PI: **Zanders, Sarah Elizabeth**	Title: Models of Selfishness: Molecular and Evolutionary Analyses of the Wtf Meiotic Drivers	
Received: 09/07/2017	FOA: RM17-006	Council: 05/2018
Competition ID: FORMS-D	FOA Title: NIH Director's New Innovator Award Program (DP2)	
1 DP2 GM132936-01	Dual: RM	Accession Number: 4087114
IPF: 4323301	Organization: STOWERS INSTITUTE FOR MEDICAL RESEARCH	
Former Number: 1DP2OD025820-01	Department:	
IRG/SRG: ZRG1 MOSS-R (70)R	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 1,500,000	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N HFT: N	New Investigator: Y Early Stage Investigator: Y
Senior/Key Personnel:	*Organization:*	*Role Category:*
Sarah Zanders	Stowers Institute for Medical Research	PD/PI

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	**State Application Identifier**

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number
6 MCB; 8 HIB

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number
GRANT12478156

5. APPLICANT INFORMATION **Organizational DUNS*:** [redacted]

Legal Name*: Stowers Institute for Medical Research
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: Michelle Middle Name: Last Name*: Lewallen Suffix:
Position/Title: Grants Information Manager
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* M: Nonprofit with 501C3 IRS Status (Other than Institution of Higher Education)

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Models of Selfishness: Molecular and Evolutionary Analyses of the Wtf Meiotic Drivers

12. PROPOSED PROJECT
Start Date* Ending Date*
09/01/2018 06/30/2023

13. CONGRESSIONAL DISTRICTS OF APPLICANT
MO-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: Dr. First Name*: Sarah Middle Name: Last Name*: Zanders Suffix:
Position/Title: Assistant Investigator
Organization Name*: Stowers Institute for Medical Research
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$1,500,000.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$1,500,000.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: David Middle Name: Last Name*: Chao Suffix:
Position/Title*: President and CEO
Organization Name*: Stowers Institute for Medical Research
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Michelle E Lewallen

Date Signed*
09/07/2017

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:1239-CoverLetterSZ1.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: Stowers Institute for Medical Research
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: MO-005

Additional Location(s) File Name:

Funding Opportunity Number: RFA-RM-17-006. Received Date: 2017-09-07T12:52:43.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ❍ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ❍ Yes ❍ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ❍ **Yes** ❍ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ❍ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ❍ Yes ❍ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ❍ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ❍ Yes ❍ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ❍ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	1236-Project Summary.pdf
8. Project Narrative*	1237-Project Narrative.pdf
9. Bibliography & References Cited	
10.Facilities & Other Resources	1238-Facilities and Other Resources .pdf
11.Equipment	

Funding Opportunity Number: RFA-RM-17-006. Received Date: 2017-09-07T12:52:43.000-04:00

Project Summary

Eukaryotic genomes are plagued with selfish DNA sequences that can have a negative impact health. Meiotic drivers are one type of these DNA parasites that exploit gametogenesis to bias their own transmission into the next generation. Instead of being transmitted to half the gametes generated by heterozygous individuals, meiotic drivers cheat the process to be found in up to 100% of the functional gametes. This selfish behavior imposes a heavy burden on the organism. Meiotic drivers can directly cause infertility by killing gametes that do not inherit them. Meiotic drivers can also contribute to diseases or infertility indirectly by promoting the maintenance and spread of deleterious alleles in a population. Although meiotic drivers are widespread in eukaryotes, including humans, few meiotic drive alleles have been cloned and very little is known about the molecular mechanisms they use to cause drive. In addition, there are few controlled experimental analyses of how these selfish genes spread within genomes and populations. This proposal exploits an innovative model system for studying meiotic drive, the *wtf* family of drivers in fission yeast. Driving *wtf* genes act by generating both a poison and an antidote from alternate transcripts. All the gametes are poisoned, but those that inherit the *wtf* allele are rescued by the antidote. The proposed experiments use a multidisciplinary approach to dissect the molecular mechanisms of how the poison protein is delivered to developing gametes, how the poison kills cells, and how the antidote neutralizes the poison. In addition, the experiments will address major questions in the evolution of meiotic drive genes. The proposed work will determine how poison and antidote specificity is maintained as the selfish *wtf* genes duplicate and diverge within a genome. This question is especially important given that disrupting poison and antidote specificity causes severe infertility, yet the selfish genes rapidly diverge. The work also develops the first assay for high-throughput experimental evolution analyses of meiotic drivers to explore questions about how these parasites (and linked variants) spread in a population. This assay will provide experimental tests of current theoretical models and test more complex real-world scenarios not currently described by such models. This work will provide essential molecular and evolutionary characterization of a model meiotic driver that will help guide discovery and analyses of analogous selfish loci in more complex eukaryotes, including humans. This expanded understanding DNA parasites should ultimately lead to improved reproductive outcomes in humans.

Project Narrative:

Meiotic drivers are selfish genes that can exploit gametogenesis to bias their own transmission into the next generation. These genomic parasites can directly and indirectly cause infertility and promote the maintenance of deleterious (e.g. disease-causing) gene variants in a population. This proposal uses a novel model system and an innovative evolution-guided molecular approach to uncover how meiotic drive alleles work and how they spread in populations.

Facilities and Other Resources:

Lab Resources: Dr. Zanders's laboratory has 1220 sq. ft. of space, including personal bench and desk space for 10 researchers. Additionally, common equipment and lab space and a cold room are available as a shared space near the laboratory.

Office Space: There are 671 sq. ft. of office space and administrative space adjacent to the laboratory, including an administrative assistant.

Stowers Institute Resources: The Stowers Institute campus is a technologically advanced biomedical research facility. The proposed research will benefit from collaboration with the following core facilities at the institute:

Cytometry
The Cytometry Center is a state-of-the-art flow cytometry laboratory that provides Stowers investigators with a resource for analytical and preparative studies of cells using flow cytometry. The cytometry facility staff provides a full range of services including routine sample processing, cell staining, data analysis and strategic planning for novel assays and custom cell sorting applications. Collaborative projects encompass a broad scope of research topics and often result from a group effort among several of the institute's core facilities.

Microscopy
The Stowers Microscopy Center strives to enable every scientific member of the Stowers Institute to get the best light microscope images technically possible. Its staff of physicists, software specialists, chemists, and biologists trains users on the state-of-the-art equipment and assists with the set up of optical experiments, the selection of the best microscope equipment for a given task and conducts microscopy experiments. In addition to supporting several image processing software packages, specialists are available to program custom solutions. The center also actively explores and develops new technologies.

Molecular Biology
The Molecular Biology Facility supports investigators in their research endeavors by providing high quality services, collaborative project potential and access to state of the art technology. Current routine services include DNA sequencing, site-directed mutagenesis, genome engineering, plasmid preparations and distributing clones/vectors from our in-house collections.

Stowers researchers also have access to the latest Illumina Next Generation Sequencing technology within the facility. The core constructs libraries, performs sequencing and assists with high-throughput genome-sequencing, RNA-seq and ChIP-seq projects.

The facility utilizes liquid handling and colony manipulation robots to automate many of the services and improve quality. In addition, the facility provides automation expertise and collaborate with researchers on custom automation projects.

Proteomics
The Proteomics Center implements a chromatography-based proteomic approach known as multidimensional protein identification technology (MudPIT). MudPIT is largely unbiased and extremely sensitive and allows for the detection and identification of low abundance proteins, as well as many different post-translational modifications. The center uses spectral counting as an effective label free proteomics quantitation tool and can apply statistical ways to compare proteomics datasets.

Typical collaborations include analyzing protein mixtures to comprehensively determine members of multiprotein complexes or to identify proteins localized to specific subcellular compartments. More in-depth analyses can be undertaken to find post-translational modifications in proteins of interest or derive probabilities of protein interactions within protein complexes when analyzing affinity purifications by MudPIT. The Proteomics Center team consists of masters- and doctoral-level scientists with backgrounds ranging from biochemistry to physics.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: Dr. First Name*: Sarah Middle Name Last Name*: Zanders Suffix:

Position/Title*: Assistant Investigator
Organization Name*: Stowers Institute for Medical Research
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: 1234-NewBiosketchSZ.pdf

Attach Current & Pending Support: File Name: 1235-Current and Pending Support b.pdf

Funding Opportunity Number: RFA-RM-17-006 . Received Date: 2017-09-07T12:52:43.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 11/16 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Sarah Elizabeth Zanders

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Assistant Investigator

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Iowa, Iowa City IA	B.S.	05/2005	Biology
Cornell University, Ithaca NY	Ph.D.	08/2010	Genetics
Fred Hutchinson Cancer Research Center, Seattle WA	Postdoc	07/2016	Evolutionary Conflict

A. Personal Statement

I have a long-standing passion for understanding the causes of infertility and congenital birth defects. I have spent my career, starting as an undergraduate, acquiring expertise on meiosis. I even organized and chaired a successful conference on meiosis. In addition, I trained with leaders in the fields to gain an exceptionally broad skill set in genetics, genomics, biochemistry, and molecular evolution approaches. I have demonstrated my ability to leverage my knowledge and skills to gain novel insights on gametogenesis. Even as a graduate student, my main research projects were largely self-designed and executed. I also designed projects and trained undergraduates both as a graduate student and post-doc. I therefore had an easy transition to becoming a principle investigator. One year into my appointment, I have already recruited a talented team and led them to make impactful discoveries. We have published our first paper describing some of these findings. The proposed research project is founded on this work. Finally, I have initiated collaborations with core research teams at my institute with expertise in microscopy, cytometry, proteomics and robotics. These collaborations, combined with my skills, will help us successfully carry out the proposed research project.

Zanders S, Alani E (2009) The *pch2Delta* mutation in baker's yeast alters meiotic crossover levels and confers a defect in crossover interference. *PLoS Genetics* 5, e1000571.

Zanders S, Sonntag Brown M, Chen C, Alani E (2011) Pch2 modulates chromatid partner choice during meiotic double-strand break repair in *S. cerevisiae*. *Genetics* 188:511-21.

Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, Smith GR, Malik HS (2014) Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. *eLife* 3:e02630.

Nuckolls NL*, Bravo Nunez MA*, Eickbush MT, Young JM, Lange JJ, Yu JS, Smith GR, Jaspersen SL, Malik HS, **Zanders SE** (2017) *wtf* genes are prolific dual poison-antidote meiotic drivers. *eLife* 6:e26033.

B. Positions and Honors

Presidential Life Sciences Fellowship, Cornell University	2005-06
NIH Training Grant support, Cornell University	2009-10 and 2007-08
Outstanding Teaching Assistant Award, Cornell University	2006
NIH Training Grant support, University of Washington	2012-13
NIH Ruth L. Kirschstein National Research Service Award (declined)	2012
American Cancer Society Postdoctoral Fellowship	2013-16
Chair, Gordon Research Seminar on Meiosis	2014
NIH K99/R00 Research Transition Award	2015-2019
Assistant Investigator, Stowers Institute for Medical Research, Kansas City MO	2016-

C. Contributions to Science

1) The origins of infertility in natural populations of fission yeasts: My research is based on the idea that identifying the origins of infertility in model natural populations should illuminate potential causes of infertility within humans. This should ultimately lead to improved human reproductive outcomes. Previously published work indicated that the fission yeast, *Schizosaccharomyces pombe*, was reproductively isolated from another fission yeast species, *S. kambucha – pombe/kambucha* hybrids are largely infertile. This observation was surprising because the two yeasts share 99.5% DNA sequence identity. To investigate the cause of this infertility, I generated an assembly of the *S. kambucha* genome and developed the first genetic tools in *S. kambucha*. I then used these tools to demonstrate that multiple selfish loci known as meiotic drive alleles and linked chromosome rearrangements cause the infertility of pombe/*kambucha* hybrids (Zanders et al. 2014). This work was the first to demonstrate that gamete-killing meiotic drive alleles exist in fission yeast and are erecting strong reproductive barriers in natural populations. It also highlighted how traditional genetic approaches using single inbred isolates are blind to detect meiotic drivers: fission yeast was intensely studied genetically for over 50 years without anyone appreciating that the genome is plagued by these parasites. Finally, the work provided experimental evidence supporting the relatively unpopular chromosomal speciation model in which meiotic drivers cause speciation by facilitating the evolution of chromosome rearrangements. I conceived this project, designed the experiments, executed most of the experiments, and wrote the paper with guidance from my mentors Dr. Smith and Dr. Malik. In addition, I trained three of my co-authors (a high school student, an undergraduate and a technician) and supervised their work on the project.

Zanders SE, Eickbush MT, Yu JS, Kang JW, Fowler KR, Smith GR, Malik HS (2014) Genome rearrangements and pervasive meiotic drive cause hybrid infertility in fission yeast. *eLife* 3:e02630.

- Featured in Perspective: Bomblies K (2014) Cheaters divide and conquer. *eLife* 3:e03371.
- Featured in Research Highlights: Zahn L (2014) Putting the genetic breaks on breeding. *Science* 345:281.

2) Characterization of the *wtf* genes as meiotic drive loci: Meiotic drive alleles are selfish DNA loci that act to bias their own transmission into gametes. These selfish genes can also directly and indirectly cause infertility, so understanding what genes can cause drive and how they work is important. The actions of many meiotic drivers have been described in a myriad of organisms, but few genes underlying drive phenotypes have actually been cloned. We used a combination of genetics and genomics to identify the *wtf4* gene from *S. kambucha* as the first yeast meiotic drive gene. We showed that *wtf4* alone is sufficient to cause drive, unlike most other described drive loci that rely on multiple genes. We showed that *wtf4* acts via a poison and antidote mechanism in which all the developing gametes are poisoned, but only those that inherit the *wtf4* locus are rescued by the antidote. The gene makes distinct poison and antidote proteins using alternate transcriptional start sites. This was a previously undescribed strategy for a selfish gene. In addition, *wtf4* is a member of a large (over 20 loci per genome) gene family and we demonstrated that meiotic drive is likely the ancestral function of the family and likely underlies its rapid expansion in gene number. Thus, our work showed that meiotic drive has been a major force shaping the evolution of fission yeast genomes. I conceived this project, mapped *wtf4*, supervised the experiments characterizing the gene, and helped my students write the paper.

Nuckolls NL*, Bravo Nunez MA*, Eickbush MT, Young JM, Lange JJ, Yu JS, Smith GR, Jaspersen SL, Malik HS, **Zanders SE** (2017) *wtf* genes are prolific dual poison-antidote meiotic drivers. *eLife* 6:e26033.

- Featured in Perspective: Shropshire and Rokas (2017) The gene family that cheats Mendel. *eLife* 6:e28567
- Recommended by F1000 as being of special significance in the field.
- This work has also been featured in several popular science forums including *The Scientist* (Selfish Yeast Genes Encode Both Toxin and Antidote) and KCUR public radio (How Yeast in Kombucha Tea 'Selfishly' Rigs The Genetic Game)

3) Identification of Pch2 as a major regulator of meiotic break repair: My graduate work focused primarily on elucidating the mechanisms underlying the regulation of meiotic DNA double-strand break (DSB) repair, which is essential for fertility in most eukaryotes. Obligate crossover formation, crossover interference, and crossover homeostasis are all manifestations of DSB repair collectively known as crossover control. Little was known about the mechanisms or relatedness of these control systems before my work demonstrated that one conserved protein, Pch2, is required for full efficacy of each aspect of crossover control in budding yeast. These results were surprising given that previous studies of Pch2 reported the protein was a checkpoint factor and had no role in recombination outside the rDNA. The work was significant because it suggested a unified and conserved mechanism underlies all known aspects of crossover control. In subsequent work, I showed that Pch2 has an even broader role in meiotic DSB repair in that it contributes to the temporal barrier to using the sister-chromatid (as opposed to the homologous chromosome) as a repair template. I designed the studies with guidance from my advisor, Dr. Eric Alani. I executed almost all the experiments and I wrote the papers with help from Dr. Alani.

Zanders S, Alani E (2009) The *pch2Delta* mutation in baker's yeast alters meiotic crossover levels and confers a defect in crossover interference. *PLoS Genetics* 5, e1000571.

- Featured in Perspective: Thacker D, Keeney S (2009) PCH'ing together an understanding of crossover control. *PLoS Genetics* 5, e1000576.

Zanders S, Sonntag Brown M, Chen C, Alani E (2011) Pch2 modulates chromatid partner choice during meiotic double-strand break repair in *S. cerevisiae*. *Genetics* 188:511-21.

4) Patterns of mutagenesis: Mutations play a key role in biological systems because they supply the variation on which natural selection acts. Although some are beneficial, most mutations in genes are deleterious and often cause or contribute to disease states. Errors made by DNA polymerase are one source of these mutations, so it is important to understand what types of errors DNA polymerase makes and where it is likely to make them. Previous analyses of polymerase errors were biased by fitness consequences of mutations or by assaying limited numbers of reporter loci. We addressed this question with limited biases using whole genome sequencing of diploid cells allowed to accumulate mutations. To do this, we developed a novel computational method to distinguish heterozygous mutations from DNA sequencing errors when sequence coverage is low. This work allowed us to calculate genome-wide rates of polymerase errors. We also confirmed previous characterizations of homopolymer (e.g. AAAAA) tracts as mutational hotspots. Finally, we made the novel discovery that the broader genomic context (within 1000 bp) of a homopolymer tract affects the probability that it will be mutated. This was a large collaborative project. I contributed to the design of the study, executed some of the experiments and wrote the first paper with the help of Dr. Alani.

Zanders S*, Ma X*, Roychoudhury A*, Hernandez RD, Demogines A, Barker B, Gu Z, Bustamante CD, Alani E (2010) Detection of heterozygous mutations in the genome of mismatch repair defective diploid yeast using a Bayesian approach. *Genetics* 186:493-503.

Ma X*, Rogacheva MV*, Nishant KT, **Zanders S**, Bustamante CD, Alani E (2012) Mutation hot spots in yeast caused by long-range clustering of homopolymeric sequences. *Cell Reports* 1(1):36-42.

5) The role of rapid chromosome motions in meiosis: During budding yeast meiotic prophase, telomeres attach to the nuclear periphery and are vigorously shaken. These movements are widely conserved in eukaryotes but are surprising given that the genome is fragmented into hundreds of pieces at this stage by induced DNA double strand breaks (DSBs). As part of a collaborative project, I explored the role of these chromosome movements. This work revealed that the motions promote timely repair of DSBs, efficient

completion of meiosis, faithful chromosome segregation, and thereby fertility. We also provided the first direct evidence supporting the experimentally elusive hypothesis that rapid chromosome movements act to break apart chromosomal interlocks, which are predicted to result from meiotic recombination and chromosome synapsis. This research gave valuable insights into the intriguing mechanism by which meiotic cells promote the integrity of the shattered genome by shaking it. I contributed to the design of the study and executed some of the experiments.

Sonntag Brown M, **Zanders S**, Alani E (2011) Sustained and Rapid Chromosome Movements are Critical for Chromosome Pairing and Meiotic Progression in Budding Yeast. *Genetics* 188:21-32.

Wanat J*, Kim KP*, Koszul R*, **Zanders S**, Weiner B, Kleckner N, Alani E (2008) Csm4, in collaboration with Ndj1, mediates telomere-led chromosome dynamics and recombination during yeast meiosis. *PLoS Genetics* 4, e1000188.

- Featured in Perspective: Dresser ME (2008) Chromosome mechanics and meiotic engine maintenance. *PLoS Genetics* 4, e1000210.

D. Additional Information: Research Support

Ongoing Research Support
R00 GM114436-03
Mechanisms of meiotic drive and the functional consequences of rapid genome evolution
The goals of this study are to 1) identify selfish DNA loci 2) to characterize the functional consequences of genome evolution on the fidelity of meiotic divisions 3) to assay how the suite of genes essential for life and gametogenesis changes over time.
Role: PI

[redacted]

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ❍ Yes ● No

*Agency-Defined Phase III Clinical Trial? ❍ Yes ❍ No

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ❍ Yes ❍ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

❍ Yes ❍ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

❍ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

Tracking Number: GRANT12479094 Funding Opportunity Number: RFA-RM-17-006 . Received Date: 2017-09-07T12:52:43.000-04:00

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ❍ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

Tracking Number: GRANT12479094 Funding Opportunity Number: RFA-RM-17-006 . Received Date: 2017-09-07T12:52:43.000-04:00

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	
3. Research Strategy*	1240-NewInnovator1.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	
12. Consortium/Contractual Arrangements	
13. Letters of Support	
14. Resource Sharing Plan(s)	
15. Authentication of Key Biological and/or Chemical Resources	1241-Authentication of Key Resources Plan .pdf
Appendix	
16. Appendix	

Tracking Number: GRANT12479094
Funding Opportunity Number: RFA-RM-17-006. Received Date: 2017-09-07T12:52:43.000-04:00

Research Plan

Project science areas

6 MCB; 8 HIB

Project Description

A detailed experimental plan and extensive preliminary data are not provided in order to comply with the FOA.

Scientific opportunity Genetic conflicts between host genomes and external parasites have shaped human evolution and our susceptibility to diseases[1]. For instance, the human sickle cell trait was likely selected in malaria-endemic populations due to its protective effect against the malaria parasite when heterozygous. This trait, however, comes at a serious cost because homozygous individuals develop sickle-cell disease[2]. Studies aimed at characterizing the molecular mechanisms and evolutionary dynamics of such genetic conflicts have revolutionized the fields of immunity and virology in recent years[3,4]. Not all genetic conflicts, however, involve external parasites. Eukaryotic genomes are also plagued with internal parasites, such as meiotic drive alleles, which exploit the process of gametogenesis to ensure their preferential transmission into the next generation[1]. These internal conflicts have also likely shaped our evolution and reproductive health, but these ideas are relatively unexplored. **I propose to use a groundbreaking fission yeast model system I developed to characterize both the molecular mechanisms by which meiotic drive alleles act and to experimentally assay how these parasites affect genome evolution.** This work will address the following questions:

1) How can meiotic drive genes destroy gametes?
2) How can drive genes maintain specificity between interacting components while rapidly evolving?
3) How can drivers shape genome evolution?

This work will lead to a greater understanding of the origins of infertility and congenital birth defects and ultimately improved reproductive outcomes.

Biomedical importance of understanding meiotic drive Meiotic drivers are selfish DNA sequences that bias their own transmission into functional gametes (*e.g.* eggs and sperm). Unlike regular loci which are transmitted to 50% of the gametes generated by a heterozygote, meiotic drive alleles are transmitted into more than 50% and up to 100% of the gametes[5]. For example, if a male carried a meiotic drive locus on his X chromosome, he could produce predominantly X-bearing sperm and father only daughters. This ability to bias allele transmission provides meiotic drive loci with significant evolutionary advantages. Drivers can theoretically quickly spread in populations, even if they are associated with significant fitness costs (described below)[6].

There are two broad types of meiotic drive loci that act in asymmetric (female) and symmetric (male) meiosis, respectively[7]. Drivers that act in female meiosis bias their segregation into the one functional gamete (*e.g.* egg) generated by each round of meiosis, while the competing locus is preferentially lost in polar bodies. Female drivers have been observed across eukaryotes from maize, where they were first identified, to humans[8]. For example, the most common type of chromosome rearrangements in humans, Robertsonian fusions, drive in women[9]. The selfish behavior of meiotic drivers often comes at a significant cost to organismal health. Robertsonian fusions, for instance, are associated with male infertility[10].

The second broad type of meiotic driver acts in male meiosis, in which all four meiotic products generally develop into gametes[7]. A male meiotic drive locus gains a transmission bias by sabotaging the viability or proper development of the gametes that do not inherit the drive locus. These drivers are also known as gamete-killers and are widespread in eukaryotes from plants to mammals. The mouse t-haplotype meiotic drive locus, for example, acts by disrupting the mobility of sperm that do not inherit the locus[11-13]. Gamete-killing meiotic drive has not yet been directly observed in humans, but the human *VCX* and *VCY* multigene families are both predicted to contain male meiotic drive genes[14,15]. As gamete-killers act by disabling gametes, it is not surprising that these loci contribute to infertility. In fact, gamete-killers are a common source of infertility within and between natural populations[15].

Despite their differing modes of action, both types of meiotic drive loci are predicted to have significant overlap in the health costs they impose on the organisms that bear them. For example, both types of drivers are implicated in increasing the frequency of aneuploid gametes, the leading cause of birth defects and infertility in humans[10,16-18]. Meiotic drivers can also contribute to disease due to their effects on genome evolution. Natural selection works best with Mendelian allele transmission in which each allele gets an equal chance of demonstrating its worth. When natural selection can test variants fairly, alleles that promote fitness are favored at the expense of those that do not. Meiotic drivers can disrupt that process because even mal-adapted variants that are linked to drive loci can persist or spread in populations because linked loci experience the same transmission advantage as the driving alleles. This process may even cause beneficial alleles to go extinct. Consistent with these ideas, many mapped drive alleles have accumulated linked deleterious and even recessive-lethal mutations[1,19,20]. Thus, meiotic drive may be affecting the prevalence of disease-associated alleles within human populations.

Meiotic drivers can also indirectly inflict health costs on organisms by fostering rapid evolution of essential cellular machinery. Because meiotic drivers are bad for fitness overall, variants that can suppress drive should be favored by natural selection. In the face of suppression, variants of the drive alleles that can evade suppression will have an advantage. In this way, the genetic conflict between meiotic drivers and suppressors causes rapid evolution of both sides[4]. The situation is analogous to the 'molecular arms races' causing the rapid evolution of viruses and host immunity factors[3]. Because meiotic drivers target critical cellular machinery, it is likely that drive suppressors will emerge from these pathways, leading to rapid evolution of essential factors. For example, a genetic conflict involving driving centromeres is thought to be causing the rapid evolution of centromere sequences and proteins required for chromosome segregation[21,22]. These molecular arms races involving essential factors could force the genome to make costly tradeoffs. For example, variants that are good at suppressing drive may be suboptimal at executing a critical cellular pathway, leading to health problems. In addition, evolution of essential factors can lead to detrimental incompatibilities between components of multi-protein complexes or pathways[23,24].

Finally, engineered drive systems are being developed to combat mosquito-borne diseases which currently kill hundreds of thousands of people each year. These 'gene drive' systems aim to use the ability of meiotic drivers to spread to modify the genomes of entire populations of mosquitoes[25]. The potential genome modifications are diverse. Some are designed to reduce the ability of mosquitoes to transmit pathogens, while others aim to eliminate populations entirely. Understanding how natural meiotic drivers work, evolve, and spread in natural populations will help inform decisions about design and appropriate use of engineered drivers.

Major challenges in the meiotic drive field The actions of many meiotic drivers in a wide range of eukaryotes have been described[1]. With growing implementation of high-throughput sequencing, more meiotic drivers are being discovered each year[26,27]. Despite the impressive numbers of potential study systems, only a handful of meiotic drive phenotypes have been conclusively mapped to the actual genes (or DNA sequences) responsible[28-31]. In addition, even in systems in which at least some of the genes responsible are known, we largely know very little about the molecular mechanisms by which the genes cause drive.

There are two major factors limiting mapping and molecular characterization of meiotic drive genes. The first is that many drive loci are genetically complex. Most loci require more than one gene to enact drive and the contributing genes are often found within complex chromosome rearrangements that impede mapping. The second major factor is that many drive loci have been discovered in non-model systems with limited experimental tractability[1,7]. These two factors have also severely limited empirical experimental analyses of how the sequences of meiotic drivers evolve and how these selfish genes spread in populations.

This historical lack of well-characterized model drive systems has prevented us from learning the potential general principles and unifying mechanisms underlying meiotic drivers and their effects on genome evolution. For example, we do not yet know if there are aspects of gametogenesis that are particularly prone to subversion by selfish meiotic drive alleles. We also do not know much about the strategies that genomes use to suppress meiotic drive or how these genes change over time. Understanding such questions in model systems is an essential first step to better predictions of how meiotic drivers could affect human gametogenesis and to ultimately alleviate drive-induced maladies.

***wtf!* A microbe from fermented tea yields an innovative model meiotic drive system** As a postdoc, I wanted to begin exploring how meiotic drive could contribute to infertility and shape genome evolution. However, I felt that the drive systems that had been described severely limited the number and types of questions that I could address due to expense, slow generation times, and/or a lack of experimental tractability. I therefore set out to discover an undescribed meiotic driver in an exceptionally tractable model system.

My quest led me to search for meiotic drive in fission yeast. The eminent yeast geneticist Amar Klar had discovered a strain of *Schizosaccharomyces pombe* (*Sp*) in a batch of fermented kombucha tea and he named it *S. kambucha* (*Sk*). Despite the two isolates being ~99.5% identical at the DNA sequence level, Klar reported that *Sp/Sk* hybrids were sterile[32]. I suspected that this infertility could have been driven by a genetic conflict, so I set out to look for evidence of meiotic drive in the *Sp/Sk* hybrids. My hypothesis was correct and I found that several meiotic drive loci were causing hybrid sterility[18]. I then mapped one of these drive loci to a member of the uncharacterized *wtf* (**w**ith **Tf** transposon) gene family, *Sk wtf4*[17].

In my own lab, we set out to uncover how *wtf4* causes meiotic drive. We showed that the single *wtf4* gene uses alternate transcriptional start sites to encode both a poison protein and an antidote protein. The short transcript includes exons 2-6 and encodes the poison. We first observed the poison before the meiotic divisions and the protein localizes inside and around all the gametes. The long transcript, which includes exons 1-6, encodes the antidote. We observed the antidote protein only after gamete individualization and the protein remains specifically enriched in the gametes that inherit the tagged *wtf4* allele. In other words, the *wtf4* selfish gene ensures that all gametes are poisoned, but only those that inherit the *wtf4* locus are rescued by the antidote (Figure 1). Furthermore, other *wtf* genes can also cause drive and we have argued that the ancestral function of the *wtf* gene family was likely meiotic drive[17,33].

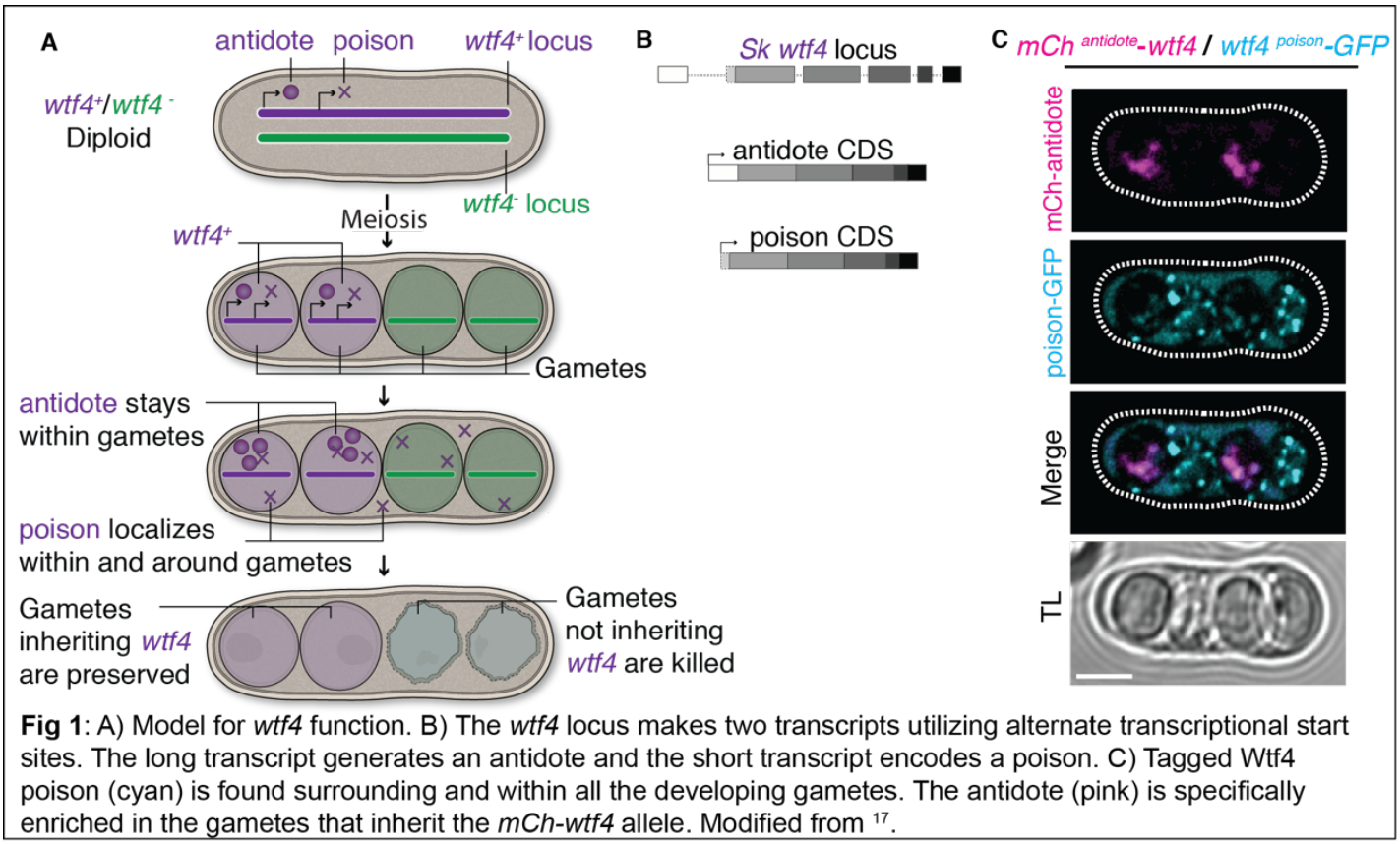

Fig 1: A) Model for *wtf4* function. B) The *wtf4* locus makes two transcripts utilizing alternate transcriptional start sites. The long transcript generates an antidote and the short transcript encodes a poison. C) Tagged Wtf4 poison (cyan) is found surrounding and within all the developing gametes. The antidote (pink) is specifically enriched in the gametes that inherit the *mCh-wtf4* allele. Modified from [17].

Identifying these *wtf* drivers in fission yeast was a major advance for the meiotic drive field[17,33]. Although *wtf* genes are not found outside of fission yeast, it is possible that drivers in other systems employ analogous strategies to enact drive. The experimental tractability of fission yeast will facilitate uncovering those mechanisms. In addition, drivers of all types, from any organism, are predicted to have overlapping consequences on genome evolution. The *wtf* model system will open the door to empirically test ideas about the evolution of drive systems previously only accessible to theoretical analyses.

We currently have only a superficial understanding of the molecular mechanism *wtf* genes employ to cause drive. We know that they each generate a poison protein, but we do not know how those proteins kill cells. Additionally, we know that each driving *wtf* gene encodes an antidote, but we do not know how the antidote neutralizes the poison[17]. The sequences of the genes have given us few clues. The *wtf* poisons and antidotes are predicted to contain between 6 and 8 transmembrane domains, but the proteins have no recognizable domains and no significant similarity to any genes outside the *wtf* family. To elucidate the mechanisms used by *wtf* drivers, I propose to use a multidisciplinary approach including cell biology, genetics and proteomics to characterize *Sk wtf4*.

How to kill the competition: cytological analyses of the poison and antidote strategy A key step to deciphering the mechanism of *wtf4* meiotic drive is to determine when the proteins are expressed during gametogenesis and where they localize within cells. We observed some expression of the Wtf4^{poison} protein in cells prior to the meiotic divisions. We suspect this early expression is how the poison enters gametes: the cytoplasm in which the gametes form is filled with poison, each gamete could encapsulate a lethal dose as it forms. To test this model, we will live image heterozygous *wtf4*^{poison}*-mEos2*/*wtf4* diploid cells as they progress through meiosis[34]. Just prior to gamete individualization, we will photo-convert the Wtf4^{poison} population (mEos2 green to red). We will then assay in time-lapse microscopy if we later detect the red Wtf4^{poison} protein inside mature gametes. If our model is correct, we will. If we do not detect red Wtf4^{poison} within gametes, our model is likely wrong, but we will be able to observe the true dynamics of Wtf4^{poison} protein production and distribution. For example, we may observe mostly green Wtf4^{poison} in the gametes. This would suggest that poison is synthesized after spore individualization and that it can traverse prospore membranes (early in gamete maturation) or even cell walls (late in gamete maturation).

We will also use cytology to better understand the mechanism employed by the Wtf4^{antidote} protein. We previously discovered that the Wtf4^{antidote} protein is made only after spore individualization and that it localizes to a subcellular compartment that is not the nucleus. We hypothesize that the compartment containing Wtf4^{antidote} is an organelle, perhaps the endoplasmic reticulum (ER), the vacuole (the yeast lysosome), or both. To test this, we will again live image cells as they progress through meiosis with Wtf4^{antidote}, Cpy1 (vacuole), and Sec63 (ER) proteins differentially tagged with fluorescent markers[35]. We will then assay for colocalization between Wtf4^{antidote} and the other factors.

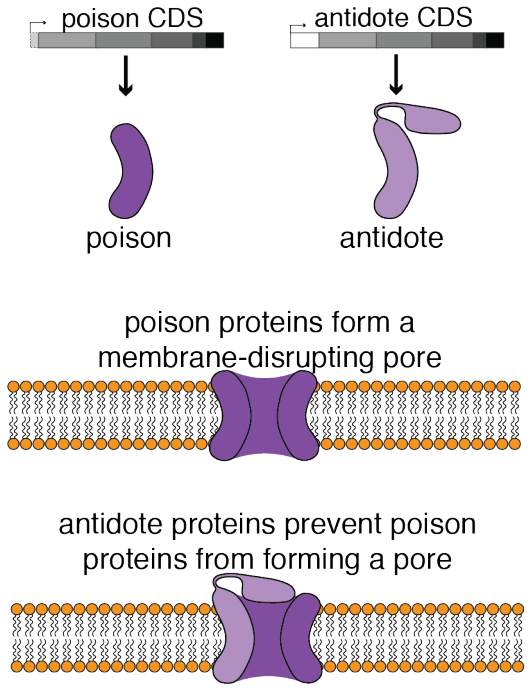

Fig 2: Model describing hypothesized interactions between the Wtf4^{poison} and Wtf4^{antidote} proteins and how these interactions could cause the poison and antidote phenotypes.

Given the large stretch of amino acids (292) shared by the Wtf4^{poison} and Wtf4^{antidote} and the colocalization of the proteins within gametes (Figure 1), we hypothesize that a physical interaction may mediate the neutralization of the poison by the antidote. For example, the poison proteins may interact with each other to form a functional complex. Given the protein is predicted to contain transmembrane domains, we hypothesize that this functional complex may form a pore that disrupts a vital membrane (Figure 2). The antidote may join this complex of poison proteins and disrupt its function, perhaps by clogging or preventing the formation of a pore. To test these ideas, we aim to explore possible physical interactions between Wtf4^{antidote} proteins, between Wtf4^{poison} proteins, and between Wtf4^{antidote} and Wtf4^{poison}. To do this cytologically, we will look for Forster resonance energy transfer (FRET) between differentially labeled protein pairs (e.g. Wtf4^{antidote}-mTurquoise and Wtf4^{poison}-YFP)[36]. Even if the proteins do interact, FRET may fail to detect it. If so, we will use yeast two hybrid and co-immunoprecipitation experiments to assay possible protein interactions. If our assays are consistent with the model in Figure 2, we will further test the model by purifying the proteins and testing the ability of the poison to form a pore in liposomes.

Functional tagged Wtf4 proteins are essential to the success of these experiments. Fortunately, we have demonstrated that both Wtf4 proteins remain functional with tags[17]. Because my expertise is in genetics, we will continue our collaborations with members of

the excellent microscopy and proteomics core facilities at the Stowers Institute to ensure our experiments and analyses are correctly executed.

Suicide suppression: a genetic approach to identifying the mechanism of Wtf4^{poison} Our cytological experiments (described above) may not fully reveal how Wtf4^{poison} kills cells. We predict the poison acts by targeting a conserved, essential aspect of cell physiology because we found that expressing Wtf4^{poison} in budding yeast mitosis kills those cells as well, despite hundreds of million years since the organisms shared a common ancestor. We have proposed the model described in figure 2, but this model may be incomplete or incorrect. There are, of course, countless number of conserved ways to kill cells, so we will use a genetic strategy to home in on the strategy employed by the Wtf4^{poison}. The conceptual basis of our approach is that if we mutate cellular factors exploited by the poison to cause spore death, cells will survive.

We discovered that we could generate what is known as a 'suicide allele' of *wtf4* (*wtf4^{poison}*) by mutating the start codon used to encode the antidote protein, while leaving the coding sequence for the poison protein intact[17]. During gametogenesis of *wtf4^{poison}* mutants, the poison is made in the absence of antidote and most of the gametes are destroyed by the poison. I propose to take advantage of this phenotype by screening for mutants that suppress this sterile phenotype. Briefly, we mutagenize haploid h^{90} (i.e. self-fertile) cells containing a *wtf4^{poison}-GFP* allele. We then copy the mutant clones in patches to starvation media (SPA) which allows them to mate to form homozygous mutants and undergo gametogenesis. We next screen the patches by replica-plating to find those which make more viable gametes than the unmutagenized *wtf4^{poison}*-GFP strain. We will further screen those hits for strains that express GFP in meiosis to help eliminate hits in which the poison allele is simply not expressed. We currently have 18 good candidate suppressors. Finally, we will identify candidate suppressor mutants using whole-genome sequencing. We will verify true suppressors by introducing the allele into the unmutagenized *wtf4^{poison}*-GFP strain and retesting. [The sequencing data we obtain will be deposited in the NCBI Sequence Read Archive upon publication of our results.]

In parallel to this mutagenesis screen, we will also introduce our suicide allele into an h^{90} derivative of the *Sp* deletion collection and look for deletion mutants that restore fertility in the presence of *wtf4^{poison}-GFP*. The biggest obstacle to overcome in these screens is the labor required. To surmount this obstacle, we are collaborating with the molecular biology core at the Stowers Institute to carry out many of the steps of each screen using robotics.

Genetics may fail to identify our proteins of interest. This is especially true if the genes exploited by Wtf^{poison} are essential. To complement potential deficiencies in our genetic approach to identifying the cellular components or pathways targeted by Wtf4^{poison}, we will use a biochemical approach. Specifically, we will induce expression of a tagged Wtf4^{poison} in mitotic cells, immunoprecipitate Wtf4^{poison} and screen for interacting proteins using multidimensional protein identification technology (MudPIT) in collaboration with the proteomics core at the Stowers Institute[37].

Avoiding friendly fire: How do Wtf poisons and antidotes ensure specificity? Consistent with their involvement in a genetic conflict, the *wtf* genes are amongst the most rapidly evolving genes in fission yeast genomes[17,33]. Between different yeast isolates, we have shown that the *wtf* orthologs can be dramatically different. For example, both *Sp* and *Sk wtf4* genes encode intact meiotic drive genes, but the antidote produced by the *Sp* gene does not protect against the poison generated by the *Sk* gene (Figure 3). We term the ability of an antidote to neutralize the corresponding poison 'specificity.' Deciphering how specificity is determined between poisons and antidotes will lead to a better mechanistic understanding of drive. This question is also very important from an evolutionary perspective because if rapid evolution yields a new poison, but not a corresponding antidote, the cell would be sterile[17]. Has this poison-induced sterility happened frequently in the evolution of fission yeasts, or does the structure of the genes mitigate this possibility?

We hypothesize that specificity between Wtf ^{poison} and Wtf ^{antidote} proteins is determined by sequence similarity between the two proteins. If this hypothesis is correct, the overlapping nature of the Wtf ^{poison} and Wtf ^{antidote} coding sequences (i.e. they share exons 2-6) would mitigate the possibility of generating a *wtf ^{poison}* suicide allele. When a mutation in a *wtf* gene does generate a novel poison, the mutation could simultaneously generate an antidote to that poison. This would be quite a clever strategy for a selfish gene because it frees the gene to diverge and/or duplicate in the genome: both are known evolutionary strategies employed by

selfish elements to evade suppression[3,4]. This hypothesis could thus help explain the rapid expansion and diversification of the *wtf* gene family in fission yeast.

To test these ideas, we will map determinants of poison and antidote specificity for a group of three *wtf* genes. We will employ separation of function alleles (i.e. poison-only and antidote-only) that we have made for each of the three genes. The ability of an antidote to neutralize a given poison can be easily assessed by assaying the phenotype of a *wtf*^{poison}/ *wtf* ^{antidote} heterozygote. If the antidote neutralizes, the *wtf* ^{antidote} allele will exhibit drive in the gametes of the heterozygote. If the antidote cannot neutralize the poison, fertility will be very low (<10% of wild-type fertility). The relationship between the poison and antidotes produced by these genes is illustrated in Figure 3A. For example, the *Sk* Wtf4^{antidote} neutralizes the *Sp* Wtf4^{poison}, but not the *Sp* Wtf13^{poison}. Those two poisons differ by one amino acid in exon 2 and by the number of units of a repetitive amino acid sequence in exon 3 (Figure 3B). We will swap those residues between the *Sp* Wtf4^{poison} and *Sp* Wtf13^{poison} proteins and then test which variants differentiate the two in terms of susceptibility to neutralization by the *Sk* Wtf4^{antidote}. We will perform analogous swap experiments to determine why neither the *Sp* Wtf4^{antidote} or *Sp* Wtf13^{antidote} proteins can neutralize the *Sk* Wtf4^{poison}. We will fully test each chimeric *wtf* we generate against the whole panel of potential agonists. For example, when we mutate the two *Sp wtf4* separation of function alleles to make exon 2 like that of *Sp wtf13*, we will test the novel antidote generated against the novel poison. My hypothesis predicts the novel antidotes should always neutralize their corresponding (those with the same exons 2-6) novel poisons. In addition, we will test the novel poisons and antidotes against the proteins generated by the wild-type genes. In this way, we will build a matrix of poison and antidote specificities that will address our hypothesis.

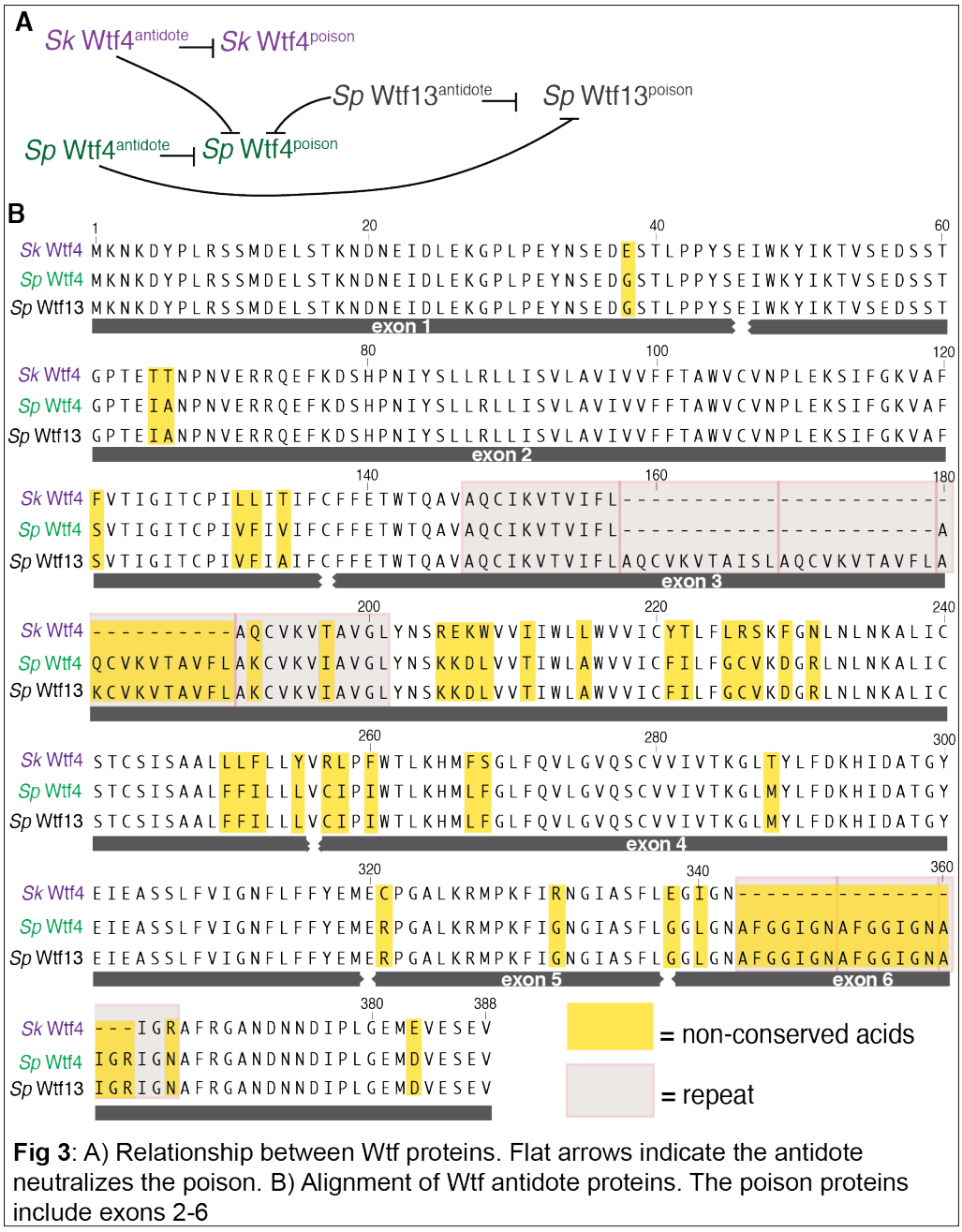

Fig 3: A) Relationship between Wtf proteins. Flat arrows indicate the antidote neutralizes the poison. B) Alignment of Wtf antidote proteins. The poison proteins include exons 2-6

A potential problem with these analyses is the fact that we perform the genetic crosses in fission yeast strains that each contain more than 25 endogenous *wtf* genes–too many to easily delete for these analyses. These genes may affect the phenotypes of our novel *wtf* chimeric genes if, for instance, an endogenous Wtf ^{antidote} can suppress a novel poison. The relationships shown in Figure 3A were uncovered in an *Sk* strain in which the endogenous *wtf4* gene was deleted and we plan to test the novel constructs in that background as well. To be

sure that novel Wtf ^{poisons} are functional in this background, we will confirm their ability to cause spore death in the absence of an added antidote. If a given novel poison cannot cause spore death in our *Sk* background, we induce its expression in budding yeast to determine if it is likely suppressed in *Sk* (the budding yeast die), or if the protein is nonfunctional (the budding yeast live).

The times they are a-changin': the *wtf* genes enable experimental analyses of meiotic drive evolution

The evolutionary dynamics of meiotic drivers have been given extensive theoretical consideration because of their profound potential to shape eukaryotic genomes[1]. Empirical tests of those evolutionary models, however, have historically been limited by the lack of cloned meiotic drive systems in genetically tractable organisms. We aim to change that using *wtf* genes.

To facilitate experimental evolution analyses of meiotic drive systems, we will first develop a high-throughput assay to reduce the time, labor and reagents needed to assay allele frequencies in a population. Analogous to other systems used in budding yeasts, our assay employs fluorescent genetic markers[38]. Briefly, alternate alleles at an experimental locus will be linked to constitutively expressed fluorescent markers (e.g. GFP and mTurquoise). The frequency of a given locus in a population can then be assayed by quantifying the frequency of cells expressing the fluorescent marker using cytometry (Figure 4). We have designed this assay in collaboration with the Stowers Institute cytometry core facility and they will continue to provide support for our experiments.

In the simplest scenario, we will use our assay to experimentally model the spread of a meiotic drive allele in a population over time. We will do this by seeding starting *Sp* populations with known fractions of *Sp* cells containing a drive gene from *Sk* (e.g. *wtf4*). We will then allow the cells in the population to reproduce and then measure the frequency of the drive locus in the progeny (Figure 4). This process will be repeated until one allele reaches fixation or until the population reaches an equilibrium. Theoretical models predict fixation of the *wtf4* allele will occur in less than 30 generations if the population is seeded with 5% *wtf4*-containing cells[6]. Due to the simplicity of the assay, we can test many duplicate populations in parallel to ensure robust results. We will then introduce variants of the simple assay to test additional hypotheses about how meiotic drivers can shape genome evolution. For example, we will test if the driver can enable the spread of linked deleterious (e.g. disease-causing) mutations or chromosome rearrangements in a population by linking them to the drive locus and repeating the protocol sketched above. It is possible that our experimental evolution analyses of the simplest scenarios described above will simply confirm predictions of the theoretical models. Even so, that would be valuable validation of a widely used, but scarcely tested, type of analysis in the meiotic drive field.

In addition, our experimental assay will also enable us to explore scenarios found in real organisms that are more difficult to model mathematically. These situations are not currently described even in theoretical models. For example, we will use the assay to address questions regarding duplications of drive loci. What happens when a meiotic driver duplicates to multiple regions of the genome? When is it advantageous for such duplicates to functionally diverge from the parent gene? What happens when alternate competing alleles of meiotic drivers are active in the same population? We will also use our assay to test if some regions of the genome are more permissive for meiotic drivers than others. For example, the *wtf* family has expanded specifically on chromosome 3 in fission yeast. In the lab *Sp*, 23 of the 25 genes are found on chromosome 3, despite it being by far the smallest chromosome. Are single or sets of meiotic drive genes more evolutionarily successful on chromosome 3 as compared to chromosomes 1 and 2?

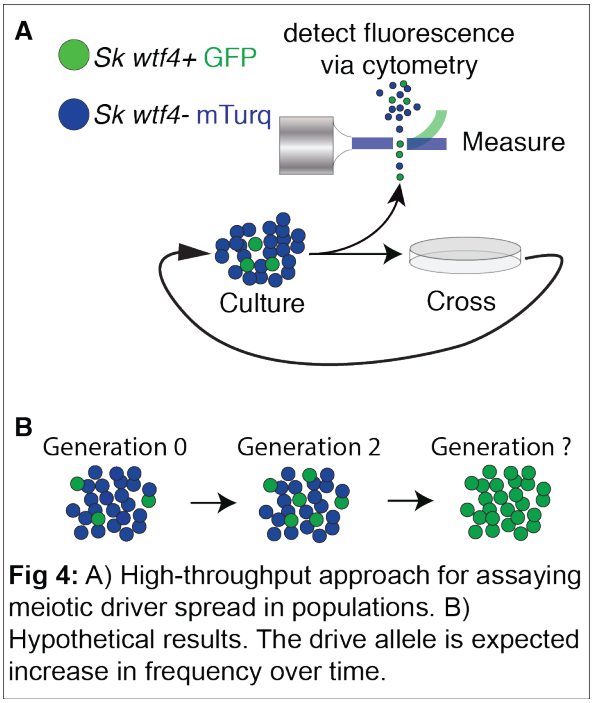

Fig 4: A) High-throughput approach for assaying meiotic driver spread in populations. B) Hypothetical results. The drive allele is expected increase in frequency over time.

Overall Impact: Genes required for gametogenesis are generally amongst the most rapidly evolving in eukaryotic genomes, including our own, even though the overall process is largely conserved[39-41]. It is likely that genetic conflicts with selfish elements, such as meiotic drive genes, underlie this rapid evolution.

Understanding the mechanisms used by selfish genes and how they shape genome evolution is, therefore, essential to understanding gametogenesis. This expanded knowledge of gametogenesis will ultimately lead to improved human reproductive outcomes. However, studies of how genetic conflicts affect gametogenesis at the molecular level are sorely underrepresented. Empirical experimental analyses of how drivers shape genome evolution are even more scarce.

The proposed research will provide characterization of the molecular mechanisms of a meiotic drive gene, *Sk wtf4*. Although meiotic drive genes are widespread, we know very little about how these selfish genes work at the molecular level. Our characterizations will facilitate discovery of emerging themes about how these selfish genes work. For instance, are there certain conserved aspects of gametogenesis that tend to be targeted by these selfish genes? This knowledge will serve as a guide to help search for meiotic drive genes in other systems, including humans. Finally, the proposed experiments will pioneer experimental evolution analyses of drive systems. This work will add critical knowledge to our understanding of how parasitic genes can invade populations and shape genome content and thereby organismal health.

Innovativeness:

This project is innovative because it bridges disparate fields of evolutionary and molecular biology to address how selfish genes act to cause infertility and how they spread in a population. This work is only now possible due to my groundbreaking discovery of meiotic drivers in yeast, which has enabled us to tackle a breadth of questions in one organism that was previously out of reach[18]. While some of the experiments, such as the genetic and proteomic screens, are risky, the approaches are complementary to maximize our chances of finding answers to our questions. In addition, we will have expert help from collaborations with the Stowers Institute core facilities for the technically challenging experiments. Our discovery of the *wtf* gene family has already gained significant attention and we anticipate the studies proposed here will become textbook examples of how meiotic drive genes work and shape genome evolution[17].

Investigator qualifications:

My career has benefited from my exceptionally high level of optimism and confidence in my abilities as a scientist. This ‘science self-esteem’ was not innate and is not (unfortunately!) mirrored by high confidence in my non-science life skills. Instead, my science self-esteem was nurtured by wonderful mentors. They helped me recognize my strengths and taught me to continually work on my weaknesses, rather than feel paralyzed by them. My self-esteem permeates how I approach science and has enabled me to challenge paradigms and make unanticipated, impactful discoveries.

As a graduate student in Eric Alani’s lab, I became interested in a conserved gene called *PCH2*. Even though mismatch repair is the focus of his lab and *PCH2* has nothing to do with mismatch repair, Dr. Alani encouraged my interest. Prior to my work, *PCH2* was reported (in *Cell* by a leading lab in our field) to have no role in meiotic crossing over outside of the rDNA[42]. I was unconvinced and stubbornly wanted to test that myself. My arrogance paid off. My work demonstrated that, not only did *PCH2* dramatically affect meiotic recombination, but that it is a major regulator of meiotic DNA double strand break repair[43,44].

As a graduate student, I was puzzled by the fact that the field investigating the molecular mechanisms of meiosis did not seem bothered by our lack of a good explanation for why meiosis genes rapidly evolve. I felt quite certain it meant there was something important about the process that we were ignoring and I decided I would endeavor to fill that gap. Even though I wanted to understand meiosis, I did not join one of the leading meiosis labs because I felt that in those labs I would continue to study meiosis using traditional approaches. Instead, I joined the lab of evolutionary biologist Harmit Malik to learn to see meiosis through an evolutionary lens. Surrounded by colleagues studying the evolution of viruses and chromatin in cell culture and fruit flies, I learned how genetic conflicts shape genomes. I used this knowledge to develop a completely novel research program in fission yeast investigating, at the molecular level, how selfish genes exploit meiosis and shape genome evolution[18].

I chose to start my own lab at the Stowers Institute, rather than a traditional university setting, to help me continue to do innovative work with the help of collaborators when needed. Specifically, I felt the core labs (microscopy, molecular biology, bioinformatics and cytometry), manned by expert scientists with the best tools,

would enable my group to easily collaborate and not be limited by our current skill sets. That has proven true so far, with our collaboration with the microscopy core already yielding a publication. The experiments proposed here will expand our network of collaborations and thus broaden our ability to address the most important questions about meiotic drive.

Suitability for the New Innovator Award program*:*

The current proposal is an ideal fit for the New Innovator award program. My overall research program and the experiments described within this proposal are truly pioneering and unique to my group. Although my work is certainly shaped by the expert mentorship I received as a postdoc under Harmit Malik, my research is not derivative of past or ongoing work in his lab. I am confident that, given my dual training in molecular mechanisms of meiosis and the evolutionary biology of genetic conflicts, my group is uniquely poised to make groundbreaking discoveries about meiotic drive. However, I am at the beginning of my appointment and have not yet accumulated extensive unpublished data required by more traditional grants. In addition, the high-risk nature of some of the experiments, such as the genetic and proteomic screens, are generally not suited to more conservative grants. Finally, the large impact selfish genetic elements have in contributing to illnesses, especially infertility, in natural populations is not currently widely appreciated. Innovative research, like that proposed in this grant, will change this.

Statement of research effort commitment:

If selected for an NIH New Innovator Award, I will commit at least 25% of my effort to the proposed project.

References:

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Authentication of Key Resources Plan:

The proposed research focuses on the *wtf* gene family of fission yeast. In unpublished work, we have discovered that this gene family is amongst the most rapidly evolving in the fission yeast genome. This leads the genes to be polymorphic, even in so-called isogenic lab strains. This polymorphism can affect our studies. To ensure that our strains are truly isogenic, we use only strains recently (in the last 5 years) derived in our laboratory from two parental isolates (one from *S. kambucha* and one from *S. pombe*). We also sequenced and assembled the genomes of these strains to know the *wtf* gene alleles present in each. If we need to use strains not recently derived from those two parent strains, we will sequence and assemble those genomes as well. Our protocols for this genotyping will be documented and published. Finally, we freeze our strains as soon as possible after generating them to allow as little time as possible for mutations to accumulate. We also minimize the time the strains are allowed to grow after being taken out from the frozen stocks prior to being used in our experiments.

The rest of the reagents to be used in these proposed studies are standard laboratory reagents and are routinely purchased from reputable biological companies. All reagents will be documented and reported by their vendor, catalog number, and lot number when available. All procedures and reagent details will be documented and included in publications.