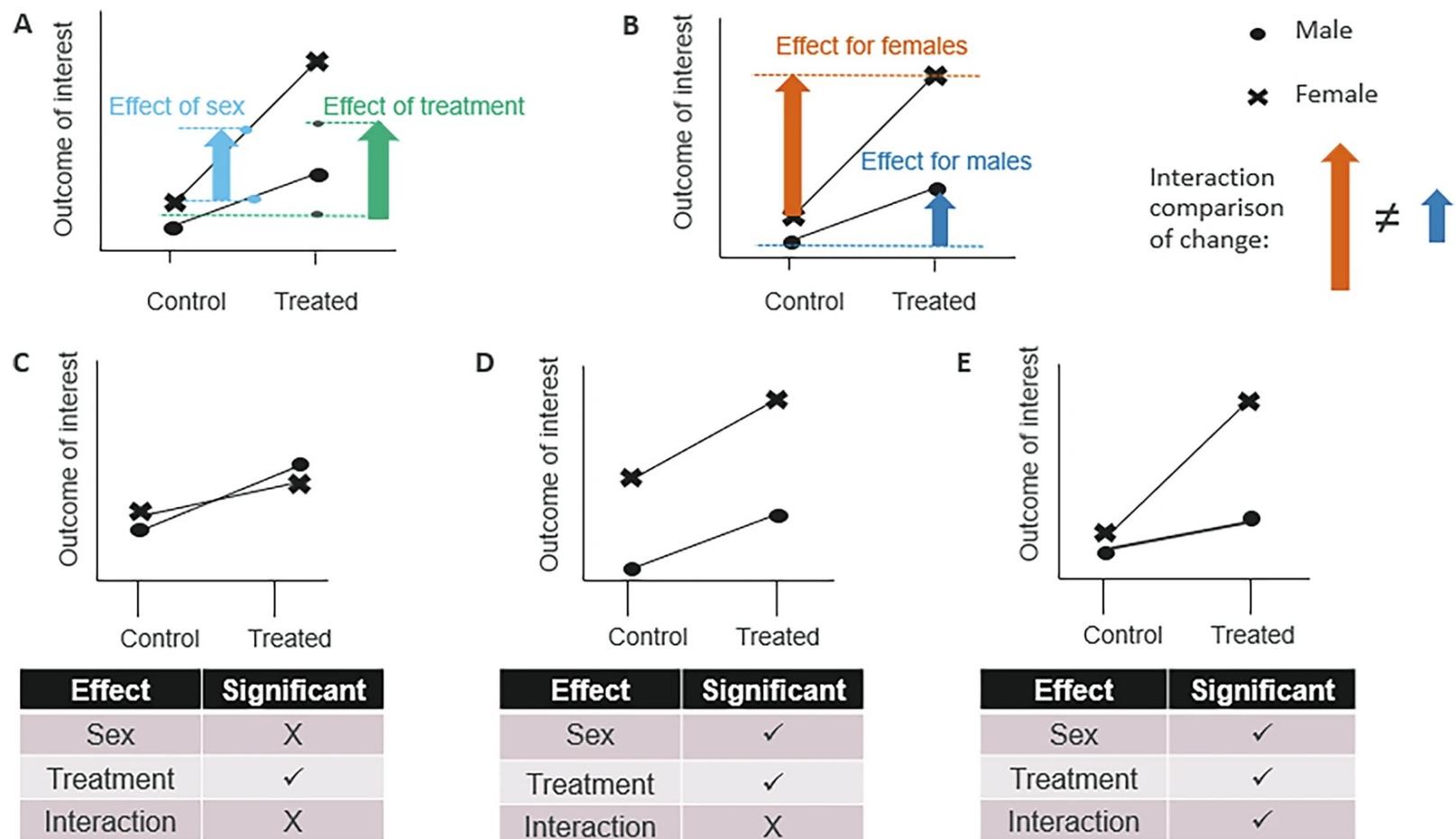
An evaluation of publicly available resources

«We evaluated three sets of **publicly available online training materials** on this topic: (1) *Integrating Sex & Gender in Health Research* from the Canadian Institutes of Health Research (CIHR); (2) *Sex as a Biological Variable: A Primer* from the United States National Institutes of Health (NIH); and (3) *The Sex and Gender Dimension in Biomedical Research*, developed as part of "Leading Innovative measures to reach gender Balance in Research Activities" (LIBRA) from the European Commission.»

«All three courses discussed the importance of including males and females to better generalize results, discover potential sex differences, and tailor treatments to men and women. The entangled nature of sex and gender, operationalization of sex, and potential downsides of focusing on sex more than other sources of variation were **minimally discussed**. Notably, all three courses explicitly **endorsed invalid analytical approaches** that produce bias toward false positive discoveries of difference.»

<https://link.springer.com/article/10.1186/s13293-024-00610-6>





<https://www.nature.com/articles/s42003-025-08118-4>

As bioinformatician

- »» When we investigate gene expression differences by biological sex, our experiments live or die by their design
- »» How to best conduct statistical analyses?
 - »» E.g., do not treat sex as a covariate to be eliminated
- »» How to avoid common pitfalls of bioinformatics studies?
 - »» E.g., sexual chromosomes often excluded from analyses
- »» Deposition in databases
 - »» “Molecular omics resources should require sex annotation: a call for action”
 - »» “Addressing sex bias in biological databases worldwide”

<https://www.nature.com/articles/s41592-021-01168-6>

https://osf.io/preprints/biohackrxiv/n9dkg_v1



Today's goals

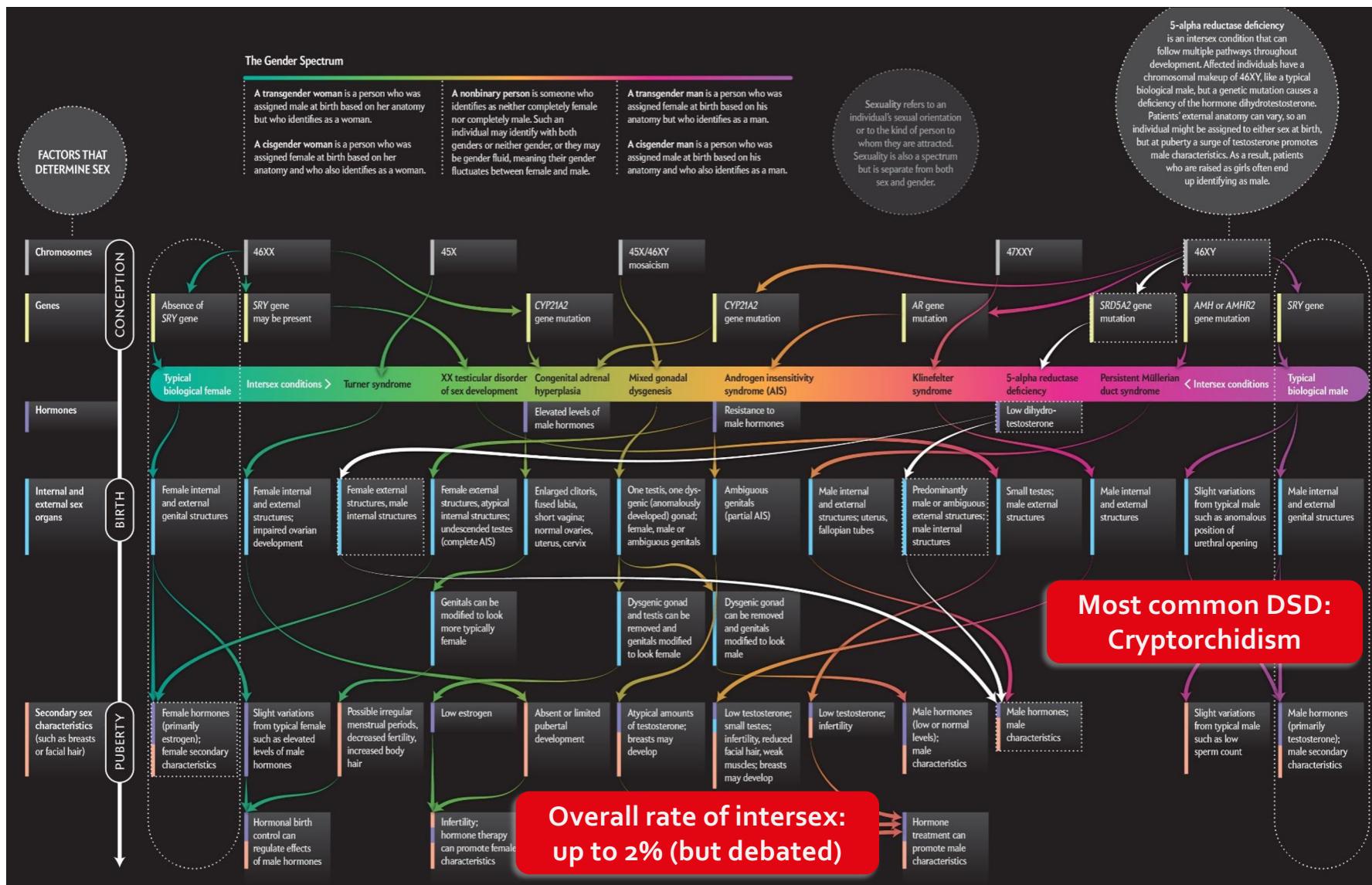
Embrace this complexity!

Learn more on how to design, analyze and report accurately

<https://www.nature.com/articles/d41586-024-01204-3>



<https://www.scientificamerican.com/article/complexity-of-sex-determination/the-extraordinary-complexity-of-xx-and-xy/>

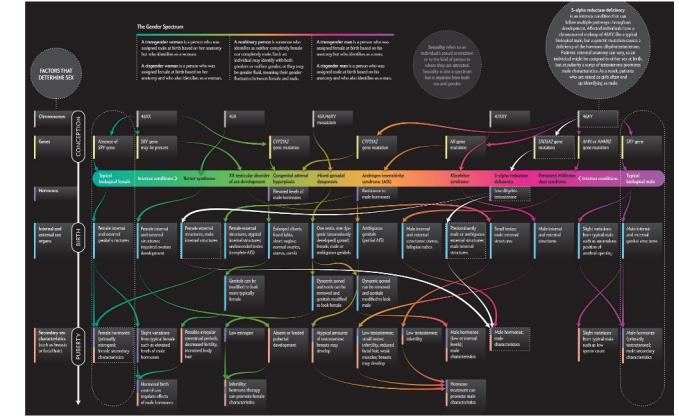


Team activity (5-10 min)

Breakout rooms, 2 by 2

Focus on one syndrome / phenotype /subcase and search more detailed information.

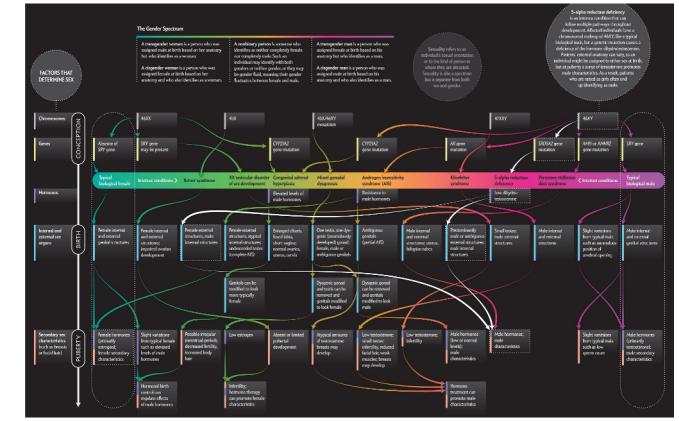
- » Sources: Wikipedia, scientific papers, blog posts, ...
- » Mechanism(s)
- » Prevalence
- » ...



<https://bit.ly/3RAkQao> 

Team activity (5-10 min)

1. Klinefelter syndrome
2. 45,X/46,XY mosaicism
3. Turner syndrome
4. XX male syndrome
5. AMHR2 mutation
6. CYP21A2 mutation
7. Androgen insensitivity syndrome
8. Denys–Drash syndrome



<https://bit.ly/3RAkQao>



SCIENCE FORUM

Best practices to promote rigor and reproducibility in the era of sex-inclusive research

Abstract To enhance inclusivity and rigor, many funding agencies now require inclusion of females as well as males in biomedical studies. These mandates provide unprecedented opportunities to discover sex differences. Educative subgroup category has lagged behind, however, resulting in a problem of sex differences, and interpretations of results are often flawed. Here, we outline best practices for studies in which sex differences are a primary focus. Our recommendations are organized within the “4 Cs of Studyin Collection, Characterization and Communication,” a framework developed by the National Institutes of Health at the National Institutes of Health in the United States. Follow these guidelines to include females and males in their studies while at the same time upholding rigor and reproducibility.

JANET W RICH-EDWARDS, DONNA L MANEY*

Perspective

Sex contextualism in laboratory research: Enhancing rigor and precision in the study of sex-related variables

Madeleine Pape,^{1,*} Miriam Miyagi,² Stacey A. Ritz,³ Marion Boulicault,⁴ Sarah S. Richardson,^{5,6} and Donna L. Maney^{7,8}

¹Institute of Social Sciences, University of Lausanne, Lausanne, Switzerland

²Center for Computational Molecular Biology, Brown University, Providence, RI, USA

³Department of Pathology & Molecular Medicine, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada

⁴Department of Philosophy, University of Edinburgh, Edinburgh, Scotland

⁵Department of the History of Science, Harvard University, Cambridge, MA, USA

⁶Committee on Degrees in Studies of Women, Gender, and Sexuality, Harvard University, Cambridge, MA, USA

⁷Department of Psychology, Emory University, Atlanta, GA, USA

“When researchers incorporate these sex-related variables into research designs, rigorous analytical methods are needed to allow strongly supported conclusions”

Research requires rigorous and precise approaches to study sex differences. First, it is important to recognize that sex is not in and of itself a causal mechanism, but rather a biological variable defined by categories, usually assigned according to a range of characteristics. Second, when researchers move beyond sex as a categorical variable and toward sex as a continuous variable, they must pay attention to how these sex-related variables matter—and do so in context-specific ways. Third, when researchers incorporate sex-related variables into their research designs, rigorous analytical methods are needed to ensure that the study is well-powered and that the presentation and reporting of sex-related variation require transparency and accountability to promote health equity for all.



nature

in × f

Sex and gender in science

How to navigate a challenging area
of research to the benefit of all

11 June 2024

March 2024: Landmark Cell focus issue centers the voices of sex and gender minorities



[nature](#) > collection

Collection | 22 August 2024

Sex as a biological variable (SABV)

Submission status

Closed

Submission deadline

22 August 2025

Sex as a biological variable (SABV), most simply defined at the chromosomal level, can have impacts ranging from gene expression to drug metabolism, and from organ shape to clinical phenotypes. Yet, historically, most basic biological or biomedical research has been carried out using only one sex, or aggregating both sexes together, for example using animal models or cell lines. This has led to foundational biases and knowledge gaps about when sex does and does not make a difference.

This cross-journal Collection aims to promote research that considers SABV. We encourage submission across all realms of biological research, from molecular and cellular biology to cognitive neuroscience, and across all levels, from *in vivo* model organisms and humans to *in vitro* and computational models including studies considering SABV in biomedical and medical research. — [show all](#)

[Collection content](#)[Participating journals](#)[About the editors](#)[About this collection](#)

<https://www.nature.com/collections/cfhhacjgbc>



Resources and guidelines (1)

- » Gendered innovations
 - » <http://genderedinnovations.stanford.edu/index.html>
- » MRC Policy on Sex in Experimental Design
 - » https://nc3rs.org.uk/sites/default/files/2022-08/MRC%20new%20expectations%20on%20the%20inclusion%20of%20sex%20in%20experimental%20design%20%28Dr%20Stella%20Child%2C%20UKRI%20-%20MRC%29_o.pdf
- » Working Group on Sex in Experimental Design of Animal Research
 - » <https://www.ukri.org/wp-content/uploads/2022/03/MRC-090322-SexInExperimentalDesign-SummaryReport.pdf>
- » Experimental design assistant
 - » <https://eda.nc3rs.org.uk/experimental-design-animal-characteristics#sex>
- » The Sex and Gender Perspective in Research Toolkit
 - » https://aquas.gencat.cat/web/.content/minisite/aquas/publicacions/2022/toolkit_perspective_sex_gender_research_aquas2022.pdf
- » Factsheet Preclinical Research UZH
 - » https://www.gendermed.uzh.ch/dam/jcr:e6ead002-e395-4126-9b22-3ee389fd358a/Factsheet_Pr%C3%A4klinische%20Forschung_Jaric_Buch_2024.pdf



Resources and guidelines (2)

- » American Thoracic Society

- » <https://www.atsjournals.org/doi/10.1164/rccm.202506-1361ST>

- » Applying the new SABV policy to research and clinical care

- » <https://www.sciencedirect.com/science/article/pii/S0031938417302585>

- » The Sex Inclusive Research Framework to address sex bias in preclinical research proposals

- » <https://www.nature.com/articles/s41467-025-58560-5>

- » Leveraging research into sex differences and steroid hormones to improve brain health

- » <https://www.nature.com/articles/s41574-024-01061-0>



Questions?



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Hands-on



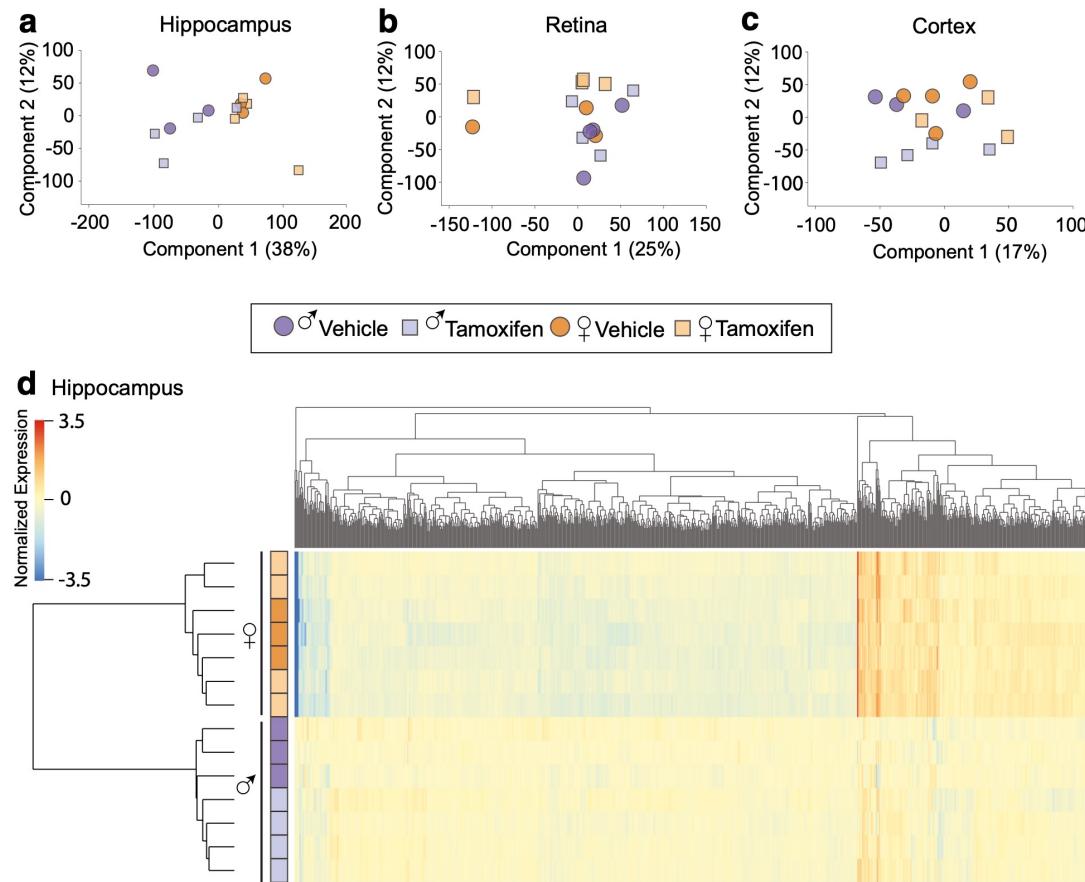
Hands-on

Installed R and RStudio before the start of the course?

Breakout rooms to help/debug / if people want to work in subgroups



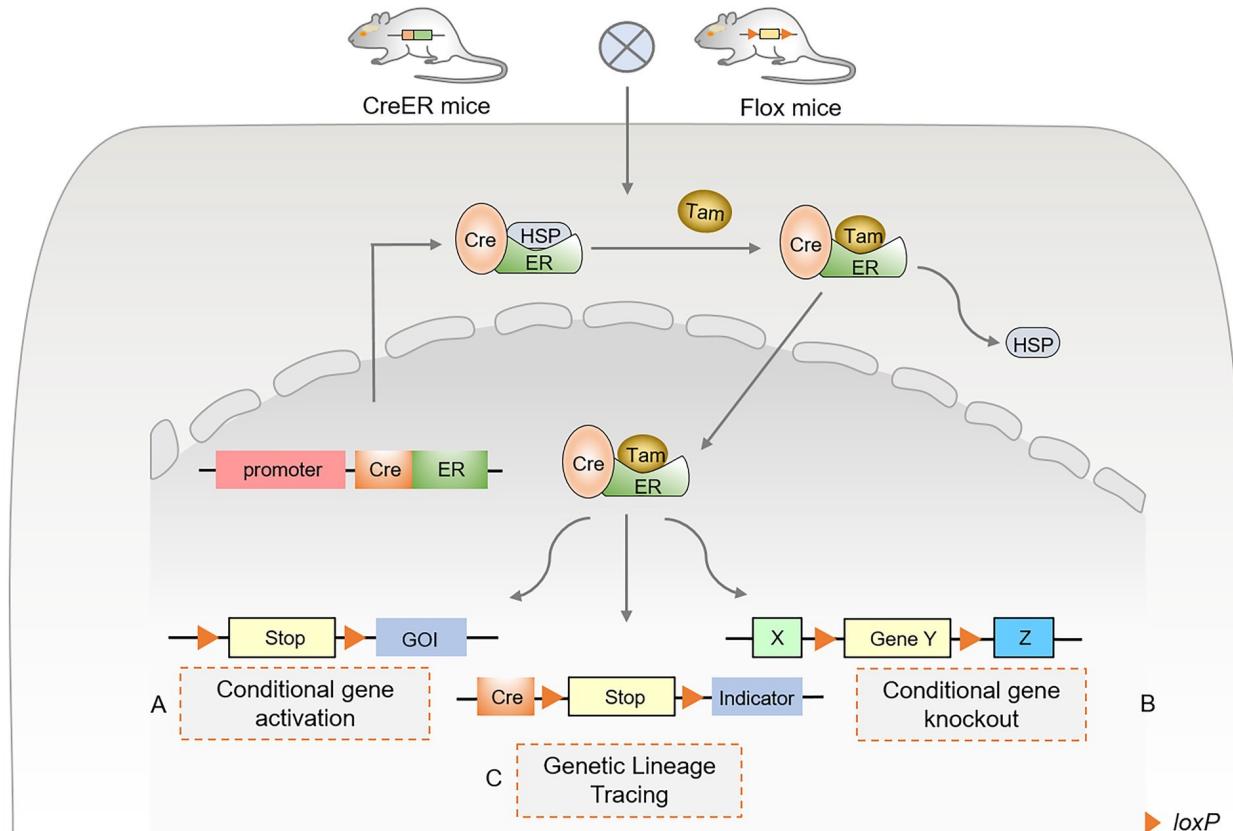
Hands-on



<https://link.springer.com/article/10.1007/s11357-019-00090-2>



Hands-on



<https://www.frontiersin.org/articles/10.3389/fcvm.2023.1085629/full>



Hands-on

 [www https://github.com/julien-roux/SIB_course_2025_best_practices_differences_biological_sex](https://github.com/julien-roux/SIB_course_2025_best_practices_differences_biological_sex)

Screenshot of a GitHub repository page for "SIB_course_2025_best_practices_differences_biological_sex". The repository contains several files and a README.md file.

Files:

- main
- hands-on
- img
- README.md
- hands_on_DESeq2.qmd
- hands_on_limma.qmd
- rse_SRP218156.rds

SIB_course_2025_best_practices_differences_biological_sex / hands-on /

| Name | Last commit message | Last commit date |
|---------------------|--------------------------------|------------------|
| .. | Add images | last month |
| img | Add images | last month |
| README.md | First commit | last month |
| hands_on_DESeq2.qmd | Add analysis script with limma | last month |
| hands_on_limma.qmd | Add analysis script with limma | last month |
| rse_SRP218156.rds | First commit | last month |

README.md

SIB course: Best Practices for Investigating Gene Expression Differences by Biological Sex -- Hands-on session

October 27th, 2025

Motivation

The past decades have seen large calls for consideration of the sex dimension into research and clinical projects. However as we progress towards more frequent inclusion of this variable, the design of experiments evolved to be much more complex, [in particular challenging the analysis steps](#). This hands-on tutorial is designed to foster a critical perspective on integrating the sex dimension in bioinformatic analyses. Through a practical exercise focusing on Differential Expression (DE) analysis, we will revisit the conclusions from a published study in light of the results of our re-analysis. We aim at rigorously testing for sex differences and notably for the presence of interactions between experimental factors. Following on Frédéric's presentation, we would like to use this hands-on to draw your attention at power issues potentially affecting conclusions, and how to best report results of your analyses.



bit.ly/4ngRUAR



Today's schedule

09:15 – 10:15 Introduction

10:15 – 10:30 Coffee break

10:30 – 12:00 Biological sex in experimental design
(Talk+hands-on by Frédéric Schütz)

12:00 – 13:00 Lunch break

13:00 – 15:00 Hands-on session in subgroups

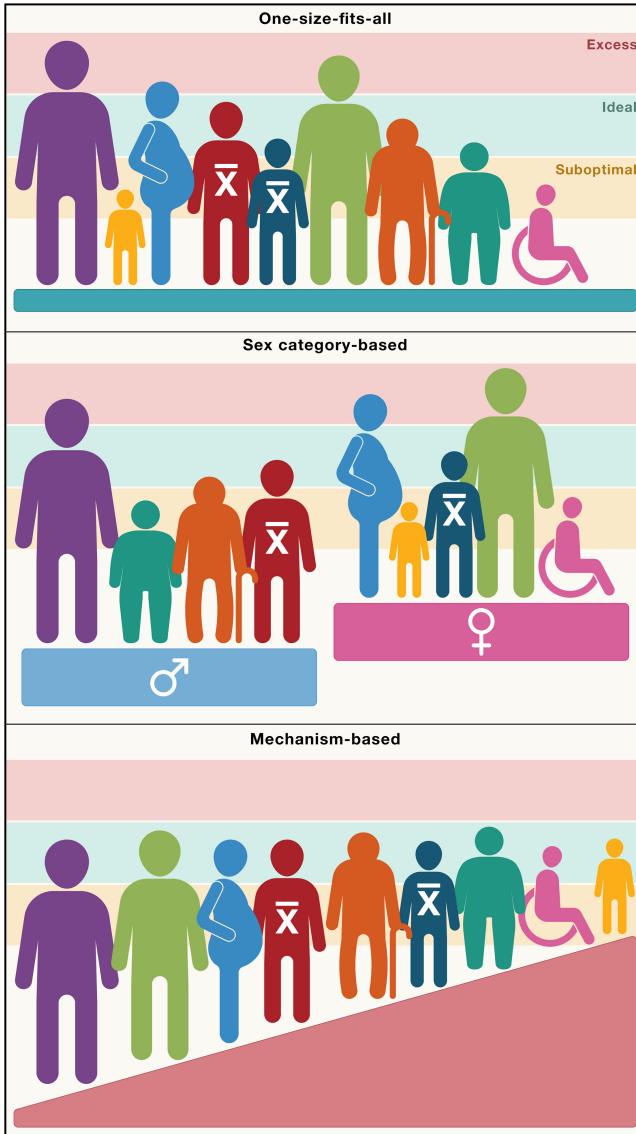
15:00 – 15:30 Coffee break

15:30 – 17:00 Finishing hands-on, wrap up and discussions



Conclusion





- » Rigorous reporting of effect of studies variables
- » Testing for interactions and attention to power issues (not significant \neq absence of effect)
- » Honestly report study limitations and beware of hype and distortion (e.g., press releases, communication to public)

Back to today's learning outcomes

- » Inform colleagues about critical issues and challenges related to sex-based considerations in biological data collection.
- » Propose suitable study design options and recognize potential biases that may arise in data collection and interpretation.
- » Apply rigorous analytical methods on -omics datasets.
- » Interpret and report results transparently and reproducibly in scientific papers.
- » Generalize methods to other variables (age, ethnicity, etc) relevant to diversity and inclusion.



Back to you

- » What did you learn that is relevant to your research project?
- » What was maybe missing?
- » Feedback, suggestions?



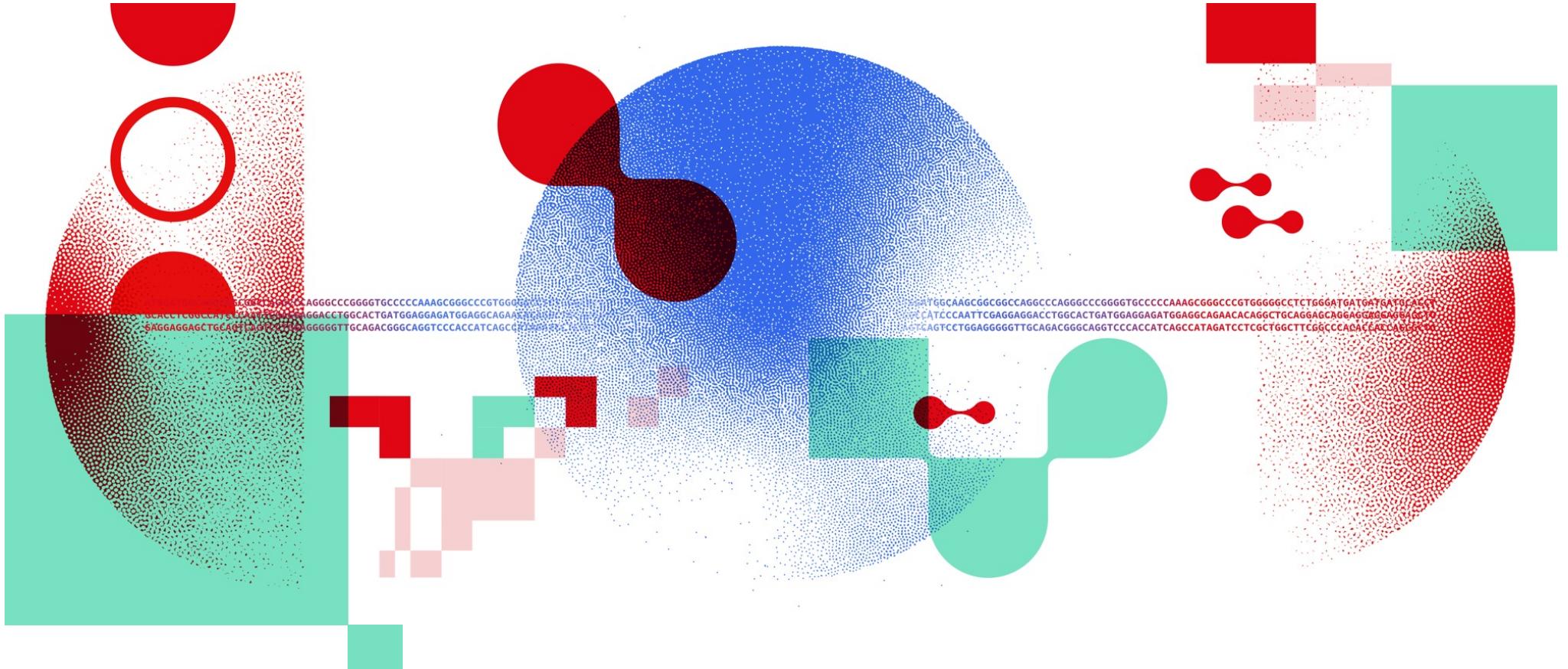
Wrap up

- » We'll share with you the slides and hands-on material
- » Exam sent in the next few days
- » Don't hesitate to get in touch with us if you have ideas or wish to contribute to the SIB Diversity focus group
[\(edi@sib.swiss\)](mailto:edi@sib.swiss)

DANKE !
THANK YOU !
MERCI !
GRAZIE !
GRACIAS !
DANK JE WEL !



Photo: <https://unsplash.com>



Thank you!

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