

# Homologous chromosomes? Exploring human sex chromosomes, sex determination and sex reversal using bioinformatics approaches

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## Abstract

Constructing a robust understanding of homologous chromosomes, sex chromosomes, and the particulate nature of genes is a notoriously difficult task for undergraduate biology students. In this lesson, students expand their knowledge of human chromosome pairs by closely examining autosomes, sex chromosomes, and the non-homologous elements of the human X and Y sex chromosomes. In this four-part guided activity, students will learn about the structure and function of human autosomal and sex chromosomes, view and interpret gene maps, and gain familiarity with basic bioinformatics resources and data through use of the National Center for Biotechnology Information (NCBI) website. (Student access to computers with Internet connectivity is required for the completion of all Investigations within this lesson.) By viewing chromosomes and gene maps, students will be able to contrast expectations for homologous autosomal chromosome pairs and sex chromosome pairs, as well as gain a deeper understanding of the genetic basis for human chromosomal sex determination. In the last part of this lesson, students can also begin to understand how genetic mutations can lead to sex-reversal. The lesson, as presented, is intended for an introductory biology course for majors, but could be modified for other audiences. In addition, each exercise ("Investigation") within the lesson can be used independently of the others if an instructor wishes to focus on only a subset of the learning objectives and provide the necessary context. Options to extend the lesson related to interpreting phylogenies, and contrasting definitions of sex and gender are also provided.

## Learning Goal(s)

Students will understand the genetic basis of human chromosomal sex determination and be able to use bioinformatics evidence (e.g., gene maps and sequence data) to defend the definition of homologous chromosomes and to contrast homologous chromosomes with sex chromosomes in humans. Additionally, students will identify the presence of the Y-linked genetic marker SRY as an important regulator of human sex determination.

## Learning Objective(s)

Students successfully completing this lesson will:

- Practice navigating an online bioinformatics resource and identify evidence relevant to solving investigation questions
- Contrast the array of genes expected on homologous autosomal chromosomes pairs with the array of genes expected on sex chromosome pairs
- Use bioinformatics evidence to defend the definition of homologous chromosomes
- Define chromosomal sex and defend the definition using experimental data
- Investigate the genetic basis of human chromosomal sex determination
- Identify at least two genetic mutations can lead to sex reversal

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**Materials and Supplemental Materials:** Table 1. Homologous chromosomes-Teaching Timeline, Supplemental File S1: Homologous chromosome-Pre-Investigation questions, Supplemental File S2: Homologous chromosome-Pre-Investigation question Debrief Options, Supplemental File S3: Homologous chromosome-Investigation 1: Homologous chromosomes? Exploring the relationship of human autosomes and sex chromosomes, Supplemental File S4: Homologous chromosome-Investigation 2: The Sex Determining Region of The Y chromosome, Supplemental File S5: Homologous chromosome-Investigation 3: Sex-reversal and other deleterious mutations of the SRY gene in humans, sex-specific diseases, Supplemental File S6: Homologous chromosome-Optional Extension 1: Sex determination varies among vertebrates, Supplemental File S7: Homologous chromosome-Optional Extension 2: Sex-linked genes and disorders, Supplemental File S8: Homologous chromosome-Optional Extension 3: Sex and Gender, Supplemental File S9: Homologous chromosome-Answer Key, Supplemental File S10: Homologous chromosome-Glossary of Terms and Supplemental File S11: Homologous chromosome-Additional Resources

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## INTRODUCTION

Constructing a robust understanding of homologous chromosomes, sex chromosomes, and the particulate nature of genes is a notoriously difficult task for undergraduate biology students (1, 2), but is essential to a complete understanding of diploid human genomes, meiotic cell division, and inheritance (1, 3).

In the current post-genomics era, it is also imperative that students of biology are exposed to the growing availability and usefulness of molecular sequence data and have opportunities to practice basic bioinformatics skills such as navigating, retrieving, and interpreting bioinformatic data (3-5). In the words of one author, “No science curriculum can remain current without a bioinformatics component” (3).

The National Center for Biotechnology Information (NCBI) curates an extensive compilation of data and databases that is freely available to any user with Internet access (6). Further, NCBI provides a great example of collaborative science in action, with the information being deposited, evaluated, annotated, and used on an ongoing basis to provide the most up-to-date, accurate, and usable information for the research community. Beyond NCBI, many freely accessible resources used by researchers in the fields of genomic, proteomics, and bioinformatics have been identified as particularly well suited for inclusion in undergraduate biology education (4-5). Several accounts have been published reporting the use of these bioinformatics resources and approaches in undergraduate education in semester-long courses focusing on genomics (5), as a series of exercises implemented in different courses across a biology curriculum (4), as a dedicated recitation section associated with a genetics course (7), or as an exercise(s) within a course (2). Assessment of courses utilizing bioinformatics approaches have demonstrated gains in student learning and positive changes in student attitudes regarding excitement about learning, abilities, perceptions of biology (8). This lesson seeks to help students solidify their understanding of large-scale chromosome structure and composition, as well as to increase students’ familiarity with basic bioinformatics data and approaches. These goals are achieved by using a scaffolded, guided inquiry based approach (i.e. process-oriented, guided inquiry learning, pogil.org) (9). If an instructor wishes to focus on only a subset of the learning objectives, each exercise or “Investigation”, can be used independently of the others, as long as the needed context is provided to the students.

### *Genomes and homologous chromosomes*

(This summary focuses on eukaryotic, linear chromosomes, although parts of the description are applicable to bacteria.) A chromosome is a single DNA molecule that, at certain times, can be condensed into a compact coiled structure visible in a light microscope. Each chromosome consists of a long arm and a short arm, defined by the location of the centromere (a sequence of DNA that binds to proteins needed for segregation of the chromosomes during cell division). The ends of linear chromosomes consist of repetitive DNA sequences known as telomeres (10). In humans, 23 pairs of chromosomes (46 individual chromosomes) constitute the full genome. Of these 23 pairs, 22 pairs are classified as homologous autosomes (non-sex chromosomes). These pairs are numbered from 1 to 22, in order of descending size of the chromosome. Thus, chromosome one is the largest, and chromosomes 21 and 22 are the smallest (10). The 23rd pair of chromosomes in humans is not autosomal, but rather is a pair of sex chromosomes,

named such because of their deterministic role in the development of human male and female sex (10).

The term “homologous chromosomes” has been, in my experience and those of others (1, 2), a very difficult concept for students to understand and explain, even after repeated exposure to the process of meiosis (i.e. students in upper division biology courses who have learned the material during an introductory biology course). Specific areas of difficulty include a lack of or incomplete understanding that homologous chromosomes will be the same size, have the same number and types of genes, and that these genes will be present in the same order along the chromosome, even though the exact DNA sequence of the homologous chromosomes will be different owing to the presence of different alleles on the maternally inherited and paternally inherited chromosomes. Further, students frequently tend to confuse or fail to understand the difference between the terms “homologous chromosomes” and “sister chromatids”. With the overarching goal of helping students construct a correct and thorough understanding of homologous chromosome pairs, I have implemented several different approaches to allow students to explore chromosomes and gene arrays on chromosomes including physical modeling (e.g. pipe cleaner and bead activities modeling genes on chromosomes) and bioinformatics approaches such as those presented here to help students visualize and solidify what can be a very abstract set of concepts. In addition to working through this lesson, instructors not familiar with DNA structure, DNA replication, and definitions of homologous chromosomes and sex chromosomes, or the bioinformatics resources used in this lesson are encouraged to read relevant chapters in an introductory biology text and make use of resources listed in Supplemental File S11: Additional Resources.

### *Intended Audience*

This lesson is intended for an introductory biology course for majors, but could be modified for a non-majors biology course (through the removal of more sophisticated questions), or revised for an upper level genetics course (through the expansion of one or more of the Investigations.)

### *Learning Time*

As presented, the entire lesson with all three Investigations could be completed during one laboratory meeting time (2-3 hours) or during two approximately 1-hour lecture sessions. The lesson includes Pre-Investigation questions that individual students complete prior to engaging in the remainder of the lesson; these questions could easily be assigned as homework so that additional class time can be devoted to discussion and review of the investigations. The remainder of the lesson (Investigations 1, 2, 3) can be completed collaboratively or independently as per the instructor’s preference.

### *Pre-requisite student knowledge*

Prior to engaging in this lesson, students should understand genomes, karyotypes, mitosis and meiosis, homologous chromosomes and the Central Dogma (gene → mRNA → protein; transcription and translation).

*Lesson Outline:*

1. **Pre-Investigation questions**
2. **Investigation 1:** Homologous chromosomes? Exploring human autosomes and sex chromosomes.
3. **Investigation 2:** Non-homologous elements: The Sex Determining Region of The Y chromosome
4. **Investigation 3:** Mapping Mutations: Sex-reversal and other deleterious mutations of the SRY gene in humans

Student access to computers with Internet connectivity is required for the completion of all Investigations within this lesson.

**SCIENTIFIC TEACHING THEMES**Active learning

Students complete Pre-Investigation Questions prior to class, then use their responses as the basis for small group discussion/comparison/debate prior to beginning the Investigations. During the Investigations, students can work collaboratively in pairs and with feedback from the instructor(s) to answer the questions posed as contemporary science inquiries through identifying and interpreting information on NCBI. Each student should be working concurrently on the question, rather than using a “divide and conquer” approach, which the instructor can reinforce and enforce by circulating among students as they work and redirecting or assisting as appropriate.

Assessment

Instructors can use various forms of formative and summative assessment relevant to this lesson. For example, the small group discussion or clicker questions reviewing the Pre-Investigation Questions (presented in Supplemental File S2) helps the instructor assess the level of knowledge students have prior to completing the lesson. These same questions could be presented post-lesson to see how student understanding has changed. Further summative assessment can be implemented by presenting any of the questions included in the investigations (or similar versions) on quizzes or exams. A further option for summative assessment would be a writing assignment that would ask students to elaborate on their understanding of how sex chromosomes differ from autosomes or to explore/report on a concept addressed in the Investigations (for example, the included Optional Extension 2: Sex-linked genes and disorders, Supplemental File S7), or explore the biological definition of sex with social definitions of gender (see the included Optional Extension 3: Sex and Gender, Supplemental File S8). Students can self-assess by exploring the resources provided within the lesson, consulting with peers, or by seeking additional clarification from instructors/facilitators.

Inclusive teaching

This lesson allows students to exchange and evaluate each other’s ideas prior to and during completion of the Investigations. The lesson also emphasizes the collaborative and interdependent nature of science by utilizing an open resource that is compiled, reviewed, and annotated by scientists from around the world investigating many different questions and utilizing these resources in many different ways. The subject matter of this lesson, namely sex determination and definitions of sex (and gender, in Optional Extension 2) also provide opportunities for exploring differences that exist

in how biologists and non-biologists may define or think about sex (i.e. definitions of maleness and femaleness). Further, discussion of sex-reversal and possible mismatches between genotypic sex and phenotypic sex provides an opportunity to extend students’ understanding of sex from a simple dichotomy to a diverse spectrum.

**LESSON PLAN**

A timeline for the delivery of this lesson can be found on Page 4, Table 1.

Presentation of this lesson should occur after students have received instruction in the central dogma of biology, including the processes of transcription, RNA processing, and translation. It would be helpful for students to have familiarity with the basic molecular structure of DNA (components of nucleotides; base-pairing rules for double stranded DNA helix) and DNA replication, as well as familiarity with the idea that genomes are comprised of chromosomes, and diploid organisms such as humans have two copies of all of their chromosomes.

In preparation for the in-class portion of this lesson, assign students the Pre-investigation questions (Supplemental File S1: Pre-investigation questions). These six pre-investigation questions probe students’ prior knowledge of homologous chromosomes and human sex chromosomes. It is not expected that students will be able to answer these questions correctly or thoroughly, and students are explicitly instructed to NOT use any outside resources to answer these questions. It is anticipated that the questions should take students ~15-20 minutes to complete. The student answers allow the instructor to assess student prior knowledge and better address student misconceptions or preconceptions as the lesson progresses. The instructor may wish to have students submit their answers to the pre-investigation questions in advance class such that the responses can be evaluated to this end. Alternatively, I have generally asked students to bring their responses to class, and we begin the class session with a short debrief of student responses to the pre-investigation questions before engaging in the Investigations. Depending on the wishes of the instructor and time constraints, this initial debrief of students’ prior knowledge can be contracted or expanded. For implementation in my classes, I include a “think-pair-share” activity in which students compile their individual responses to the pre-investigation questions and highlight areas of disagreement or ambiguity to be clarified throughout the course of the Investigations or in the final debrief with the instructor. A second pre-investigation debrief option that can also serve as a pre- and post-instruction assessment is the use of audience response “clicker” style activity for a subset of the pre-investigation questions. Supplemental File S2 “Pre-investigation question debrief options” includes possible student instructions for both approaches.

Students may then begin Investigation 1. I provide paper handouts to students (Supplemental File S3 is the handout for Investigation 1) to enable them to easily follow the instructions while navigating through the online resources at NCBI, rather than having to switch between the instructions and NCBI on their computer screens. I instruct students to work in pairs to complete the steps for Investigation 1, discussing the questions as they go along. As this is the first time students will be navigating the online database and identifying the information relevant to their investigation, I plan ample time (reflected in the timeline provided in Table 1) for students to become familiar with this resource and the type of data being used. If

**Table 1: Homologous chromosomes-Teaching Timeline**

Activity	Format and Approximate Time
<b>Pre-class</b>	
Pre-class assignment	Completed individually ~15 min
<b>In-class</b>	
Review of responses to pre-investigation questions	Possible formats: Pair-Share; Group List; Audience Response Questions 10 min
Investigation 1: Homologous Chromosomes?	Completed in pairs or groups 40 min
Investigation 2: Non-homologous Elements	Completed in pairs or groups 15 min
Investigation 3: Mapping Mutations	Completed in pairs or groups 20 min
Wrap-up	Possible formats: Pair-Share; Revisit Group List; Class Discussion; Mini Lecture 20 min
Assessment	Formative: Repeat audience response questions Summative: Writing assignment; Exam questions

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students have already been exposed to NCBI or similar online databases, the time for Investigation 1 can likely be truncated from the 40 minute period in the time line. As the students work to answer the investigation questions, the instructor(s) can circulate among students to ensure they are able to find the correct information, stay on task, and arrive at appropriate conclusions from the data presented. Instructor input and prompting to thoroughly consider the bioinformatics data in relation to the investigation questions posed (or, playing devil's advocate) also helps to keep "minds on" in addition to "hands on" throughout the lesson.

Rather than doing an extensive whole-class debrief after Investigation 1, I generally wait until students have completed all three investigations before revisiting the outstanding questions that students had from the Pre-investigation questions. I control student progression through the lesson by providing the handout for the next Investigation according to the timeline established for the lesson (example timeline provided in Table 1; student handouts for each of the three investigations are Supplemental Files S3, S4 and S5). At the conclusion of the three investigations, several different wrap-up strategies can be used to ensure the learning goals of the lesson have been achieved: revisiting the pre-investigation questions and the student responses provided prior to instruction and challenging students to build on their previous responses and provide more sophisticated and thorough responses is way to come full circle and provide closure.

### *Options for Extending the Lesson*

Three possible options for extending the lesson have been provided; I have used each variation in my courses, sometimes in conjunction with the delivery of this lesson. I have implemented Optional Extension 1: Sex Determination Varies Across Vertebrates (Supplemental File S6) following Investigation 1, after students have been able to identify differences between homologous chromosome pairs and sex chromosome pairs. To help students place the mammalian X-Y sex determination system in a broader biological context, I present a phylogenetic tree of major vertebrate clades (for examples, see figures in references 12 and 13).

Optional Extension 2 (Supplemental File S8) presents one option for a follow up assignment in which students can further investigate sex-linked genes and disorders in humans. This extension option may also fit well as an element of a lesson on pedigrees, as it asks students to identify inheritance patterns of diseases and to differentiate between sex-linked (i.e. genes that are located on sex chromosomes, either X-linked or Y-linked) and sex-specific (i.e. specific to either males or females).

The courses in which I have implemented this lesson are part of an integrated curriculum, so it is an additional learning goal for students in these courses to consider concepts (e.g. definitions of sex) from multiple disciplinary perspectives. Thus, I have included Optional Extension 3 (Supplemental File S9) as an example of how biological definitions of sex can be compared and contrasted with social definitions of sex and gender. In this example assignment, students are asked to consider both perspectives in proposing approaches to defining eligibility for athletic competitions that have male and female categories for competitors.

## **TEACHING DISCUSSION**

This lesson was designed to help students achieve the biology learning goals of (1) developing and reinforcing

a robust understanding of the relationships between and among homologous and sex chromosome pairs in a diploid organism; and (2) refine definitions of biological sex through identification of genetic mutations that can lead to sex reversal (i.e. a mismatch between chromosomal and phenotypic sex). Further, this lesson also provides an investigative context for students to begin to develop skills in using bioinformatics research databases and accessing genetic sequence data that can be found there.

One of the challenges in teaching this lesson is to help students avoid "pointing and clicking" through the lesson without reflecting on the meaning of the data they are finding. Focusing on the embedded questions in each investigation, and ensuring that pairs or groups of students work collaboratively and simultaneously on all of the questions, will help students to actively engage with the concepts, rather than click through on auto-pilot. Instructors may wish to tailor the delivery of the Investigations to more stringently regulate the progress of students through the lesson and ensure adequate time for reflection and strengthening of understanding.

There are three general areas addressed in this lesson that appear to be surprising to students and lend to the effectiveness of the lesson:

1. Substantial differences exist in the size and numbers of genes present on different human chromosomes, and indeed the absolute size of chromosomes. Students appear to more readily grasp the differences in size and genetic composition of chromosomes after they find quantitative information, such as number of base pairs and number of genes present on different chromosomes. Discussing the size and gene number on homologous chromosome pairs helps to solidify the similarities between homologous chromosomes, which share the same genes in the same order, versus the differences between non-homologous chromosomes, which have entirely different genes. Further, understanding that homologous chromosomes contain the same genes but not the exact same sequence (owing to the presence of different alleles on the maternally and paternally inherited chromosomes) can also be contrasted with the identical nature of sister chromatids in mitosis and pre-crossing over sister chromatids in meiosis.
2. Vast amounts of genetic information are publicly accessible. First year college students seem impressed with the ready availability of so much sequence data (e.g., "This is really the sequence of the SRY gene in the human genome?") and also respond well to the visual display of individual genes on chromosomes.
3. There is some unexpected complexity within the concepts of sex and gender, and sex chromosomes and sex phenotype. Investigations 2 and 3 allow students to think about their familiar, but often limited definitions of what it means to be a human male or a human female. This understanding can be enriched if they have had the opportunity to think about sex vs. gender from a different disciplinary context (e.g. from a sociological or psychological perspective rather than strictly biological). Understanding the sex determination pathway starting with SRY is aligned with their simplistic, dual definitions of sex, i.e. males have Y chromosomes. Encountering translocations of the SRY

transcription factor gene to a different chromosome that lead to sex reversal provides opportunities for students to confront and revise simple definitions of sex (see Supplemental File S8: Sex and Gender for an example of such an extension activity.).

Students enrolled in the courses in which I have implemented this lesson are accustomed to student-centered, active learning instructional strategies described in the lesson plan; they generally do not require much instructor intervention to keep them on task or to make sure they are having constructive discussions with peers as they navigate through the resources and answer the questions presented.

However, if this lesson is being implemented in a course for which the instruction is generally more instructor-centered (e.g. class time is mostly used for lecture), then more instructor intervention or guidance may be required to keep students on task and productive in their investigations, or the instructor may wish to only have students complete one Investigation, and supplement with more instructor-focused delivery for other components and learning objectives address by the other Investigations not implemented.

*A last implementation note:* because of the timely and ongoing revision of the data that populates the NCBI databases, the exact layout of the website and information presented may change; hence it is a highly recommended best practice for instructors to review and attempt the lesson in full prior to disseminating to students to ensure navigation instructions (and indeed, answers provided in the instructor key!) are still accurate and helpful. The answer key was revised and represents data that was available as of November 14, 2014. The ongoing revision of the data available at NCBI and other online research databases provides an authentic opportunity to reinforce to students (who are really, scientists-in-training) the idea that scientific knowledge is always open to revision as new information comes to light, better tools are created for looking at data or measuring phenomena, and understanding is advanced.

## SUPPLEMENTAL MATERIALS

- Table 1. Homologous chromosomes-Teaching Timeline
- Supplemental File S1: Homologous chromosome-Pre-Investigation questions
- Supplemental File S2: Homologous chromosome-Pre-Investigation question Debrief Options
- Supplemental File S3: Homologous chromosome-Investigation 1: Homologous chromosomes? Exploring the relationship of human autosomes and sex chromosomes
- Supplemental File S4: Homologous chromosome-Investigation 2: The Sex Determining Region of The Y chromosome
- Supplemental File S5: Homologous chromosome-Investigation 3: Sex-reversal and other deleterious mutations of the SRY gene in humans, sex-specific diseases
- Supplemental File S6: Homologous chromosome-Optional Extension 1: Sex determination varies among vertebrates
- Supplemental File S7: Homologous chromosome-Optional Extension 2: Sex-linked genes and disorders
- Supplemental File S8: Homologous chromosome-Optional Extension 3: Sex and Gender
- Supplemental File S9: Homologous chromosome-Answer Key

- Supplemental File S10: Homologous chromosome-Glossary of Terms
- Supplemental File S11: Homologous chromosome-Additional Resources

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