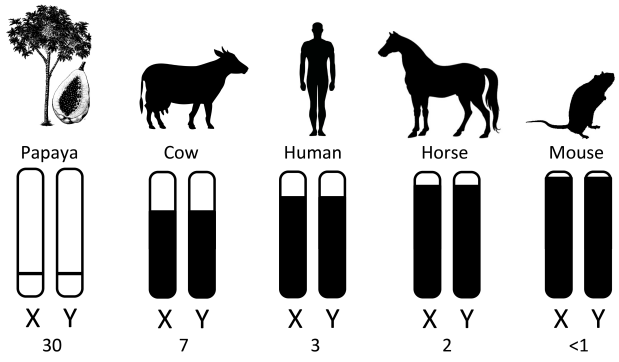
How will we advance human health over the next 10 years? One way that we can do this is to build our understanding of the underlying causes of errors during spermatogenesis that lead to reproductive health problems (e.g. Turner and Klinefelter syndromes). Understanding the evolutionary forces acting on sex chromosomes is essential to our understanding of these diseases. The development of separate sexes is a common trait in animals and can be accomplished by environmental cues or genetic mechanisms [1]. One of the most common forms of genetic sex determination is the XX/XY chromosomal sex determination system that humans and many of our model organisms possess [2]. In this system there are two alternative versions of the sex chromosome (X and Y). If an individual is homogametic (XX) they develop as female, while heterogametic (XY) individuals develop as males.

Though the X and Y chromosome begin as a pair of identical autosomes, in most species they diverge over time as the Y chromosome loses most of its genes. In humans this process has led to an X chromosome with approximately 2,000 genes but a Y with only a few dozen. The process of Y chromosome degeneration, the loss of shared genes with the X chromosome, is driven by reduction in recombination between the X and Y chromosome [3]. In fact, the sex chromosomes can be clearly divided into two regions based on whether they recombine in males. The pseudoautosomal region (PAR; Box 1 white) recombines with the X and does not decay. The sex specific region (SSR; Box 1 black) no longer recombines with the X in males and suffers from decay. When we look across species we find that the size of the PAR ranges from almost the entire chromosome to less than a megabase (Box 1).

Box 1. Species vary greatly with regard to the amount of the sex chromosomes that can recombine. The pseudoautosomal region (PAR; white) and the sex specific region (SSR; black) are shown for five species. The size of the PAR region in megabases is indicated below each illustration. In all cases the entire X chromosome pairs and recombines normally during female meiosis. However, in males the X and Y can only recombine in the PAR region, and must do so for spermatogenesis to proceed successfully.



But what drives reduced recombination between the X and Y? Available evidence suggests it is sexual antagonism – where alleles have different fitness in males and females [4]. For instance, if a new allele arises in the PAR region of the Y chromosome that provides a special benefit to males, selection will favor reducing recombination between the SSR of the Y (which is always in males) and the new mutation that benefits males. Studies in primates have revealed a pattern consistent with this, where a series of inversions have shifted more and more of the Y from the PAR into the SSR. Conventional wisdom holds that the PAR avoids complete loss because it is essential during spermatogenesis [5]. The faithful segregation of either an X or a Y into each sperm requires that the PAR portion of the sex chromosomes pair and recombine during spermatogenesis [6]. However, a handful of groups (e.g. Drosophila, Marsupials, and several rodents) are able to segregate sex chromosomes despite a complete lack of recombination in males. These species possess achiasmatic meiosis where protein complexes hold the homologous chromosomes together until they progress to opposite poles during meiosis.

Failure to properly segregate the sex chromosomes leads to the production of sperm that either have no sex chromosome or have both an X and a Y. If a sperm lacking either sex chromosome fertilizes an egg it produces an "XO" offspring exhibiting Turner syndrome. Fertilization by a sperm carrying both an X and a Y chromosome produces an "XXY" offspring exhibiting Klinefelter syndrome. In humans Turner syndrome is seen in approximately 1 in 2000 live female births and is responsible for roughly 10% of spontaneous abortions [7]. Other species are also affected by Turner syndrome. In fact, it is one of the most common genetic abnormalities in horses. Equine industries generate in excess of $39 billion in direct economic impacts in the United States [8], yet we don't know whether there are differences within or among breeds. More broadly, comparative analysis suggests that transitions from XY to XO are common. In an analysis of 700 species of beetles I inferred approximately 69 independent losses of the Y chromosome [9], and of the 13,917 species with chromosomal sex determination 16% lack a sex specific chromosome (e.g. Y chromosome) [2]. A strong indication that sex chromosome evolution often culminates in the complete loss of the Y chromosome.

Current models of sex chromosome evolution fail to address possible underlying differences in the rate at which XO offspring are produced, and do little to explain why some clades maintain a highly decayed Y chromosome for millions of years while other clades possess many species that have lost the Y chromosome [10, 11]. **Fully understanding the complex behavior of sex chromosomes requires the integration of empirical data gathered using molecular methods with understanding from theoretical population genetics**.

My previous work shows that clades of beetles with small PARs have more species that have lost the Y chromosome, and that in general clades that evolve achiasmatic meiosis have fewer species that lose the Y chromosome [12, 13]. These observations led to the development of the Fragile Y hypothesis, which can explain both variation in rates of producing XO offspring as well as variation in the frequency that species completely lose the Y chromosome. Specifically, it states that recurrent selection to reduce the size of the PAR leads to greater difficulty in segregation of the sex chromosomes during spermatogenesis, or put simply having a small PAR makes it hard to reliably segregate the sex chromosomes. This is a challenging hypothesis to evaluate because available data (i.e. frequency of XO offspring) confound the mutation rate (how many XO zygotes are created) and fitness (how many XO offspring live to term). For example, the high incidence of XO offspring observed in horses relative to cattle could be due either to the production of many more XO zygotes in horses or survival of a higher percentage of these zygotes to term in horses. Furthermore, it is currently unclear what degree of sexual antagonism and range of aneuploidy rates would allow for the Fragile Y effect to be an important force in the evolution of genome architecture.

Research Plan

The proposed research will advance our understanding of sex chromosome evolution on two fronts simultaneously. First, from a theoretical side I will explore a simplified two-locus model of sex chromosome evolution. This model has one locus that determines sex and a second that has alleles with different finesses in males and females. I will use this model to determine when a mutation that expands the SSR but increases sex chromosome aneuploidy will be favored by selection. I have already performed preliminary simulations under this model (Figure 2) that indicate that even with weak levels of sexual antagonism, mutations that increase aneuploidy can be positively selected.

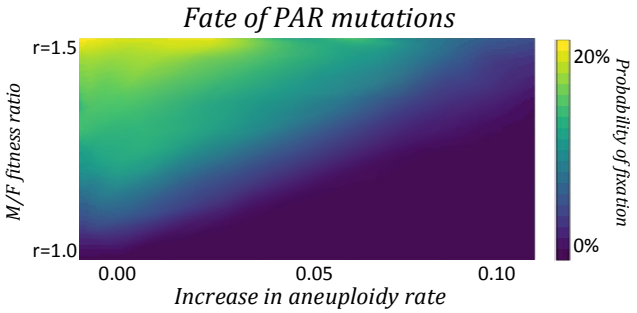


Figure 2. The fate of PAR mutations: The color in the plot reflects the probability of an inversion in the Y PAR fixing in a population of size 1,000. The y-axis shows the ratio of male fitness to female fitness caused by the sexually antagonistic locus in the PAR. The x-axis shows the rate of aneuploidy during male spermatogenesis for individuals with the new mutation.

The second avenue of my research plan is to collect the empirical data necessary to distinguish the role of mutation and fitness in the production of XO offspring. The approach that I will use is FISH based sperm genotyping. This is an approach that uses fluorescently labeled probes that target sequences on the X and Y chromosome. It has been established in mice [14], and will allow me to genotype of thousands of sperm. I will use this approach to measure the proportion of aneuploid sperm (sperm that have neither an X or Y) in a variety of species. This data will disentangle the contribution of fitness and mutation in the incidence rate of Turner and Klinefelter syndromes.

I will measure the frequency of aneuploid sperm in two lab strains of mice, C57BL/6 and PAF. C57BL/6 is the standard lab mouse strain that was used as the reference strain for genome sequencing. The PAF strain contains a spontaneous inversion on the X chromosome that decreases the size of the PAR [15]. These two strains of mice will allow me to determine the relationship between PAR size and aneuploidy rate within a single species. I will also measure the frequency of aneuploid sperm in horse and cow. These two species have been used as an example of the difference in fitness effects of the XO genotype [11]. However, measuring the frequency of aneuploid sperm will quantify the degree to which mutation or fitness is responsible for difference in the proportion of XO offspring observed in these two species.

Selection of advisors

Dr. Zarkower in the department of Genetics, Cell Biology, and Development and Dr. Brandvain in the departments of Plant Biology/Ecology, Evolution, and Behavior will be my faculty advisors for this research project. I chose Dr. Zarkower as the advisor for the empirical side of this project because of his interest in sex determination and his experience with molecular protocols especially FISH [16-18]. The funds provided by this grant along with the use of his equipment will make it possible to complete all objectives in the empirical portion of this project. I chose Dr. Brandvain as the advisor for the theoretical side of this project because of his experience in theoretical population genetics [19, 20]. Dr. Brandvain has developed models for the evolution of systems involving sexual antagonism, and should be a valuable source of guidance. The project that I have described will provide a link between campuses and departments in CBS and will foster collaboration among new and established labs. This work will also broaden my research experience helping me prepare for a transition into a faculty role in the future. Furthermore it will bring together two different avenues of investigation to better understand one of the most dynamic portions of our genome and provide insights in to the health of humans and domestic animals.

Work Cited

1. Bachtrog, D., et al., *Sex determination: why so many ways of doing it?* 2014.
2. Consortium, T.o.S., *Tree of Sex: A database of sexual systems.* Scientific data, 2014. **1**: p. 140015.
3. Charlesworth, D., B. Charlesworth, and G. Marais, *Steps in the evolution of heteromorphic sex chromosomes.* Heredity, 2005. **95**(2): p. 118-128.
4. Charlesworth, B. and D. Charlesworth, *The degeneration of Y chromosomes.* Philos Trans R Soc Lond B Biol Sci, 2000. **355**(1403): p. 1563-1572.
5. Bachtrog, D., *Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration.* Nat Rev Genet, 2013. **14**(2): p. 113-24.
6. White, M.J.D., *Animal cytology & evolution*. 3rd ed. 1977, Cambridge: University Press.
7. Bojesen, A., S. Juul, and C.H. Gravholt, *Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study.* The Journal of Clinical Endocrinology & Metabolism, 2003. **88**(2): p. 622-626.
8. Deloitte-Consulting, *The Economic Impact of the Horse Industry State Breakout Reports*. 2005, American Horse Council: Washington D.C.
9. Blackmon, H. and J.P. Demuth, *Coleoptera Karyotype Database.* The Coleopterists Bulletin, 2015. **69**(1): p. 174-175.
10. Raudsepp, T. and B. Chowdhary, *Chromosome Aberrations and Fertility Disorders in Domestic Animals.* Annual Review of Animal Biosciences, 2015. **4**(1).
11. Raudsepp, T. and B.P. Chowdhary, *The horse pseudoautosomal region (PAR): characterization and comparison with the human, chimp and mouse PARs.* Cytogenet Genome Res, 2008. **121**(2): p. 102-9.
12. Blackmon, H. and J.P. Demuth, *Estimating tempo and mode of Y chromosome turnover: explaining Y chromosome loss with the fragile Y hypothesis.* Genetics, 2014. **197**(2): p. 561-572.
13. Blackmon, H. and J.P. Demuth, *The fragile Y hypothesis: Y chromosome aneuploidy as a selective pressure in sex chromosome and meiotic mechanism evolution.* BioEssays, 2015. **37**(9): p. 942-950.
14. Dumont, B.L., et al., *No Evidence that Infection Alters Global Recombination Rate in House Mice.* PloS one, 2015. **10**(11): p. e0142266.
15. Burgoyne, P. and E. Evans, *A high frequency of XO offspring from XPafY* male mice: evidence that the Paf mutation involves an inversion spanning the X PAR boundary.* Cytogenetic and Genome Research, 2000. **91**(1-4): p. 57-61.
16. Gamble, T., et al., *Anolis sex chromosomes are derived from a single ancestral pair.* Evolution, 2014. **68**(4): p. 1027-1041.
17. Gamble, T. and D. Zarkower, *Sex determination.* Current Biology, 2012. **22**(8): p. R257-R262.
18. Raymond, C.S., et al., *Evidence for evolutionary conservation of sex-determining genes.* Nature, 1998. **391**(6668): p. 691-695.
19. Brandvain, Y. and G. Coop, *Scrambling eggs: meiotic drive and the evolution of female recombination rates.* Genetics, 2012. **190**(2): p. 709-723.
20. Brandvain, Y. and G. Coop, *Sperm should evolve to make female meiosis fair.* Evolution, 2015. **69**(4): p. 1004-1014.