## The relationship between ploidy, sex chromosomes, and sexual condition in Syntrichia






## Syntrichia

- A diverse genus of mosses, with $\sim 100$ named species
- A large number of dryland specialists
- Low rates of sexual reproduction, extreme female population bias common


Moss Reproduction


## Presentation Outline

## Presentation Outline

Moss Reproduction



## Presentation Outline

Moss Reproduction<br>Sex Chromosomes



## Presentation Outline

Moss Reproduction
(\%) Sex Chromosomes
(4ᄃ) Polyploid Phylogenetics


## Presentation Outline

Moss Reproduction(\%) Sex Chromosomes
(4ᄃ) Polyploid Phylogenetics
(P) Future Research


## Mosses are not like other plants

Moss Reproduction

## Mosses are not like other plants

- Many species exhibit vegetative desiccation tolerance (Proctor et al. 2007; Stark 2017)


Moss Reproduction

## Mosses are not like other plants

- Many species exhibit vegetative desiccation tolerance (Proctor et al. 2007; Stark 2017)
- Haploid-dominant, free-living gametophyte


## Mosses are not like other plants

- Many species exhibit vegetative desiccation tolerance (Proctor et al. 2007; Stark 2017)
- Haploid-dominant, free-living gametophyte
- Spore-bearing and reproduce with swimming sperm


Moss Reproduction

## Most moss species ( $\sim 60 \%$ ) produce unisexual gametophytes



Moss Reproduction

Bisexual gametophytes produce both sperm and egg


Moss Reproduction

## Bisexual gametophytes produce both sperm and egg

Many bisexual species can self, resulting in a sporophyte that is homozygous at all loci and spores that are clones of the parent

BISEXUAL



## Evolutionary transitions in sexual condition

- McDaniel (2013) et al. found least 133 transitions between unisexuality and bisexuality in mosses
- Rate of bisexuality to unisexuality was $2 x$ higher than the reverse
- Net diversification rates higher in bisexual lineages

Moss Reproduction

## Sex determination

 occurs in the haploid gametophyte stage via a single sex chromosome

## Sex determination

 occurs in the haploid gametophyte stage via a single sex chromosome

Sex determination occurs in the haploid gametophyte stage via a single sex chromosome

BISEXUAL


Sex Chromosomes

How does bisexuality evolve?

## Hypothesis

## Hypothesis

BISEXUAL


UV?

## Hypothesis

Allopolyploids have both maternal and paternal progenitors, so are expected to have both U and V chromosomes

## Hypothesis

Allopolyploids have both maternal and paternal progenitors, so are expected to have both U and V chromosomes


Only 1 copy of a sex chromosome needed for sexual function

## Hypothesis

Allopolyploids have both maternal and paternal progenitors, so are expected to have both U and V chromosomes


Only 1 copy of a sex chromosome needed for sexual function

UV gametophytes may be bisexual

## Inferring sex chromosomes

## Inferring sex chromosomes

- Genome skimming for 80 accessions of Syntrichia and close relatives
- Mapped reads to two reference genomes:


## Inferring sex chromosomes

- Genome skimming for 80 accessions of Syntrichia and close relatives
- Mapped reads to two reference genomes:
- Female (U) S. caninervis (Silva et al. 2020)
- Male (V) S. ruralis (Zhang et al. in press)
- Counted reads that differentially mapped to U and V


## Inferring sex chromosomes

## Inferring sex chromosomes



## Inferring sex chromosomes

- > $75 \%$ of reads preferentially mapping to one chromosome to call it
- Read mapping ratios of $40 \%-60 \%$ called UV


## Inferring sex chromosomes

- > $75 \%$ of reads preferentially mapping to one chromosome to call it
- Read mapping ratios of $40 \%-60 \%$ called UV


## Inferring sex chromosomes

- > $75 \%$ of reads preferentially mapping to one chromosome to call it
- Read mapping ratios of $40 \%-60 \%$ called UV
- Read mapping ratios of 60\%-75\% considered ambiguous and not called



## Inferring sex chromosomes

- > $75 \%$ of reads preferentially mapping to one chromosome to call it
- Read mapping ratios of $40 \%-60 \%$ called UV
- Read mapping ratios of 60\%-75\% considered ambiguous and not called


## Association between <br> bisexuality and sex chromosomes?

Syntrichia caninervis Syntrichia pseudohandelii Syntrichia rigescens Syntrichia subpapillosa Syntrichia calcicola Syntrichia montana Syntrichia echinata Syntrichia amphidiacea Syntrichia ammonsiana Syntrichia papillosissima Syntrichia subpapillosissima

Syntrichia cainii Syntrichia fragilis Syntrichia latifolia Syntrichia norvegica

Syntrichia bartramii Syntrichia sucrosa Syntrichia campestris Willia austroleucophaea Syntrichia virescens Syntrichia lithophila Syntrichia buchtienii Willia brachychaete Syntrichia breviseta Syntrichia sinensis Syntrichia geheebiaeopsis Syntrichia submontana Syntrichia laevipila Syntrichia christophei Syntrichia obtusissima Syntrichia anderssonii Syntrichia percarnosa Syntrichia intermedia Syntrichia princeps Syntrichia norrisii Syntrichia antarctica


UV

## Association between

bisexuality and sexchromosomes?

- Bisexual species more commonly have UV and V chromosomes
- Fisher's Exact Test $P=1.004 \times 10^{-6}$


## Association between bisexuality and sex chromosomes?

- Bisexual species more commonly have UV and V chromosomes
- Fisher's Exact Test $P=1.004 \times 10^{-6}$
- Doesn't account for phylogenetic non-independence!



## Association between bisexuality and sex chromosomes?

- Bisexual species more commonly have UV and V chromosomes
- Fisher's Exact Test $P=1.004 \times 10^{-6}$
- Doesn't account for phylogenetic non-independence!

(4ᄃ) Polyploid Phylogenetics


## Possible polyploidy in Syntrichia

- Polyploidy (having 2 or more genomes in an individual) is common in mosses, and polyploid moss species tend to be bisexual (Crawford et al 2009, and refs therein)



## Polyploid Phylogenetics

## Possible polyploidy in Syntrichia

- Polyploidy (having 2 or more genomes in an individual) is common in mosses, and polyploid moss species tend to be bisexual (Crawford et al 2009, and refs therein)
- Several notoriously difficult species complexes in Syntrichia



## Polyploid Phylogenetics

## Possible polyploidy in Syntrichia

- Polyploidy (having 2 or more genomes in an individual) is common in mosses, and polyploid moss species tend to be bisexual (Crawford et al 2009, and refs therein)
- Several notoriously difficult species complexes in Syntrichia
- Many intra-specific chromosome series in the genus ( $\mathrm{n}=\sim 12,24,36$, etc.; Fritsch 1991)


Polyploid Phylogenetics


Polyploid Phylogenetics

## Hybridization +

Polyploidization
= Allopolyploidy


## Hybridization +

Polyploidization
= Allopolyploidy


Hybridization +
Polyploidization
= Allopolyploidy


## Multree

1 tip per
subgenome


Hybridization +
Polyploidization
= Allopolyploidy


## Multree

1 tip per
subgenome



Hybridization +
Polyploidization
= Allopolyploidy


## Multree

1 tip per
subgenome


## Network

1 tip per species/ individual


Hybridization +
Polyploidization
= Allopolyploidy


Haploid 2

## Multree

1 tip per
subgenome


## Network

1 tip per species/ individual



## Approach

Polyploid Phylogenetics


## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups

Polyploid Phylogenetics


## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups
- de novo assembly of raw reads with strict parameters in SPAdes (Bankevich et al. 2012)



## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups
- de novo assembly of raw reads with strict parameters in SPAdes (Bankevich et al. 2012)
- Manual selection and curation of alignments



## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups
- de novo assembly of raw reads with strict parameters in SPAdes (Bankevich et al. 2012)
- Manual selection and curation of alignments
- Identified 8 potential polyploids to investigate



## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups
- de novo assembly of raw reads with strict parameters in SPAdes (Bankevich et al. 2012)
- Manual selection and curation of alignments
- Identified 8 potential polyploids to investigate
- Based on presence of $>\mathbf{1}$ sequence per locus



## Approach

- HybSeq/Target Capture; GoFlag probe set (Breinholt et al. 2021)
- 8o accessions of Syntrichia + outgroups
- de novo assembly of raw reads with strict parameters in SPAdes (Bankevich et al. 2012)
- Manual selection and curation of alignments
- Identified 8 potential polyploids to investigate
- Based on presence of $>\mathbf{1}$ sequence per locus
- Phase gene copies and build tree with hOMOLOGIZER (Freyman et al. 2023) in RevBayes (Höhna et al. 2016)




Polyploid Phylogenetics


Polyploid Phylogenetics


Polyploid Phylogenetics


Polyploid Phylogenetics

(2)

Polyploid Phylogenetics





## S. Iaevipila

$$
\mathrm{n}=12,15,26
$$

(Patel et al., 2021)

- A species complex
- Worldwide distribution, but primarily in the N . Hemisphere
- Bark epiphyte
- Bisexual; some populations reported to be unisexual



S. obtusissima

$$
\mathrm{n}=?
$$

- Southwest US and MexicanAndean disjunction
- Bisexual; some populations are reported to be unisexual


Polyploid Phylogenetics


Polyploid Phylogenetics


## S. princeps

$$
\mathrm{n}=12,24,26,28,36
$$

(Patel et al., 2021)

- A species complex
- Worldwide distribution, primarily in N . Hemisphere
- Bisexual



Polyploid Phylogenetics

## Conclusions

There seems to be something going on!

## Conclusions

There seems to be something going on!

8 suspected polyploids were tested in polyploid phylogenetic framework:

- All 8 appear to be allopolyploid


## Conclusions

There seems to be something going on!

8 suspected polyploids were tested in polyploid phylogenetic framework:

- All 8 appear to be allopolyploid
- Of those, 5 were bisexual and have UV sex chromosomes


## Conclusions

There seems to be something going on!

8 suspected polyploids were tested in polyploid phylogenetic framework:

- All 8 appear to be allopolyploid
- Of those, 5 were bisexual $\mathbf{\Delta}$ and have UV sex chromosomes
- One is bisexual $\mathbf{A}$ with only a V sex chromosome
S. sinensis
S. submontana
S. obtusissima
S. rubella
S. laevipila

Tortula inermis
S. princeps
S. glacialis (bisexual, unknown
chromosomes)

## Conclusions

There seems to be something going on!

8 suspected polyploids were tested in polyploid phylogenetic framework:

- All 8 appear to be allopolyploid
- Of those, 5 were bisexual $\mathbf{\Delta}$ and have UV sex chromosomes
- One is bisexual $\mathbf{\Delta}$ with only a $V$ sex chromosome
S. sinensis
S. submontana
S. obtusissima
S. rubella
S. laevipila

Tortula inermis
S. princeps
S. glacialis (bisexual, unknown
chromosomes)
S. rubella (unknown sexual condition and unknown chromosomes)
(P) Future Research


## Next Steps



## Next Steps

- Check and validate assembly pipeline for accuracy with allopolyploids of known ancestry
- Investigate more potential polyploids
- Chromosome and sexual condition correlation analyses in phylogenetic network framework


# Join my lab! MEEPLab Moss Eco-Evo-Physio 

The MEEP Lab will be opening THIS MONTH at San Francisco State University.

Now recruiting undergraduate and master's students to start Fall 2024.


Spread the word! www.meep-lab.com

Ecology



Genomics

## Acknowledgments

- Thank you to Northern California Botanists for inviting me to speak at this symposium
- My collaborators; my current and past advisors Carl Rothfels, Rebecca Dikow, and Brent Mishler; and my support systems in the Mishler, Dikow, and Rothfels Labs.
- NSF CAREER Award \# 204413 (to C. Rothfels), The Smithsonian Data Science Lab Biodiversity Genomics Fellowship, NSF Dimensions of Biodiversity grants \#1638956 and \#1638972.



## Thank you

