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Coexistence Theory and its Implications for Eco-evolutionary Dynamics

A dissertation submitted in partial satisfaction
of the requirements for the degree

Doctor of Philosophy
in
Ecology, Evolution and Marine Biology

by

Kelly Marie Thomasson

Committee in charge:

Professor Stephen Proulx, Chair
Professor Cherie Briggs
Professor Bruce Kendall

September 2019

The Dissertation of Kelly Marie Thomasson is approved.

Professor Cherie Briggs

Professor Bruce Kendall

Professor Stephen Proulx, Committee Chair

September 2019

Coexistence Theory and its Implications for Eco-evolutionary Dynamics

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by

Kelly Marie Thomasson

This dissertation is dedicated to my parents, Gary Lynn and Monta Marie Thomasson, who have always supported me with love, enthusiasm for my career goals, interest in my research and the occasional rent check. I know that I am lucky to have you and recognize how difficult and rare it is for people to complete a doctorate without support like that which you have given freely.

This dissertation is also dedicated to Dr. Samuel Sweet, who has been a supportive and encouraging friend and mentor over the last seven years, and, without whom, I would have surely quit the program three times over.

Acknowledgements

This dissertation would not have been completed without the help of many many people. I would like to thank and acknowledge my committee for their patience and help on several aspects of this dissertation and for agreeing to accompany me on this journey. Thank you, also, to my honorary committee member, Dr. Roger Nisbet, for his kindness and patience with me as I attempted to become a mathematician. Thank you also to Dr. Mark Wilber for his patient explanations of math, modeling techniques and coding. I would like to offer my gratitude Alexander Franks for his help with Bayesian analyses in Chapter 3. To Simone Dupuy, a huge thank you for keeping the lab tidy and organized for nearly four years, and for keeping me sane and supported as well. Thank you to Kate McKee for allowing me to drag her into the world of academic research and for becoming my friend despite that. My greatest appreciations go to Fernanda Pett for her fantastic artwork in chapters 3-4; there is no way anything I would have drawn would look as professional as they do. This dissertation would have looked so much worse without the help of Dr. Matthew Tucker-Simmons. Thank you for your extensive help with Latex, Overleaf, teaching me about the ink to information concept (although I clearly did not follow it here) and battling your way through a multitude of red Latex errors. To Dr. Lisa August-Schmidt, thank you for reminding me I have friends and keeping me focused on what is important (pst...it's signatures). Thank you to Stephanie and Erik Lucero for being so supportive in the beginning of my program, and to Niko Hensley for help with phylogenies and being a supportive friend at the end of my program, and gym companion when I became a dissertation-writing slug. Thank you all so much; things would have been far worse without you. Finally, thank you to the 60 undergraduates that I had the privilege to work with. I learned more from you than I did in my seven years as a doctoral student.

Curriculum Vitæ

Kelly Marie Thomasson

Education

2019	Doctorate in Evolution, Ecology and Marine Biology (Expected), University of California, Santa Barbara.
2018	Certification of College and University Teaching, University of California, Santa Barbara.
2016	Master of Arts in Animal Behavior and Ecology, University of California, Santa Barbara.
2011	Master of Science in Biological Conservation and Ecology, California State University, Sacramento.
2002	Bachelor of Arts in Linguistics and Minor in French, University of California, Los Angeles.

Publications

Manuscripts in Advanced State of Preparation

Thomasson, K.M. and W.E. Avery. An Analysis of the Efficacy of the Marine Protected Areas in Maui County in consideration of Tourism.

Thomasson, K.M., K.M. McKee and S.R. Proulx. Quantifying the probability of survivorship through insect-gut vectoring in dispersed yeast communities.

Thomasson, K.M. and S.R. Proulx Long-term insect-gut vectoring selects for faster sporulation rates in *Saccharomyces cerevisiae*.

Manuscripts in Early State of Preparation

Thomasson, K.M., T. Sakal and S.R. Proulx. Evaluating models of coexistence in insect-vectored populations of *Saccharomyces cerevisiae*.

Thomasson, K.M. and S.R. Proulx Review: Coexistence mechanisms and their role in species divergence.

Thomasson, K.M., S.L. Dupuy and S.R. Proulx. Regional variation in adaptive trajectories in *Saccharomyces cerevisiae* strains subjected to repeated gut-vectoring by *Drosophila melanogaster*.

Thomasson, K.M., K.M. McKee, and S.R. Proulx. Genetic Analysis of experimentally evolved strains of *Saccharomyces cerevisiae* from diverse genetic backgrounds.

Awards and Honors

2019 Graduate Student Association: Excellence in Teaching Award Nominee, University of California, Santa Barbara.

2018 Graduate Student Association Excellence in Teaching Award Nominee, University of California, Santa Barbara.

American Society of Naturalists Student Travel Award, Asilomar, California.

Graduate Student Association Travel Grant, University of California, Santa Barbara.

Academic Senate Graduate Travel Grant, University of California, Santa Barbara.

2017 Academic Senate Distinguished Teaching Award Nominee, University of California, Santa Barbara.

Graduate Student's Association Travel Grant, University of California, Santa Barbara.

Block Grant Award Recipient, University of California, Santa Barbara.

Graduate Student Association Excellence in Teaching Award Nominee, University of California, Santa Barbara.

2016 Worster Award Recipient, University of California, Santa Barbara.

Graduate Student Association Excellence in Teaching Award Nominee, University of California, Santa Barbara.

Dixon Levy Community Service Award Recipient, University of California, Santa Barbara.

Fiona Goodchild Mentorship Award Recipient, University of California, Santa Barbara.

Academic Senate Distinguished Teaching Award Nominee, University of California, Santa Barbara.

2015	Graduate Student Association Excellence in Teaching Award Nominee, University of California, Santa Barbara.
	Graduate Student's Association Travel Grant, University of California, Santa Barbara.
2014	S.T.E.M. Support Fund, University of California, Santa Barbara.
	Fiona Goodchild Mentorship Award Nominee, University of California, Santa Barbara.
2013	Graduate Departmental Fellowship, University of California, Santa Barbara.
2011	2nd Place, University Graduate Research Competition, California State University, Sacramento.
2009	Biological Conservation Scholarship, California State University, Sacramento.
	Marda West Scholarship, California State University, Sacramento.
2002	Chancellor's Service Award, University of California, Los Angeles.
2001	Bruin Belles Stellar Service Award, University of California, Los Angeles.

Meetings

2018	Thomasson, K.M. U. Rajpurkar, C. Kwon and S.R. Proulx. **Adaptation in regionally diverse, experimentally evolved strains of *Saccharomyces cerevisiae*: the effects of similar selection pressures on varying genetic backgrounds**. 2nd Joint Congress on Evolutionary Biology, Montpellier, France. Abstract and Poster Presentation.
	Rajpurkar, U. K.M. Thomasson and S.R. Proulx. **The diversity of adaptive trajectories in regional strains of *Saccharomyces cerevisiae* in response to the selective pressure of pseudo-digestion**. 2nd Joint Congress on Evolutionary Biology, Montpellier, France. Abstract and Poster Presentation.Dupuy, S.L., K.M.
	Thomasson, K.J. Luxmore, H. Plett and S.R. Proulx. **Early Stage competition of post-traumatic vectored founder colonies of**

yeast. 2nd Joint Congress on Evolutionary Biology, Montpellier, France. Abstract and Poster Presentation.

Thomasson, K.M. **SnapScience: Accessing the Benefits of Popular Social Media for Education**. Society for the Advancement of Biology Education Research, Minneapolis, Minnesota. Abstract and Poster Presentation.

Thomasson, K.M. **SnapEd!: Accessing the Benefits of Popular Social Media for Education**. Graduate Student Teaching Symposium, Santa Barbara, California. Contributed Talk.

Thomasson, K.M **Long-term evolution of *S. cerevisiae* subjected to frequent dispersal by insect gut-vectoring**. American Society of Naturalists, Asilomar, California. Contributed Talk.

Thomasson, K.M. **Social Media Platforms as Educational Tools in STEM**. Society for the Advancement of Biology Education Research (SABER) West Conference, Irvine, California. Abstract and Poster Presentation.

2017

Thomasson, K.M. and S.R. Proulx. **A model for coexistence and divergence in insect-vectored yeast populations**. Society for Evolutionary Biology, Portland, Oregon. Contributed Talk.

Thomasson, K.M. **Coexistence mechanisms as promoters of divergence**. Society for Evolutionary Biology, Portland, Oregon. Abstract and Poster Presentation.

Thomasson, K.M. and S.R. Proulx. **Genetic divergence and phenotypic plasticity in yeast strains subjected to repeated fly-gut vectoring**. Society for Molecular Biology and Evolution, Austin, Texas. Abstract and Poster Presentation.

Thomasson, K.M. **Regional differences in the competition colonization trade-off in dispersed communities of *Saccharomyces cerevisiae***. Ecological Society of America, Portland, Oregon. Contributed Talk.

2015

Thomasson, K.M. **The role of depth in diversity indices and marine protected area resilience**. International Conference of Conservation Biology, Montpellier, France. Abstract and Poster Presentation.

2013	Thomasson, K.M. **Tourism's Impact on Policy and MPA regulation**. International Conference of Conservation Biology. Baltimore, Maryland. Abstract and Poster Presentation.
2011	Thomasson, K.M. and W.E. Avery. **An Analysis of the Efficacy of the Marine Protected Areas in Maui County in consideration of Tourism**. California State University, Statewide Research Competition. Fresno, California. Abstract and Oral Presentation.
	Thomasson, K.M. and W.E. Avery. **An Analysis of the Efficacy of the Marine Protected Areas in Maui County in consideration of Tourism**. California State University, Sacramento Research Conference. Sacramento, California. Abstract and Oral Presentation.
2009	Lewis, B.B., K.M. Thomasson, B. Wachocki and M. Sondossi. **Calcium oxalate crystals in leaves of *Halogeton glomeratus* and their possible contribution to alteration of soil chemistry**. American Botanical Society Conference, Snowbird, Utah. Abstract and Poster Presentation.
	Thomasson, K.M., B.B Lewis, B. Wachocki and M. Sondossi. **Evaluation of Soil Microbiota and Soil Chemistry in Competitive Success of *Halogeton glomeratus***. Weber State University Undergraduate Research Symposium, Ogden, Utah. Abstract and Poster Presentation.
	Lewis, B.B., K.M. Thomasson, B. Wachocki and M. Sondossi. **Mycorrhizal Associations in *Halogeton glomeratus***. Weber State University Undergraduate Research Symposium, Ogden, Utah. Abstract and Poster Presentation.

Field Work Experiences

2009-2010	Coral Reef Biodiversity and Conservation, Maui County, Hawaii Collected 500 photographic quadrat samples in both unprotected and protected marine areas. Measured and compared diversity of benthic epifauna based on diversity and percent cover indices.
2008	*Halogeton*-Winterfat competition, Great Basin National Park, Nevada An analysis of the mechanism for out-competition of native species by *H. glomeratus*. A description of fungal interactions with *H. glomeratus* and the chemical reactions within the surrounding soils in the Nevada Great Basin.

2007	Red Abalone Fisheries, North California Coast An analysis of the Randomized response technique on Fisheries management of California Red Abalone. Master's Thesis work of Sara Grace Blank, University of Wellington, New Zealand.
2005-2006	Humpback Whale Behavior, Maui County, Hawaii An observation of Cow/Calf and Boat interactions and the possible resultant changes in behavior and migration routes.
2004	Water-treatment, Placer County, California A recreation of the Toha System of waste water management for developing countries. An assessment of the function ability of the Tohá system in a Mediterranean climate.

Laboratory Research Experiences

2013-2018	Competition-Colonization Tradeoffs and long-term experimental evolution in the yeast *Saccharomyces cerevisiae*. University of California, Santa Barbara, California. Protocols included microbial culture, Drosophila melanogaster husbandry and sorting, P.C.R. tagging, gel electrophoresis, plasmid extraction and transformation, sporulation protocols, optical density calculations and genomic sequencing.
2009-2010	Mammalian cell culture technician, California State University, Sacramento, California. Protocols included aseptic technique for mammalian culture, reseeding of cell culture flasks, confluence assessment and cell quantification.
2008-2009	*Halogeton*-Winterfat competition root and soil analysis, Weber State University, Utah. Protocols included root propagation, microscopy and tissue staining, microbial culture and serial dilutions, IR and NMR spectroscopy, and soil analysis.

Workshops

2019	Dynamic Energy Budgets Course: Applying DEB to ecology and evolution. Université de Bretagne Occidentale, Brest France.
2018	A Meta Cognitive Approach to Reading Science Text. SABER-West Education Conference, Irvine, California.
	Using Whiteboards to Leverage Learning in Multiple Settings. SABER-West Education Conference, Irvine, California.

Biology Education Research at Community Colleges – Exploring Opportunities, Challenges, and Support Strategies. SABER-West Education Conference, Irvine, California.

2017 — Fitting Non-Linear models in R using the mgcv package: Ecological Society of America Conference. Portland, Oregon.

Data visualization using R and ggplot: Ecological Society of America Conference. Portland, Oregon.

College and University Teaching —from theory to practice. University of California Department of Instructional Development, Santa Barbara, California.

Activating students and content: converting standard materials into active learning modules: SABER-West Education Conference, Irvine, California.

Transforming how we teach is good, but transforming what we expect students to learn is better: SABER-West Education Conference, Irvine, California.

Process Oriented Guided Inquiry Learning (POGIL): A student-centered approach to science instruction: SABER-West Education Conference, Irvine, California.

STEM Essentials Teaching Workshop, Backwards Design: UCSB Instructional Development, Santa Barbara, California.

2015 — R and Git Hub statistical computing. UCSB EEMB Graduate Student Advisory Committee, Santa Barbara, California.

Discipline-based Research Education Seminar Series: UCSB department of Molecular, Cell and Developmental Biology, Santa Barbara, California.

2014 — Lead TA Institute: UCSB Instructional Development, Santa Barbara, California.

2013 — Panel on International Students: UCSB Instructional Development, Santa Barbara, California.

2012 — Plagiarism and Academic Integrity: AAAS Conference, Vancouver, British Columbia.

Invited Lectures

2019	Guest Lecture, Animal Behavior, Ethology and Evolution (EEMB 138) University of California, Santa Barbara Title: “Predator-Prey Interactions” Instructor: Stephen Proulx
2016	Guest Lecture, Animal Behavior, Ethology and Evolution (EEMB 138) University of California, Santa Barbara Title: “Genetics, Ontogeny and Behavior” Instructor: Jonathan Pruitt
2011	Guest Lecture, Coastal and Marine Policy Class (ENVS 150) University of California, Santa Cruz Title: “An Analysis of the Efficacy of the Marine Protected Areas in Maui County in consideration of Tourism” Instructor: Sara Lewis
2010	Ecology Seminar, Department of Biological Sciences Lunch Seminar Simone Fraiser University, Burnaby, British Colombia The Role of Depth in biodiversity indices and robustness in coral reefs exposed to eco-tourism. Invited by Arne Mooers
2006	Seminar, Pacific Whale Foundation Lecture Series, Maui, Hawaii Title: “Humpback whale and commercial boat interaction: Curiosity or Avoidance?” Seminar Chair: Greg Kaufman
	Marine Seminar Series, Carnival Cruise Lines, Hawaiian Islands Marine Systems Seminar Series of 5 lectures titled: “Island Biogeography and Culture,” “Mammal Mammals,” “Humpback Whales,” “Coral Reefs,” and “Tropical Fishes”.

Teaching

Courses taught as Instructor of Record

Summer 2019	EEMB 102: Macroevolution University of California, Santa Barbara Course on evolutionary history of earth and major evolutionary eras as well as phylogenetic techniques and macroevolutionary processes
Fall 2017	EEMB 157: Cell Physiology University of California, Santa Barbara Course on basic cellular function, cellular diversity and specialization within and across taxa
Winter 2015	EEMB 138: Behavioral Ecology and Ethology University of California, Santa Barbara Course on Evolutionary Principles of Behavior, Behavioral Economics and Game Theory.

Courses taught as a Teaching Assistant

EEMB 3: Introduction to Diversity. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Spring 2014

EEMB 101: Molecular Evolution. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Summer 2016

EEMB 102: Macroevolution. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Spring 2015

EEMB 113: Vertebrate Ecology and Evolution. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Fall 2015

EEMB 138: Ethology, Animal Behavior and Evolution. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Winter 2013, Winter 2014, Winter 2016, Winter 2019

EEMB 146: Biometry. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Spring 2016, Spring 2017, Spring 2019

EEMB 154: Integrative Physiology. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Winter 2016

EEMB 157: Cell Physiology. Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara. Fall 2013, Fall 2014, Fall 2016

MCDB 1: Introductory Biology: Introduction to biochemistry, cell biology and development, and genetics. Department of Molecular, Cell and Developmental Biology, University of California, Santa Barbara. Fall 2018

Mentorship

As a Ph.D. student at UCSB, I directly mentored 60 undergraduate and high school students. They worked as undergraduate researchers, biological illustrators and student lab managers in the Proulx Lab.

2017	Biological Illustration Worked with students to create illustrations and diagrams for publication and presentation. Discussed digitization and scale for intended use as well as figure goals for papers and presentations. Total students mentored: 2
2016-2017	Worster Program Mentor The Worster program is a grant-based program designed to fund and undergraduate and graduate student for the summer. The goal is that the graduate student will mentor and assist the undergraduate with an independent research project of their own. The student had the opportunity through this program to experience what it was like to run a research project from the aspect of the principle investigator. He oversaw materials, protocol, quality control and had a research staff of three undergraduates and four high school volunteers. Total Worster students: 1
2014-2016	Research Mentorship Program The Research Mentorship Program is a summer program in which gifted and motivated high school students attend college classes, stay in freshman housing and complete a summer research project in a field of their choosing. Mentors work with the students to design a project, complete data collection, analyze the data present their findings as a paper, a poster and a talk. Total students mentored: 8
2014-2017	Undergraduate Research and Creative Activities (URCA) students Worked with students to experience the complete professional scientific process including experimental design, grant writing, pilot studies, materials and through-put estimation, data collection, data analysis, manuscript writing and poster presentation skills. Some of these students attended professional conferences to present their research. These students also received mentorship about conference etiquette and networking skills. Total Students Mentored:16 Total research projects completed: 22
2012-2017	Lab Technician and Lab Management Worked with students to understand the basic and advanced skills of managing a lab, including: accounting, inventory, materials storage, reagent preparation, task and project delegation and running a lab meeting. Total students mentored: 5
2012-2017	Undergraduate Research Assistants Taught students aseptic technique, good lab notebook practices, basic wet-lab skills and careful protocol design as well as data organization, data analysis, most importantly how to get back up after failing in science. Total students mentored: 28

Posters Resulting from Mentorship Projects

2018

Rajpurkar, U., K.M. Thomasson and S.R. Proulx. **Adaptive Trajectories of *Saccharomyces cerevisiae* as a result of pseudo-digestion**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Uma Rajpurkar)

Luxmore, K.J., H.M. Plett, K.M. Thomasson and S.R. Proulx. **How selection affects fitness of the model organism *Saccharomyces cerevisiae***. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researchers: Hannah Plett and Kathleen Luxmore)

2017

Brantz, H.A., A. Thompson, K. McKee, S. Dupuy, P. Shindgikar, J.A. Leibreich, K.J. Luxmore, K.M. Thomasson and S.R. Proulx. **Fly sex and its biochemical effect on survivorship of vectored yeast**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Helena Brantz)

Brantz, H.A., A. Thompson, K. McKee, S. Dupuy, P. Shindgikar, J.A. Leibreich, K.J. Luxmore, K.M. Thomasson and S.R. Proulx. **Fly sex and its biochemical effect on survivorship of vectored yeast**. Ecological Society of America, Portland, Oregon. Abstract and Poster Presentation.

Dupuy, S., K.M. Thomasson, P.J. Woo-Sam and S.R. Proulx. **Early stage competition of post-traumatic vectored founder colonies of yeast**. Ecological Society of America, Portland, Oregon. Abstract and Poster Presentation.

Dupuy, S., K.M. Thomasson, P.J. Woo-Sam and S.R. Proulx. **Early stage competition of post-traumatic vectored founder colonies of yeast**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Simone Dupuy)

Fisher, J., A. Thompson, K.M. Thomasson and S.R. Proulx. **Preference driven co-evolution of *Drosophila melanogaster* and *Saccharomyces cerevisiae***. Society for Evolutionary Biology, Portland, Oregon. Abstract and Poster Presentation.

McKee, K., S. Dupuy, H. Brantz, J. Leibreich, P. Shindgikar, K.M. Thomasson and S.R. Proulx. **Regional variation and alternative survival strategies in Vectored yeasts**. Society for Evolutionary Biology, Portland, Oregon. Abstract and Poster Presentation.

McKee, K., S. Dupuy, H. Brantz, A. Thompson, J.A. Leibreich, P. Shindgikar, K.M. Thomasson and S.R. Proulx. **Identification of an alternative survival mechanism in fly-vectored yeast**. Society for Molecular Biology and Evolution, Austin, Texas. Abstract and Poster Presentation.

McKee, K., S. Dupuy, H. Brantz, K.M. Thomasson and S.R. Proulx. **Regional variation and alternative survival strategies in vectored yeasts**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Kate McKee)

Seagrave, S.L., G. Minsky, L. Bartkó, T. Sakal, K.M. Thomasson, S. R. Proulx. **Evidence of genetic adaptation in frequently vectored yeasts**. Society for Molecular Biology and Evolution, Austin, Texas. Abstract and Poster Presentation.

2016

Dupuy, S., K.M. Thomasson and S.R. Proulx. **Analyzing competitive implication of lag growth periods in vegetative and spore forms of the yeast *Saccharomyces cerevisiae***. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Simone Dupuy)

Gable, C., K.M. Thomasson and S. R. Proulx. **Computational approaches to sympatric speciation of interacting polymorphic populations of a species**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Cameron Gable)

Leibreich, J.A., P. Shindgikar, S. Dupuy, K. Kurtz, K..M Thomasson and S.R. Proulx. **The examination of spore survival in *Saccharomyces cerevisiae* through fly digestion**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation: (Undergraduate Researcher: Julie Leibreich)

Seagrave, S.L., K.M. Thomasson, J.A Leibreich, P. Shindgikar, and S.R. Proulx. **Vegetative-form yeast survival mechanisms for**

ingestion by *Drosophila melanogaster*. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Sarah Seagrave)

Shindgikar, P., J.A. Leibreich, S. Seagrave, K.M. Thomasson, S.R. Proulx. **Long-term Experimental Evolution based on phenotypic trade-offs in *Saccharomyces cerevisiae***. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Priyanka Shindgikar)

2015

Baker, T.S., C.A. Gable, V.H. Quach, S. Seagrave, K.M. Thomasson, S.R. Proulx ***Drosophila melanogaster*'s feeding preferences to unique cell-surface molecules of *Saccharomyces cerevisiae* vegetative and sporulated states**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Turner Baker)

Gable, C.A., V.H. Quach, T.S. Baker, K.M. Thomasson and S.R. Proulx. **Insect vectors as a mechanism of divergence and sympatric speciation in microbial communities**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Cameron Gable)

Leibreich, J.A., P. Shindgikar, S. Dupuy, S. L. Seagrave, K.M. Thomasson and S.R. Proulx. **The examination of phenotypic trade-offs in sympatric populations of the yeast *Saccharomyces cerevisiae***. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation. (Undergraduate Researcher: Julie Leibreich)

Shindgikar, P., S. Dupuy, J.A. Leibreich, K.M Thomasson and S.R. Proulx. **Understanding the effects of HPH1 and HPH2 regulatory genes on the sporulation efficiency of *Saccharomyces cerevisiae* in pH-adjusted environments**. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation: (Undergraduate Researcher: Priyanka Shindgikar)

2014

Kurtz, K., N. Allen, K.M. Thomasson and S.R Proulx. **Investigating the role of Uric acid in Saccharomycotina species'**

competitiveness. UCSB Student Research Symposium, Santa Barbara, California. Abstract and Poster Presentation: (Undergraduate Researcher: Keifer Kurtz)

University and Public Service

2018	Transportation and Logistics for the UC STEM Faculty Learning Community Conference
2017	Manuscript Reviewer: Current Zoology
2016-2017	UCSB EEMB Graduate Curriculum Committee Member: Worked with three faculty and three graduate students to revise and improve the current graduate curriculum.
2016	Santa Barbara County Science Fair judge: Judged grade school and high school entries from Santa Barbara County.
2015-2017	Honorary Faculty Advisor of Hungry Fish: Student Founders: Victor Quach and Michael Devano
2015-2016	Eco-Coffee Departmental Coordinator: Purchased supplies, organized weekly duties and sent weekly emails for departmental coffee hour.
2015	UCSB TA Orientation Expert Panel Member: Answered questions regarding teaching experiences and advice from incoming university cohort of graduate students.
2014-2016	EEMB Graduate Student Advisory Chair: Organized and delegated responsibilities of the departmental Graduate Student Advisory Committee and ran meetings.
2014-2015	EEMB Seminar Series Chair: Worked with seminar series coordinator for travel and lodging logistics for speaker. Organized host schedule and send announcement emails as well as thank you cards to speakers.
2013-2014	EEMB Seminar Series Coordinator: Organized schedule and travel arrangements for visiting seminar speakers. Coordinated graduate student lunch with the speaker and post-seminar dinner with speaker, faculty and students.

2012-2014	EEMB Graduate Student Advisory Committee Cohort Representative: Organized and executed several professional development events designed to prepare graduate students for careers after completion of their graduate programs. Also organized social events to increase comradery among graduate students in the department.
2012-2013	EEMB Graduate B.E.E.R.S Coordinator: Purchased supplies, organized weekly duties and sent weekly emails for departmental happy hour.
1998-2001	Educational Docent and Assistant Aquarist, UCLA Ocean Discovery Center, Santa Monica, California Taught Ocean Discovery Center visitors about the individual species represented as well as the ecology of the Santa Monica Bay and the human impacts on that bay. Worked both with animals in an interactive setting and in a classroom setting. 10 hours a week
1999	Research Assistant, UCLA Marine Sciences Department, Los Angeles, California Collected and recorded survey data off the Catalina Islands and the Southern California Coast. Worked underwater using SCUBA gear at depths down to 30 meters. Part of both certification course and cooperative research projects at UCLA. Up to 20 hours a week.

Abstract

Coexistence Theory and its Implications for Eco-evolutionary Dynamics

by

Kelly Marie Thomasson

Until recently, the sub-fields of ecology and evolution have existed as separate entities in the broader field of biology. Ecologists focused their attention on community dynamics, species interactions, abiotic contributions to the biological system, and conservation of these systems. Evolutionary biologists preferred to devote their studies to population genetics, selective pressures and the definition, identification and mechanism of evolutionary events, including speciation. When viewing these topics from a broader vantage point, it is clear there is discernible overlap between these topical niches. Community dynamics are the phenotypic, multi-species versions of population genetics and species interactions and abiotic contributions are, frequently, the selective agents of evolution and speciation. Competition between and among species is one field that is overtly shared by ecologists and evolutionary biologists. Competitive pressures both shape the species composition of a community and the gene frequency of a population. Coexistence, the process by which two or more species evade competitive exclusion of one of the species through implementation of a coexistence mechanism, is an important component of the study of competition; however, the mechanisms described in coexistence theory are not as readily accepted as both ecological *and* evolutionary mechanisms. In this dissertation, I aim to reconcile the disconnect of these fields with respect to coexistence theory, illustrating that not only are coexistence mechanisms vital in preventing competitive exclusion of one species or deme, so too are they facilitators of evolutionary events such as divergence or even sympatric speciation. I first attempt to exemplify this by reviewing the literature

that discusses coexistence mechanisms (both ecologically and molecularly derived) that also show evidence of facilitating evolutionary events. Next, I attempt to substantiate these two outcomes of coexistence and divergence mechanisms empirically, by evaluating one known coexistence mechanism, the competition-colonization trade-off, in microbial communities. Using the yeast *Saccharomyces cerevisiae* as model of a species that exhibit different competitive behavioral strategies, and the fruit fly *Drosophila melanogaster* as an exemplary insect which participates in the gut-vectoring of yeasts and other microbes, I describe how behavioral trade-offs of the yeast can allow two or more different phenotypes to coexist. Further, I illustrate that this same behavioral trade-off can promote change in the differing populations and may lead to further divergence of these two populations. By expanding on Tilman's competition-colonization trade-off model, we can evaluated whether one possible coexistence mechanism functions in the model organism *Saccharomyces cerevisiae*, using parameters derived from empirical work with the species *S. cerevisiae* and *D. melanogaster*. Results from this dissertation will help to formulate a mathematical model of the yeast-insect dispersal system based on Tilman's competition-colonization model and may offer breadth to the new field of eco-evolutionary dynamics and evaluate coexistence theory's applicability to species divergence and sympatric speciation.

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Chapter 1

Introduction

1.1 Community and Species Diversity

Diversity and the maintenance of diversity is a topic of great interest to both ecologists and evolutionary biologists [Cardinale et al., 2012, Cardinale, 2011, Hooper et al., 2012]. On the ecological side, diversity levels are both drivers of and driven by community structure and interaction [Chesson, 2000, Rueffler et al., 2006]. Diversity levels of a community are driven by abiotic factors —water, temperature, substrate, disturbance, and distance to biotic and abiotic resources— [Rosenzweig et al., 1995, Macarthur and Levins, 1967, Rueffler et al., 2006], as well as biotic factors such as inter- and intra-species competition for resources, prey diversity and abundance, sexual selection and adaptive mutations to the environment [Oliveira et al., 2008, Orr, 2005, Chesson, 2000, Roughgarden and Feldman, 1975]. The community structure is in turn driven by diversity due to niche partitioning, habitat restructuring and speciation events [Sage and Selander, 1975, Bush, 1969, Carstens and Richards, 2007]. From an evolutionary perspective, research on diversity tends to be focused on the adaptability of traits, mutation, selection and the genetic drivers of diversification and speciation [Hou and Schacherer, 2016, Gonçalves et al.,

Figure 1.1: Yeast populations are found in cool oak woodlands and hot dry vineyards and at the interface of these two disparate environments. Drawing Credit: Stansilav Cuseac

2011]. While mutation provides the initial material for diversity, it is how these mutations interact –by regulation, alternative splicing and epigenetic factors—that reveal phenotypic diversity [Phillips, 2008, Price et al., 2003, Hayden et al., 2011, Barbosa-Morais et al., 2012]. Speciation increases diversity and counteracts the continuous extinction events on a global scale [Bush, 1975]. The mechanisms of speciation –not to mention the definition of the term species—is a contested issue among evolutionary biologists [Coyne and Orr, 2004, Chan et al., 2012, Mayr et al., 1963]; however, although we may not be able to agree on where to draw the line of speciation in a diverging population, we can all agree that the mechanism of speciation is some level of genetic separation, with the

exception of one type of speciation: sympatric. Sympatric speciation is different from all other mechanisms of speciation because it lacks a geographic or physical barrier between the two populations [Smith, 1966, Mayr et al., 1963]. Because there is no physical boundary between populations in sympatry, they are subject to direct competition for resources [Tilman, 1982]. Furthermore, due to the ecological similarity between these the populations, the intensity of competitive pressure is increased and it is likely that one population will competitively exclude the other [Roughgarden, 1974, Leimar et al., 2013, Macarthur and Levins, 1967, MacArthur, 1970].

1.2 Competitive Exclusion and Coexistence Theory

Wright's concept of the fitness landscape generated discussion of ecological similarity, fitness and competition. These conversations gave rise to the theoretically based Competitive Exclusion Principle. The Competitive Exclusion principle presents a theoretical-empirical paradox of sorts. The competitive exclusion principle states that when two species interact, one of these species will be slightly more fit than the other and that individual will be able to competitively exclude the other [Gause, 1932, Hardin, 1960]. In other words, because of the axiom of inequality, one species will perform more poorly in a direct competition and go extinct in that community [Hardin, 1960, Armstrong and McGehee, 1980]. Despite theoretical support for the exclusion principle, many species coexist in spatially similar habitats and in close proximity to one another, both spatially and in niche alignment [Sage and Selander, 1975, Tilman, 1994, Roughgarden, 1974]. The concept of the competitive exclusion principle was first empirically supported by Gause, although he was not the first to conceptualize it, and the topic has been debated by mathematicians and biologists since then [Hardin, 1960, Hubbell, 2005]. For example, Hubble finds no evidence for the necessity of competitive exclusion, claiming that by

tenets of neutral theory, most species are functionally equivalent. Functional equivalence implies that species may vary by morphology, niche space, or many other traits but do not vary in their population dynamics [Hubbell, 2005]. It is based on a community-level application of neutral theory, postulated by Kimura, who stated that based on the predicted number of mutations in a genome, most mutations must be neutral in order for species to persist [Kimura et al., 1968]. Hubbell's main argument with the competitive exclusion principle is the dearth of observed, empirical evidence of absolute competitive exclusion [Hubbell, 2005]. However, the lack of evidence may not indicate competitive exclusion principle is invalid, but rather that there are other mechanisms counteracting that competitive pressure Chesson [2000]. There is substantial evidence of competitive exclusion in nature, however, this competitive exclusion is limited to the niche area in which the species over lap and because these species are different, by definition, they should not occupy identical niches, so complete competitive exclusion should be unlikely [Mayr et al., 1963]. The limiting similarity of two species is the maximum amount of niche overlap that will allow those two species to coexist [Macarthur and Levins, 1967]. Roughgarden investigated the types of limiting similarity that might allow two or more species to coexist based on the shape of their resource curves. She found that the ability for two or more species to coexist depends, not on the overlap, but on the distribution of each species resource utilization curve. If the tails of the curve are thin, indicating there is a low probability that resources came from the outer bounds of the resource area, then the community will be closed to invading species attempting to coexist [Roughgarden, 1974]. Roughgarden's work is an example of how resource variation—not just genetic variation—plays a role in the ability for two species to coexist. Coexistence theory attempts to explain how two species or populations of different fitnesses might evade the fate predicted by the competitive exclusion principle. Coexistence theory remains a paradox among evolutionary theorists. Some have suggested that what we deem coexistence

is a short term observation of a long term competition where one of the “coexisting” populations will eventually be extinct [Chesson, 2000, Hardin, 1960, Geritz, 2005, Macarthur and Levins, 1967]. Others have suggested that resolving this paradox is not a matter of time scale but more so a matter of spatial scale [Bell and Gonzalez, 2009a, Grimm, 1994, Tilman, 1994]. Based on the strength, but limited scope of each of these postulates, it is far more likely that there are several contributing factors to coexistence rather than just one. Chesson describes the effects of these coexistence mechanisms on population models of competing species with respect to limiting similarity and community dynamics. Using several mathematical models of competitive scenarios, Chesson explains that competitive exclusion can be nullified with the addition of coexistence terms. With the addition of these terms, there are many models that result in stable or unstable coexistence [Chesson, 2000]. Several mechanisms of coexistence have been proposed. Much of the work published on coexistence discusses the coexistence of different species with overlapping niches within a community; however, by Haldane’s logic, mechanisms of coexistence are just as necessary in the case of two phenotypically different populations of the same species. If differences between individuals of the same species are the same as differences between individuals of different species [Haldane, 1957] then coexistence models simulating between species competition can be expanded for use in genetically diverging, within-species competition. The molecular mechanisms that can promote coexistence in these diverging populations do so by reducing the phenotypic manifestation of the genetic mutation and variation. These can include epigenetic silencing, epistasis, or duplication [Hou and Schacherer, 2016, Hou et al., 2014, Qian et al., 2010] Ohno 1970. The ecological mechanisms of coexistence include niche portioning [Chesson, 2000, Kremer and Klausmeier, 2013, Sage and Selander, 1975, Bush, 1969, Stamps et al., 2012, Price et al., 2010], density dependent selection [Cao et al., 2008, Roughgarden and Feldman, 1975, Nee and May, 1992, Bull and Harcombe, 2009] and the formation of trade-offs

[Tilman, 1994, Garland, 2014]. As it is in density dependent mechanisms, in an ecological trade-off, the fitness of an individual's phenotype is context dependent; it depends its current environment including abiotic factors and community interaction and competition. Trade-offs are defined in ecological terms as the possession of a beneficial trait that can be harmful in some circumstances (Garland 2014). Trade-offs may elicit coexistence between two populations because it halts complete competitive exclusion of one population by the other because each population is better than the other in certain circumstances. These coexistence mechanisms may additionally promote reproductive isolation because many of these mechanisms discourage hybridization (create a gene flow barrier) or promote in-group divergence (cryptic genetic variation) allowing for more rapid reproductive isolation [Price et al., 2010].

1.3 Levins-Tilman Model

Both Chesson and Kremer and Klausmeier ([Kremer and Klausmeier, 2013]) cite the ecological trade-off as a key mechanism of coexistence. Indeed, many examples of niche partitioning and frequency-dependent selection can be viewed as a trade-off [Chesson, 2000], and Chesson's compensatory variables in his coexistence equations can be view as trade-offs when considering the values are positive for one species and negative for the other [Coluccio et al., 2008, Tilman, 1994, Chesson, 2000]. Hastings [Hastings, 1980] and, later, Tilman described [Tilman, 1994] the coexistence mechanism as a tradeoff between the two behavioral strategies of being a superior competitor for resources within the current environment or being superior at colonizing new areas where competition is less intense [Hastings, 1980, Tilman, 1994]. In order for these two strategies to engender coexistence, they must exist as a trade-off: one individual cannot have the advantage of being good at both. Tilman's model was based on the Levins model which was designed

(1969) in order to describe and predict the rate in which a species will fill a given number of patches. The model (Equation 1.1) can be described in two terms: the colonization term (green) and the extinction term (red). The colonization term describes the increase in occupied patches, where colonization rate, c, is multiplied by the current percent occupied patches, p, which is then multiplied by the percent available patches, $1 - p$. The extinction term describes mortality or decrease in occupied patches by multiplying the mortality rate, m, by the percent occupied patches, p. The resultant value is the change in patch occupancy; this value could be adjusted to represent absolute population size by incorporating a carrying capacity value, K, which illustrates that the competitor's equation is simply the logistic growth equation (Hollings type II response; Equation 1.2).

$$\frac{dp}{dt} = cp(1 - p) - mp \tag{1.1}$$

$$\frac{dn}{dt} = c_1 n_1 (\frac{1 - n}{K}) - m_1 n_1 \tag{1.2}$$

Tilman adjusted the model by assuming the original model would apply only to the competitively superior species because this competitively superior species would not be affected by species interactions (Equation 1.3). Tilman used this concept to describe the effect of competitively superior species interacting with competitively inferior species. Assuming that the competitively superior species would always outcompete the competitively inferior species, Tilman added a term that subtracts from the total based on competitive displacement by a competitively superior species (Equation 1.4). The competitive displacement term (blue) is indicated by colonization rate, c_1, of the more fit species multiplied by the percent occupancy of the competitively superior species and then by the percent occupancy of the competitively inferior species. This term can be added repeatedly, as competitively inferior species are added to the system (Equa-

tion 1.5).

$$\frac{dp_1}{dt} = c_1p_1(1 - p_1) - m_1p_1 \tag{1.3}$$

$$\frac{dp_2}{dt} = c_2p_2(1 - p_1 - p_2) - m_2p_2 - c_1p_1p_2 \tag{1.4}$$

$$\frac{dp_i}{dt} = c_ip_i(1 - \sum_{j=1}^{i} p_j) - m_1p_1 - (\sum_{j=1}^{i-1} c_jp_jp_i) \tag{1.5}$$

Calcagno and colleagues addressed models of the competition-colonization trade-off and some of the criticized aspects of Tilman's model. Most contentious, was the absolutism of Tilman's model: there was absolute competitive dominance by the more fit population in any competitive interaction. Calcagno and colleagues point out that this is unrealistic but also unnecessary to achieve stable coexistence. In their adaptation of Tilman's model, a scalar variable, α, was added that would adjust the competition strength of the competitors allowing them to determine the minimum required α value that would allow coexistence. The results showed stable coexistence but with greater limitations on number of interacting populations and fitness disparities [Calcagno et al., 2006].

1.4 Species and Speciation

Scientists have long debated over how to define the term species. At least 26 definitions of the word species exist [Coyne and Orr, 2004], the existence of these many definitions reflects the need for specific elements that were lacking in other definitions, but are necessary for a particular field of biology [Coyne and Orr, 2004]. The biological species concept is a widely accepted definition of the term species. It states that two individuals are separate species when they experience some form of reproductive isolation, resulting in no offspring or non-viable offspring [Mayr et al., 1963]. Even within the biological species concept, there is debate as to what constitutes a breeding population.

For example, some evolutionary biologists want to define a species as any populations than could interbreed [Dobzhansky et al., 1956] whereas others want to define species as only the distinct populations that will interbreed. Mayr points out the biological species concept is about reproductive isolation in all forms, including two populations that do not mate, even if they could. He is careful to clarify that reproductive isolation does not equate to sterility. It is possible that two different species are able to interbreed and produce a hybrid offspring; however, this offspring may be sterile, or not capable of mating with one or both of the parental species [Mayr et al., 1963]. Despite its widespread use, the biological species concept is not accessible or functional across all taxa or within every community. In microbial communities, in which offspring are often produced clonally, sexual recombination is not necessary and therefore clear cases of reproductive isolation are less common. In purely asexual lineages and in taxa where sexual recombination is not essential for perpetuation of the lineage, scientists find other species definitions more adequate. The genetic species concept defines species as a genetically compatible, interbreeding group of populations that is genetically isolated from other groups. Percent genetic similarity is used to determine species, and this percentage is specific to taxonomic group [Baker and Bradley, 2006]. In bacteria, 16s ribosomal RNA is often used to determine genetic similarity because it is present in most bacteria, its function is conserved across bacteria, and it is large enough that it can be genetically informative. However, this method is still lacking because it has low phylogenetic power at the species level and many bacterial strains are uncultivable and therefore unable to be analyzed (Janda and Abbott 2007). Mayr highlighted that the reproductive species concept and other biological species concepts are centered on the concepts of distinctive differences, population based groups and isolation from non-conspecifics rather than closeness to conspecifics [Mayr et al., 1963]. Haldane points out that the differences we use to describe individuals in two different species are the same as those used to describe variation within

a species, just at a different scale and this change in scale can vary widely across taxa [Haldane, 1957, Dobzhansky et al., 1956]. Thus, on the spectrum of genetic similarity, where we chose to define individuals as two different species –rather than variants within one species— is often a matter of opinion based on our intended use of the definition. Speciation, the process by which, one species becomes two or more, is also a topic with long-term points of contention. There are multiple mechanisms which can result in the reproductive isolation of two individuals [Mayr et al., 1963]. In the modern synthesis, the two factors that change the gene frequencies in a population —that promote evolution— are genetic mutation and natural selection. If these changes, over short or long term, result in the reproduction isolation of a population within the species, by the biological species concept, this population has speciated. The manner by which these populations arrive at a reproductively isolated state is defined by the type and strength of gene flow barriers separating them [Bush, 1975]. Allopatric Speciation is defined as speciation that occurs as a result of completely obstructed gene flow due to a geographical barrier. This geographic barrier can be described as a landmass or distance beyond an individual's vagility (migration range ability). Conversely, Sympatric Speciation shows no geographic separation or barrier; in fact the populations will often regularly interact. Sympatric speciation is defined as speciation between two populations when there is no genetic isolation [Bush, 1975]. Parapatric Speciation exhibits intermediate gene flow between diverging populations and is defined by a narrow zone of interaction referred to as a hybrid zone [Bush, 1975]. While it is fairly uncontroversial as a speciation type, parapatric speciation is often difficult to confirm with certainty because these hybrid zones could indicate historical sympatry that has separated or allopatry that has rejoined [Coyne and Orr, 2004]. While the three widely-accepted categories of speciation are allopatric, parapatric, and sympatric speciation, these three categories are often subdivided or redefined. In order to differentiate between two types of allopatric speciation,

Bush divides the allopatric mechanism into Allopatric 1A and 1B. These two types are separated based on the strength of the strength of the geographical barrier separating the two populations. Bush also points out that different groups of taxa are more likely to take part in certain type of speciation. Factors like reproductive rate, vagility and whether the organism is an r- strategist or a K strategist may predispose that organism to a certain type of speciation [Bush, 1975].

1.5 Sympatric Speciation

While Allopatric speciation, is the commonly described mechanism of speciation, Bush suggests that far more speciation events are likely to be parapatric or sympatric. He speculates that this inaccurate allocation of speciation events is due to our poor understanding of the mechanisms and processes of sympatric and parapatric speciation [Bush, 1975]. Allopatry and sympatry have been thought to be on a spectrum of gene flow but perhaps it would be better to define them based on the order of the two events taking place in any speciation process: some level of genetic and phenotypic divergence and a reproductive isolation. In Allopatric circumstances, we describe the divergence as a product of the populations being separated, with the process culminating in eventual reproductive isolation. Under sympatric circumstances, the two events are reordered; the reproductive isolation occurs first and this isolation drives the population to further diverge [Bush, 1975]. Because of this, the process of sympatric speciation is often considered rapid while the process of allopatric speciation is considered slow [Bush, 1975]. There is, however, the possibility that sympatric speciation can occur as the result of some genetic divergence prior to establishment of reproductive isolation [Rundle et al., 2000, Sage and Selander, 1975]. The following sections represent three generally accepted mechanisms of sympatric speciation: polyploidy, separation of morphological extremes

in a population, and the formation of sexual preferences.

1.6 Yeasts as a model organism

Yeasts are single-celled members of the fungal phylum Ascomycota within the subphylum of Saccharomycotina. The Saccharomyces sensu stricto complex includes the six species within the genus *Saccharomyces*: *S.cerevisiae*, *S. paradoxus*, *S. bayanus* , *S. caricanus*, *S. mikatae* and *S. kudriavzevii* [Sniegowski et al., 2002, Warringer et al., 2011]. These six species are considered to be closely related and possibly recently speciated [Warringer et al., 2011]. Despite their large distribution range, these species also are commonly found in similar environments –typically oak and broadleaf woodlands will often coexist with other members of the complex [Sniegowski et al., 2002]. Yeast cells normally reproduce asexually by budding. This process can occur in both the haploid and diploid states. Because of this, hybrid (allopolyploid) sterility is escapable by selfing, but this sterility is still observable. Despite their ability to proliferate asexually, species within yeasts are defined by the biological species concept and therefore function as a model organism for other speciation mechanisms [Bush, 1975, Neiman, 2011, Tsai et al., 2008]. Although yeasts can reproduce in both haploid and diploid states, in nature, yeasts are constitutively diploid [Tsai et al., 2008]. The proximate mechanism of this diploidy is the cell's ability to change from one mating type to another. If the cell, by poor luck, is in proximity to only cells of the same mating type, it is capable of changing to the other mating type and then combining with that cell to form a diploid [Neiman, 2011]. The ultimate cause of this diploidy in nature likely stems from the need to sporulate which can only be done in diploid form [Tsai et al., 2008]. Yeast traits have also been studied extensively. Sporulation, the meiotic phase of reproduction in yeast, is initiated by changes in pH, nitrogen, and acetate as a result of increased metabolic processes

[Pizarro et al., 2008, Neiman, 2005]. Sporulation in yeasts is considered a quantitative trait but the main genes that influence the initiation of the sporulation pathway are well known and well-described [Tomar et al., 2013]. Additionally, evidence of gene duplication events, chromosomal rearrangements, and key loci leading to reproductive isolation events in yeasts have also been described [Yona et al., 2012, Hou et al., 2014, Charron et al., 2014]. The sporulation pathway in yeast is initiated by environmental stressors but the threshold of pathway initiation is genetically based [Yona et al., 2012, Neiman, 2011]. Within one yeast community there is substantial variation between individual sporulation rates and it is thus, sporulation rate within a yeast community is subject to selective pressures. To what degree is this selective pressure due to insect ingestion and digestion? Is the selective pressure of insect digestion sufficient to cause divergence and speciation in the yeast Saccharomyces cerevisiae? Yeast has been widely touted as the next big thing in experimental evolution because of its single-cellular eukaryotic state, its high number of conserved genes with larger vertebrates and its simple culturing protocols. The genome of S. cerevisiae has been completely sequences and many aspects of its genetic infrastructure have been analyzed. It contains 35-55 transposable elements [Pretorius, 2000], is capable of single-gene, multiple-gene and whole genome duplication [Yona et al., 2012], as well as chromosome rearrangement [Charron et al., 2014] and epistatic regulation. Epistasis is currently estimated to account for approximately 9% of the phenotypic variation. Epistasis in yeast is still not well understood and epistasis' role in reproductive isolation is also poorly studied. However, Hou and Schacherer report evidence of reproductive isolation events stemming from laboratory observations from populations of 27 natural isolates of Saccharomyces cerevisiae due to negative epistasis. The analysis yielded two Dobzhansky-Muller incompatibilities, both involving the respiratory abilities of these yeasts. They concluded that negative epistasis could lead to reproductive isolation in yeasts in a condition-specific manner [Hou and Schacherer,

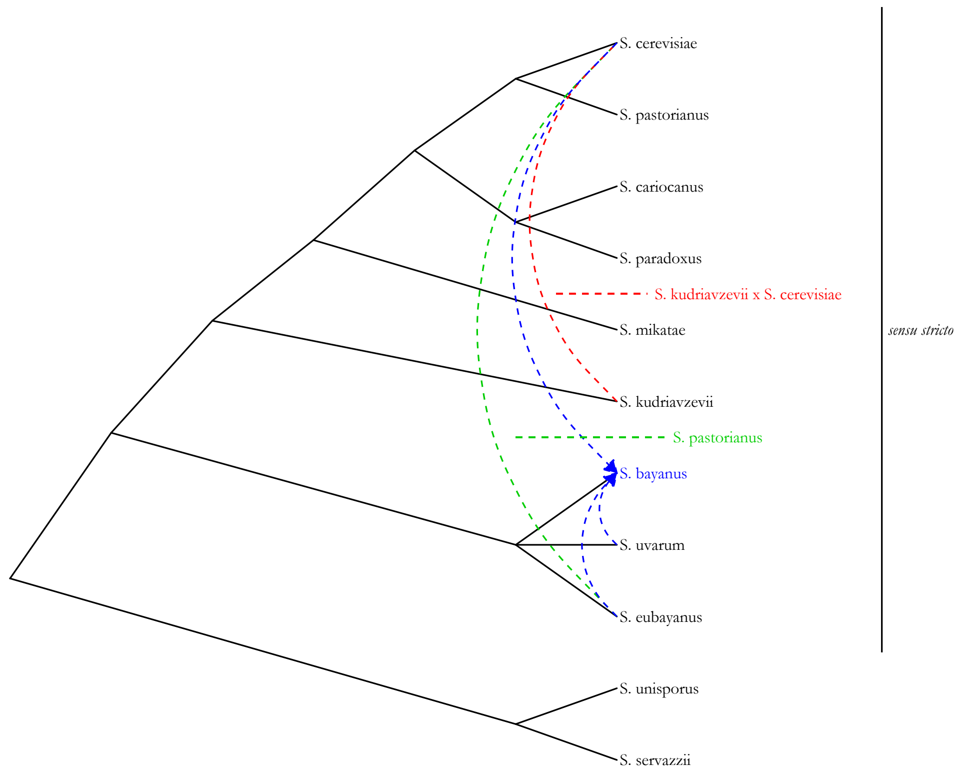

Figure 1.2: Cladogram of the *Saccharomyces sensu stricto* complex and its well-studied industrial hybrids. [Hittinger, 2013, Yu et al., 2017, 2018]

2016, Dettman et al., 2007]. In yeasts, there are copious examples of both coexistence and sympatric speciation as a result of divergence of some spatial aspect of the natural histories of these two populations [Murphy and Zeyl, 2012, Gonçalves et al., 2011, Sniegowski et al., 2002]. Speciation promotes further speciation by changing selection pressures due to range partitioning. Once reproductive isolation has occurred, the need for coexistence mechanisms does not dissipate. Rather, if possible these mechanisms continue until the two incipient species have managed to carve out new niches for themselves, reducing competitive interaction.

1.2

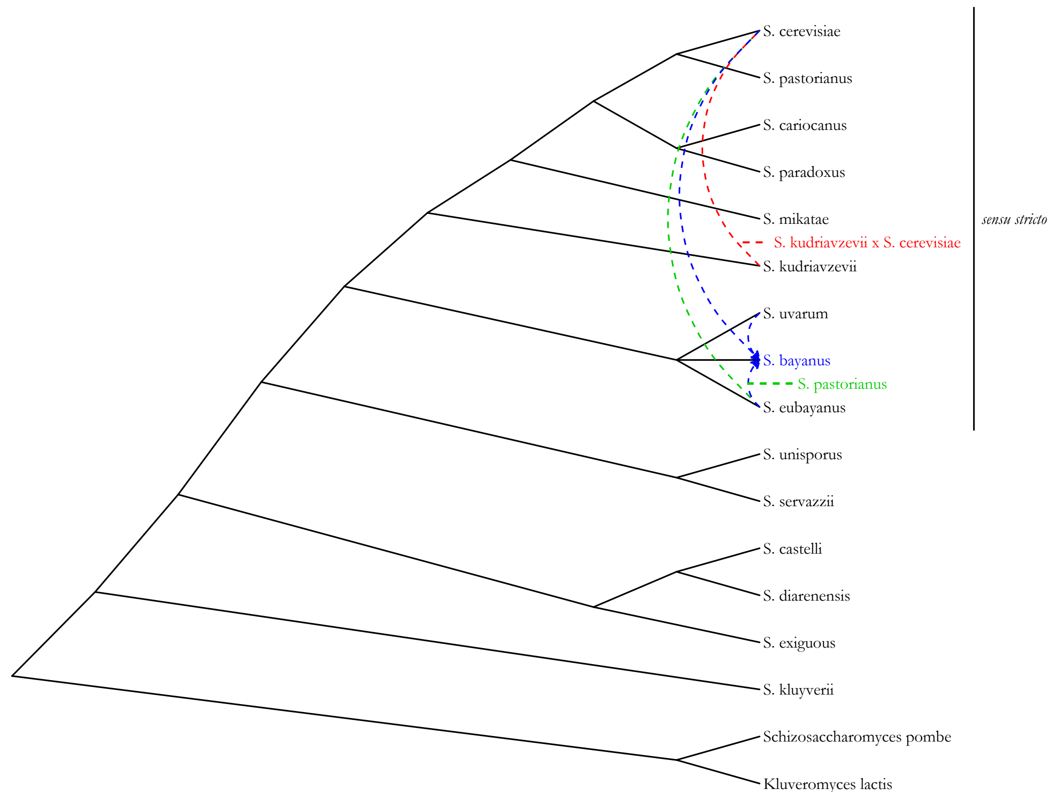

Figure 1.3: Cladogram of the *Saccharomyces sensu stricto* complex and its well-studied industrial hybrids. Hittinger [2013], Yu et al. [2017, 2018]

1.7 Behavioral Strategies in the yeast *Saccharomyces cerevisiae*

Saccharomyces cerevisiae has two constitutive behavioral strategies, the timing of which varies across strains and environments [Pizarro et al., 2008, Chopra et al., 1999]. The vegetative stage is what one would typically consider the "normal" phase, in which the cell respires, grows, is reactive to stimuli, and is capable of both asexual budding and sexual recombination [Madhani, 2007, Bergman, 2001]. In the more quiescent spore phase, the cell performs minimal to no respiration, is less reactive to stimuli, and exists

as a tetrad of four haploid spores within an ascus surrounded by a thick, protective cell wall [Madhani, 2007, Codon et al., 1995, Neiman, 2005, 2011, Orlean, 2012, Tomar et al., 2013]. These two phase strategies exist as a tradeoff; in order to be good at one strategy, the individual must be less efficient at the other strategy.

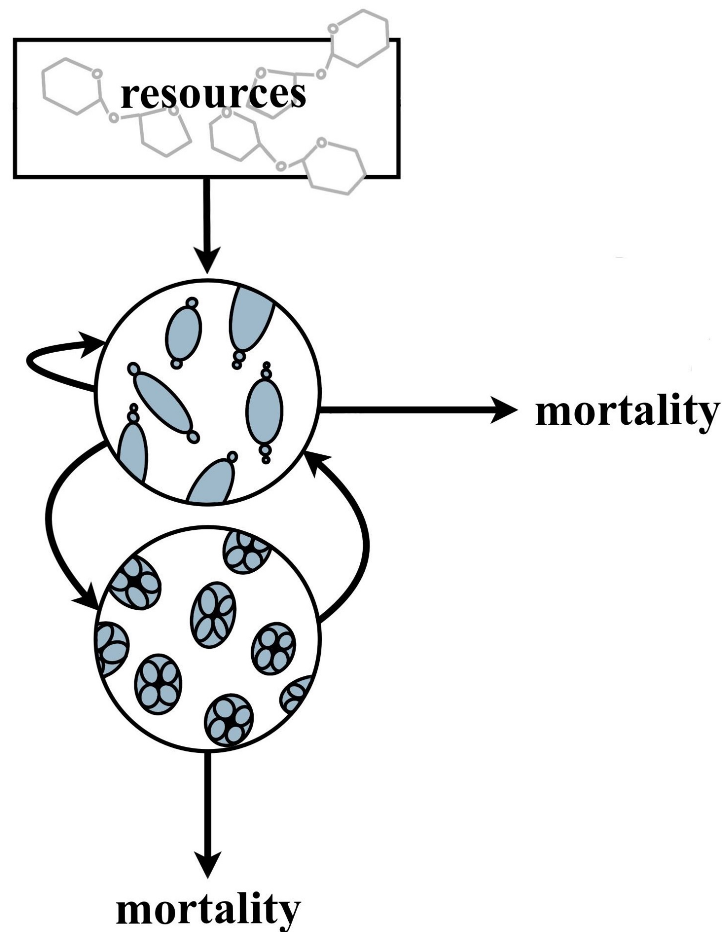

Figure 1.4: Flow diagram of the yeast *Saccharomyces cerevisiae* moving between states and interacting with available resources. Arrows represent state changes between the resource pool, the vegetative state and the sporulated state.

The yeast, *S. cerevisiae*, serves as a potential example of the competition-colonization trade-off in nature. In a given environment, it may be advantageous to sporulate earlier than later especially if the organism is not the most competitive in the vegetative state.

Sporulation offers two possible advantages: it allows the cell to shut down metabolically, preserving itself in a dormant mode before starvation and it allows the cell to move through less than ideal environments during transport and germinate only in suitable conditions. In the case of the natural yeast environment, there is evidence that mechanisms and rates of sporulation are environment dependent [Anderson et al., 2004, Gerke et al., 2006, Gancedo, 2001, Magwene et al., 2011, Gonçalves et al., 2011, Warringer et al., 2011, Sniegowski et al., 2002], indicating that sporulation rate is both under selection and potentially adaptive in the right environment. Applying this species to the concepts of coexistence theory, the cells with superior growth rates (competitors) may be able to out-compete those with inferior growth rates; however, these slower growing cells may be better at sporulating and colonizing new areas. Upon colonizing a new area, the slow growing yeast has reduced competitive pressure and can proliferate with minimal competition [Tilman, 1994, Wright, 1932].

1.8 Natural Interactions between *S. cerevisiae* and *D. melanogaster*

The natural distributions of the yeast S. cerevisiae and the fruit fly Drosophila melanogaster overlap ([Ort et al., 2012, Morais et al., 1994]. Drosophila feed on sugars and microbia that are typically found in or around ripe and rotting fruits ([Anagnostou et al., 2010]. Evolutionary evidence exists to support the long-standing relationship between Drosophila and Saccharomyces ([Barker and Starmer, 1999, Dobzhansky et al., 1956, Schiabor et al., 2014, Masek and Scott, 2010, Hill and Otto, 2007, Ito et al., 1995, Deutschbauer et al., 2002]. There is even evidence that flies may play a role in the community assemblage of yeasts by vectoring and propagating specific types of yeast

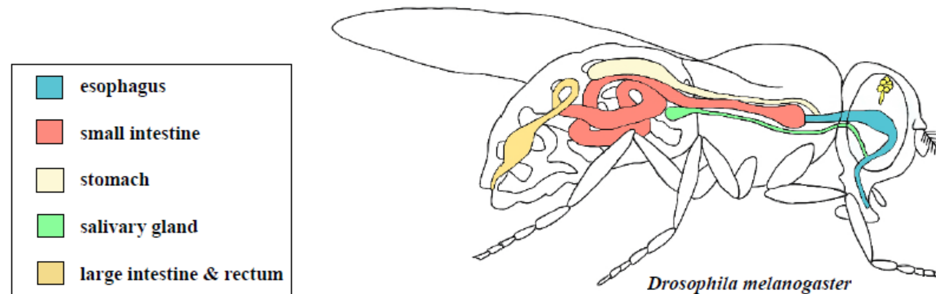

Figure 1.5: Digestive tract of *D.melanogaster* which ingests and digests– to some degree – yeasts including *S. cerevisiae*. When the fly defecates, it deposits yeast that have survived the digestion process in a new area, resulting in a yeast dispersal event by the fly. Figure credit: Fernanda Pett

near egg-laying sites [Stamps et al., 2012, Anagnostou et al., 2010]. Coluccio's study highlighted a trade-off in sporulation rate for yeasts that were frequently subjected to ingestion and digestion by insects. Yeasts with slow sporulation rates have higher reproductive success because they spend more time actively dividing rather than sporulating; however, yeast survivorship was increased in sporulated cells relative to vegetative cells when transferred through the gut of a fly. However, from a community perspective, these survivorship values, it was unclear that differential consumption was not a factor in survivorship [Coluccio et al., 2008]. As an extension of this study Reuter and colleagues suggested that this trade-off held and additional advantage for the spores because their outcrossing rate increased as a result of digestion by the fly [Reuter et al., 2007, Otto and Lenormand, 2002]. Coluccio and colleagues [Coluccio et al., 2008] explored the differential survival rates of vegetative yeast cells relative to sporulated yeast cells and found that vegetative yeasts tend to not survive the flies digestive tract, while some spores are capable of surviving although their asci were well degraded by the time they passed through the gut [Coluccio et al., 2008]. Again, applying these observations

to theory, the colonization events are more successful as spores, which by the definition of yeast competitive ability are no longer competing [Gause, 1932]. Coluccio went on to analyze the elements of the tetrad itself, stating that vegetative cells were digested except for their cell walls, while the cell walls of the spore were thinned, but the asci remained intact [Coluccio et al., 2008]. Might this ingestion and digestion process allow for a faster initiation of growth and a more competitive relative growth rate in the new habitat where the digested spores are deposited? Reuter and colleagues [Reuter et al., 2007] further investigated the phenomenon of flies vectoring yeast spores and found that not only do the spores have a higher probability of surviving relative to vegetative cells, but the rate of out-crossing between spores, seems to increase when vectored through the fly gut [Reuter et al., 2007]. This increased rate of out-crossing may act as an adaptive behavior as the recombination events may ensure at least some success in the newly colonized environment [Coyne and Orr, 2004]. In other words, this ability to out-cross upon deposition is a type of bet-hedging against the environment roulette of colonization. Is it possible that the ingestion of yeast by flies is an adaptive trait for the yeast? If ingested, the yeast cell, normally lacking in motility, gains mobility using the fly as a vehicle, as long as it survives the process. Applying this phenomenon to the previously discussed theoretical work, the fly becomes the vehicle of colonization for the yeast cell. In light of the preceding literature review, I would like to explore the evolutionary implications of competition-colonization trade-offs as a theoretical model and with empirical research in the model organism *Saccharomyces cerevisiae*.

1.9 Dissertation Goals and Chapter Layout

This dissertation aims to formulate a comprehensive analysis of one ecological system by quantifying a critical aspect of the mechanism, evaluating possible empirical outcomes

and fitting these outcomes to a model of coexistence. It begins with a comprehensive review of the literature involving coexistence mechanisms. It postulates that coexistence mechanisms may not only help evade competitive exclusion of one deme by another, but these mechanisms may also promote divergence of the groups and incipient speciation. Chapter 2 of this dissertation expands on the concept, introduced above, that coexistence mechanisms often also promote divergence within incipient species. This review paper address three key molecular and three key ecological mechanisms and discusses how they play dual roles in this eco-evolutionary process of diversification and diversity maintenance.

Chapter 3 focuses on the role insects play on the community composition of microbial communities. Beyond confirming the result of previous studies in a way that reduces confounding sources of noise in the insect-yeast system, this chapter begins to quantify the differential mortalities of yeasts in the two life-stages of actively dividing vegetative cells and metabolically quiescent spores using a survivorship assay of these cell types passing through the gut of the fruit fly *Drosophila melanogaster*. Chapter 3 of the dissertation aims to quantify the differential survival in yeasts of different life-history stages passing through though the gut of an insect.

Chapter 4 of the dissertation reports on the results of a long-term evolution experiment in which the yeast *S. cerevisiae* was repeatedly subjected to ingestion and digestion selection pressures when being gut-vectored by the insect *D. melanogaster*. Results from this experiment may be utilized as an example of microbe-insect interactions on a larger scale. outcomes on microbial communities frequently vectored by insects. Chapter 4's goal in this dissertation is to infer the effects of long-term selective pressure on a phenotypically diverse or diverging population, and refer back to the focus of chapter 1: that coexistence mechanisms can play a role in species divergence.

Lists of tables and figures are on a per chapter basis in the appendix. Appendices

and references are together at the end of the dissertation.

1.10 Permissions and Attributions

1. This dissertation was produced using LaTeX and Overleaf. The statistics were performed using R studio (version 3.5.3). All data and code for analysis will be archived at `Dryad.org`

2. The content of chapter 3 and associated supplementary material in appendix A is the result of a collaboration with Alexander Franks who developed the Bayesian analysis for this work.

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Chapter 2

Speciation and Coexistence Theory: Connections to and Considerations on Evolutionary Mechanisms

2.1 Introduction

Speciation and extinction and competition are vital components to community diversity. Competition between two species vying for the same resource or other aspect of an overlapping niche space can set the population sizes of each species or the allelic frequency within a species. If competitive pressure is strong enough, or if two competing species differ that drastically in competitive fitness, one species may competitively exclude the other from the community. On a global or regional scale, this competitive exclusion may result in extinction. Extinction and competition reduces species diversity and genetic diversity while speciation and mutation increases species and genetic diversity [Alfaro et al., 2009]. Species are commonly defined based on their ability to interbred with other conspecifics, but there are a multitude of definitions depending the

purpose of the definition[Wilkins, 2006, Bush, 1975]. Just as the definition of species is contested, so too is the process of speciation. The manner in which speciation is defined (allopatric, parapatric, sympatric, or peripatric) is on a spectrum of gene flow allowance [Bush, 1975]. How we chose to define speciation, therefore, can be taxon specific or based on our scientific goals. To date there is not a master equation for the term species or the process of speciation. We will use the biological species concept as the definition of species and discuss the two processes that delineate a new species: genetic divergence and reproductive isolation.

2.2 Drivers of differentiation and speciation.

In order to acquire a reproductively isolating mechanism that separates one population from another, any one or a combination of a multitude of events must occur. The process towards this isolation may be slow or rapid [Bush, 1975] and the isolation mechanism may be defined as mechanical, physiological, behavioral, pre-zygotic or post-zygotic [Coyne and Orr, 2004]. The following is brief overview of some of the key molecular and ecological factors thought to promote reproductive isolation.

2.2.1 Molecular

Reproductive incompatibility at the molecular level begins with mutation. According to Drake mutation rate is estimated to be 10^{-3} to 10^{-4} per base pair per genome per generation (replication) for many organisms, including prokaryotes. A more recent estimate by Drake explains that there is large variation in the mutation rate ranging from 10^{-4} in RNA viruses to a mean of 10^{-10} in *H. sapiens*, *C. elegans*, *D. melanogaster* and *S. cerevisiae*. He also noted these values varied by locus in the eukaryotic genomes. When considering that each cell represents a genome, it is easy to understand 1) why

sophisticated proof-reading protein complexes evolved and 2) why, regardless of these proof-reading protein complexes, there is still such a diversity of multi-cellular organisms (Drake 1998). Even without the advantage of proof-reading protein complexes, the rate of replacement of one allele in a species is thought to be very slow. Haldane made the estimate that one single substitution may take 300 generations to replace the original base pair in a population and the possibility of it not being immediately selected against or removed stochastically is low [Haldane, 1957]. The rate at which the accumulation of mutations leads to reproductive isolation is highly variable but may also be quite slow –if the two species are able to both persist– or occur over as few as thirteen generations [Hendry et al., 2000] or can be immediate [Ohno, 1970]. As an organism's genome becomes larger and more inter-reliant on regulatory mechanisms and networks, the rate and consequence of mutations become more dependent on genetic architecture [McDonald et al., 2009] but these mutations may also arise de novo, shifting genetic architecture or the regulation of the current genetic motif [Gresham, 2015]. Mutations may also give rise to alternative splicing, which allows the same genetic architecture to be used to transcribe multiple genes [Barbosa-Morais et al., 2012]. There have even been attempts in the field of evolutionary biology to locate speciation genes: genes that both promote reproductive isolation and are repeatedly involved in speciation events across taxa. Many of the initial candidate genes for speciation genes involved intrinsic hybrid sterility; however, Nosil and Schluter's findings indicate even genes causing reproductive isolation are polymorphic and the affect size of any one of these genes on reproductive isolation is difficult to quantify in isolation. In some cases, the genes that cause divergence are separate from the mutations that cause reproductive isolation [Nosil and Schluter, 2011]. More tractable than a speciation gene, and a strong driver of genetic diversity is the mutator gene. Mutator genes are genes that promote mutation within a genome. Often these genes inhibit the abilities of proof-reading complexes but by definintion, a mutator gene

can be any gene that increases the mutation rate within an organism. Investigation by Taddei and colleagues speculates that these mutator alleles may become fixed by genetic hitchhiking [Taddei et al., 1997]. Raynes and colleagues expanded on this confirming that mutator alleles will persist in asexual (clonal) populations because of genetic hitchhiking with beneficial mutations, but the frequency of mutator genes declines rapidly in sexual populations. Recombination can often reduce the frequency of mutator genes as they are not putatively linked to these beneficial mutations [Raynes et al., 2011].

2.2.2 Ecological

One ecological factor of speciation is a change in community composition. An example of this is migration. Migration plays a substantial role in the definition and successful completion of speciation [Mayr et al., 1963, Bush, 1975]. Migration can be viewed in a similar manner to mutation: a new genetic variant invading the resident population [Gaggiotti and Hanski, 2004]. Normally migrating individuals are thought to have lower fitness than the resident population because it is expected that the resident population would have adapted to its environment [Hendry et al., 2000]. The effects of migration on speciation type and outcome are dependent on both the rate of migration [Mayr, 1970], the relative fitness of the immigrant and the resident population [Bolnick and Nosil, 2007], and presence of any selective preferences within the resident group. Bush explains that in order speciation to occur between two groups, the rate of migration must be low or the degree of homoselection must be high [Bush, 1975]. Community composition change can promote rapid evolution, and with it, resultant speciation especially with populations have specialized or coevolved with other species in their community. The establishment of a preference for these other species or the removal of that species can have dramatic fitness effects on the reliant population. One well-documented example is pollinator

loss, where removal of pollinators results in the restructuring of the plant and animal community. Bodbyl-Roels and Kelly examined the evolutionary effects of pollinator loss in an empirical study on Mimulus guttatus. In this experiment, requisite out-crossing flowers were denied their natural pollinators and the fitness of any propagating plants was tracked. Researchers found that the loss of a pollinator, promoted rapid adaptive divergence and that within two generations, selfing versions evolved from the normally out-crossing plant [Bodbyl Roels and Kelly, 2011].

Polyploidy

A commonly accepted mechanism of sympatric speciation is instantaneous reproductive isolation by polyploidy [Coyne and Orr, 2004]. Polyploidy is the duplication of the whole genome [Ohno, 1970]. It can occur spontaneously in single-celled organisms like yeast [Yona et al., 2012], or in somatic cells within the body. In an assessment of copy number variants (evidence of duplication), Gillooly states that at any given time, the human body has cells with multiple levels of ploidy within it, and that this multiploid state is possibly common among many multi-cellular organisms [Gillooly et al., 2015]. When polyploidy arises in a gametic cell as a result of autopolyploidy [Ohno, 1970], or non-disjunction [Ohno, 1970], the individual can become immediately isolated from other conspecifics. For this reason, Ohno believed polyploidy was well described in hermaphroditic populations that are capable of selfing because in many cases the only genetically compatible mate is itself [Ohno, 1970]. However, in analysis of polyploid populations and self-compatible populations, Mable found no association with the two, indicating that either there are many self-compatible plants that do not engage in polyploidy or selfing is less essential for continuation of a polyploid lineage than previously thought [Mable et al., 2005]. Complete polyploidy, while far more observed in plant communities, is not constrained to plant communities. In animal taxa, polyploidy is

incompatible with many species because of their chromosome-based sex determination mechanisms [Ohno, 1970]. In vertebrates with genomes possessing regulatory feedback networks, there is the added complication of dosage effects in the regulatory genes of a duplicated genome. Because vertebrate genomes often consist of highly connected gene networks with multiple sources of regulation, a change in gene dosage of a regulatory gene may have multiple downstream affects in these systems, and so the possibility of a normally developing polyploid becomes less likely [Ohno, 1970]. Tandem-duplication is far more possible and is, in fact, ubiquitous and variable by taxonomic group [Ohno, 1970], tissue type [Gillooly et al., 2015] and environmental state [Yona et al., 2012]. There is evidence of some animal groups experiencing whole genome duplications. Many of these examples are thought to be cased of autopolyploidy, when polyploidy occurs as a result of abnormal gametogenesis, and many of the animal taxa that been observed to be polyploid have less absolute chromosomal requirements for sex determination. Ohno provides the example of amphibians and fish, which have multiple shared loci on the pairs of sex determining chromosomes. He speculates that these sex chromosomes have not differentiated from each other as much as mammalian sex chromosomes, resulting in fewer complications as the result of polyploid events [Ohno, 1970]. Polyploidy plays a role sympatric speciation because it creates an immediate reproductive barrier either by requiring the organism to self or by limiting the reproductive options the organism has [Ohno, 1970, Otto, 2007]. A mechanism that results in fewer reproductive options seems like a poor strategy from the standpoint of fitness, but it is believed that polyploidy plays a role in the organism's survivorship when this process is activated. In yeasts, polyploidy may enable yeast to harbor genes that incur fitness benefits for multiple environments [Pretorius, 2000] or may function as a temporary and reversible response to stress [Yona et al., 2012].

Separation Extremes in Morphological Variation

In sympatric speciation not associated with instantaneous reproductive isolation (such as polyploidy), divergent selection drives separation of two or more populations but gene flow counteracts this separation, therefore it may be difficult to establish different niches until the reproductive isolation event occurs [Kisel et al., 2012, Rundle et al., 2000]. In some cases, however, there is evidence that niches are established before reproductive isolation is complete [Price et al., 2010, Sage and Selander, 1975, Rundle et al., 2000]. The divergence and reproductive isolation of populations by morphological variation within the larger population is less understood and more controversial [Rundle et al., 2000]. These morphological changes can be the result of gene duplication [Ohno, 1970] but can also stem from selection or separation of normal variation within a population, or phenotypic plasticity [Sage and Selander, 1975, Rundle et al., 2000, Price et al., 2010]. Sage and Selander analyzed the four ecomorphs of cichlids existing sympatrically in a lake. These four populations were thought to have speciated sympatrically, based on their behavior and morphology [Sage and Selander, 1975]. Genetic analysis showed that these populations were nearly identical to each other. Mating trials indicated that despite lower fitness of the hybrids, all four populations were able to inter-mate with each other [Sage 2016, personal communication]. Mouth morphology is considered a key contributor to fish diversification and speciation [Price et al., 2010]. These morphological differences are often plastic at first, but play a role in incipient speciation. These four populations of cichlids exist sympatrically as populations of the same species, but exhibit minimal interaction. For this reason, they may be considered incipient species [Sage and Selander, 1975] but by Mayr's definition can also be considered separate species because they do not mate [Mayr et al., 1963]. Even if they are not currently species, these cichlids may eventually establish a reproductively isolating mechanism. There is

sufficient evidence from nature that sympatric speciation is possible by this method. There are especially many cases of this pattern of divergence and speciation in fish, and many of these fish exhibit complete reproductive isolation [Coyne and Orr, 2004]. One well-known example is the diversification in jaw morphologies is found in Parrotfish in the family, labridae. Price and colleagues assessed the evidence of divergence in this family because it demonstrates evidence of a radiation event in sympatry. Price and colleagues concluded that the jaw morphologies split into three groups based on the available food substrate types. Once these three feeding groups were established, the groups further diverged [Price et al., 2010]. Most evidence of divergence in sympatric species of fish point to divergence by selection rather than drift and this evidence is supported by the diversity of niches these fish now occupy [Hendry et al., 2000, Rundle et al., 2000]. A key factor in this example was the reduced fitness in the hybrid form. This hybrid inferiority may reinforce the separation of two populations [Coyne and Orr, 2004] because the hybrids contribute fewer and less-fit offspring. It may also promote behavioral mutations for preference within each population.

Preference and Behavioral Changes

Sympatric Speciation can also result from behavioral changes within a population, such as the formation of preferences for food or mates. The textbook example of this is Bush's paper on *Rhagoletis*, in which members of two sibling species exist in sympatry but show positive correlations between both food preferences and mates with similar food preferences [Bush, 1969]. Although this is the commonly used, introductory example to sympatric speciation, there are several additional interesting and informative examples of how preference and behavior play a role in sympatric speciation. Sexual preference behaviors may also evolve in the cases of diverging morphological variants, as well as food preference formation. In Rundle and colleagues' study, in addition to finding that

Stickleback divergence was due to selection events, they believed this sympatric speciation was reinforced by the evolution of preferences for self within each population. In other words, the reproductive isolation was not simply due to divergence of genetic traits alone, but included a behavioral (pre-zygotic) isolating mechanism prior to a genetic (post-zygotic) one [Rundle et al., 2000]. The empirical evidence of Rundle and colleagues is supported theoretically by Geritz and Kisdi, who analyzed simulations of divergence models in sympatry and found that the establishment of mating preferences in a diverging population, stabilized the post-divergent populations and resulted in only one possible outcome rather than multiple as was seen when there was no mating preference [Geritz and Kisdi, 2000].

2.3 Coexistence and Sympatric Speciation

2.3.1 Coexistence Theory

The competitive exclusion principle states that when two species interact, one of these species will be slightly more fit than the other and that species will be able to competitively exclude the other [Gause, 1932]. The concept was first empirically supported by Gause, although he was not the first to conceptualize it, and the topic has been debated by mathematicians and biologists since then [Hardin, 1960, Hubbell, 2005]. For example, Hubble finds no evidence for the necessity of competitive exclusion, claiming that by tenets of neutral theory, most species are functionally equivalent. Functional equivalence implies that species may vary by morphology, niche space, or many other traits but do not vary in their population dynamics [Hubbell, 2005]. It is based on a community-level application of neutral theory, postulated by Kimura, who stated that simply based on the predicted value of mutations in a genome, most mutations must be neutral in order for

species to persist [Kimura et al., 1968]. Hubbell's main argument with the competitive exclusion principle is the dearth of observed, empirical evidence of absolute competitive exclusion [Hubbell, 2005]. However, the lack of evidence may not indicate competitive exclusion principle is invalid, but rather that there are other mechanisms counteracting that competitive pressure [Chesson, 2000]. There is substantial evidence of competitive exclusion in nature, however, this competitive exclusion is limited to the niche area in which the species over lap and because these species are different, by definition, they should not occupy identical niches, so complete competitive exclusion should be unlikely [Mayr et al., 1963]. This idea of niche overlap is what is often referred to as limiting similarity. The limiting similarity of two species is the maximum amount of niche overlap that will allow those two species to coexist [Macarthur and Levins, 1967]. Roughgarden investigated the types of limiting similarity that might allow two or more species to coexist based on the shape of their resource curves. She found that the ability for two or more species to coexist depends, not on the overlap, but on the distribution of each species resource utilization curve. If the tails of the curve are thin, indicating there is a low probability that resources came from the outer bounds of the resource area, then the community will be closed invading species attempting to coexist [Roughgarden, 1974]. Roughgarden's work is an example of how resource variation—not just genetic variation—plays a role in the ability for two species to coexist. Chesson describes the effects of these coexistence mechanisms on population models of competing species with respect to limiting similarity and community dynamics. Using several mathematical models of competitive scenarios, Chesson explains that competitive exclusion can be nullified with the addition of coexistence mechanisms. With the addition of these mechanisms, there are many equations that result in stable or unstable coexistence. Stable and unstable coexistence may also be referred to as fluctuation-independent or fluctuation-dependent coexistence and refers to the stability of the system over a range of normal population

fluctuations due to environmental cycles or demographic stochasticity [Chesson, 2000]. In Hubbell's 2005 paper on functional equivalence, he discusses neutral theory's validity by drawing an analogy to Boyle's law and Lotka-Volterra equations: these two equations are both approximations but considered valuable concepts despite their lack of precision in describing real systems [Hubbell, 2005]. Interestingly, the same can be said for the very concept Hubbell argued against: the competitive exclusion principle. If the competitive exclusion principle always proceeded in biological systems as purported, there would be far fewer extant species and each would occupy only one, well-delimited niche space [Hardin, 1960]. However, this is not the case. It is more likely that the competitive exclusion principle acts as a null theorem, much like the Hardy-Weinberg theorem or the antecedent of Newton's 1st law. Perhaps the competitive exclusion principle may be more aptly worded: two species competing for exactly the same resources cannot stably coexist, unless acted on by compensatory coexistence traits. These coexistence traits may be defined as any trait that promotes a mechanism of coexistence. Given the abundant evidence of coexistence in natural ecosystems, many ecologists set out to explain these mechanisms that allow for coexistence, despite the apparent validity of the competitive exclusion principle [Chesson, 2000, Roughgarden, 1974, Roughgarden and Feldman, 1975, Tilman, 1994]. Much of the work published on coexistence discusses the coexistence of different species with overlapping niches within a community; however, by Haldane's logic, mechanisms of coexistence are just as necessary in the case of two phenotypically different populations of the same species. If differences between individuals of the same species are the same as differences between individuals of different species [Haldane, 1957] then coexistence models simulating between species competition can be expanded for use in diverging, within-species competition. In the case of incipient species in sympatry, coexistence mechanisms are vital in maintaining the two populations prior to reproductive isolation and after reproductive isolation when gene flow between the two

populations has ceased but the two sister species still occupy a similar if not identical niche. Just as the mechanisms of extinction can be grouped into molecular and ecological types [Gaggiotti and Hanski, 2004], the mechanisms of speciation and coexistence can be caused by molecular processes, ecological processes, or some combination of the two.

2.3.2 Molecular Mechanisms of Coexistence in sympatric incipient species

At the molecular level, coexistence mechanisms involve genetic variation which is minimally expressed in the phenotype. Because competition and selection occurs at the level of the phenotype of the individual [Mayr et al., 1963], two individuals with similar phenotypes will experience equivalent competitive fitness and selective pressures. Furthermore, in some cases the genes that cause divergence are separate from the mutations that cause reproductive isolation Nosil and Schluter [2011], so it is possible for populations to speciate with minimal divergence genetically or phenotypically. There is already evidence of this type of speciation, between two phenotypically identical individuals. Cryptic species are species that are morphologically indistinguishable but are reproductively and genetically isolated from each other. Hayden and colleagues describe several cases of cryptic genetic variance [Hayden et al., 2011]. This concept of genetic neutrality has also been described at the theoretical level [Kimura et al., 1968] as well as the community level [Hubbell, 2005]. The following three molecular mechanisms have the potential to both allow coexistence between incipient species and promote reproductive isolation between those species [Coyne and Orr, 2004].

Hybridization

Allopolyploidy is form of polyploidy, also known as hybridization. This can be interspecies hybridization or hybridization between two members of the same species with differing chromosomal ploidies. There are observed cases of hybrid offspring in mammals. The offspring are sexed (non-hermaphroditic) and so it appears these hybrids have found a way to overcome the initial obstructions with sex chromosome determination found in standard polyploidy. The chromosomal compatibility in these hybrids is likely because these cases are often between parents from two closely related species. However, all observed cased are still sterile because of chromosomal pairing difficulties during meiosis. Hybridization is, however, common in some vertebrate taxa such as fish, amphibians and reptiles, and invertebrate animals as well as plants [Ohno, 1970]. Hybridization of two species may generate new species that are reproductively isolated from their parental phenotypes. Hybridization is common among closely related species, especially when these two species have some degree of habitat overlap [Mayr et al., 1963]. The ability for two species to reproduce viable offspring is commonly thought of as evidence that the two species are actually conspecifics; however, Mayr [Mayr et al., 1963] points out that there are numerous examples of hybridization among species and that “cross-fertility does not prove conspecificity”. Hybridization can play a role in divergence of two populations. Hybridization may also play a role in seemingly instantaneous reproductive isolation among sympatric incipient species. In this case, however, the reproductive isolation does not occur between the two parental populations but rather between the parental populations and their offspring. Grant referred to this type of speciation as recombinational speciation and because of the requirement of interaction in order to hybridize, these events take place in sympatry or parapatry only [Grant, 1959]. Coyne and Orr, go on to point out, however, that post-isolated species are more likely to experience

stronger competitive exclusion pressures in sympatry than in parapatry because this new hybrid species will more-frequently interact with the resident parental species when the three are in sympatry [Coyne and Orr, 2004]. Hybridization may play a secondary role in speciation: as a promoter of assortative mating when the hybrid offspring of the two parental population have marked reduced fitness [Nagel and Schluter, 1998]. If the two populations possess significant genetic differences, despite possibility limited phenotypic differences, hybridization may result in a Dobzhansky-Muller incompatibility, when the hybridization of two genotypes leads to reduced fitness because of new genetic interactions between recombined genes [Nosil and Schluter, 2011]. Because of the potential outcome of instantaneous reproductive isolation, hybridization plays its part in allowing coexistence of diverging species.

Duplication

In 1970, Susumu Ohno wrote his work on Gene Duplication and its role in Evolution. Since then, Gene Duplication has been considered of the most dramatic mutations with the potential for a great deal of diversification [Mileyko et al., 2008, Marques et al., 2008], if the duplication is sustainable [Ohno, 1970]. Gene duplication can be limited to one gene or region (Tandem Duplication) or it can be genome wide (Polyploidy). One or both forms of duplication are common across living taxa and are said to be complementary to each other, meaning: when one form is not advantageous, the other form is. [Ohno, 1970]. While gene duplication can have substantial effects on protein concentration, development and gene network motifs [Mileyko et al., 2008, Ohno, 1970], duplication events can also occur in regions of D.N.A. that are expressed irregularly or in a manner such that redundant expression has little effect on fitness [Ohno, 1970]. Duplications may also be co-opted into new genes at which point the phenotype would likely be affected [Ohno, 1970, Marques et al., 2008]. Joseph and Hall point out that duplicated genes may

be able to mask new mutations because the duplicated pair would function in a manner similar to a heterozygous trait: the new gene product exists but at low levels and not in absence of the original gene product [Joseph and Hall, 2004]. In some cases, gene duplication serves as a mechanism of both coexistence and sympatric speciation because it can initiate sources of silent variation, but can also be the impetus for reproductive isolation. Duplication events are also present in yeast. These duplications are initiated by the yeast as a response to stress [Yona et al., 2012, Kondrashov, 2012]. In extreme cases, yeast cells have opted to duplicate their entire genome and this has led to subsequent lineage divergence [Kellis et al., 2003]. It is possible that yeast are capable of a range of duplication events and types because they exhibit complete lifecycles in both haploid and diploid states [Neiman, 2011]. Yeast are also capable of reducing expression of one of the duplicated genes, an ability that allows the duplication to become effectively silent [Qian et al., 2010].

Epistasis

The phenotypic effects of gene duplication may be neutralized by epigenetic silencing or epistasis. Epistasis may also act as a mechanism of coexistence. Epistasis is defined as the interaction of non-allelic genes, specifically in the regulatory sense. Much of the research done on epistasis has focused on its role in genetic enhancements. Some of the earliest work on epistasis addresses its synergistic effects on deleterious alleles in Drosophila [Mukai, 1969]. Jasmine and Lenormand recently assessed the role of epistasis in mutation accumulation experiments in yeasts and found epistasis did not have a significant effect on the accelerated fitness decline experienced by these lines [Jasmin and Lenormand, 2016]. In a separate experiment, however, epistatic interactions between yeast nuclei and mitochondria were found to promote diversity within a wild population of yeast [Paliwal et al., 2014]. Epistasis makes it possible to silence mutations and for

that mutation to therefore proliferate in the population [Dettman et al., 2007]. Epistasis may allow differing mutations to accumulate as they would in allopatrically diverging populations, but without detection in an individual's phenotype. This makes it possible for growth of two populations in sympatry without direct competition between the two because phenotypically they are still similar. As long as the accumulated mutations remain neutral there should be no differential application of selective pressure [Kimura et al., 1968, Hubbell, 2005].

Rodin and Riggs recognized the potential for epistatic silencing to play a role in divergence events when they assayed the prevalence of the larger regulatory field of epigenetics in divergence events and found that duplication events were masked by cytosine methylation in many taxa that were capable of DNA methylation [Rodin and Riggs, 2003]. While epigenetics involves regulation by non-DNA elements, it is likely that like epigenetic silencing, antagonistic epistasis may reduce phenotypic traits that would normally experience heavy selective pressure. In the yeast, *Saccharomyces cerevisiae*, epistasis is currently estimated to account for approximately 9% of the phenotypic variation. Epistasis in yeast is still not well understood and epistasis' role in reproductive isolation is also poorly studied. However, Hou and Schacherer report evidence of reproductive isolation events stemming from laboratory observations from populations of 27 natural isolates of *S. cerevisiae* due to negative epistasis. The analysis yielded two Dobzhansky-Muller incompatibilities, both involving the respiratory abilities of these yeasts. They concluded that negative epistasis could lead to reproductive isolation in yeasts in a condition-specific manner [Hou and Schacherer, 2016, Dettman et al., 2007].

2.3.3 Ecological Mechanisms of Coexistence

If phenotypic differences are apparent between two populations, it is possible that the less fit competitor within these two populations may prevent its extinction by migration or dispersal. Dispersal alone may promote coexistence [Berkley et al., 2010] even if the separation of the two populations is only temporary [Tilman, 1994]. However, if the two populations remain together there are several mechanisms that may prolong time to extinction or even promote stable coexistence.

Reduction of Limiting Similarity

One possible mechanism of coexistence is to reduce the limiting similarity of the two incipient species. This is accomplished by splitting the niche that the two populations (incipient, cryptic or sister species) occupy or formation or movement by one of the populations to a new niche [Chesson, 2000]. Referred to as resource partitioning Chesson demonstrates that this mechanism can yield stable coexistence of two populations, but notes that the niche differences must be substantial and that not all niche differences will yield stable coexistence. For example, if the niche difference intensify intraspecies competition, while reducing interspecies competition, then the coexistence will be stable. Additionally, if the niche differences discourage hybridization between the two populations, resulting in reduced separation, then the coexistence will become unstable [Chesson, 2000]. Kremer and Klausmeier also note that in order for niche-partitioning to promote coexistence, the segregation has to happen within a population-specific time frame: the segregation must occur and begin to become effective before the populations drop too far below effective population size [Kremer and Klausmeier, 2013]. These adjustments in the niche overlap of these two populations can be spatial or temporal [Chesson, 2000] and may be based on the manifestation of behavior preferences or formation of

morphological or physiological adaptations [Sage and Selander, 1975, Bush, 1969]. In their work on yeast communities within fly fecal pools Stamps and colleagues indicate that several members of the yeast group Saccharomycotina are capable of coexisting at stable frequencies by utilizing different resources within the fecal pool [Stamps et al., 2012]. While this example shows coexistence between species, it is analogous to coexistence between populations of the same species [Hubbell, 2005, Hayden et al., 2011]. Niche partitioning based on temporal variability, such as adjusting pollination times to different sections of a larger season or choosing to sporulate within a different thermal range has been demonstrated as a mechanism of coexistence as well as a driver of evolution [Kremer and Klausmeier, 2013] and speciation [Murphy and Zeyl, 2012]. These niche separation events can also promote coexistence, in yeasts, there are copious examples of both coexistence and sympatric speciation as a result of divergence of some spatial aspect of the natural histories of these two populations [Murphy and Zeyl, 2010, Gonçalves et al., 2011, Sniegowski et al., 2002]. There are also notable examples where spatial separation of niches lead to coexistence and potential incipient speciation [Sage and Selander, 1975] or complete sympatric speciation [Price et al., 2010]. This mechanism of coexistence reduces the competitive pressure between the two populations but also promotes further divergence and acts as a potential isolating mechanism. This limited separation of these two populations in even one aspect of their natural history is sufficient to elicit reproductive isolation. If the separation of the two populations is dramatic or absolute, these once sympatric species may be considered allopatric or parapatric, with habitat segregation occurring despite a very short distance between the two [Mayr et al., 1963]. In the case that the separation between populations is not absolute, the two populations may still experience what Mayr refers to as ethological barriers to mating, more modernly referred to as sexual preferences or sexual selection [Mayr et al., 1963, Bush, 1969]. Speciation promotes further speciation by changing selection pressures due to range partitioning.

Once reproductive isolation has occurred, the need for Coexistence mechanisms does not dissipate. Rather, if possible these mechanisms continue until the two incipient species have managed to carve out new niches for themselves, reducing competitive interaction.

Density-dependent selection

The ability for two populations to coexist can also be attributed to the relative density of the two population and the resultant effects of those densities on their environment and other species within the community. In a predator prey cycle, as the population of predators increase, the population of predators decrease [Cao et al., 2008]. Roughgarden and Feldman investigated the effects of two prey coexisting and consumed by the same predator. They found that this type of community composition can promote coexistence of the prey types as long as the niche overlap between the two prey species is substantial [Roughgarden and Feldman, 1975]. One explanation for this may be that the strong niche overlap and the predation pressure results in these two species being treated as one. The primarily selection pressure in the system is predation and this selection pressure increases for the population with the higher density. Resources are not likely to induce strong selective pressure on the two populations because the populations are kept below carrying capacity by predation. Another variation of density-dependent selection is habitat destruction, which Nee and May point out can also lead niche partitioning or niche creation [Nee and May, 1992]. When population densities increase past their carrying capacities there is the potential for temporary or long-term habitat destruction because of exhausted resources or excessive waste product accumulation. This can be an opportunity for selection of new phenotypes capable of utilizing the current resources or finding new ways to extract and use previously unused resources [Bull and Harcombe, 2009]. In diversity studies, habitats with intermediate levels of disturbance typically have the highest biodiversity, and opportunities, due to minor habitat destruction, are

believed to be the causative factor [Rosenzweig et al., 1995]. Bull and Harcombe observed of coexistence between two microbial species based on temporal niche separation due to habitat disturbance caused by density-dependent selection. Because resources were depleted by high population density, each species adapted to consuming some of the waste by-products of the other species, thereby providing the two populations with different resource needs. The resultant population dynamics were such that as one population increased, its resources were depleted, and the amount of resource for the other population increased and the frequencies of the populations oscillated [Bull and Harcombe, 2009]. This oscillation may eventually result in a stable coexistence with no change in population frequencies, or it may coexist as an oscillation [Chesson, 2000]. This type of waste-resource temporal niche partitioning is analogous to one of the most predominant coexistence examples: the coexistence of autotrophs and heterotrophs.

Trade-offs

A third possible mechanism of coexistence between two populations is the existence of phenotypic trade-offs. Like density dependence, trade-offs, the fitness of an individual's phenotype is context dependent; it depends its current environment including abiotic factors and community interaction and competition. Trade-offs are defined in ecological terms as the possession of a beneficial trait that can be harmful in some circumstances [Garland, 2014]. Trade-offs may elicit coexistence between two populations because it halts complete competitive exclusion of one population by the other because each population is better than the other in certain circumstances. A well-known trade-off is between egg size and clutch number [Guisande et al., 1996] Often known as the Smith-Fretwell trade-off, this ecological trade-off describes the inverse relationship between the number of eggs a parent can lay and the amount of resources that parent can allocate to that offspring (egg size). This value is bounded by an egg-size maximum (based on physical

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Figure 2.1: If Hamlet were a yeast cell..."To spore or not to spore, that is the question!" Illustration Credit: Stansilav Cuseac

traits of the parent) and a resource maximum (based on the efficiency of the parent's metabolic system to take in nutrients and the maximum amount of resources a parent could conceivably acquire). Using this model, this trade-off, like many trade-offs, can be optimized based on environmental conditions and ideal resource values for the offspring [Messina et al., 2013]. Colonization ability as a polymorphic trade-off can also allow for coexistence between two competing species. Tilman suggested that a trade-off between colonization ability and competitive fitness with multiple species of grass in one community could promote stable coexistence between two or more species. In his model, populations were ranked based on their competitive ability an assigned a population dynamics equation based on that ranking. The competitive ability of the population was inversely correlated with the colonization ability of the population, representing a trade-off between these two characteristics. Tilman's model was one of the first to illustrate that the compettion-colonization trade-off could establish stable coexistence across multiple species, given the right colonization rate and mortality rate parameters [Tilman, 1994]. Calcagno and colleagues addressed models of the competition-colonization trade-off and some of the criticized aspects of Tilman's model. Most contentious, was the absolutism of Tilman's model: there was absolute competitive dominance by the more fit population in any competitive interaction. Calcagno and colleagues point out that this is unrealistic but also unnecessary to achieve stable coexistence. In their adaptation of Tilman's model, a scalar variable was added that would adjust the competition strength of the competitors allowing for some demographic stochasticity. The result was still stable coexistence but will greater limitations on number of interacting populations and fitness disparities [Calcagno et al., 2006]. Coluccio's study highlighted a trade-off in sporulation rate for yeasts that were frequently subjected to ingestion and digestion by insects. Yeasts with slow sporulation rates have higher reproductive success because they spend more time actively dividing rather than sporulating; however, yeast survivorship

was increased in sporulated cells relative to vegetative cells when transferred through the gut of a fly. However, from a community perspective, these survivorship values, it was unclear that differential consumption was not a factor in survivorship [Coluccio et al., 2008]. As an extension of this study Reuter and colleagues suggested that this trade-off held and additional advantage for the spores because their outcrossing rate increased as a result of digestion by the fly [Reuter et al., 2007, Otto and Lenormand, 2002]. Both Chesson and Kremer and Klausmeier cite the ecological trade-off as a key mechanism of coexistence. Indeed, many examples of niche partitioning and frequency-dependent selection can be viewed as a trade-off [Chesson, 2000], and Chesson's compensatory variables in his coexistence equations can be view as trade-offs when considering the values are positive for one species and negative for the other [Coluccio et al., 2008, Tilman, 1994]. These mechanisms of coexistence aim to reduce competitive interaction between the two populations of limiting similarity. The molecular mechanisms accomplish this by reducing the notable phenotypic differences of the two populations, while allowing the genotype to continue diverging. These three molecular mechanisms also make it possible for reproductive isolation to occur with limited direct competition between the incipient species. The ecological mechanisms reduce competitive interaction by decreasing limiting similarity and thereby the selective pressures driven by competition for resources. These ecological mechanisms promote speciation by reducing the amount of interaction between the two populations, even though there is no geographical barrier between the two.

Of course, these mechanisms of coexistence may in fact just be a mechanism of prolonging the inevitable: competitive exclusion. In an analysis of species distribution across vertebrates, Alfaro and colleagues concede that the three groups of slowly evolving lineages did display higher species diversity over evolutionary time than they do currently, that rather than experiencing an evolutionary event that slowed down the rate of diversification, the diversity within these groups may have competitively excluded each other

over deep time [Alfaro et al., 2009]. The applicability of these mechanisms is taxon-specific and case-specific for the mechanism's role in coexistence and in reproductive isolation.

2.4 Sympatric Speciation and Coexistence: The Missing Pieces

In order for sympatric speciation to occur, two phenotypically similar populations of the same species must coexist long enough to diverge into reproductively isolated populations. Because of the unlikeliness of this successfully occurring, it is often believed that many sympatric speciation events are the result of allopatry [Bush, 1975]. With new information available regarding genetic regulation and genome sequencing and new evidence of sympatry and coexistence, we should be evaluating our definitions of speciation and its processes once more. With this new information, also comes new questions and new ways to answer old questions.

2.4.1 Genetic Questions

Based on Sage and Selander's discovery that many morphologically diverse species were actually the same species and that these incipient species exhibited morphological differences due to phenotypic plasticity, how much of a role does plasticity play in sympatric speciation? What role does alternative splicing, epigenetics or epistatic interaction play in this phenotypic plasticity? Also in need of further study are the mechanisms hybridization and the levels of introgression in sympatrically occurring species and whether these events indicate sympatric species or incipient species [Sage and Selander, 1975, Coyne and Orr, 2004]. Additionally, is it possible that repeated introgression or hy-

bridization could lead to a completely new speciation event? Given that hybridization can result in incomplete speciation or rapid speciation, [Ohno, 1970] are there traits other than the ability to self that promote functional and viable hybrids? What other trait incompatibilities or compatibilities steer reproductive isolation? Both Kellis and Yona and colleagues point out that duplication events in yeast can result in lineage divergence in yeasts [Kellis et al., 2003, Yona et al., 2012]. What is unclear is the level to which epistasis may have played a role in the maintenance of these duplications within diverging species. There has been some work on the effects of negative epistasis on reproductive isolation [Hou and Schacherer, 2016], but what role does positive epistasis play, particularly in epistatic silencing of duplication events [Rodin and Riggs, 2003]? Beyond duplication and epistasis, yeasts are capable of chromosomal rearrangement. Several studies have indicated chromosomal rearrangements as a significant factor in the reduced viability of hybrid offspring [Hou et al., 2014, Charron et al., 2014]. Do chromosomal rearrangements complement epistatic regulation in yeasts similar to Ohno's description of Polyploidy and Tandem Duplication? Reuter and colleagues expanded on Coluccio's yeast survivorship study by measuring the difference in out-crossing rate of digested and undigested spore cells. They found that out-crossing rates were increased and this genetic recombination could lead to increased diversity in the founding colony where these surviving yeasts are deposited [Reuter et al., 2007]. Reuter and colleagues findings on increased out-crossing in yeasts promotes new questions in relation to the role of out-crossing as a mechanism of blocking polyploidy. Despite a century of work on this topic, it is still not clear if problems with dosage compensation are the primary drivers in the inability for some taxa to out-cross between different ploidies [Coyne and Orr, 2004]. Additionally, Hendry addressed the rapid divergence possible by Salmon colonizing new habitats [Hendry et al., 2000]. How might dispersal enhance the effects of out-crossing in a yeast cell that has been recently digested and dispersed by an insect?

2.4.2 Ecological Questions

Hendry and colleague's study was also substantial because it looked at the mechanisms of divergence and speciation in natural habitats. Sniegowskii and colleague's study of coexistence and speciation in *S. paradoxus* also allows us to look at the processes speciation and coexistence within a natural system and begin to draw conclusions of the affect size of the system variables. In their study, *S. paradoxus* was in the processes of allopatric speciation from a diverging second population while coexisting with a population of *S. cerevisiae*, which was isolated from its own second population, and showed little evidence of divergence or speciation [Sniegowski et al., 2002]. In order to confirm the validity of our theoretical and empirical findings, more work in natural habits are necessary. In the case of yeasts and sporulation, it is unclear as of yet, if sporulation rate is plastic, conditional or genetically constant. Sporulation timing is driven by the environment [Neiman, 2011], but does the rate in a single yeast lineage fluctuate with environmental conditions, does the timing per strain stay constant in one lineage despite the environment, or does the yeast participate in a bet hedging strategy where some offspring sporulate earlier and some offspring sporulated later [Chesson, 2000]. Finally, despite evidence to support the idea [Tilman, 1994, Chesson, 2000, Coluccio et al., 2008, Reuter et al., 2007], it is still unclear if the sporulation rate of yeasts act as a trade-off with growth rate and whether this trade-off, if present, is a possible mechanism for sympatric speciation. This trade-off may be one of many mechanism that explain the high rate of observed sympatric speciation events in this genus.

2.5 Conclusions

In their 2014 Nature debate, Laland and colleagues and Hoekstra and colleagues debated whether the tenets of evolutionary biology and the key factors of the modern

synthesis should be redefined given the plethora of new information and new understanding that evolutionary biologists now possess about genetics, epigenetics and epistasis and its effect on phenotype and variation. These developmental idiosyncrasies may not be genetically constant across taxa or communities, but they can change the outcome of selection events [Laland et al., 2014]. The same is true in tracking the mechanisms of sympatric speciation. One mechanism may not work on its own, but many mechanisms may, in combination, produce a reproductive barrier between two incipient species. The effects of these mechanisms may not be constant in each speciation process, but they can change the outcome of selection events by allowing for coexistence or promoting competitive exclusion. Perhaps, in light of these findings, we should redefine our understanding of the mechanisms of sympatric speciation. The molecular and ecological mechanisms described above can promote both coexistence between species as well as divergence of these species and potentially the formation of a reproductive barrier. The scope and effect of each of these mechanisms is limited by the organism's genetic architecture and the other mechanisms at work as well as the level of competition between two populations. The overarching mechanism of all of these mechanisms is reduction of competitive pressure and promotion of divergent traits or genetic variation.

Chapter 3

An Assessment of Differential Survivorship in insect-vectored *Saccharomyces cerevisiae*

3.1 Introduction

3.1.1 Ecological Factors of Community Composition

Community composition, the number of species populations and the relative size ratios of these populations to each other, is a contributing factor to community stability and resilience [Chesson, 1986, Rosenzweig et al., 1995, Kaneko, 2012]. It is controlled by a multitude of factors including competitive pressures [Edwards et al., 2010, Gause, 1932, Hardin, 1960, Roughgarden and Feldman, 1975], migration rates [Molina-Montenegro et al., 2012] and resource and climate fluctuations [Abrams, 2000, Chesson, 1986, Amarasekare and Nisbet, 2001]. Disturbance events facilitate the opportunity to change the composition of a community because they allow new niche spaces to become available.

Species richness is maximized, for example, when disturbance in a community occurs at intermediate levels [Rosenzweig et al., 1995]. Dispersal can be considered a form of migration to meta-communities; however, the nature of the dispersal can determine whether the dispersed group of individuals can coexist with the residents of the new environment or if one group will competitively exclude the other [Berkley et al., 2010].

In microbial communities, the role of insect phoresis, dispersal by insect vectoring, may also play a role in each founder colony's composition and the successive dynamics of that community by selecting specific taxa to vector or inflicting differential mortality on those vectored species [Stamps et al., 2012, Coluccio et al., 2008]. Insects that disperse microbes by ingestion of one individual or population in one location and defecation of that individual or population in another location, have evolved preferences to specific microbes as well as to specific stages of the microbe [Schiabor et al., 2014, Barker and Starmer, 1999]. A diversity of insects, ranging from vespids to coleopterans [Stefanini et al., 2012, Klepzig and Hofstetter, 2011] to tephrids and Drososphila [Ito et al., 1995, Reuter et al., 2007] are known to vector these immobile microbiota and each microbial group may adapt to be more proficient at this vectoring process by physiological mechanisms such as the development of protective coverings or excretions [Codon et al., 1995, Orlean, 2012, Neiman, 2011], or through behavioral mechanisms such changing the timing of sporulation or avoiding ingestion [Coluccio et al., 2008, Begon et al., 1982, Madhani, 2007]. Insect phoresis is a common method of dispersal among sessile invertebrates, microbes and plant gametes. This long-standing relationship between microbe and insect is often beneficial for both participants [Anagnostou et al., 2010, Barker and Starmer, 1999], but could affect the evolutionary trajectories of each population as well [Reuter et al., 2007, Hyma and Fay, 2013]. Indeed, the coevolution of these interacting organisms is so evident that many insect species have become dependent specializes to specific microbia which they disperse [Morais et al., 1994, Ort et al., 2012].

3.1.2 Competition and Coexistence

Once dispersed, the maintenance and richness of the existing microbial propagules are determined by competition and competitive exclusion. Competition plays a substantial role in community composition and phenotypic variance within a species [Stier et al., 2013, Amarasekare, 2003, Bolnick]. In species with a high degree of limiting similarity, competitive pressures can be reduced through functional equivalence [Hubbell, 2005] or implementation of a coexistence mechanism [Chesson, 2000, Amarasekare and Nisbet, 2001]. These coexistence mechanisms will determine the ratio of one species to another at equilibrium [Tilman, 1994] or the diversity of phenotypes within one species[Stamps et al., 2012, Amarasekare and Nisbet, 2001]. For example, Stamps and colleagues found that some species of the yeast genus *Pichia spp.* were found at higher-than-expected frequencies in microbial communities that interacted with *Drosophila*. When communities of similar species composition did not interact with *Drosophila*, these species existed at lower frequencies. The ability for *Pichia* to utilize uric acid as metabolic resource allowed these normally competitively-inferior species to gain the competitive advantage in insect fecal pools. Thus, the species that normally might be competitively excluded are capable of coexisting at different frequencies with this coexistence mechanism: the trade-off.

3.1.3 Ecological Trade-offs

The trade-off is one type of ecological mechanism that has been empirically and theoretically shown to elicit coexistence between two competing species [Tilman, 1994]. Trade-offs occur when an individual or population is competitively superior to its competitor in one aspect its life-history, but competitively inferior to its competitor in another aspect of its life-history. Often, trade-offs occur when both life-history scenarios occur at regular or alternating frequencies [Kneitel and Chase, 2004, Tilman, 1982]. Tilman's

competition-colonization trade-off has been shown to elicit universal coexistence between two or more species of limiting similarity. The competition-colonization trade-off can be initiated when two species differ inversely in their propensities to disperse and their abilities access nutrients [Tilman, 1994].

3.1.4 Yeast

Members of the family Saccharmytina, commonly referred to as the yeasts, are examples of populations that both exhibit coexistence between two species of limiting similarity [Frenkel et al., 2015, Nissen et al., 2004, Sniegowski et al., 2002] and utilize insects as a method of dispersal. Yeasts, such as *Saccharomyces cerevisiae*, may have also adapted to the process of insect vectoring by altering their phenotype to increase their chances of survivorship when ingested and digested by insects such as *Drosophila*. Because colonization of a new patch space can be beneficial by reducing competitive pressure on a less-fit competitor, it could be adaptive for microbia, like *S. cerevisiae* to become better at colonization at the cost of reduced replication time [Bohannan et al., 2002].

While the fruit fly, *Drosophila melanogaster* is not the only insect that vectors yeast, there is substantial evidence of its long history of doing so as well as its adaptation to preferences for feeding from well-established metabolically late-stage yeast communities. This preference may be due to specific metabolic products that the fly has evolved to find attractive [Schiabor et al., 2014]. Insect biochemistry, including digestive processes, has also been shown to be different based on the sex of the insect [Magwere et al., 2004, Buchon et al., 2013, Dutta et al., 2015]. Furthermore, female insects may benefit differently from the incomplete or complete digestion of the yeast that it ingests, relative to the benefits a male insect may receive. For example, a female *Drosophila* that ovaposits and defecates in the same area may have developed the strategy to allow surviving

yeast to pass through the gut, so these yeasts can proliferate and become a resource for the *Drosophila*'s hatching larvae [Barker and Starmer, 1999]. Conversely, it may be advantageous for female flies to digest as much of the ingested yeast as possible in order to convert this resource to energy to make more eggs [Anagnostou et al., 2010, Begon et al., 1982]. Males may also benefit from the complete digestion on food in order to have more energy for courtship rituals [Yuval et al., 1998].

Coluccio and colleagues [2009] established the potential of differential survivorship of yeasts processed through the gut of fruit flies. This differential survivorship was based on life-history phase of the yeast cell: metabolically active vegetative cell or metabolically quiescent spore. Coluccio found that yeasts in the sporulated state where 4 times more likely to survive the gut of a fruit fly than were yeasts in the vegetative state [Coluccio et al., 2008]. What is unclear, however, is whether differential consumption of the two yeast cell types affected this outcome. Is the extent to which a spore more effectively traverses the insect gut relative to a vegetative cell and its differential survival the result of yeast cell physiology or fly preference for spores?

3.1.5 The Sporulation Rate Trade-off as a behavioral strategy

It is possible that spores function as a mechanism of colonization in that they prevent the yeast from being digested in the gut of insects that ingest the yeast. The sporulated yeast cells are able to survive the gut at a higher rate relative to that of vegetative yeast cells, and are deposited with higher frequency at founder sites. We set out to further investigate the possibility of a competitive trade-off in yeasts. Based on the findings of Coluccio and colleagues [Coluccio et al., 2008], we expected that yeast cells that have undergone sporulation before ingestion and digestion of insects were more likely to survive the process and be successfully dispersed by insect vectoring.

Figure 3.1: Yeast behavioral strategies to survive fly gut ingestion: Live to die another day... Drawing Credit: Hannah Plett

While there is sufficient empirical evidence to support the adaptive character of spores when transferred through the fly gut [Coluccio et al., 2008, Reuter et al., 2007], the extent to which this difference in survivorship incurs a competitive advantage is still poorly understood. We first confirmed the result of Coluccio and colleagues using a novel method of food administration known as the Capillary Feeder (CaFe) vial [Ja et al., 2007, Deshpande et al., 2014]. We then quantified survivorship and used these numbers to calculate the predicted likelihood of survivorship in each of the two states. We predicted that differential survival in gut vectoring will result in sporulated strains in a higher ratio to vegetatively growing strains relative to the ratio of the original 1-to-1 sample. Moreover, our goal in this study was to directly quantify the probability of survivorship of each cell type to explore the possibility of an existent trade-off between

fast and slow sporulating strains of *S. cerevisiae*.

3.2 Methods

In order to test the relative survivorship of *Saccharomyces cerevisiae* in its sporulated and vegetative states, an equal ratio of spores and vegetative cells were quantified and then fed to *Drosophila melanogaster*. The surviving yeast were then collected from the flies' frass deposits, quantified and compared to the predigested values. Twenty to thirty replicates of each of five regionally diverse strains were tested using male or female flies and alternating fluorescent markers (Figure 3.4 figure) totaling in 800 independent trials.

3.2.1 Strain preparation

Using the strain set of *S. cerevisiae* acquired from five global regions and prepared by Louvel and colleagues [Louvel et al., 2014, Cubillos et al., 2009], we homologously tagged diploid strains with the fluorescent protein, GFP and Mcherry, by tagging haploids of compatible mating types from the same strain set each with GFP and MCherry markers [Amberg et al., 2005, Bergman, 2001]. The two GFP compatible strains were then mated, yielding a homothallic diploid strain with homologous markers for GFP [Amberg et al., 2005]. This mating process was then repeated for the MCherry strains of the same strain and then for GFP and MCherry-transformed strains for four additional strain sets from four other regions. These five regional strains were constructed from five regionally diverse samples, using the same techniques to diversify and genetically tag new sub-strains within each region. Each region contains newly derived laboratory strains that have been adapted identically, so that each member of one region has four sister strains (experimentally identical in laboratory adaptations), one from each other region. These five regional strain groups were chosen both because of their diverse eco-

logical backgrounds and because their phenotypic and genetic characters have been well documented and studied. Additionally, because this strain set was derived by the same working group at the same time [Louvel et al., 2014] we can be more certain that the laboratory adaptations to these strains were performed uniformly across all five regional groups. Because of this consistency in alteration, we can therefore have increased confidence that the regionally-based differences from this spore-vegetative survivorship assay (SVSA) are due to regional backgrounds and not different degrees of laboratory artifact [Elena and Lenski, 2003, Rice and Hostert, 1993].

For each regional strain set, one diploid fluorescent transformant was sporulated to over 95% sporulation using standard protocols with 0.6% Potassium Acetate (KAc) at a pH value of 6.8 [McCusker and Haber, 1977] and refrigerated at 4°Celsius [Amberg et al., 2005]. The other diploid fluorescent transformant of the same strain was grown vegetatively overnight at 30°Celsius with shaking (230 RPM). In order to ensure the strain cultured was the focal strain, vegetative cultures were always grown in Yeast-Peptone-Dextrose (YPD) media with G418. As an additional protective measure, the bacterial antibiotics tetracycline and ampicillin were added to the YPD with G418. This antibiotic YPD (YPDA) helped ensure both the propagated and collected yeast culture was the focal yeast and free from possible microbial contaminants.

Previously sporulated yeast cells with a different fluorescent protein marker were then removed from refrigeration. To maintain the two cell groups in their sporulated or vegetative states, the optical densities of the vegetative cells (in YPDA) and the sporulated cells (in KAc) were pelleted, washed and re-suspended in spent YPDA (SYPD) which is YPD media depleted of the majority of its accessible Carbon and Nitrogen sources and then amended with the antibiotic cocktail [Bergman, 2001, Amberg et al., 2005]. Both the vegetative and sporulated strains were then adjusted to equal optical densities of 0.05, using the Tecan optical density reader and Magellan (V 7.2) software

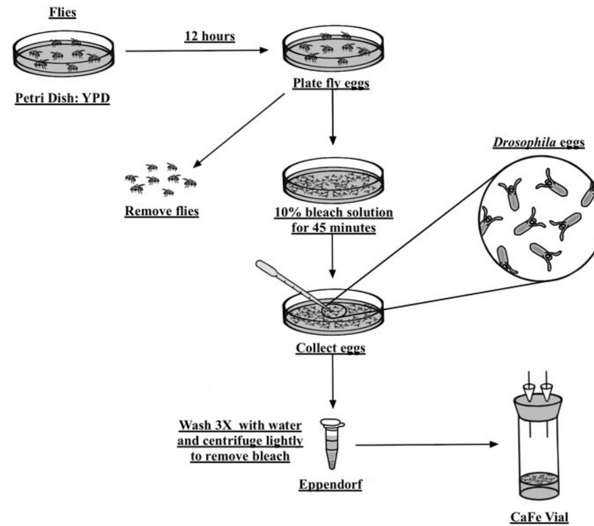

Figure 3.2: Fly bleaching method to reduce fly fungal and bacterial load.

set to a wavelength of 600 nanometers. Equal parts of each strain were mixed to create a 1-to-1 solution of vegetative-to-spores [Mytilinaios et al., 2012, Seidel]. To ensure an evenly proportioned mixture of spores and vegetative cells, serial dilutions of this 1-to1 mixture were plated on YPDA plates. These serial dilutions also acted as a pre-treatment measurement, referred to as the *Baseline*. Antibiotics were included in this pre-treatment measurement to maintain consistency with the post-treatment measurement, ensuring that the presence of antibiotics did not play a role in any resulting changes.

3.2.2 Fly preparation

D. melanogaster stocks were created by out-crossing strains from isogenic Al-Ral, Taiwanese, Santa Barbarian and Malaysian lines. This was done to increase robustness of the fly lineage [Begon et al., 1982, Buchon et al., 2013]. Adult flies were allowed to lay eggs on YPD agar plates. These flies were then removed and the eggs were bleached

using a 10% bleach solution for 40 minutes. Fly eggs were then gathered by pipette, washed with sterile water and, transferred using sterile technique to clean media that was free of anti-fungal factors such as Tegosept ™ (Figure 3.2). Clean flies were reared and propagated on this media so that other yeasts, fungi and bacteria were minimized and did not confound the results [Reuter et al., 2007], but also so that the ingestion of antifungal elements did not reduce the viability of living yeasts traveling through the gut [Buchon et al., 2013].

CaFe apparatus tops were assembled per design by Ja and colleagues [Ja et al., 2007, Figure 3.3] using four 200 μL pipette tips which were cut to increase opening size, rubber stoppers (See Appendix A for complete list of materials) and standard rubber bands. These CaFe tops were then affixed to narrow fly vials each containing 3mL of 2% solidified agarose solution to maintain humidity within the vial. The pipette tips within the CaFe top were fitted to one 5μL capillary tube each; however, during treatment, only two capillary tubes were used and two were left open for airflow. 24 hours prior to treatment, four clean, sexed flies were added to each of four vials per replicate. This starvation period was necessary to ensure sufficient consumption of yeast by the flies during treatment [Reuter et al., 2007, Buchon et al., 2013].

3.2.3 Treatment

Using the CaFe apparatus, male only or female only flies were offered this one-to-one mixture of spores and vegetative cells which were differentially tagged with fluorescent markers and resistance to G418. Capillary tubes holding 5μl of the 1-to-1 mixture of spores and vegetative cells were dispensed to four, clean, sexed flies that had been starved for 24 hours in vials containing only 2% agarose. The flies were allowed to eat for 48 total hours. At 24 hours the initial yeast consumption by flies was measured and at 48 hours,

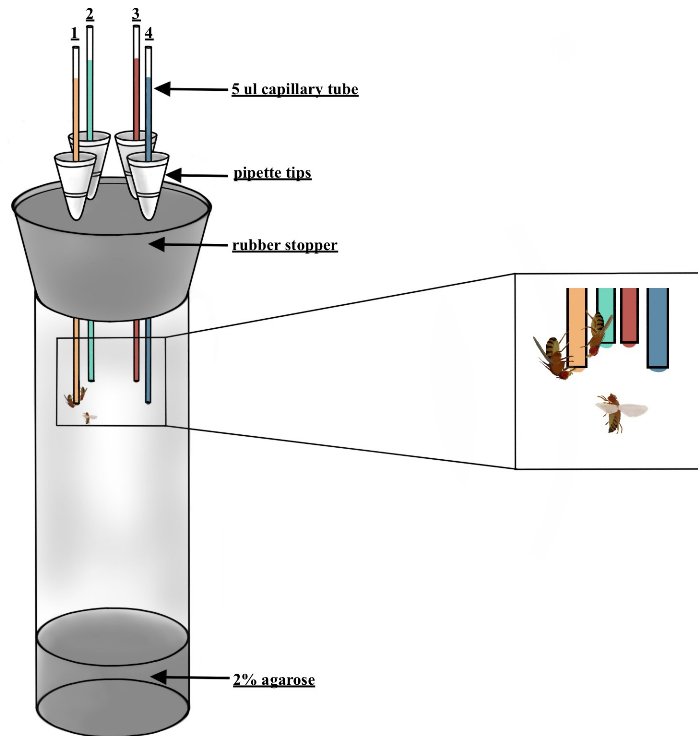

Figure 3.3: Diagram for the Fly Capillary Feeding (CaFe) vial designed by Ja and colleagues [Ja et al., 2007] and adapted for this survivorship assay.See Appendix A for complete list of materials. Capillary feeding vial contains four $5\mu L$ capillary tubes affixed to the rubber stopper, so that flies can feed directly from the tube rather than walking through the feeding substrate. The vial also contains $3mL$ of 2% agarose to maintain the humidity in the vial but prevent the flies from eating any other substrates.

the total yeast consumption by flies was measured and the flies were removed from the vial using sterile technique. Vials were rinsed with $500\mu L$ of water and the rinse solutions were then collected in Eppendorf tubes. The tubes were then mixed by pipetting and 100μ L of three serial dilutions (10^{-3}, 10^{-4}, 10^{-5}) of each rinse were plated on antibiotic (YPDA) plates.

After serial dilutions were made and plated, a 100μ L sample of each vortexed collection Eppendorf was inoculated into fresh, sterile YPDA media and allowed to grow

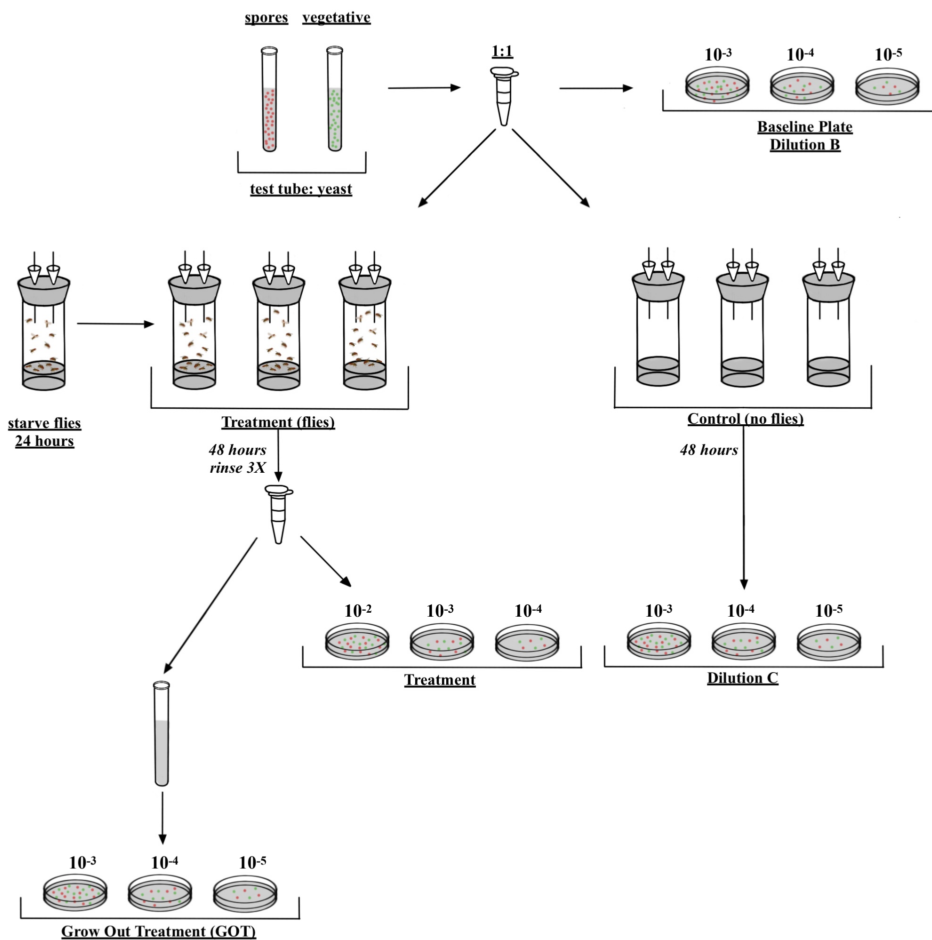

Figure 3.4: Diagram of the experimental procedure for the spore-vegetative survivorship assay (SVSA). Diagram moves from top to bottom and events in the same horizontal space occur at the same time. Four plating events at three time points and the transitions to the three stages of this experiment. On top, a fluorescent sample of sporulated yeast cells (red) and a fluorescent sample of actively growing yeast cells (green) are mixed together in equal densities in an Eppendorf tube and 3 dilutions of this solution are then plated on YPDA plates. These are the *Baseline plates*. The Eppendorf is then used to inoculate the *Treatment* vials (containing 4 sexed *D. melanogaster* and agarose) and *Control* vials (containing only agarose) which proceed at room temperature for 48 each. These vials are then serially diluted and plated. At the same time the Treatment vials are diluted and plated, a sample of the treatment vial solution is transferred into fresh YPDA and grown for 24 hours, then diluted and plated (*Grow out Treatment* or GoT). This process was repeated using fluorescently tagged samples from strains from different regions and using red as spores and green as vegetative cells to account for the possibility that the color change was a factor in the change in outcome.

for 24 hours at 30°Celsius without shaking in culture tubes [Mytilinaios et al., 2012]. Samples were then mixed by pipetting and serial dilutions were made (10^{-4}, 10^{-5}, 10^{-6}) and plated.

Plated dilutions of all treatments were then incubated for 24 hours from the time of plating in order to allow any viable cells time to germinate and grow to a visible colony forming unit (CFU). Each CFU on a plate represents one surviving and viable cell. While there may have been some viable cells on the plate that required more than 24 hours to be visible to the counter, there is an upper boundary to acceptable incubation time. Incubation time longer than 24 hours would increase the cell density on the plate and the individual CFUs would no longer be distinguishable. Additionally, these slow-starting cells would likely not have been competitively viable in natural systems because they would have been quickly competitively excluded by other fast-germinating or fast-growing cells.

3.2.4 Controls

To control for the effect of capillary tubes and ambient growth time, two capillary tubes containing 5μL of the one-to-one mixture of spores and vegetative cells were inserted into a CaFe apparatus containing no flies. These tubes were allowed to sit adjacent to the treatment vials for the 48 hour treatment period. For consistency and to confirm vectoring was not occurring due to gravity, the change in capillary tube menisci were measured for the controls as well at 24 and 48 hours. At the end the treatment time, these capillary tubes were then dispensed into 550μL of water and serial dilutions (10^{-4}), 10^{-5}, 10^{-6}) were plated to confirm a ratio similar to the pre-treatment ratio.

3.2.5 Quantification of Consumption and Survival

Consumption of the yeast mixture was measured twice over the 48 treatment period: at 24 hours by raising – but not removing– the capillary tubes from the CaFe vials, measuring change in meniscus and lowering capillary tubes and 48 hours by removing the capillary tubes, measuring total change in the meniscus and discarding the capillary tubes. As a metric of consumption, these two consumption values were then averaged to estimate the amount able to pass through the gut as a product of early and late consumption. Consumption was calculated by converting millimeters in the capillary tube to microliters of liquid to approximate number of cells [Deshpande et al., 2014]. This number was then divided by two under the assumption that approximately half of the consumed cells made it through the gut [Buchon et al., 2013, Deshpande et al., 2014]. To determine the number of each cell type consumed, the resulting value was divided by two again under the assumption that each cell type represented 50% of the total because the baseline solution was adjusted to a 1-to-1 mixture. Colony forming units (CFUs) were then counted and spore to vegetative cell line ratios were determined. Capillary tubes were checked for changes in spore and vegetative densities to eliminate the possibility that one-to-one ratios were not administered evenly or that interactions with the capillary tube alone –perhaps polarity-based– were responsible for the changes found in the treatment.

Survival was calculated by comparing the estimated number of digested cells by the time of collection to the cell counts on each plate scaled to appropriate dilution values. These consumption values were then compared to the scaled approximation of the cells of each type that survived the process, by counting colony forming units (CFUs) on each plate and quantifying the number of GFP and Mcherry colonies.

Quantification of Survivorship was performed by colony counts of serial dilutions from the post-treatment vial wash. Red and green cells were counted using double-blind, analogue counting, and each count was performed by two technicians.

3.2.6 Post-digestive Growth Assessment

To gain a more realistic description of the competitive interactions of the system in its entirety, both vegetative and sporulated cells were assessed for their competitive ability in a newly founded community, where spores must germinate from spores to vegetative cells and vegetative cells must recover from potential damage incurred in the vectoring process. Using a direct empirical assessment of the cells that survived the survivorship assay treatment, we assessed the recovery time of these cells [Mytilinaios et al., 2012]. After the rinsing procedure of the post-treatment processing of the survivorship assay, 1mL of the remaining swish solution was transferred into 1mL of double strength YPDA. These culture tubes were then allowed to grow for 24 hours, and then they were diluted and plated. Counts relative to the immediately post-treatment plating were then assessed for changes.

3.2.7 Assessment of the effects of fly sex on spore or vegetative survival

In our analysis of the effects on survivorship based on the sex of the *Drosophilae* in each tube, we compared the total counts and the ratios of spores to vegetative cells between the trials using only male flies and the trials using only female flies, using a logistic regression [Zuur et al., 2009, Townend, 2013].

3.2.8 Statistical Analysis

The data set consisted of 690 independent trails (60 per week, except weeks 10 and 11 which had 75 trials per week) each containing 3 serial dilutions each of a baseline (pre-treatment), control (CaFe apparatus with no flies)and treatment (CaFe apparatus with fly consumption) as a plate set. Therefore the total plates that were processed (photographed and counted) was 6243. Contaminated plates and high-density lawn culture plates (which were not countable) were removed from the data set. With this reduction, the data set totaled about 40 replicates a week, or 3960 plates. Quantification of survivorship was performed by colony counts of serial dilutions from the post-treatment vial wash. Red and green cells (CFUs) were counted using hand counters and transilluminators, and without knowledge of which color represented spores or vegetative cells. Each plate was photographed before counting and these photographs were filed for future validation and analysis. Once colony forming units (CFUs) were counted, these values were entered into a digital file where the known color associations per trial were listed and colors were assigned to cell types. The spore to vegetative cell line ratios were then determined as percent spores of total cells counted.

A model of the system was described using both sampling probability and three functions of each potential treatment effect (capillary tube, fly ingestion, and rich media) on differential mortality. The conceptual design of the experiment is described below as a series of events and random draws from binomial and normal distributions. The initial population, in which there is approximately a 1-to-1 ration of sporulated cells to vegetative cells (each denoted as red or green) is named the baseline and is described as the true population .

$$[\![P_{\text{true}}]\!] \rightarrow \text{Baseline}$$

This true population is then passed through the first function. The first function describes the effect of the capillary tube on each cell type's mortality and is given by the differences between the benchmark plates and the control plates. The 15 control vials are inoculated with a random sample from a normal distribution of the $[\![P_{\text{true}}]\!]$ with some degree of deviance from that distribution (σ_c). P_{ci} is the effect of the capillary tube and is the central selective pressure in this function. The resulting solution (post-capillary tube) is then plated based on a random draw from a binomial distribution with some degree of error (φ).

$$
\begin{aligned}
[\![P_{\text{true}}]\!] &\rightarrow \text{Control}_{i=15} \rightarrow P_{\text{ci}} \sim N(P_{\text{true}}, \sigma_c) \\
&\rightarrow f(P_{\text{ci}}, \varphi) \\
&\xrightarrow{\text{plate}} \text{Bi}\left(N_{\text{count}}, f(P_{\text{ci}}, \varphi\right)
\end{aligned}
$$

The second function describes the effects of the fly ingestion and digestion on mortality of each yeast cell type and is given by the differences between the control plates and the treatment plates. The output of the first function, f (Control) is passed through the treatment function, g, which is the effect of fly ingestion and digestion on the mortality of the two cells types. Again, the resulting solution (post-digestion) is plated based on a random draw from a binomial distribution with some degree of error (φ).

$$
\begin{aligned}
[\![P_{\text{true}}]\!] &\rightarrow \text{Treatment}_{j=60} \rightarrow P_{tj} \sim N(P_{\text{true}}, \sigma_c) \\
&\xrightarrow{\text{capillary}} f(P_{\text{ci}}, \varphi) \\
&\xrightarrow{\text{treatment}} g(f(P_{\text{ci}}, \varphi), \varphi) \\
&\xrightarrow{\text{plate}} \sim \text{Bi}(N_{\text{count}}, g(f(P_{\text{ci}}, \varphi), \varphi))
\end{aligned}
$$

The third function, h, describes the effects of grow out time and nutrients on the mortality of each cell type post digestion and is given by the differences between the treatment plates and the grow out treatment plates. The resulting solution (post-incubation) is then plated based on a random draw from a binomial distribution with some degree of error (φ).

$$
\begin{aligned}
[\![P_{\text{true}}]\!] &\rightarrow \text{Grow out Treatment}_{k=60} \rightarrow P_{gk} \sim N(P_{\text{true}}, \sigma_c) \\
&\xrightarrow{\text{capillary}} f(P_{\text{ci}}, \varphi) \\
&\xrightarrow{\text{treatment}} g(f(P_{\text{ci}}, \varphi), \varphi) \\
&\xrightarrow{\text{media}} h(g(f(P_{\text{ci}}, \varphi), \varphi), \varphi) \\
&\xrightarrow{\text{plate}} \sim \text{Bi}(N_{\text{count}}, h(g(f(P_{\text{ci}}, \varphi), \varphi), \varphi))
\end{aligned}
$$

These three functions (labeled d_1, d_2 and d_3 below) can then be compared to observe the dynamics of the complete, experimental yeast-fly-interaction system from initial decision to sporulate (or not), through digestion and germination to re-establishment of the population and including effects of the experimental design (capillary tube).

$$f(P_{\text{ci}}, \varphi) = d_1 = \text{effect of capillary tube (control)}$$
$$g(f(P_{\text{ci}}, \varphi), \varphi) = d_2 = \text{effect of fly gut (treatment)}$$
$$h(g(f(P_{\text{ci}}, \varphi), \varphi), \varphi) = d_3 = \text{effect of post-treatment growth time (Grow out Treatment)}$$

A Bayesian analysis of this 3-model system was then performed using the R programming language (version 3.5.3) and R packages dplyer, tidyr, hexbin, rstan, and ggplot2. (See supplement for complete R Markdown of statistical analysis). Additionally, frequentist student's t-tests were performed on the variables of fly sex and the post-hoc biochemical analysis of aggregating and non-aggregating strains [Townend, 2013, Whitlock and Schluter, 2015].

In the Bayesian analysis, the response rate of sporulation rate was written as a reflection of the percent vegetative cells counted on the plates per treatment, and like the conceptual models above, each model describing Baseline, Control, Treatment, and Grow out Treatment, adapts the model that preceded it to reflect the added factor of that treatment. The Baseline model was adapted in the Bayesian analysis to

$$\theta_B(n) = logit^{-1}(\alpha(n))$$

where θ_B represents the percent of vegetative cells in the total number of cells, α represents a function of the initial level of bias towards vegetative cells in the starting dilution per number of observations, n. This could be a factor of dilution error or mixing error, but this value establishes the assumed *true* ratio for the remainder of the processes. The value of n represents the number of observations in any given week or data grouping. These observations are based on the number of weeks in the data group analyzed, mul-

tiplied by and the number of regions analyzed that week. This function is logistically transformed to reflect a percentage of the total cells (Colony Forming Units or CFUs) counted.

The treatment models were then written as the preceding model multiplied by the effect of the treatment. This was defined in the model as the relative rate of growth, e, raised to the power of the selection pressure of the treatment factor, rho_x. This value was then divided by the total, represented by the cells affected by the treatment $(\theta_{x-1}(n) \times e^{\rho_x})$ and the cells not affected by the treatment $(1-\theta_x(n))$. The control model was then written

$$\theta_C(n) = \frac{\theta_B(n) \times e^{\rho_C}}{(\theta_B(n) \times e^{\rho_C} + (1-\theta_B(n))}$$

where θ_C represents the percent of vegetative cells in the total number of cells, and rho_C represents the effect of the capillary tube on growth, e, of the θ_B sample. The treatment model was written as

$$\theta_T(n) = \frac{\theta_C(n) \times e^{\rho_T}}{\theta_C(n) \times e^{\rho_T} + (1-\theta_C(n))}$$

where θ_T represents the percent of vegetative cells in the total number of cells, and rho_T represents the effect of the fly ingestion and digestion on θ_C. The Grow out Treatment model was written as

$$\theta_G(n) = \frac{\theta_T(n) \times e^{\rho_G}}{\theta_T(n) \times e^{\rho_G} + (1-\theta_T(n))}$$

where theta, θ_G, represents the percent of vegetative cells in the total number of cells, and rho_G represents the effect of rich media on the germinating cells of θ_T.

These four equations which represent the complete experimental design, are accessed in the Bayesian model, based on four digit indicator vector that determines the treatment level (B,C,T,or G) of each data point. The Bayesian model updates as more values are added to each equation over three iterations but the outputs of each, above-described,

sub-model are recorded. To account for effects of dilution and strain color (red or green) as a source of bias, the parameters of β and Γ where respectively assigned to these factors and were included in the overall model. Our main focus is on the effect of the fly treatment and grow out treatment on the fraction of colonies from vegetative vs sporulated cells. We examine the posterior distributions of ρT and ρG to see if they overlap with 0 and if not in what direction they are biased.

3.3 Results

3.3.1 Quantification of Consumption

Consumption values averaged $2.48\mu L$ (27.29 mm of capillary tube volume). The initial solution solution concentration can be calculated to contain approximately 5550 cells available to the flies in each vial. (OD 0.05 = $0.055x10^7$ cells mL^-1, $\approx 0.055x10^4$ cells μL^-1, 2 capillary tubes of 5μ L each). Each micro-liter (μL) theoretically contains 550 cells per muL [Seidel, Deshpande et al., 2014].

The average consumption of the flies per vial was, therefore, approximately 1364 cells (per vial of 3 or 4 flies, see appendix A for per fly rates). The average ratio of cells on the baseline plates were 36 % spores (variance = 2826.76) and 64% vegetative cells (variance = 5304.69) so we would expect that of the 1364 theoretical cells that passed through the fly, approximately 491 were spores and 873 were vegetative. The average number of cells on the treatment plates were 71% spores (or initially sporulated cells; variance = 35243) and 29% vegetative cells (or initially vegetative cells; variance = 8858), meaning we can approximate the expected number of cells to be 968 cells from the spore group and 396 cells from the vegetative group. Taking into consideration the dilutions, we can approximate the probability of survival of spores passing through a fly gut is

Table 3.1: Descriptive Statistics of the Spore-Vegetative Survivorship Assay. Mean values of the total colony forming units (CFUs), CFUs representing the initially sporulated group, CFUs representing the initially vegetative group and the mean percent spores, per treatment plate type. Each CFU represents 1 suriving cell in the initially plated culure (post-treatment).

Plate type	mean total cells	var	mean spores	var	mean vegetative	var	mean % spores	var
Baseline	83	15266	30	3255	53	5906	39.5	11.0
Control	311	218643	134	67241	176	105245	36.1	14.1
Treatment	96	51081	68	35243	28	8858	63.2	19.2
GOT	202	1116614	84	34735	118	54342	43.6	14.7

between 71% and 91.6% whereas the probability of survival of vegetative cells passing through a fly gut is between 0.1% and 8%. These values are based on maximum and minimum survival estimates that would yield the observed percentages on the treatment plates, relative to the baseline percentages.

3.3.2 Quantification of Survival

An overall comparison of the four plate types, representing the four stages of this experiment (see Table 3.5, 3.7) indicated high variance of the cell numbers counted, but an overall trend of reduced vegetative cells in treatment, relative to the baseline and control, and increased vegetative cells in control and grow out relative to the baseline and the treatment, respectively.

3.3.3 Bayesian Analysis of Treatments

Bayesian analysis of the four treatments over all eleven weeks indicated a clear shift from the baseline ratios of vegetative to spores in each sample to the treatment, as well as a compensatory shift from the treatment to the grow out treatment (Figure 3.5). All baseline samples exhibit at least a ratio of one-to-one vegetative-to-spores with some

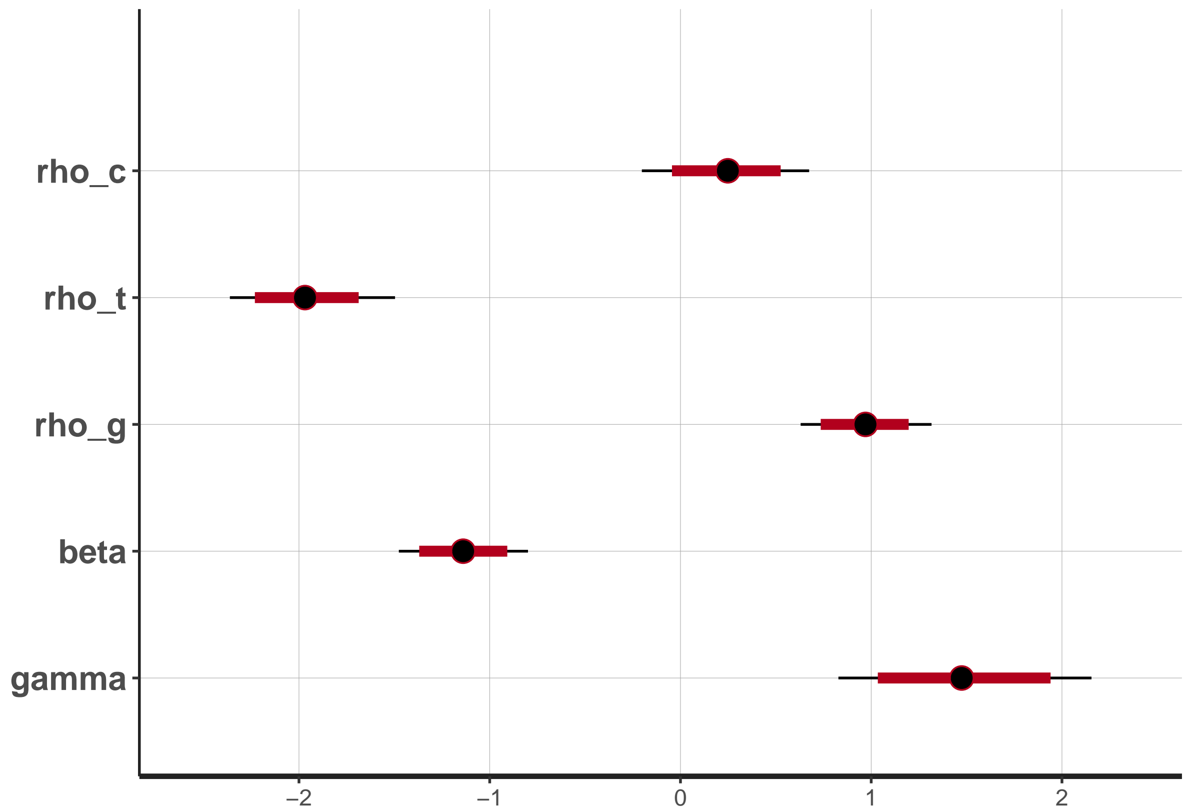

Figure 3.5: Bayesian Analysis of regionally based response from baseline plating to experimental control ρ_c (rho_c), fly ingestion treatment (rho_t), and grow-out treatment (rho_g). The effect of color assigned to the vegetative and sporulated cell(gamma, γ) and the effect of dilution level (3 orders of magnitude between 10^{-3} and 10^{-6}, beta, β. The y-value ρ (rho) represents the treatments while the x axis lists the values of ρ ratio to spores on a natural log scale where the center value, 0, represents a 1-to-1 ratio of cells that started as spores to to cells that started as vegetative cells. Increased values (up to 2) represent increased percentages of the vegetative group represented, while decreased values (down to -2) represent increased percentages of the sporulated group represented

Parameter	Estimate	sd	2.5% (lower bound)	97.5% (upper bound)	$\widehat{R}$
ρ Control	0.24	0.22	-0.20	0.67	1.03
ρ Treatment	-1.96	0.22	-2.36	-1.50	1.00
ρ Grow out Treatment	0.97	0.18	0.63	1.32	1.05
β	-1.14	0.18	-1.48	-0.80	1.06
γ	1.49	0.35	0.83	2.15	1.01

Table 3.2: Bayesian Analysis of Survivorship of vegetative and sporulated *S. cerevisiae* cells passed through the gut of *D. melanogaster* relative to those *S. cerevisiae* cells that were passed through a capillary tube only (control) and initially mixed and plated (baseline). 95% confidence boundaries are included as well as Bayesian estimates of each variable.

strains exhibiting a higher number of vegetative cells than a one-to-one ratio. The control group maintained this ratio range with a slight reduction of the vegetative values in the more extreme ratios. The treatment values drastically shifted to favor spores over vegetative cells in all regional groups. After the 24-hour grow out period, these strains returned to values closer to the baseline or control. The data set, however, exhibited high variance and contamination across the control, treatment and grow out treatment data points, specifically in weeks 1-6 (Figure 3.5). Furthermore, weeks 1-9 tested only 1 or 2 regional strains per week.

In order to improve consistency in the experimental design, the data set was reduced to include the data collected over two weeks when all 5 regions were used. Other weeks showed higher contamination and only two regions per week. This inconsistency in the first 9 weeks made it difficult to parse whether differences were due to regionality of strain or to the trial week. This reduced data set contained 1350 data points maintained the main effects of treatment but saw increased in the effects of dilution and color. (Figures 3.7 and 3.6).

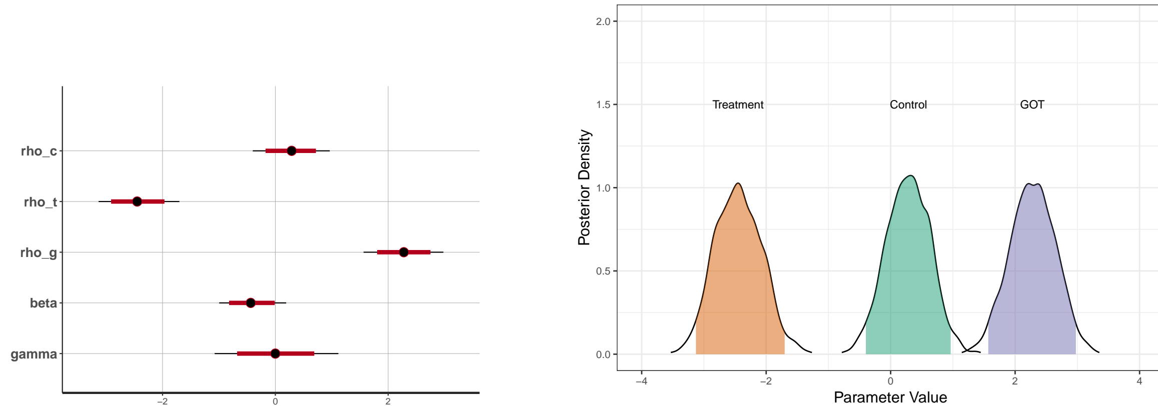

Figure 3.6: Left panel: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Beta and gamma values represent the dilution and strain color respectively. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for all strain regions, distributions represent data distributions for each treatment.

3.3.4 The effect of yeast Region on Survival

A Bayesian analysis of the effects of yeast region on the survivorship of the vegetative cells, the spores, and the total cell count indicated there was a diverse response to the treatments based on these regional differences. In the subsetted data that contained all regions tested during the same weeks, the response of each strain to the treatment was different in both direction and magnitude.

This variance began at the baseline assay, in which there were differences between the starting ratios of the Wine strains, West African strains, and North American strains near 50%. Malaysian strains and Japan Sake strains closer to a 2:1 ratio favoring vegetative. (Figure 3.7).

In the subsetted data group, the wine strain slightly dropped to favor spores in the control group but returned to a one-to-one ratio in the treatment group. Overall, the wine strains in the larger data set was minimally responsive to all treatments (Control, Treatment and GOT). In the subsetted group containing only weeks that analyzed all strain regions together, the European wine strain elicited a response similar to the overall

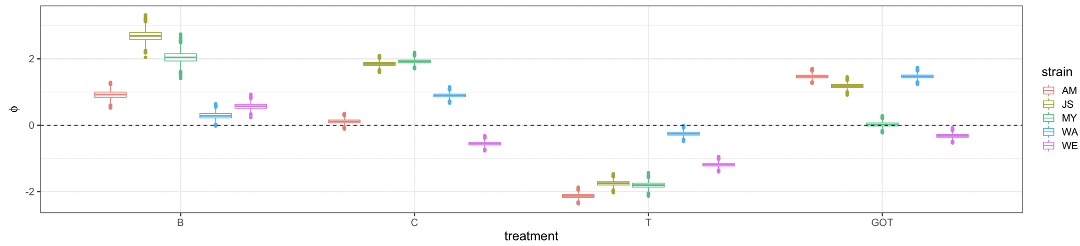

Figure 3.7: Box-plot of the sub-setted data (weeks 10 and 11) of the four stages of the survivorship assay. B represents Baseline (one-to-one mixture), C represents Control (effect of capillary tube and transfer, no flies), T represents Treatment (effect of capillary tube and flies), and GOT represents Grow out Treatment (paired effect of post-digestion growth in rich media). The y-value ϕ represents the ratio to spores on a natural log scale where the center value, 0, represents a 1-to-1 ratio of cells that started as spores to to cells that started as vegetative cells. Increased values (log scale, up to 2) represent increased percentages of the vegetative group represented, while decreased values (log scale, down to -2) represent increased percentages of the sporulated group represented

response. The control group showed slightly lower numbers of vegetative cells than the overall response and a reduced response to the grow out treatment, having fewer overall cells and only a two-old increase of vegetative cells rather than a four-fold increase as seen in the larger data-set (See Appendix A).

The West African strain showed an increase in vegetative group strains in the control group, but recovered back to a one-to-one ratio in the treatment group. In the grow out, the vegetative cells again increased to favor the vegetative cell group. The response was dramatic but began favoring the vegetative group. In the subsetted data set, the West African strain showed more dramatic responses to both the treatment, favoring sporulated strain groups, and the grow out treatment, favoring vegetative strain groups (See Appendix A).

In the larger data set, the North American strain was minimally responsive to the control group but dramatically responsive to both the treatment and the grow out treatment groups showing a increase in the spore group in the treatment and an increase in the vegetative group in the grow out treatment. North American strains in the subsetted

group showed minimal response to treatments of the capillary tube (control) or ingestion of the fly (treatment) but while the magnitude of the response was reduced, the direction of the response was similar to the main effect: the control values raised to favor vegetative cells in the ratio; treatment values lowered to favor sporulated cells in the ratio; and grow out treatment values raised to favor vegetative cells in the ratio. In the North American strain, there was no notable effect of dilution or color on the counts (See Appendix A).

The Japanese Sake strain's control value dropped slightly toward a more equal ratio of spores to vegetative cells in the larger data set as it began with higher relative values of the vegetative cell group. The treatment set showed a decrease of the vegetative cell group which rebounded to about a one-to-one ratio in the grow out treatment. In the subsetted data, this strain showed some response to the treatment by reducing its ratio value to favor the sporulated group, but this strain also responded to the grow out treatment by returning to values almost identical to the control values (See Appendix A).

The Malaysian strain exhibited a minimal response to treatment in the larger data set. There was some increase of the vegetative cell group in the control set but this was reduced to about a one-to-one ratio in the treatment set. In the subsetted data set, the Malaysian strain also showed a limited response to all treatments, with control and grow out treatments remaining at the 1-to-1 ratio mark (rho=0, x axis) and the treatment value reducing slightly to favor the sporulated strain group. (See Appendix A).

3.3.5 The effect of fly sex on survival

We found that the percent spores in the yeast treatment sample did not differ significantly between the two sexes of flies ($t = 0.66462$, $df = 1261.7$, p-value $= 0.5064$). There was also no significant difference between the total number of cells plated in the treatment plates ($t = -1.503$, $df = 1105.7$, p-value $= 0.1331$).

3.3.6 Grow out Treatment

The grow out treatment was an assessment of the ability of the cells that had passed through the gut of the fly to recover and grow in rich media. In the Bayesian Analysis, the main effect was that the mean total number of cells in the system doubled from the post-digestion frass collection. The ratio of initially vegetative to initially sporulated cell groups changed from a spore favored ratio to a vegetative-favored ratio, and closely resemble the ratio of the control (Figure 3.7). When sub-setted into regions, the grow out treatment effects varied in terms of both rate of growth (total number), ratio of spore to vegetative groups (differential fitness) and variance of response as described above in the regional analysis(Tables in Appendix A).

3.4 Discussion

3.4.1 The effect of Treatment

The limited change between the percent spores on the baseline plates and the control plates is an indication that the change seen in sporulation percentage on the treatment plates is due to the presence of the fly and not the time in the capillary tube. If anything, time in the capillary tube seemed to increase the number of vegetative cells which is likely due to some individuals in the vegetative group attempting to replicate despite extremely limited resources.

The clear increase in the percent spores between the baseline or control and the treatment plates substantiates Coluccio and colleagues' claim that there is a strategic advantage to being in the sporulated state when passing through the gut of the fly. It may also support the work of Reuter and colleagues in that one spore, which is a tetrad of haploid cells, may have become up to four free cells if the tetrad was freed from its

ascus because of digesting [Reuter et al., 2007]. In short, the process of digestion capable of quadrupling the number of cells in sporulated state, regardless of survival differences.

The overall increase in the vegetative cell group relative to sporulated cell group in the grow out treatment is an indication that there is likely a lag in the germination time of spores relative to re-initiated mitotic division of already vegetative cells. This lag time may be small, but sufficient to allow one more doubling event in the vegetative cell group. The grow out treatment results counter the benefits of survival of spores in that they show that vegetative cells that do survive the gut –or perhaps are vectored to the new patch space by corporeal transfer– are capable of proliferating in the new patch space rapidly, surpassing the once-sporulated cells, despite their recent germination. This oscillation of fitness values based on the varying environmental conditions of two events (dispersal or growth) may facilitate a trade-off through the storage effect [Warner and Chesson, 1985] and therefore coexistence between the two, fast-sporulating and slow-sporulating ecotypes. The relative population sizes on the treatment and grow-out-treatment plates also suggest that the timing and frequency of the fly interaction with, and the ingestion of, the yeast is a formative factor in the composition and coexistence of these two yeast phenotypes in one community. It may be the case that there are communities, like vineyards, that experience high rates of ingestion where slow-growing (fast-sporulating) yeasts are the dominant phenotype in the species composition because they have a higher probability of surviving frequent ingestion and deposition within the same community. Conversely, yeast residing in Oak woodlands which may not experience frequent visitation of and ingestion by insects may exhibit a community composition in which the slower sporulating cells are dominant, or at least seasonally dominant. Thus, we would expect that insect-facilitated coexistence between slow and fast-sporulating strains of yeast will occur within a range of insect visitation frequencies.

In discussing the results of this experiment and how they apply to natural systems,

it is important that we address the unnatural state of this experiment. The goal of this experiment was determine whether one aspect of the yeast cell's phenotype: the cell's states of vegetative or spore, helped in survival of the yeast during gut vectoring. These cells, were therefore, only different based on cell state. In nature, cells that sporulate faster may have additional differences than cells that sporulate slower; thus, this study can only infer outcomes based on cell state and not the physiology of the two different phenotypes.

3.4.2 Regional effects

The regional strains that showed baseline deviations from the calculated one-to-one ratio may have been due to experimentor error but also may have been a factor of the individual strains. The Japan sake strain, for example, is a strain that has adapted to evade sporulation and may be less efficient at germination.

The change in spore to vegetative ratio between baseline and control remained the same for Malaysian strains. It is likely that these Malaysian strains remained in stasis, waiting out the period of low resources as they would in nature. The Malaysian strain is well-described for its atypically large amount of cellular aggregation [Louvel et al., 2014]. The Malaysian strains ability to aggregate may also increase its resilience to these low resource environments, similar to the events of quorum sensing and bio-film formation. The North American strain, which was expected to be the most responsive environmental cues for sporulation, showed an increase in the number of spores even from baseline to control. When considering that these cell types were already in their sporulated and vegetative states and the end results do not represent necessarily who sporulated and who was vegetative but who started as a spore and who started as vegetative, this result requires more analysis, controlling for both starting state and monitoring of state

throughout the protocol.

Both European (wine) and Japanese (sake) strain are commercial strains, artificially selected to grow in minimal resource conditions without sporulating [Borneman et al., 2013]. Whereas many strains might initiate sporulation or cease mitotic -entering quiescent latent stage, but not sporulating- in the absence of sufficient resources, these two strains often attempt to remain metabolically active, even mitotically dividing pseudo-hyphally [Zaragoza and Gancedo, 2000, Gancedo, 2001]. This trend might result in an increase in vegetative cells; however, this limited additional pseudo-hyphal growth would likely not be apparent as the pseudo-hyphal cells are only partially separated and thus, would likely stay together on the plate, resulting in one C.F.U. counted for many pseudo-hyphal divisions. At the same time, we might also expect a resultant increase in spores in these commercial systems with already limited resources. The impetus of sporulation is the presence of higher concentrations of metabolic byproducts and Nitrogen as well as low concentrations of glucose. Even though this spent media is low enough to prevent germination of spores, it is typically not low enough to initiate sporulation in the vegetative cells, only to severely reduce further vegetative reproduction in most strains [Neiman, 2005]. For these strains that are able to glean a few more reproductive events in this media (like the commercial strains), the biochemical environment is further altered by this metabolic activity and these changes may be enough to initiate some sporulation in the vegetative cells. Such a scenario might be the case in the Japanese sake strain, in which some of the vegetative cells may have sporulated, preserving resources and promoting viable perceived vegetative cells at the time of plating. Thus, the resultant high, but reduced value of vegetative to spore ratio in the Japanese Sake strain may be caused by either or both of these idiosyncratic characteristics which could reduce the perceived number of cells representing the vegetative group.

The European wine strain also shows a reduction in its relative number of vegetative

cells, rather than an increase. This may be due to pseudo-hyphal growth misrepresentation (mentioned above), but may also be the result of weakening of the vegetative cells who have used reserve stores of energy that normally would be used to sustain each cell to germination. Therefore, in the case of the wine European strain, sustained metabolic activity may have reduced the viability of the cells, resulting in few vegetative cells in the control group.

Grow out treatment values were relatively consistent across regions, but there was a regional difference in the rate of response from treatment to grow out treatment, indicating there may be regional differences in the ability for these yeast strains to participate in storage-effect-based coexistence [Snyder and Adler, 2011] The most rapid recovery of vegetative cells from the treatment to the grow out treatment was in the North American strain, a strain that is wild-derived and potentially more robust to harsh conditions such as ingestion and digestion regardless of cellular state. Conversely, the commercially adapted European Wine, for which selective pressures of this type have been relaxed over its generations of artificial selection, showed the slowest recovery of vegetative cell ratio. The other three regional strains showed similar vegetative response rates.

Regional differences suggest that differences in the genetic background have some effect on the ability to survive a fly gut beyond the ability to sporulate [Louvel et al., 2014, Mortimer, 2000, Pronk, 2002, Warringer et al., 2011]. Evidence of greater numbers of vegetative cells in some strains may be an indication that fewer spores of that strain survived overall or that more vegetative cells in that strain were able to survive. In certain strains, where insect ingestion is a common selective pressure, some strains may have evolved a strategy to reduce gut-passage mortality while preserving their ability to proliferate. This adaptation would by-pass the potential sporulation rate trade-off we propose typically occurs as a coexistence mechanism in phenotypically diverse yeast communities. We expect this strategy to arise in yeasts regions that experience insect

interaction at an intermediate or unpredictable frequency.

The true effects of regionality may be apparent in differences in sporulation rate or each regional strains adaptability to change this rate. In this study, sporulation was forced on cells and thus, the strategy of each region to sporulate earlier or later is not addressed. It is well-documented, however, that these five strains do exhibit substantial differences in their sporulation efficiency [Cubillos et al., 2009, Louvel et al., 2014, Liti et al., 2009].

Another characteristic that Louvel and colleagues note in their analysis of these five regional strains was the differing levels of aggregation ability [Louvel et al., 2014, Vallejo et al., 2013]. Could this mysterious and variable secretion by the cells of different regions be adaptive for protection against environmental hazards such as passage through the fly gut?

Yeast cells, like most of their fungal relatives possess chitin-based structural molecules and rely on chitin to provide protection and structure in their sporulated state [Orlean, 2012, Briza et al., 1990]. It is more likely that this aggregating secretion is at least partially composed of chitin, given its already prolific usage in the yeast cell wall [Madhani, 2007, Neiman, 2005, 2011]. Rubin and Waite have shown the ubiquity of chitin as a protective structure in many taxonomic groups [Rubin et al., 2010]. In marine organisms, these structures are often rigid externally but can be excreted in the dermal tissues as a gelatinous liquid. We performed a post-hoc analysis on the aggregating cells to explore the relationship to chitin and this protective excretion. In the case of the Malaysian strain, which has the highest propensity to aggregate and previously described evidence of changes in cellular chemistry relative to other regions [Lee et al., 2013], a comparative analysis of the cells' biochemistry was performed to indicate any differences in the Nitrogen-to-Carbon ratios Comparison to a non-aggregating strain (Wine/European) indicated that there was not a significant difference in Carbon values (two Sample t-test,

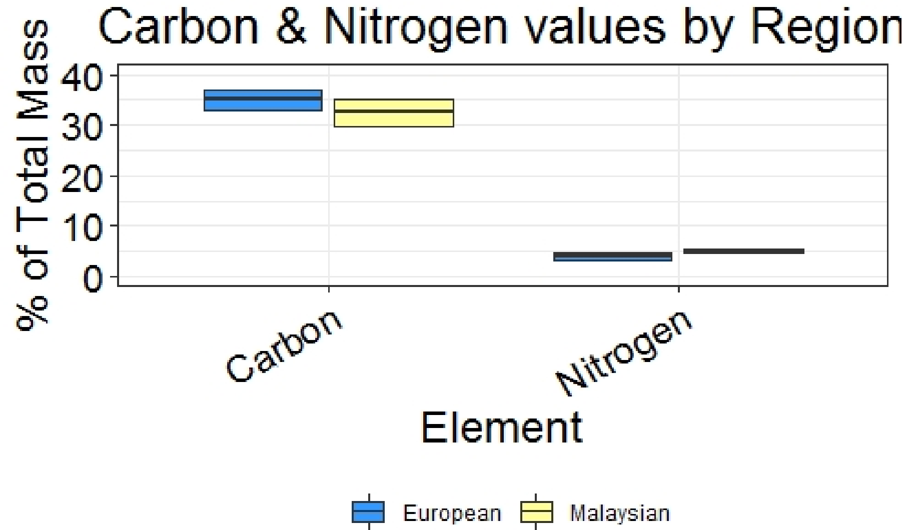

Figure 3.8: Carbon and Nitrogen Analysis of severely aggregating (Malaysian, yellow) and non-aggregating (European/Wine, blue) strains.

$t = -1.9933$, $df = 6$, $p-value = 0.09329$) or Nitrogen values (two Sample t-test, $t = 1.2526$, $df = 6$, $p-value = 0.257$) when assessing the values as percentages of the total; although, there were significant differences in both Carbon and Nitrogen (Figure 3.8) when considering only mass values (See Appendix A for table)

3.4.3 The effect of fly sex on survival

Insect biochemistry, including digestive processes, has been shown to be different based on the sex of the insect [Ballard et al., 2007]. Furthermore, female insects may benefit differently from the incomplete or complete digestion of the yeast that it ingests, relative to the benefits a male insect may receive. For example, a female *Drosophila* that ovaposits and defecates in the same area may have developed the strategy to allow surviving yeast to pass through the gut, so these yeasts can proliferate and become a resource for the *Drosophila*'s hatching larvae [Barker and Starmer, 1999]. Conversely, it may be advantageous for female flies to digest as much of the ingested yeast as possible

in order to convert this resource to energy to make more eggs [Alpatov, 1932]. Males may also benefit from the complete digestion on food in order to have more energy for courtship rituals [Yuval et al., 1998]. While there was no significant differences between survivorship of spores and that of vegetative cells alone, when factoring in differences in consumption, there may be a difference in these values. Overall, the female flies consumed twice as much volume of food as the male flies, but the number of CFUs on the treatment plate were not significantly different. It is likely that larva are capable of consuming both live and dead yeast cells for nutrition as well as the raw materials digested by the mother fly. Given the rapid replication rate of a yeast cell, even post-digestion, as shown by the grow out treatment and our knowledge of the development time of a larval fruit fly, it may not be necessary for female flies to adapt to allow yeast cells to survive the gut as just one surviving cell can propagate sufficiently by the time the *Drosophila*'s eggs hatch.

3.5 Conclusions

In consideration of these results, it is clear that both genetic background and current environment of an *S. cerevisiae* cell affect its ability to survive fly-gut vectoring. The work of Coluccio and colleagues and Reuter and colleagues give a more narrow, and perhaps inaccurate, estimate of a yeast cell's probability of survival. The results of this study further support the results of many ecological and evolutionary studies [Calcagno et al., 2006, Yu and Wilson, 2001]that illustrate eco-evolutionary processes are idiosyncratic to the the system. The dynamics illustrated over the four sections of this study also illustrate that the fly-gut vectoring process is adaptive to different yeast states at different stages of the process. This is not an essential factor, but a common aspect of successfully implemented ecological trade-offs that elicit coexistence [Tilman, 1990]. Essential in

determining the level of coexistence capable of in this system is the lag time from spore to dividing vegetative cell post-digestion. Based on the grow out time, it is clear that despite the increased mortality in the vegetative cell group, that group was capable of recovering its numbers in 24 hours, resulting in another coexistence strategy: the storage effect [Warner and Chesson, 1985, Snyder and Adler, 2011]. Along with further analysis of the germination lag-time of the spores, the frequency of ingestion also plays a clear role in the the population's phenotypic composition. If these yeasts are ingested frequently, the system may already be exhibiting its coexistence frequencies, as the strategy for slow-sporulating yeast cells may be to reproduce as much as possible allowing for increased mortality as kin selection. Alternatively, the fast-sporulating yeast cells may utilize the strategy of always having fewer overall cells in the population but with the benefit of decreased mortality. These questions may be, and will be, further analyzed in a model of coexistence with varying system parameters such as sporulation rate, growth rate and resource utilization.

3.6 Acknowledgements

We would like to acknowlege Fernanda Pett for her exquisite diagrams of our experimental design. We would also like to extend our sincerest appreciation to Kathleen J. Luxmore, Helena Brantz, Kate McKee, Francis Wang and the 35 undergraduate researchers that helped with this work.

Chapter 4

Experimental Evolution: Evolutionary Effects of Long-term insect vectoring in Yeasts of Regionally Diverse Backgrounds

4.1 Introduction

Eco-evolutionary dynamics is the intersection of ecological and evolutionary processes; they describe how ecological change affects evolutionary processes [Kremer and Klausmeier, 2013, Bolnick], and how evolutionary events reshape ecosystems [Webb et al., 2002, Fussmann et al., 2007]. Rather than considering the fields of Ecology and Evolution separately, considering the two fields as one inter-connected system may improve purely ecological or evolutionary viewpoints and provide scientists with answers to questions not answered when addressing their study field in isolation [Webb et al., 2002], but are these two fields always paired? At what point do ecological processes become evolution-

ary process, or are they always both? Specifically, ecological mechanisms of coexistence, those that deter or reduce competitive exclusion of one group by another, may contribute to evolutionary processes such as divergence and speciation. Coexistence mechanisms are diverse in their function and can reduce competitive pressures through niche portioning [Sage and Selander, 1975, Levine and Rees, 2002], prey-switching [Kuang and Chesson, 2008, Roughgarden, 1974], and natural history effects such as trade-offs or the storage effect [Tilman, 1982, Warner and Chesson, 1985, Chesson, 1986], but does this reduction in competitive pressure always indicate a change in selection pressure, and if so, shouldn't all coexistence mechanisms lead to selection events?

Ecological trade-offs are one type of mechanism used to explain coexistence between species with substantial niche overlap (limiting similarity) and populations of competitive conspecifics which would, in the absence of a coexistence mechanism, competitively exclude the inferior population [Tilman, 1982, Macarthur and Levins, 1967]. Trade-offs in life-history traits – such as abilities to find or process food or the ability to successfully disperse – can allow two or more types of competing species (or genotypes within one species) to be competitively superior to the other in alternative environmental conditions, so that when the environment itself fluctuates then both populations can increase in density when rare [Cadotte et al., Kremer and Klausmeier, 2017]. Trade-offs can be physiological, such a limitation of body size as a result of energy invested in a new feature, or behavioral, such as using a mating strategy that increases mating opportunities but decreases lifespan Alonzo and Warner [1999]. The competition-colonization trade-off, as described by Tilman within a narrow range of parameter values, allows for universal, stable coexistence between two or more species in a grassland community [Tilman, 1994]. Trade-offs, like other ecological mechanisms of coexistence, may also promote evolutionary processes such as divergence or speciation.

Yeasts are one group that may utilize trade-offs to maintain coexistence among species

of limiting similarity in microbial communities. Yeasts are able to reproduce by mitotic division in both their haploid and diploid states. Both haploids and diploids exist in nature but only diploids are capable of initiating sporulation, the process by which the cell undergoes meiotic recombination and forms a protective outer layer around the tetrad of newly derived haploid cells [Neiman, 2005]. Sporulation is initiated in diploid yeast cells when the cell senses both decreased levels of glucose and increased levels of Nitrogen [Madhani, 2007, Neiman, 2011]. The sensitivity to changes in these resources–and the resource level at which the individual spore initiations sporulation– is genetically derived and varies across individuals in the population. Furthermore, yeasts in the genus *Saccharomyces* live frequently with other species within the genus and are all recently diverged sister taxa, indicating a high likelihood of sympatric divergence. Thus, yeast are ideally characterized to explore the intersections of ecological and evolutionary processes [Botstein and Fink, 2011, Hittinger, 2013].

Yeasts exhibit a potential ecological trade-off based on their sporulation strategy in that cells which transition through meiosis into the spore state do not continue to consume available resources and divide, but spores are resistant to some negative environmental conditions, including the gut environment of insects [Neiman, 2011]. Coluccio and colleagues found that yeast cells in their sporulated state are over three times as likely to survive ingestion by an insect [Coluccio et al., 2008]. Reuter and colleagues also suggested that spores ingested by insects gain a genetic benefit from higher rates of out-crossing, finding that yeasts passsaged through flies had a ten-fold increase in the number of out-crossed individuals in the population [Reuter et al., 2007]. This significant increase in out-crossing in spores passed through the fly gut may contribute to the population's adaptability by allowing diversification in the event of dispersal to new environments [Berkley et al., 2010, Bell and Gonzalez, 2009b, Kremer and Klausmeier, 2013]. The studies of Coluccio and colleagues, and Reuter and Bell illustrate how ecological

species interactions can result in differing selection pressures on the same species, and promote changes in recombination within those species. Yeast, in particular, are likely to show effects of these differing selection pressures through their evolutionary trajectories. Yeasts are capable of clonal reproduction in both haploid and diploid states but are mostly diploid under natural conditions [Tsai et al., 2008]. Furthermore, haploid yeasts in the *Saccharomyces* complex, such as *Saccharomyces cerevisiae* are capable of switching between the two mating types (α and A) during replication, thereby producing a viable mate with which to form a diploid [Madhani, 2007]. This ability to both self and recombine allows individual yeast cells that have become isolated to persist without needing to find a mate, but also allows the biological species concept to be used to determine new species Mayr et al. [1963].

There is evidence of frequent divergence and speciation within the group of yeasts in the *Saccharomyces* complex exhibited by a high degree of limiting similarity between yeast species, with only one or two key differences in life-history, behavior or physiology that separate them [Sweeney et al., 2004, Murphy and Zeyl, 2012]. It is likely that they diverged in sympatry based on their closely aligned genetic and ecological profiles [Gonçalves et al., 2011, Dettman et al., 2007]. These recently diverged sister taxa have evolved different optimal growth temperatures [Gonçalves et al., 2011], or in the case of *S. paradoxus*, have evolved to initiate different timing of reproductive events [Murphy and Zeyl, 2012]. Both of these adaptations may allow the two populations to gain competitive ground depending on the fluctuating environmental conditions [Bohannan et al., 2002]. This differences may have initially functioned as mechanisms of coexistence, but eventually gave rise to divergence and eventual reproductive isolation [Bush, 1994]. In natural settings, insects can act as vectors of yeasts and other microbes [Stefanini et al., 2012, Klepzig and Hofstetter, 2011, Gibbs and Stanton, 2001]. Because genotypes that sporulate earlier have fewer cell divisions but produce more spores (See section 3.1),

there is a natural ecological trade-off that depends directly on the timing of sporulation. The ecological connection between insects and microbes is well-studied and ubiquitously-observed [Stefanini et al., 2012, Ort et al., 2012, Morais et al., 1994]. If spores survive more often, the insect gut acts as a sufficient source of selective pressure, and insect ingestion is frequent enough then the ability of a yeast genotype to sporulate faster will be under selective pressure and is expected to evolve analogously to a behavioral strategy.

Members of the *Drosophila* genus of tephrid flies have evolved to interact with yeasts. They show differential preference for yeasts within the Saccharomytina clade [Dobzhansky et al., 1956, Barker and Starmer, 1999], and preference for yeasts at specific life history stages of the yeast [Schiabor et al., 2014]. The fly's preferences for different yeast types also change as the fly progresses from larval to adult stages itself [Morais et al., 1994]. The yeast can affect the fly's development and adult food preferences [Anagnostou et al., 2010]. The fly, in turn, affects the yeast species composition [Coluccio et al., 2008], its out-crossing rate [Reuter et al., 2007] and the community composition of that yeast [Stamps et al., 2012]. Although there is much evidence of the ability of insects to shape these microbial communities [Stamps et al., 2012, Orlean, 2012], less is known about how passaging through insect guts drives evolution of traits within a single species of yeast. As Coluccio and her colleagues observed, there is differential survivorship between spores and vegetative cells as they pass through the gut of *D. melanogaster*. This differential survivorship facilitates a trade-off between being a better competitor and being better at dispersing by gut-vectoring. This competition-colonization trade-off was described by Tilman as a promoter of universal coexistence between groups [Tilman, 1994]. Moreover, in populations that exhibit differential sporulation rates, the increase in recombination rates in early sporulating portions of that population, paired with the differential success of spores to vegetative cells, may promote divergence within the yeast population. In order for increased sporulation rate to evolve in response to fly ingestion there must be a

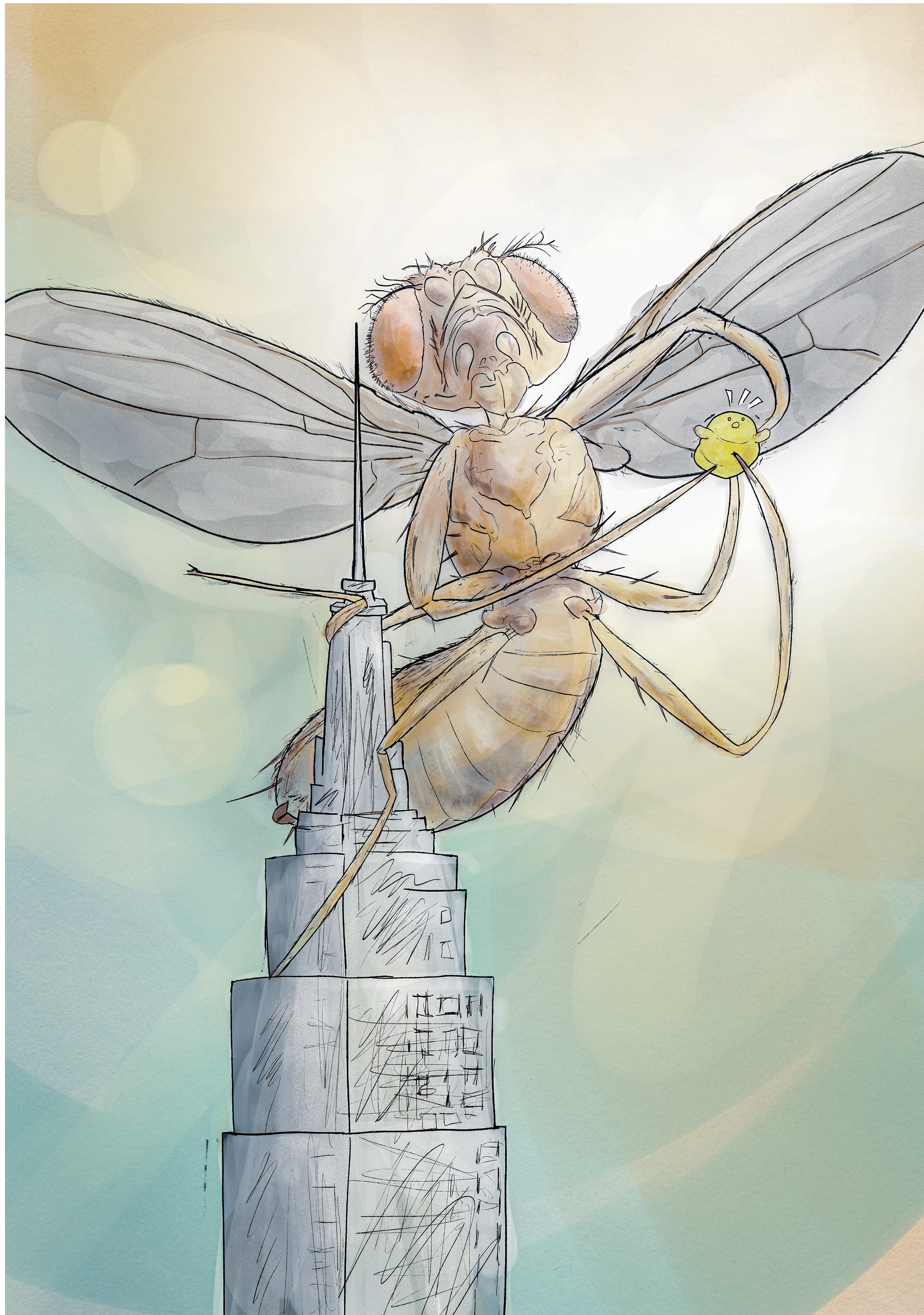

Figure 4.1: Yeast vectoring by insects can be traumatizing and dangerous, especially if the yeast is not in its sporulated state. Fly-Kong and his yeast victim. Drawing Credit: Taom Sakal

net benefit of early sporulation as well as genetic variation for the sporulation strategy.

In order to explore the connection between ecologically-based coexistence mechanisms and evolutionarily-based divergence processes, we performed evolution experiments assessing if a trade-off in *S. cerevisiae* would reduce competitive pressure just enough to promote coexistence, or enough to change the selective pressures in the system, facilitating evolution and divergence. We repeatedly exposed yeast cells to ingestion and digestion by the fruit fly *Drosophila melanogaster*, over the course of 31 one-week cycles and measured the phenotypic response in terms of sporulation timing. We used a set of regionally diverse and genetically distinct strains of *Saccharomyces cerevisiae* with different historical selection pressures cultured and adapted by Liti and Louvel [Cubillos et al., 2013, Liti et al., 2009, Louvel et al., 2014]. We compared phenotypic characteristics of yeast using multiple replicates of each of these regional strain lineages repeatedly exposed to an insect vector (treatment) relative to those same characteristics of the same strains repeatedly transferred by pipette (control).

4.2 Methods

4.2.1 Ancestral Strain production

We used a set of five genetically distinct strains of *S. cerevisiae* that were collected from five global regions, haploids were transformed with a genetic barcode and antibiotic marker, then cloned, and back-crossed to their transformed clones to form homozygous diploids. All strains were wild isolates or wild-derived (commercial) isolates transformed with resistance to Geneticin (G418) a yeast orthologue to Kanamycin [Louvel et al., 2014]. These five strains were chosen both because of their diverse ecological backgrounds and because their phenotypic and genetic characters have been well documented and studied.

Additionally, because this strain set was derived by the same working group at the same time [Louvel et al., 2014] we can be more certain that the laboratory adaptations to these strains were performed uniformly across all five regional groups. Because of this consistency in alteration, we can be more certain that the regionally-based differences from this evolution experiment are due to regional backgrounds and not laboratory artifact [Elena and Lenski, 2003, Rice and Hostert, 1993]. The five regional strains came from an Oak woodland in the Northeast united states (North American strain, AM), a winery in Western Europe (Wine European, WE), a recently formed brewery in West Africa (West African, WA), a Sake brewery in Japan (Japanese Sake, JS) and a palm blossom in Malaysia (Malaysian, MY).

For each ancestral strain, we created four replicates by inoculating into YPD (Yeast-Peptone-Dextrose) broth culture and growing for five days with shaking (230 rpm) at 30° C. This allowed the population to utilize the majority of metabolic resources in the rich media and created an environment where individuals in the population began to sporulate based on biochemical cues of low glucose, high nitrogen and high metabolic byproducts. The cells in each population are likely in one of three states at the end of 5 days: late growth phase, when the cells are still attempting to divide mitotically (vegetatively) but a slower rate because of limited resources, stationary phase, when cells have ceased division and may be entering the meiotic process of sporulation but have not formed spores yet, and the sporulated phase when the meiotic process is completed and the protective outer covering of the spore is forming or formed. These five populations were expected to exhibit different ratios of these three stages at five days of growth time. These different ratios are based on the different genetic backgrounds of each strain. However, each regional strain showed at least 20% but no more than 70% sporulated cells at this five day period, and these percentages varied by strain. We chose to use five days of incubation because based on observations from a pilot study and because

of previous reports of the strains' sporulation efficiencies [Liti et al., 2009, Louvel et al., 2014]. In order to reduce chances of contamination from other yeasts or bacteria, cultures were always grown in YPD media with G418, tetracycline, and ampicillin added. While haploid cells are often used in long-term experimental evolution [Frenkel et al., 2015], we used diploids because sporulation is only initiated in diploid cells [Madhani, 2007, Neiman, 2011], and diploidy more accurately describes yeast in their natural state [Tsai et al., 2008]. Additionally, using diploid cells in long-term evolution allows us to explore the role of out-crossing in this system. In typical yeast systems, out-crossing plays a role in approximately 1% of all reproductive events [Murphy and Zeyl, 2010, Magwene et al., 2011], but in systems where yeast is vectored and dispersed by insects, this number may be increased [Reuter et al., 2007] and therefore out-crossing may play a larger role in the population genetics of vectored yeast communities [Ruderfer et al., 2006].

4.2.2 Fly rearing and CaFe apparatus assembly

D. melanogaster stocks were created by out-crossing strains from isogenic Al-Ral, Taiwanese, Santa Barbarian and Malaysian lines. This was done to increase robustness of the fly lineage [Wagner, 2000, Shull, 1948]. Flies were allowed to lay eggs on YPD agar plates. Adult flies were then removed and the eggs were bleached using a 10% bleach solution for 40 minutes at 22° C (See Figure 3.2). Fly eggs were then collected by sterile pipette, washed with sterile water, and transferred using sterile technique to clean media that was free of anti-fungal factors such as Tegosept ™. Clean flies were reared and propagated on this media so that other yeasts, fungi and bacteria were minimized and did not confound the results [Reuter et al., 2007], but also so that the ingestion of antifungal elements did not reduce the viability of living yeasts traveling through the gut.

Fly Capillary Feeder (CaFe) apparatus tops were assembled per design adapted from Ja and colleages ([Ja et al., 2007] (Figure 3.3) using four 200μL pipette tips which were cut to increase opening size, rubber stoppers and standard rubber bands (See appendix A). These CaFe tops were then affixed to narrow fly vials each containing 2ml of 3% solidified agarose solution to maintain humidity within the vial. The pipette tips within the CaFe top were fitted to one 5μL capillary tube each; however, during treatment, only two capillary tubes were used and two were left open for filtered airflow. 18 hours prior to treatment, four clean, sexed flies were added to each of four vials per replicate. This starvation period was added to ensure sufficient consumption of yeast by the flies during treatment [Reuter et al., 2007]

4.2.3 Selection Experiment

Diploid G418 resistant strains were grown in 2 milliliters of antibiotic media over a 120-hour (5 day) period. Samples of these initial strains were then frozen in 15% glycerol solution at -80°C. Each strain's optical density was measured using the Tecan optical density reader and Magellan (V 7.2) software set to a wavelength of 600 nanometers. These values were then recorded for analysis. All replicates were then appropriately diluted to an optical density of 0.3 in an effort to both ensure adequate population size throughout the experimental procedure [Shaffer, 1981] and to prevent blockage in the capillary tube caused by high cell density. The dilution process was performed using YPD that had been depleted of its sugar and Nitrogen by culturing yeast in the media for 2 weeks, filter sterilizing, re-innoculating with yeast for 1 week and filter sterilizing again. This spent YPD solution (SYPD) was then amended with the same antibiotic cocktail of G418, Ampicillin and Tetracyclin (SYPDA). SYPDA was used instead of YPDA to mimic carbon and nitrogen sources in a late growth stage population and to

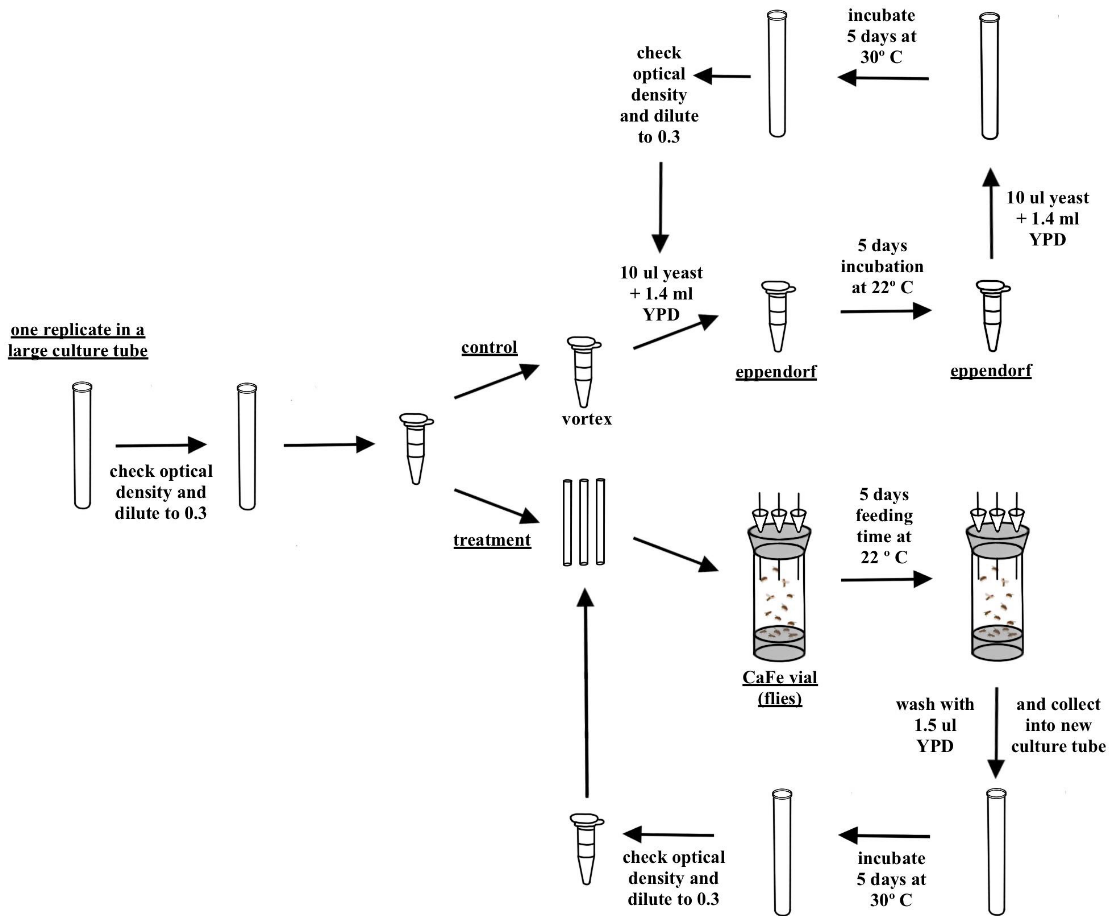

Figure 4.2: Experimental design of the Long-term Experimental Evolution Experiment (LTEE). Each lineage was grown in nutrient-rich broth and then the initial growth sample was adjusted to an optical density of 0.3 and split into control and treatment Eppendorf tubes. From that point, the control and treatment went through parallel procedures lasting 7 days: 2 days of exposure to treatment (control: 22°C incubation; treatment: 22°C exposure to flies) and 5 days of growth at 30° C. Initial (Ancestral) generations and all odd generations were frozen in 15% glycerol at −80° C.

prevent initiation of state change of any of the cells [Madhani, 2007, Neiman, 2005].

The five initial concentration-adjusted samples of the regional strains were then proportioned into 4 replicates each. Each replicate was then split into two Eppendorf tubes: a control sample and a treatment sample, and two cryotubes: a control sample and a treatment sample, for freezing. Each control sample remained in the Eppendorf tube and was placed in proximity to the treatment vials (same ambient conditions) for the duration of the treatment time. After 48 hours, each control tube was vortexed and 10μL of each control was moved to 1.49 mL of YPDA in a new culture tube (1.5 mL total volume). This new culture tube was labeled with experimental replicate number (lineage), control label, and the proceeding generation value ($G + 1$).

Each concentration-adjusted treatment was offered to 3-4 clean, sexed flies using a CaFe apparatus. Flies were allowed to feed for 48 hours and then removed from the vials. Measurements of total fly food consumption were taken by recording the change in meniscus of the two capillary tubes. The vials containing fly fecal material (frass) and body transfer yeast were then rinsed with 1.6 mL YPDA media and the supernatant (1.5 mL total volume because some volume is reabsorbed into the agar in the vial) was collected. This new culture tube was labeled with experimental replicate number (lineage), treatment label, and the proceeding generation value ($G + 1$).

Each new generation of control and treatment tubes were then incubated at 30° C for 120 hours without shaking to allow yeast to form diploids and grow. After incubation, the optical density adjustments, mentioned above, were repeated using established lineages (1-20) of all strains, both control and treatment. This process was repeated for 30 generations (treatment cycles) freezing every odd sample (G_1, G_3 etc...) of both the treatment and control lineages after the initial (G_0) samples in 15% glycerol at $-80°$ C.

4.2.4 Assessment of Sporulation Rate

Single colony isolates of the Ancestral (G_0), and evolved treatment and control (both at G_{31}) strains were grown for 6 hours without shaking and then assessed for their optical densities. Equal concentrations (2 mL of media at an Optical Density of 1.5) of each ancestral, control, and treatment replicate were then washed and sporulated in $2mL$ of Potassium Acetate (2% KAc at pH $\approx$ 6.7), and incubated at 30° C with shaking (230 rpm). Sporulation percentage was checked and recorded at 2.5 days (midpoint) and 5 days (endpoint).

The sporulation assay was performed in two experimental blocks with each block containing samples of all treatment and control replicates at 31 generations. Two single colony isolates for each of 20 experimental replicates (evolutionary lineages) were taken for both the control and the treatment evolved strains. Two technical replicates were taken of each colony isolate for a total of 160 samples. For the ancestral strain, three single colony isolates were taken for each of the 5 represented lineage backgrounds, which were previously frozen at the start of the experimental evolution procedure to ensure comparison of a true ancestral strain. Two technical replicates were taken for each of the single colony isolates, these samples were also repeated across two dates for a total of 60 samples. At two time points in the sporulation process, midpoint and endpoint, each sample was diluted to $10^{-}2$ concentration ($5\mu L$ in $95\mu L$), photographed at $40X$ magnification and assessed for its sporulation rate by counting the number of spores and vegetative cells in each similarly dense objective frame. The sporulation rate of the evolved treatment and control strains were quantified by counting both the spores and vegetative cells in a similarly diluted concentration of cells, which was photographed at $40X$ magnification. These counts were then compared to samples of the ancestral strain which had been frozen at the start of the experimental procedure, and was quantified in

the same manner as the evolved strains.

4.2.5 Analysis of Morphological Change

Single colony isolates of the ancestral, midpoint and evolved strains were grown at 30° Celsius with shaking and colony morphology, cell size and cultural character were observed at 12 hours, 24 hours and 36 hours. At 2 hours, 100μL of each sample was plated on YPDA, to observe colony formation on a solid surface.

4.2.6 Statistical Analysis

We used a binomial-linked logistic regression model to estimate the evolved sporulation efficiency in the 5 experimental strains. We utilized the R packages lme4, car, multcomp, and emmeans (See supplement for complete code of the analysis) The full model was written as described in the experimental design and then variants of this model (with systematically removed terms) were assessed for their validity in the model.

$$[\text{sporulated count}\,|\text{vegetative count}] \sim r + t + (r * t) + d + \epsilon \tag{4.1}$$

where r represents region and each r is composed of the isolates and technical replicates of each of 4 experimental lineages originating from the same regional strains for each of five regions (See figure 4.7), t represents the two experimental treatments (control and treatment) and the ancestral samples (considered a treatment level), the interaction of region and treatment $(r * t)$ and the date, d on which the sporulation assay took place (2 possible dates), plus un-modeled error (ϵ).

To determine the effect of fly sex on the resulting sporulation rates in the treatment, a second weighted binomally-linked logistic regression model was assessed using Aikake Information Criteria (AIC) values to optimize model fit. This model included region (5

regions), sex of the consuming fly (2 sexes), and assay date (2 dates) and utilized only the data from treatments that contained flies (treatment, not control or ancestral). We analyzed the effects of fly sex using data from only the experimental treatment (not the experimental control or the ancestral data) and the model:

$$[\text{sporulated count}\,|\text{vegetative count}] \sim r + s + (r * s) + d + \epsilon \tag{4.2}$$

where s represents the sex of the flies feeding on the yeast cells and the interaction of the region and sex terms is $r * s$.

The variables tested within the experimental design (represented in these models as terms) included the experimental treatment (3 levels), strain region of origin (5 regions), the assay date (2 dates) and interactions between the variables. Each region included 4 experimental lineages (20 lineages over 5 regions). Each lineage contained 8 data points of 4 isolates with 2 technical replicates of each isolate. These data points were all nested within the regional term in the model. This model was used to determine the effects of treatment, region, assay date, and interactions between these variables on the resulting sporulation rates of the yeast.

We used an ANOVA of the fitted logistic regression model to test for the effects of treatment type and strain region. Post-hoc pairwise analyses were performed using Tukey's method for correcting p-values [Whitlock and Schluter, 2015, Zuur et al., 2009].

4.3 Results

4.3.1 Descriptive Results and Model Analysis

Over the 320 paired data points representing the experimental samples and the 60 samples from the ancestral populations of the five focal regions, the density of the samples

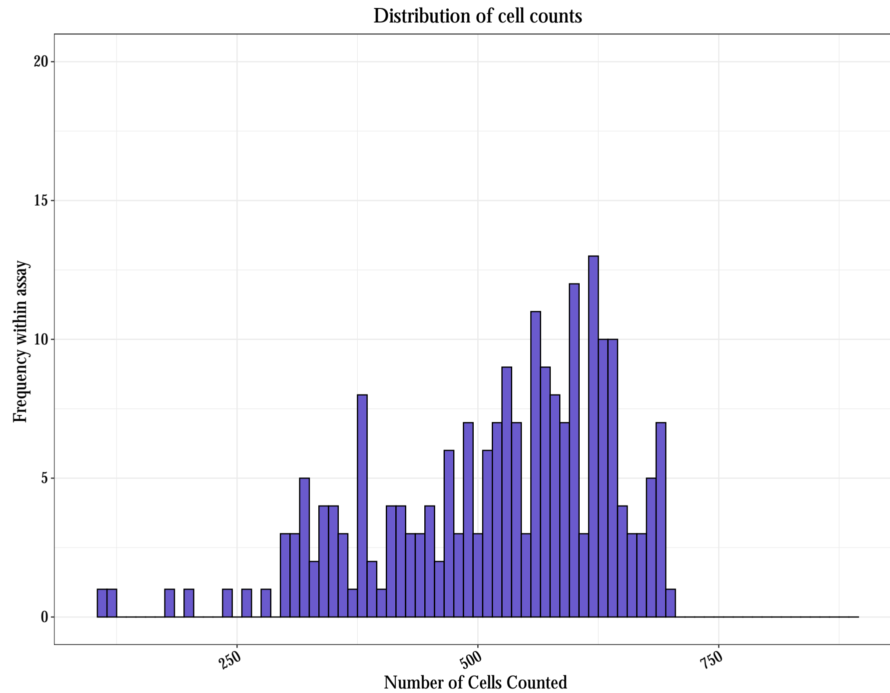

Figure 4.3: Distribution of total counts of cells (vegetative and sporulated) in sporulation assay across all three sample types: Ancestral, Experimental Control and Experimental Treatment. Roughly equal densities were achieved by starting with equal concentrations at the time of inoculation into sporulation media, and subsequent dilution of $5\mu L$ of sample into $95\mu L$ of sterile water. Once sample settled in flat-bottom 96-well plate well, where photographs were taken at 40X magnification, the viewing field was adjusted to an area with high density but limited cellular overlap. This introduces a source of experimenter artifact but this potential bias was mitigated by random counting of the data files while keeping the sample type obscured from the counter.

were relatively similar within region groups and ranged from 266 to 765 (263-869) total cells per sample (Figure 4.3). Values for the percent spores varied from nearly 0 (0.006 at 2.5 days and 0.014 at 5 days) to nearly 100% (0.915 and 1.00, respectively) and showed separations between strain regions and experimental treatment (See Appendix A for complete descriptive statistics table).

We tested for uncontrolled experimental effects by including a random effect of strain isolate and technical replicate, and assessed these by comparing the AIC of models with and without these random effects. Based on this analysis, we were able to conclude that

Table 4.1: Aikake Information Criteria for Model 1, analyzing the effect of treatment, region, interactions, and date. Model column defines either the Full model as described by the experimental design, or a model with a missing term or terms, noted by the minus (-) sign. AIC values are for the binomal data of vegetative and sporulated counts of cells at day five of the sporulation assay.

Model	AIC
Full	3489.1
-region (r)	16901.0
- interaction	6199.3
-treatment (t)	35539.4
-assay date (d) -interaction	6248.7
-treatment (t) -region (r)	41532.8
-region -assay date (d)	16986.0
-treatment (t) -assay date (d)	35611.7
-assay date (d)	3539.6

the random effects could be left out of the model (Figure 4.7;Table A.4). We decided on a logistic regression model based on the count data as a percentage of a total being used to asses the response variable of sporulation rate.

Aikake Information Criteria (AIC) Analysis of this model (Table 4.1) indicated that the lowest AIC value was associated with the full model and therefore all the variables were kept for analysis. A second model was described to test the effect of fly sex on the sporulation rate of the yeast. This second model was necessary because it was based on subsetted data containing only the treatment data points. A logistic regression model was, again, used because the type of data measuring the response variable is the same.

Aikake Information Criteria (AIC) Analysis of this model (Table 4.2 indicated that the lowest AIC score was associated with the model that included all parameters of the experimental design.

Table 4.2: Model analysis based on Akaike Information Criteria for Model2, analyzing the effect of fly sex, region and date on the treatment data's sporulation rate.Model column defines either the Full model as described by the experimental design, or a model with a missing term or terms, noted by the minus (-) sign. AIC values are for the binomal data of vegetative and sporulated counts of cells at day five of the sporulation assay.

Model	AIC
Full	840.8
-assay date (d)	840.6
-assay date (d) -interaction	1107.7
-interaction	1108.4
-region (r)	11672.0
-region (r) -assay date (d)	11672.7
-fly sex (s)	1154.3
-fly sex (s) -assay date (d)	1154.7

Table 4.3: Treatment effects on sporulation response

treatment	estimate	SE	z value	p-value
ancestral	-0.95935	0.02819	-34.036	< 0.0001
control	-0.16371	0.03720	-4.401	< 0.0001
treatment	4.46351	0.07444	59.962	< 0.0001

4.3.2 Effects of Fly

There was a significant effect of fly ingestion and digestion on the sporulation rate of the yeast cell (ANOVA estimate = 4.46351 se = 0.07444, z value = 59.962, p value < 0.0001)

Repeated exposure to *D. melanogaster*(via ingestion) had a significant effect on the mean sporulation rate of the *S. cerevisiae* strains (midpoint ANOVA x^2 = 12728.1, df = 2, $p < 0.0001$; endpoint ANOVA x^2 = 29344.1, df = 2, $p < 0.0001$). This effect was already significant at the midpoint of the assay and maintained significance by the endpoint of the assay but the effect size increased from the midpoint (3.0) to endpoint (4.6). Across all strains the main affect was an increase in sporulation rate in the treatment when compared to the ancestral strains, and no significant change in

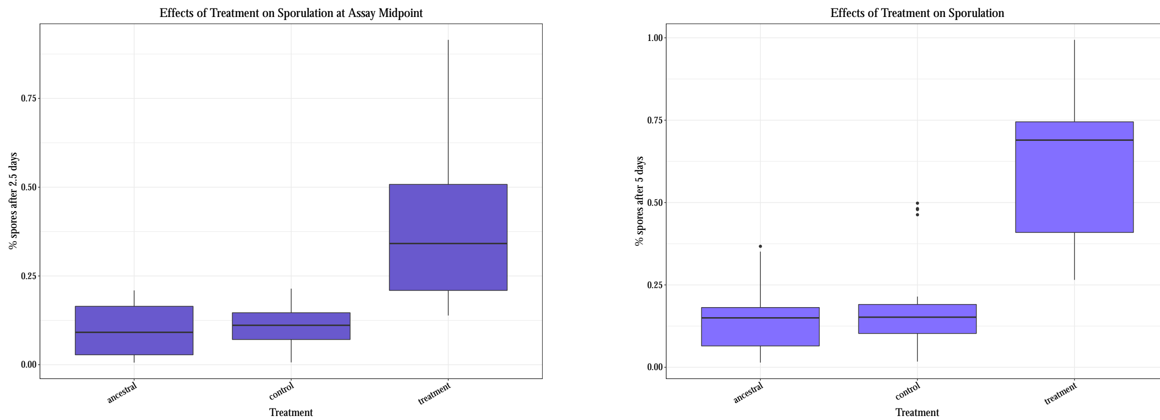

Figure 4.4: The effects of treatment type on sporulation rate measured at 2.5 days (left panel) and 5 days (right panel) of the sporulation assay. Box plots show percent spores at these two time points for each treatment type: Ancestral, Control and Treatment.

Table 4.4: Treatment effects of Wine European strain at midpoint and endpoint of the sporulation assay. Differences between any two treatments in one column indicates effect size.

	Estimate at 2.5	SE at 2.5	Estimate at 5	SE at 5
Ancestral	0.01564	0.00913	0.0296	0.0178
Control	0.05938	0.00878	0.0671	0.0165
Treatment	0.20271	0.00878	0.3640	0.0165

sporulation rate in the control when compared to the ancestral. This was true overall (Figure 4.4) and when the strains were analyzed by region (Figure 4.5).

The effect of treatment was evaluated by region. While the effects remained significant, the smaller regional sub-groups exhibited responses characteristic to their regions (midpoint: ANOVA $x^2 = 1081$, df = 3, $p < 0.0001$; endpoint: ANOVA $x^2 = 601$, df = 3, $p < 0.0001$). The European- wine strain showed a similar pattern of effect size increase from the midpoint (0.14) to the endpoint (0.33) but both assay points were significantly different in their sporulation percentages based on treatment (Table 4.4). This strain, which is a commercially derived strain, was also the most reluctant to sporulate: with the ancestral strain showing 1.8% spores at the endpoint assay and only reaching 36.4% spores in the evolved treatment endpoint assay.

Table 4.5: Treatment effects of West African strain at midpoint and endpoint of the sporulation assay. Differences between any two treatments in one column indicates effect size.

	Estimate at 2.5	SE at 2.5	Estimate at 5	SE at 5
Ancestral	0.14835	0.00651	0.0943	0.0143
Control	0.14185	0.00618	0.0869	0.0136
Treatment	0.71034	0.00618	0.4472	0.0136

Table 4.6: Treatment effects of North American strain at midpoint and endpoint of the sporulation assay. Differences between any two treatments in one column indicates effect size.

	Estimate at 2.5	SE at 2.5	Estimate at 5	SE at 5
Ancestral	0.1746	0.0154	0.2673	0.0352
Control	0.1548	0.0147	0.2459	0.0335
Treatment	0.8315	0.0147	0.9710	0.0335

Conversely, the effect size of the West African strain decreased from the midpoint in the sporulation assay (0.57) to the endpoint (0.36) in the sporulation assay, but both assay points were significantly different in sporulation percentage based on treatment (midpoint: ANOVA $x^2 = 1779$, df = 3, $p < 0.0001$; endpoint: ANOVA $x^2 = 19863$, df = 3, $p < 0.0001$ Table 4.5). From a morphological perspective, these cells also reduced greatly in overall vegetative cell size after treatment.

The wild-derived North American strain exhibited a decreased effect size at the midpoint (0.68) in the sporulation assay relative to the endpoint (0.72) at which point treatment samples reached over 95% sporulated cells (Table 4.6). This region also showed a more varied response to treatment overall (midpoint: ANOVA $x^2 = 5847$, df = 3, $p < 0.0001$; endpoint: ANOVA $x^2 = 1225$, df = 3, $p < 0.0001$).

The commercially-derived Japanese-Sake strain indicated a significant difference in percent spores based on treatment at both the midpoints (ANOVA $x^2 = 199$, df = 3, $p < 0.0001$) and endpoints (ANOVA $x^2 = 635$, df = 3, $p < 0.0001$) of the sporulation

Table 4.7: Treatment Effects of Japanese Sake stain at midpoint and endpoint of the sporulation assay. Differences between any two treatments in one column indicates effect size.

	Estimate at 2.5	SE at 2.5	Estimate at 5	SE at 5
Ancestral	0.0518	0.0194	0.0929	0.0271
Control	0.1204	0.0189	0.1460	0.0267
Treatment	0.1898	0.0189	0.3730	0.0267

Table 4.8: Treatment effects of Malaysian stain at midpoint and endpoint of the sporulation assay. Differences between any two treatments in one column indicates effect size.

	Estimate at 2.5	SE at 2.5	Estimate at 5	SE at 5
Ancestral	0.15599	0.00713	0.20503	0.00918
Control	0.13655	0.00618	0.18858	0.00861
Treatment	0.35117	0.00618	0.71116	0.00861

assay; however, the effect sizes for both the midpoint (0.07) and the endpoint (0.28) were much smaller than the effect sizes of other regional strains, and the treatment lineages were still resistant to sporulation, ending the assay at only 37% spores (Table 4.7).

The Malaysian strain showed the biggest effect size change between time points in the sporulation assay with the midpoint in the sporulation assay showing an effect size (0.21) that was much lower than the effect size at the endpoint (0.51), although both were significant (midpoint: ANOVA $x^2 = 4198$, df = 3, $p < 0.0001$; endpoint: ANOVA $x^2 = 8293$, df = 3, $p < 0.0001$). Most striking in the morphological differences in the treatment strains relative to the evolves was the notable reduction of aggregation in the cell matrix (Table 4.8).

Pairwise analysis of the effect of treatments separated by region confirmed that, within each regional group, the assessed sporulation rate of the treatment group varied significantly from both the ancestral and control groups but the ancestral and control groups did not significantly differ from each other (Figure 4.5 and Table 4.9).

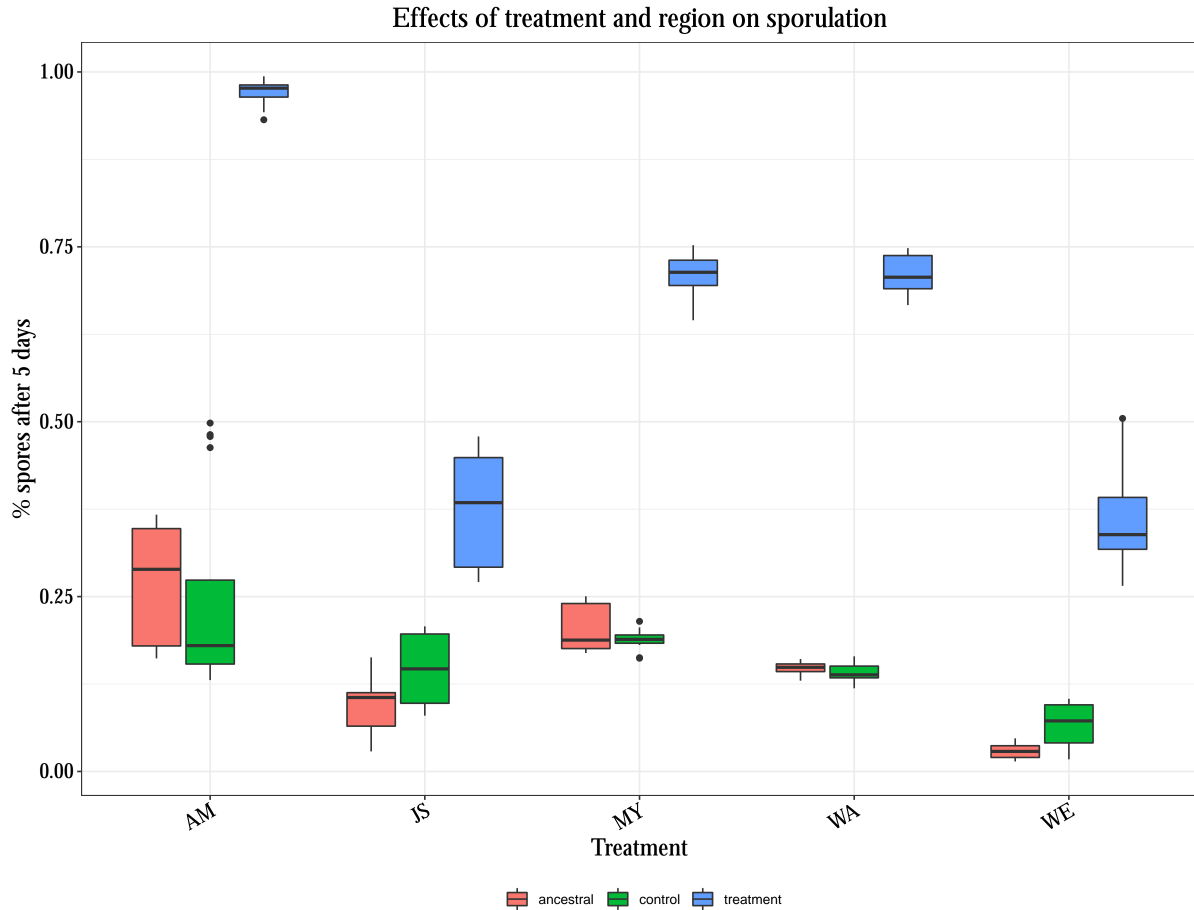

Figure 4.5: The effect of Treatment when separated into regional subgroups. AM represents the North American group, JS represents the Japanese/Sake group. MY represents the Malaysian group. WA represents the West African group. WE represents the Wine/European group. For a complete description of each strain's idiosyncratic characteristics and trait values, see the tables section of Appendix A

4.3.3 Regional Analysis

There was a significant effect of region on sporulation rate at both the midpoint of the sporulation assay (ANOVA $x^2 = 25540.5$, df= 5, p-value < 0.001) and the endpoint of the sporulation assay (ANOVA $x^2 = 22843.7$, df= 5, p-value < 0.001)

Regional background significantly affected both the response to treatment and the variance of that response across treatments (Tables 4.12, 4.13 and 4.11). Pairwise analysis revealed that in the ancestral group the there were three comparisons that were signif-

Table 4.9: Pairwise analysis of differences in treatment by regional group

contrast	estimate	SE	z value	p-value
JS -AM	-1.29670	0.06072	-21.356	< 0.001
MY -AM	-0.37958	0.04198	-9.043	< 0.001
WA -AM	-0.77863	0.04493	-17.331	< 0.001
WE -AM	-2.57672	0.08942	-28.817	< 0.001
MY -JS	0.91712	0.06211	14.767	< 0.001
WA -JS	0.51807	0.06413	8.078	< 0.001
WE -JS	-1.28002	0.10042	-12.746	< 0.001
WA -MY	-0.39905	0.04679	-8.528	< 0.001
WE -MY	-2.19714	0.09036	-24.317	< 0.001
WE -WA	-1.79809	0.09176	-19.597	< 0.001

Table 4.10: Regional effects on sporulation response

region	estimate	SE	z value	p-value
North American	-1.02273	0.02955	-34.613	< 0.0001
Japanese Sake	-2.31943	0.05427	-42.736	< 0.0001
Malaysian	-1.40231	0.03217	-43.594	< 0.0001
West African	-1.80136	0.03586	-50.235	< 0.0001
Wine European	-3.59945	0.08504	-42.326	< 0.0001

Table 4.11: Variance of the logit-transformed percent spores at the 2nd assay point in the sporulation assay. These variances are listed per strain and per treatment for comparison

Region	variance	ancestral variance	control variance	treament variance
All Regions	3.1614	0.9485	0.4859	2.5599
North American	5.9099	0.2030	0.4920	0.4145
Japanese Sake	0.8208	0.3760	0.1886	0.1325
Malaysian	1.3036	0.04211	0.0084	0.0210
West African	1.7123	0.0050	0.0101	0.0165
Wine European	1.8396	0.1624	0.3836	0.1272

icantly different from each other based on region (Tukey adjusted p values, df = 45.9; Figure 4.6).

- North American strains to Japanese strains (ratio = 4.983, p-value = 0.008)
- North American strains to Wine European strains (ratio = 6.789, p-value < 0.001).
- Malaysian strains to Wine European strains (ratio = 5.009, p-value =< 0.001).

After treatment, however, the number of significantly different comparisons between strains increased from only three to eight (all p-values < 0.001). Only two pairs showed no significant difference in sporulation rate based on region (Tukey adjusted p values, df = 38.8; See appendix A for tables of pairwise analysis):

- Japanese strains to Wine European strains (ratio = 0.265, p-value = 1.00)
- Malaysian strains to West African strains (ratio = 0.024, p-value = 1.00)

Individual lineage (experimental replicates 1-20) was evaluated for effects on sporulation rate. Analysis of a model that used separate lineages (See Appendix A) instead of those lineages combined into their respective regions, reported that lineage alone had a significant effect on sporulation percentage (Estimate = 0.00478, SE = 0.00133, t-value = 3.59, p-value = 0.0004). However, AIC values for models with lineages nested within their respective regions indicated these lineages should not be considered independently from their parent region. Closer analysis of the pairing of the significantly and non-significantly different lineages showed that the lineages' independent significance was likely caused by the effect of their respective regions (Type I error) and therefore, lineage was considered a factor of region that did not have effects independent of region. (Figure 4.7 and Appendix A).

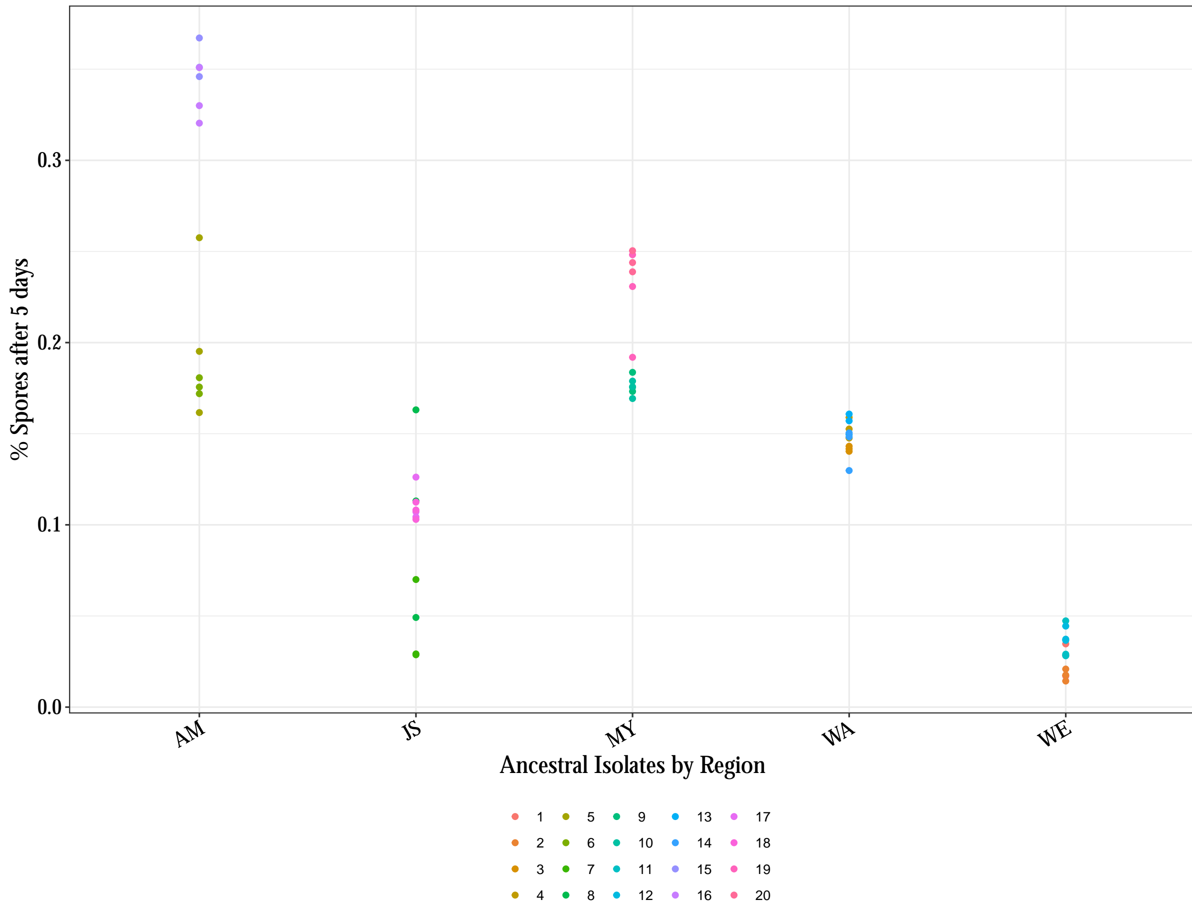

Figure 4.6: Ancestral sporulation rates separated by region. Individual points represent end point assay values of ancestral strains when subjected to KAc sporulation media. Colors indicate different lineages, which at the ancestral level is expected to be the same.

4.3.4 Interactions between Region and Treatment Response

There was a significant interaction of the yeast strain region and the treatment in the response of sporulation rate at both the midpoint and endpoints of the sporulation assay. The response was significant at the midpoint (ANOVA $x^2 = 25540.5$, df $= 8$, $p < 0.0001$) and the endpoint of the sporulation assay (ANOVA $x^2 = 2726.3$, df $= 8$, $p < 0.0001$).

There was an effect of region on the response rate to the treatment. In the sporulation assay the change in % spores from the midpoint of the sporulation assay to the endpoint

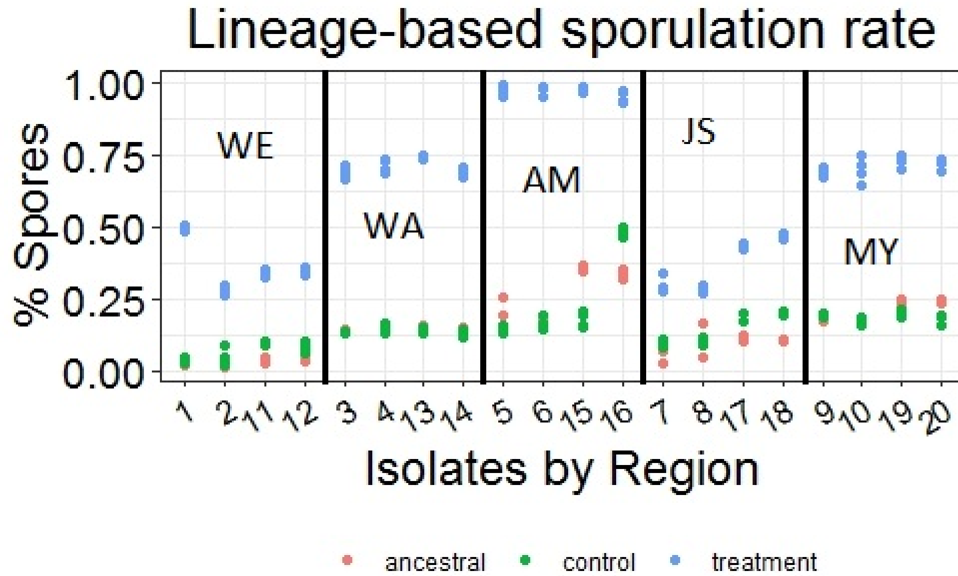

Figure 4.7: The three treatments and their variances displayed as a function of both region and lineage. Lineages of the same region are grouped together (AM: 5, 6, 15 & 16; JS: 7, 8, 17, 18; MY: 9, 10, 19, 20; WA: 3, 4, 13, 14; WE: 1, 2, 11 & 12) Colors differentiate the three treatment types: Ancestral, Control and Treatment.

Table 4.12: Response to sporulation assay by region and treatment. Percent change of sporulation rate (% spores) between midpoint and endpoint of the sporulation assay.

	European Wine	West African	North American	Japanese Sake	Malaysian
Ancestral	89.26%	57.32%	53.09%	79.34%	31.44%
Control	13.00%	63.23%	58.85%	21.26%	38.10%
Treatment	79.57%	58.84%	16.78%	96.52%	102.51%

was a 53.13% increase for the ancestral (17.46% to 26.34%) and a 58.88% increase for the control samples (15.48% to 24.59%), but only a 16.77% increase for the treatment (83.15% to 97.10%). At a regional level these changes were not consistent with the main effect (Table 4.12).

An analysis of the change between Ancestral and Control from the midpoint and the endpoint revealed an 11.34% decrease in % spores (17.46% to 15.48%) at the midpoint of the assay but only an 8.01% decrease in % spores (26.73% to 24.59%) at the endpoint

Table 4.13: Response to sporulation assay by region and treatment. Percent change of sporulation rate between Ancestral and Control (A → C), between Ancestral and Treatment (A → T) and between (C → T) at the assay midpoint (2.5 days) and the assay endpoint (5 days)

	A→C 2.5	A→T 2.5	C→T 2.5	A→C 5	A→T 5	C→T 5
European Wine	279.7%	1196.1%		126.7%	1129.7%	
West African	-7.85%	374.2%		-4.38%	378.8%	
North American	-11.34%	376.2%		-8.00%	263.3%	
Japanese Sake	132.4%	266.4%		57.16%	301.5%	
Malaysian	-12.46%	125.1%		-1.64%	246.8%	

of the assay. The change in % spores between the ancestral and treatment groups in the midpoint of the sporulation assay was 376.2% spores (17.46% to 83.15%) and 263.2% change (26.73% to 97.10%) in % spores at the endpoint. Regional analysis of these percent changes of sporulation rate indicated variability by region of these response strengths (effect sizes) and response timing (or when, during the sporulation assay, did the biggest change happen, Table 4.13).

4.3.5 The effect of fly sex as a differential selective pressure

The role of the fly's sex as a factor of selective pressure on the yeasts' survival through the gut was significant for both the endpoint of the assay (ANOVA $x^2 = 47.9$, $df = 1$, $p < 0.001$) and the midpoint of the assay (ANOVA $x^2 = 70.0$, $df = 1$, $p < 0.001$; Figure 4.8).

4.3.6 The effect of assay on sporulation date

Assay date had a small but significant effect on the sporulation rate results from the assay. This was true for both the endpoint of the sporulation assay (ANOVA $x^2 = 52.5$, $df = 1$, $p < 0.0001$) and the midpoint (ANOVA $x^2 = 32.6$, $df = 1$, $p < 0.0001$)

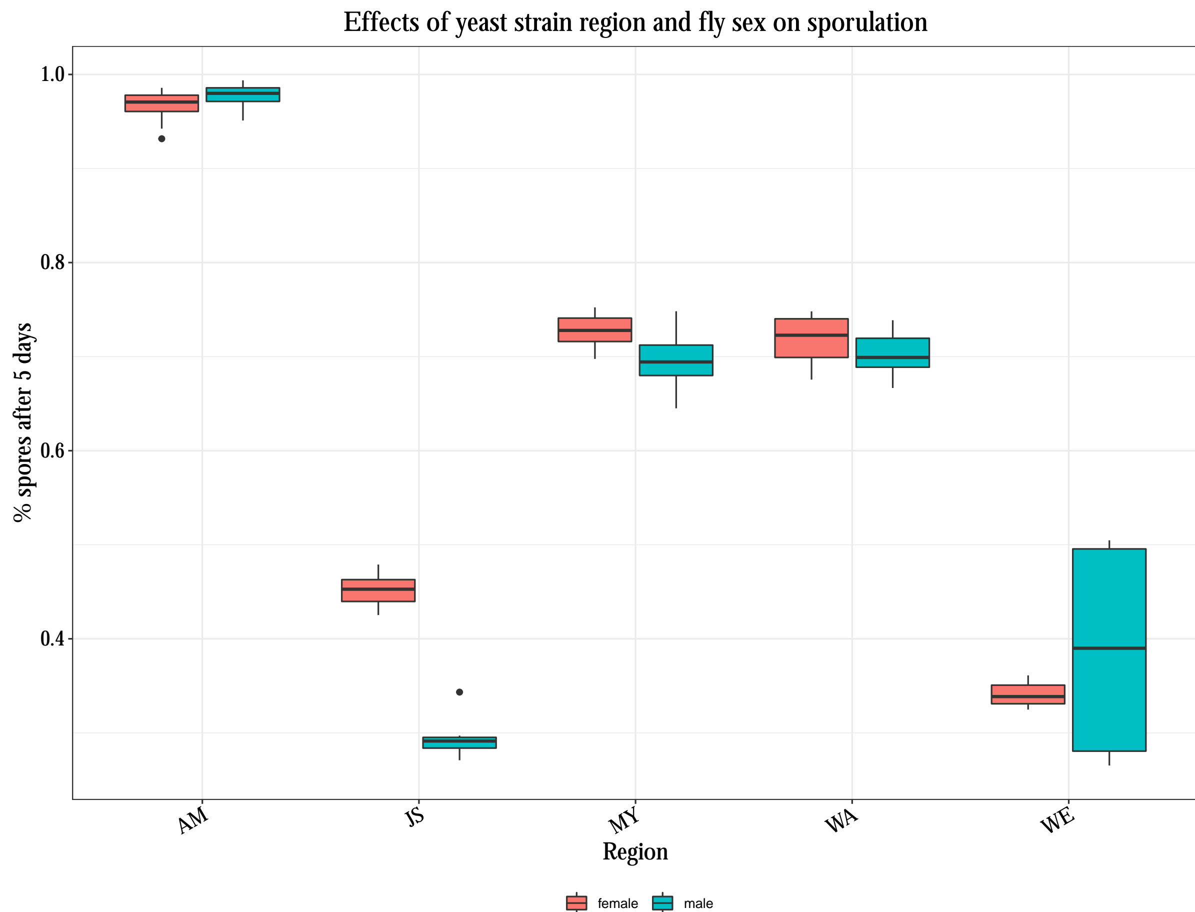

Figure 4.8: The effect of fly on sporulation rate. Data is separated by strain region. Blue represents yeast passaged through male flies and red represents yeast passaged between female flies.

4.4 Discussion

4.4.1 Adaptation to Insect Ingestion and Vectoring

The effects of ingestion support Coluccio and colleagues' conclusions that the insect gut is a type of selective pressure that yeasts are capable of adapting to survive through sporulation. The changes in sporulation rate in the treatment lineages relative to the control and ancestral lineages indicate that this selective pressure can shape the phenotypic landscape of future population of yeasts. Although the four isolates per lineage

were originally from the same laboratory clone, this experiment indicates that despite a lack of direct out-crossing prior to the experiment, there may have been some standing variation between the regions, and even within the regions, or these differences may have been from de novo mutations [Wright, 1932, Wagner, 2000].

The lack of significant change in sporulation rate between the control group and the ancestral group indicated that the effect of the capillary tube and serial transfer of the yeast sample offered little selection pressure, or the selection pressure of those factors did not elicit a change in sporulation rate. It is possible that further testing of these groups may reveal other selection-driven phenotypic or genotypic changes (such as temperature preferences, metabolism, or growth rate) but these controlled factors did not shape the behavioral strategy of sporulation rate with any significance.

Given that the state of yeast cell determines the degree of mortality within the ingested group, this behavioral strategy to sporulate early or late may meet the necessary requirements of a trade-off. A yeast cell that sporulates before ingestion by insects is more likely to survive the process of ingestion, digestion and transfer, but loses the opportunity to replicate up to the point of ingestion. The yeast cell that remains vegetative up to the point of ingestion will gain more reproductive divisions, but has a higher chance of mortality. This divergence of two ecotypes with either superior competitive abilities (slow sporulators) or superior survival abilities during colonization of new areas (fast sporulators) set up a framework for the coexistence strategy described by Tilman (Tilman [1994] in which a competition-colonization trade-off pair of grass species exhibited stable coexistence. This same dynamical system may exist within populations of the same species (Haldane [1957], allowing two ecotypes of one species to coexist. With the yeast system, the stability of this coexistence, however, is dependent on the frequency of the visitation and ingestion by the insect. If the insect visits a yeast patch with high frequency, then we expect that the yeast population ratios of fast and slow sporulators will shift to a

fast-sporulator-dominated population with very few cells remaining in their vegetative state for longer periods. In this case, we would also expect a theoretical upper bound to the frequency of insect ingestion, as the population must have time to germinate, form diploids from their haploid spore states and replicate a few times before sporulating if the population is to persist during the next insect ingestion. As frequency of insect visitation and ingestion decreases, we would expect the slower sporulating cells to increase increase in number because the benefit of reproductive time now outweighs the cost of the infrequent mortality events associated with insect ingestion. Coexistence for this yeast system would likely be achieved in cases when insects visited yeast patches with intermediate frequency. It is also likely that the regularity (predictability) of the visits may determine the rate of sporulation, as previous work by Dey and colleagues have shown that uncertain environments effect reproductive (in this case sporulation) events [Proulx and Teotonio, 2017]. If the yeast community is actually a meta-community of diverse patch space types that experience different degrees of fly interaction, then at the meta-community level, coexistence is likely. Spatial heterogeneity through both dispersal and parapatry is well-documented as a promoter of coexistence [Amarasekare and Nisbet, 2001, Berkley et al., 2010].

4.4.2 Regional and Lineage differences

For anyone who has worked with yeasts in a laboratory setting, the idea that different strains show different sporulation rates is not surprising. With substantially different genetic backgrounds it should also not be surprising that these five strains showed different evolutionary trajectories. In the ancestral group, the paired strains with significant differences were between strains that we know to come from wild sources (North American or Malaysian) or recently industrialized, or semi-wild sources (West African) and indus-

trialized strains that have been artificially selected to resist sporulation (Wine European and Japanese Sake). These results are consistent with the findings of Louvel who assessed the recent ancestors of these strains for their sporulation efficacy and found these strains elicit sporulation rates based on their degree of industrialization with wild strains sporulating most rapidly, industrialized commercial strains sporulating most slowly, and the semi-wild West African strain sporulating at an intermediate rate (See Appendix A for tables by [Louvel et al., 2014]).

The treatment group did not separate into different sporulation rate groups in the same manner as the ancestral group; however there was a link to this same wild versus industrial pattern. The three pairs that showed significant differences from in the ancestral group were the three to show the most striking differences in sporulation rate after treatment. These changes were likely due to the resistance of the commercial strains to increase sporulation rate paired with the instinct to respond to environmental stress in the wild strains. These differences in response rate are due to the differences in genetic background or standing genetic variation within each group.

The regionality of each strain also plays a role in achieving coexistence between fast and slow sporulating ecotypes. Regional differences in the strains may indicate the presence of preexisting coexistence strategies in some regions and the predominance of one species eco-type over the other the species eco-type in other regions. This may be directly influenced by the presence and frequency of insect interaction in these areas, or it may be a response to other environmental factors acting as selection pressures that have shaped each region genetic background.

The regionality of the strain affected both the response to treatment and the variation to that response. This variation within the treatment and control may hold information as to the environment's variability in the region which each strain came from. For example, in the European Wine strain, ancestral strains have been selected to persist in

vegetative states even at low levels of nutrients. This is evident in the ancestral and control samples of the sporulation assay, in which the sporulation rates remained low but increased in variance in the control group. In the European Wine treatment group, we see a strong response to fly ingestion relative to the responses of other treatments based on percent change from original, but the sporulation rate remains lower than that of other treatments. This persistence is likely due to the long-term selection pressures that established the genetic background of this strain in comparison to the short-term selective pressures of this experimental evolution trial. In the wild-derived North American strain, we see a different response: a rapid and dramatic adaptation to the current environment and large variance in the ancestral strain. Large variance may indicate a historical selection pressure for variability or more generalist adaptations in a fluctuating environment. If the variability is small in the regional strain (as it was in the European Wine strain), we might expect that the strain typically experiences a narrow range of environmental variation and has thus, specialized to be successful in this narrow range of environmental parameters. These effect the coexistence of the two strains because if a region's selective pressures dictate specialization, this narrow range of ecotypes is likely not going to elicit stable coexistence between two ecotypes: there is really only one ecotype if the range of acceptable phenotypes is narrow.

Lineage Differences, although not considered in this study, may possess interesting genetically-based facilitators of divergence. Especially in the case of a strong selection pressures where populations repeatedly experience bottle-necking, these once identical lineages may opt to rapidly diversify as a survival strategy, in consideration of kin selection. Small populations should elicit multiple paths up a fitness peak for a single repeated environment, whereas larger populations should be robust to a path based on selection [Lachapelle et al., 2015], and in an environment where individuals have a high degree of relatedness to others, diversification from your relatives to ensure one of you

survives is both bet-hedging and kin selection [Otto and Lenormand, 2002, Otto, 2003]. This diversification strategy is also well-documented in species that commonly experience new environments through dispersal or migration [Birdsell and Wills, 2003, Lenormand and Otto, 2000]. The type of landscape also acts as a factor in each replicates evolutionary trajectory: in a rugged fitness landscape with several fitness peaks, the evolutionary history of the strain (the region in which it evolved) matters as it is constrained to the closest peak. If the fitness landscape is smooth, we would expect to see one or multiple paths (depending on population size) up the same fitness peak, despite evolutionary history [Lachapelle et al., 2015].

4.4.3 The effect of fly sex

The sex of the ingesting insect may shape the composition and evolutionary events of microbial communities. In this study we measured the changes in the sporulation rates when subjected to male or female flies and found a small, but significant effect, but this analysis does not take into account the possibility of differential consumption by the two sexes, or the change of this consumption rate as the lineages evolved throughout the treatment. In other words, the effects of fly sex may be much larger than observed in this experiment.

The sex of the insect ingesting the yeast could also affect the ability for two population eco-types of yeast to coexist. If the two sexes of fly show differential selection pressure on the yeast, this narrows or widens the range of phenotypes that can exhibit coexistence based on which sex more commonly visits a patch of cells . The sex-based behavior of the insects then dictates the ability for the two eco-types to coexist. If female or male flies visit patches more frequently than the other sex, this could affect the range of acceptable sporulation time frames. Conversely, if successful germination of surviving yeast cells is

more beneficial to one insect-sex relative to the other, we might expect to see a change in the range of the acceptable sporulation rates that incur coexistence.

4.4.4 Evolution, Evolutionary Rescue and Divergence

Rapid evolution can occur due to evolutionary rescue events as the result of repeated extreme bottlenecking of the populations of each lineage [Bell and Gonzalez, 2009b]. Throughout the duration of the experimental evolution procedure, population sizes (determined by optical densities) dropped and rebounded precipitously. In the later iterations of the experiment, many five day growth samples did not reach the minimum optical density to move forward with the next fly trial and these lineages had to be stepped back to the previous generation's sample and regrown. This experiment, thus, exhibited evidence of extreme bottlenecking by selection pressure or stochasticity, but eventually all populations recovered to a sufficient optical density and most populations increased geometrically after one rescue by regrowth. This steep decline in population size, followed by a bottleneck and then geometric increase follows the U-shaped recovery curve discussed by Gomulkiewicz and Holt [Gomulkiewicz and Holt, 1995] and Bell and Gonzalez [Bell and Gonzalez, 2009b], and may indicate evolutionary rescue-like processes occurring during this experiment.

The result of this study clearly indicate genetic background, and life history phase (spore or vegetative cell) are factors in the survival and transmission of gut-vectored *S. cerevisiae*. This differential survival and resultant selective pressure may facilitate coexistence through a trade-off mechanism centered around the alternating benefits of the two life-history phases. These ecological events may also drive evolutionary events such as population divergence. Exploitation of novel behavioral strategies have been hypothesized as potential mechanism for divergence [Wilson and Turelli, 1986]. Reuter

and colleagues indicated an increase in the rate of out-crossing in yeast spores that had been vectored by flies. They demonstrated that the digestive process of the fly facilitated deterioration of the outer spore wall, allowing the asci to break up more often, and haploids to encounter and recombine with non-self haploids, rather than the inter-ascus selfing that is typical in spores not ingested by insects [Reuter et al., 2007]. So in a community of spores and vegetative cells that are experiencing fly-gut ingestion and dispersal, there is the potential for reduction of gene flow between fast sporulators and slow sporulators. Slow sporulators that remain vegetative will reproduce clonally by mitosis, remaining diploid and will survive in fewer numbers during vectoring, while fast sporulators are likely to recombine to form new haploids, then diploids at each dispersal event [Tsai et al., 2008] and thanks to the digestive power of the fly, will out-cross a higher than normal rate [Reuter et al., 2007, Tazzyman et al., 2012, Lang et al., 2011]. In other words, these two populations have, by choice of behavioral strategy reduced opportunities to recombine with each other. Due to the reduction of gene flow between these two populations (fast sporulators and slow sporulators) throughout the vectoring process, insect vectoring may also promote the genetic divergence of these populations.

As Dey and colleagues described, new and uncertain environments often promote recombination in order to hedge one's bets for the diverse environmental options the offspring might encounter [Dey et al., 2016]. In the case of two population states, the digestive process of gut-vectoring may encourage differential rates of recombination among each population. Populations that sporulate may experience more recombination than populations that remain vegetative because only the sporulated population has the potential to out-cross with other sporulated populations. If the yeast cell remains vegetative and then manages to survive the fly ingestion process, it will not out-cross because it has remained diploid throughout the process. A sporulated cell, on the other hand, has gone through meiosis to become a spore and is thus, in the haploid state and capable of

recombining by either method: out-crossing or selfing [Madhani, 2007, Murphy and Zeyl, 2010, Tazzyman et al., 2012, Lang et al., 2011].

4.5 Conclusions

Repeated vectoring of yeast cells by insects may promote coexistence by exploitation of a sporulation rate trade-off as a behavioral strategy. In microbial habitats where insects frequently pick up and vector cells, the selective pressure of digestion may play a key role in the community and genetic composition of the species. In extreme cases, this selective pressure may lead to divergence of an ancestral population into niche-distinctive populations and the eventual reproductive isolation of these populations. As meta-population theory predicts, some lineage replicates in this study experienced multiple near-extinction events before being rescued artificially, and some of these never showed rapid population recovery: we were able to isolate very few yeast cells for the final assay. Part of this difficulty in isolation was due to direct competition with bacterial strains from the fly gut that had apparently evolved YPDA resistance over the course of the experiment, but this antibiotic resistance was both expected and unavoidable. It was unexpected because insects, like humans, must maintain a normal flora of gut bacterial in order to maintain healthy functions that we would see in nature. Removing these gut bacteria would have likely affected the behavior of the fly, the appetite of the fly, and the duration of the experimental trial because of increased fly mortality [Brooks, 2012]. None of these changes would have improved the outcome of this protocol. A second option would have been to remove the bacteria as it was released from the fly by selecting out the yeast in a plating of each generation. This strategy, however, would have introduced substantial bias, even artificial selection into the experiment because the yeast that grew large enough to be visible on the plate would likely not represent all the yeast in the culture. Differences of

growth rate or random sampling error may have unintentionally selected the populations toward fast growth or conspicuous appearance and confounded the results. Despite these setbacks, we were able to evolve and isolate these strains and reach the conclusion that genetic background and current environmental conditions interact with varying strengths and elicit both rapid, more gradual, and alternative responses to selection.

4.6 Acknowledgements

We would like to acknowledge Fernanda Pett for her fantastic diagrams of the experimental design. We would also like to thank Uma Rajpurkar and Tracy Yu for their dedicated work in isolating and cataloguing the evolved strains of this experiment.

Concluding Remarks

As we understand more about evolutionary processes, it becomes more apparent that the process of speciation is not definable by a unifying theory, but rather the unifying theory must remain basic enough to encompass all possible mechanisms. Accurate predictability of the pathway or process of speciation in any one system is unlikely; however, with our growing knowledge of the mechanisms of biologically similar outcomes, we should in time be able to predict the probability of known mechanisms of speciation based on ancestral extant species, and trends in environmental dynamics. The same is true for theory relating to coexistence to interacting species: one unifying set of parameters and circumstances will not elicit coexistence for every set of competing populations, but as we further our understanding of the mechanisms of coexistence, we should eventually be able to predict the stability and ratio of any pair of competitively interacting populations.

The four chapters of this dissertation represent the broader fields of biology, illustrating ecological, evolutionary, theoretical and empirical. I have attempted to describe and connect the fields of ecology and evolution, not as two related fields but as elements of the same interconnected larger field of eco-evolutionary dynamics.

I reviewed key elements of the two fields of ecology and evolution and described processes that can lead to coexistence of two ecotypes in some cases and facilitate divergence events in other cases. Chapter 3 empirically supported the findings of previous work but added valuable information on the effects of the genetic background as a factor of sur-

vivabiliy. Regionally-based strain differences affected a variety of response factors from the ability for a strain's vegetative cells to survive to the ability of both the vegetative and sporulated cells to rebound and grow post-ingestion and digestion, to the magnitude of response of these factors. These regionally-based differences in genetic background were, again, important in the experimental evolution chapter (4), where these five strains adapted differently to the repeated exposure to the fruit fly gut. Based on regional differences, both the direction response, the rate of response and the variability of this response to each treatment was different, resulting in the potential for many possible evolutionary trajectories for some strains and limited options for others. Further, I illustrated that the same behavioral trade-off can promote coexistence between the differing populations and may lead to further divergence of those populations. The results of this dissertation will serve as the building blocks of a developing model of coexistence and divergence. Using parameters and results from chapters 3 and 4, we intend to evaluate a model of the yeast system that interacts with flies and defining the parameter values at which coexistence is achieved, the parameter values where population divergence initiates, and evaluating the validity of the competition-colonization trade-off as a mechanism for coexistence and divergence in this system.

While there is clear evidence that the fields of ecology and evolution share many facets of the same biological niche space, it is still unclear if this overlap constitutes a limiting similarity of attributes in which these interacting fields must compete or coexist. Results from this dissertation may offer breadth to the new field of eco-evolutionary dynamics and evaluate coexistence theory's applicability to species divergence and sympatric speciation.

Appendix A

Reference Diagrams, Figures and Tables

The following diagrams describe the yeast fly system of chapters 2,3, and 4 as well as chapter 3 and 4's materials and methods including the larger experimental design, the CaFe apparatus used in both chapters and the data layout for both chapters.

A.2

The following figures are additional references for chapters 3-5 of the dissertation. They are based on additional analyses of the data that was not focal to the dissertation.

Bayesian Analysis of regionally based response from baseline plating to experimental control (rho_c), fly ingestion treatment (rho_t), and grow-out treatment (rho_g). The effect of color assigned to the vegetative and sporulated cell(gamma, γ) and the effect of dilution level (3 orders of magnitude between $10^{-3} and 10^{-6}$), beta, β.

The following tables represent the complete analysis of chapters 2-4 of this dissertation. Some tables are complete versions of abridged versions in text. Other tables are referenced in the text but not directly provided in the text.

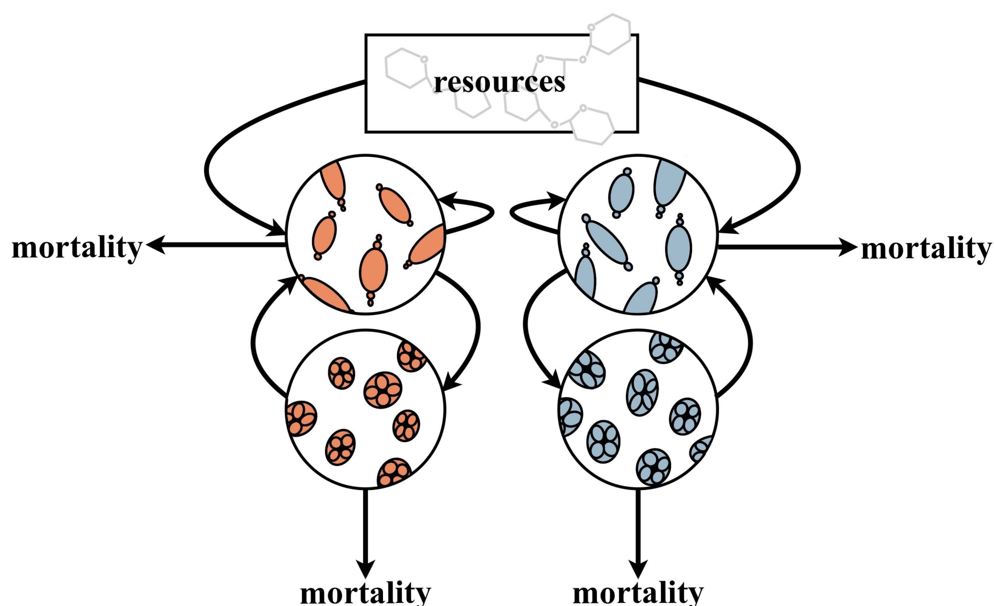

Figure A.1: Graphical depiction of the complete yeast system without parameters for two differing phenotypes within one population.

Table A.1: Estimated generation time (G) and growth rate (μ) for each clean lineage from [Louvel et al., 2014]

Origin	Strain	G (hrs.)	SD	µ(h-1)	SD
Wine/European	YLF 186	1.19	0.07	0.59	0.03
West African	YLF 188	1.16	0.08	0.6	0.04
North American	YLF 189	1.1	0.06	0.63	0.03
Japan/Sake	YLF 192	1.03	0.01	0.67	0.00
Malaysian	YLF 194	ND	ND	ND	ND

Table A.2: Estimated level of aggregation and sporulation efficiency for each clean lineage from [Louvel et al., 2014]

Origin	Strain	estimated level of aggregation	sporulation efficiency
Wine/European	YLF 186	+++	1-5 %
West African	YLF 188	++	10-30 %
North American	YLF 189	+++	100 %
Japanese/Sake	YLF 192	++	1-5 %
Malaysian	YLF 194	++++++	100 %

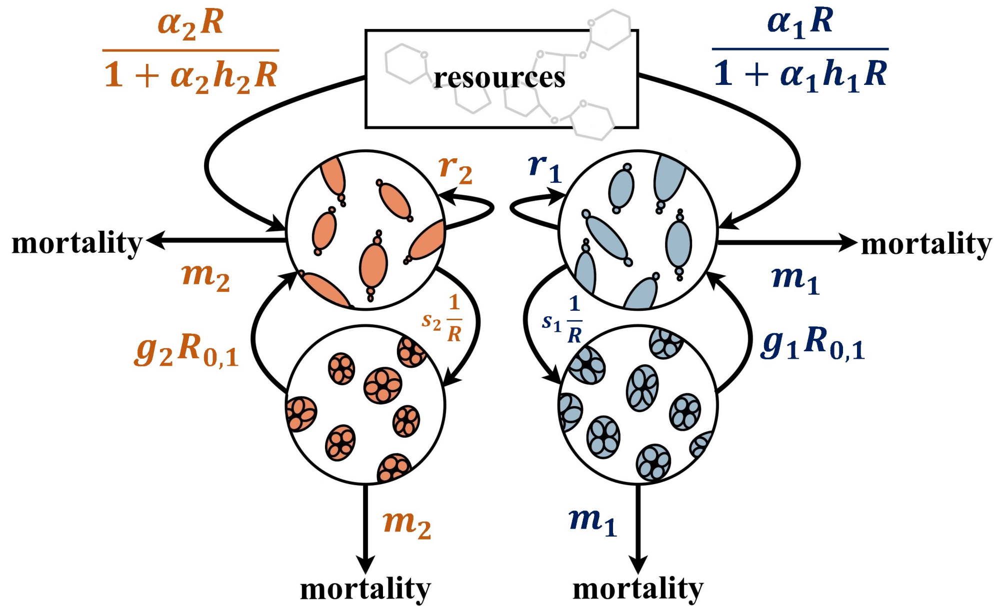

Figure A.2: Graphical depiction of the complete yeast system with parameters for two differing phenotypes within one population. Phenotypes differ in their colonization rates which is described by their sporulation rate and mortality rates in spore and vegetative states. Phenotypes also differ in their competitive abilities which are described by their resource utilization and reproductive rate. Resources affect the rates of sporulation, germination and reproduction and are pulled from a single pool for both phenotypes. Thus, the rate of utilization by one phenotype affects the rates of several aspects of the other phenotype.

Table A.3: Results of Two Sample t-test of Carbon and Nitrogen differences between aggregating (Malaysian) and non-aggregating (European) strains.

Parameter	t-value	df	p-value
Mass N by region	-7.8041	6	0.0002334
Mass C by region	-4.6198	6	0.003616
N as Percent of total by region	-1.9933	6	0.09329
C as Percent of total by region	1.2526	6	0.257

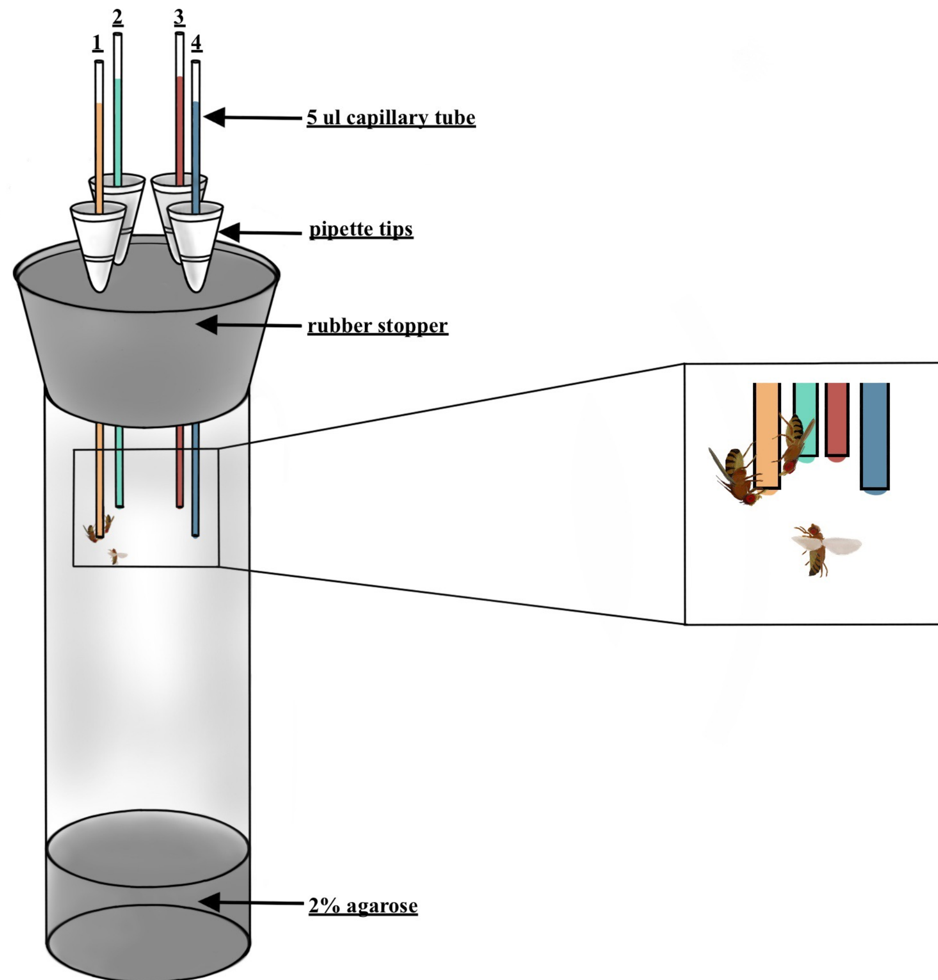

Figure A.3: Diagram of the Fly Capillary Feeder (CaFe) vial including its assembly. List of materials available in supplement.

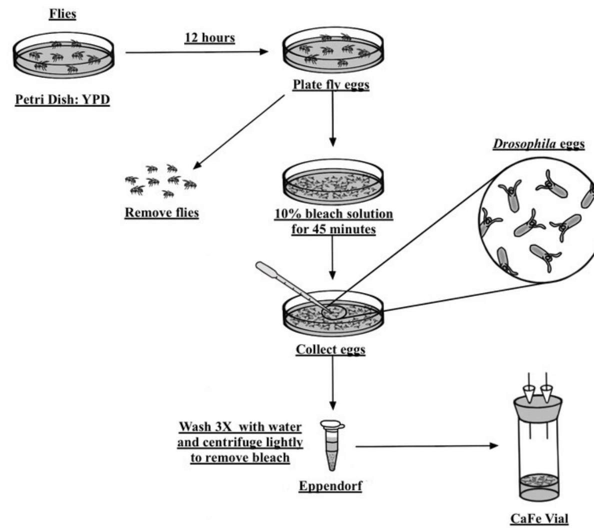

Figure A.4: Fly bleaching method to reduce fly fungal and bacterial load. Flies were allowed to lay eggs on agar plates. Adult flies were then removed and the eggs were covered in a bleach solution. The eggs were then dislodged from the agar and collected using a $1mL$ pipette and rinsed with sterile water. The clean, rinsed eggs were then added to sterile media and allowed to hatch and proliferate.

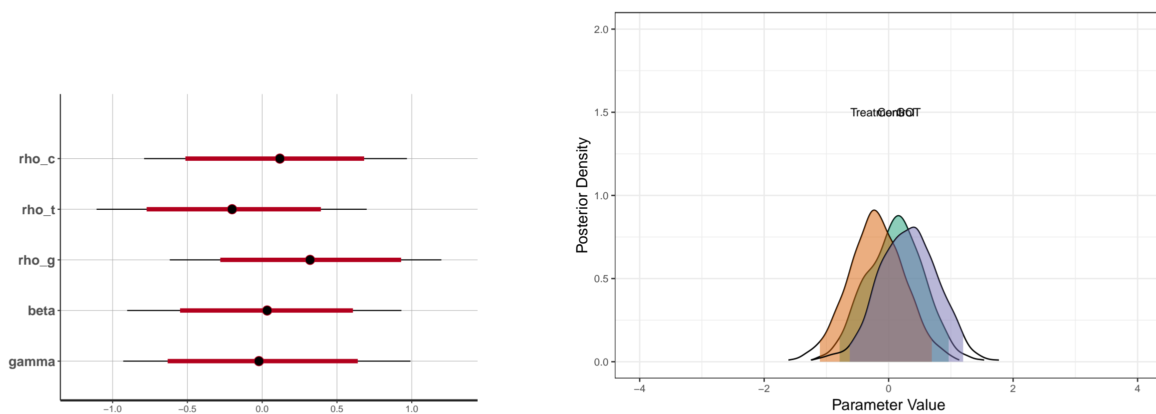

Figure A.5: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for the North American strain.

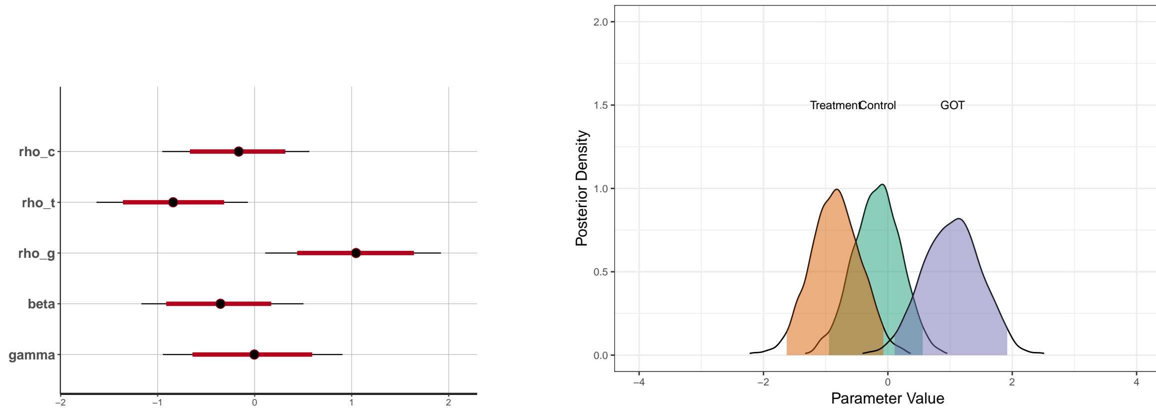

Figure A.6: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for the European/Wine strain.

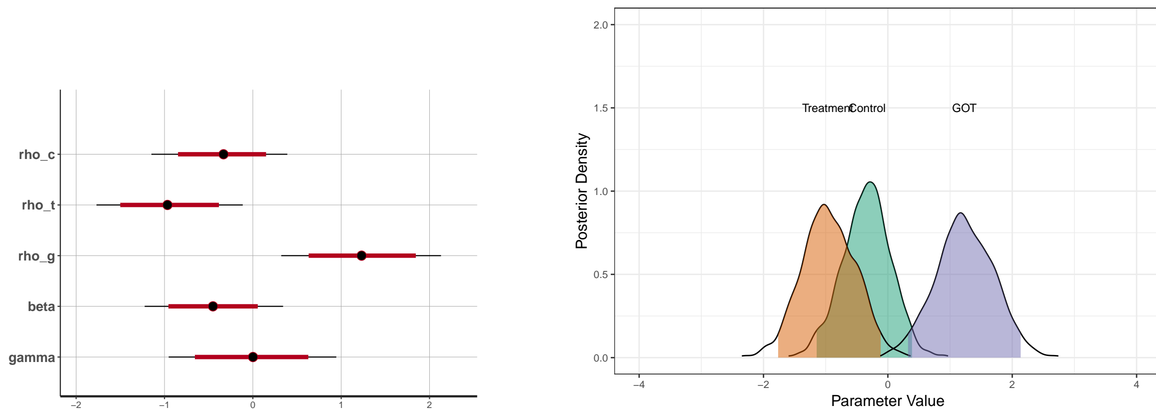

Figure A.7: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for the West African strain.

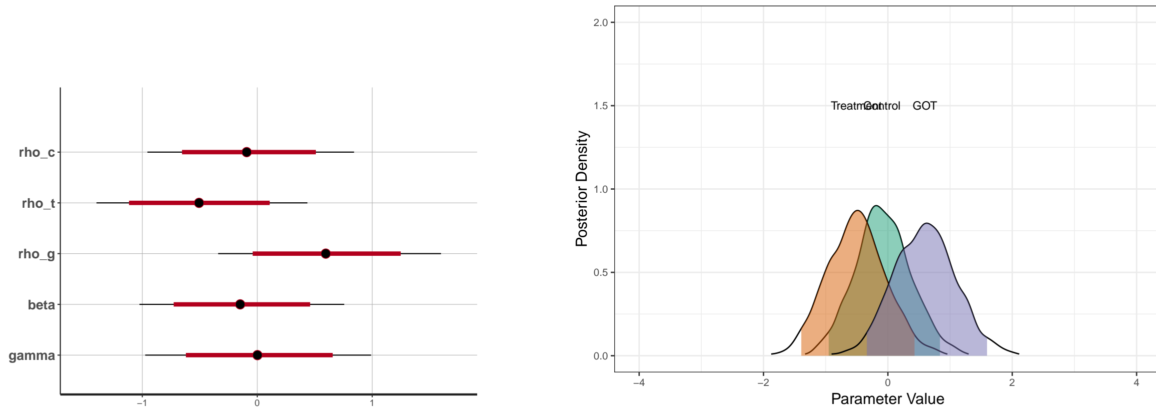

Figure A.8: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for the Japanese/Sake strain.

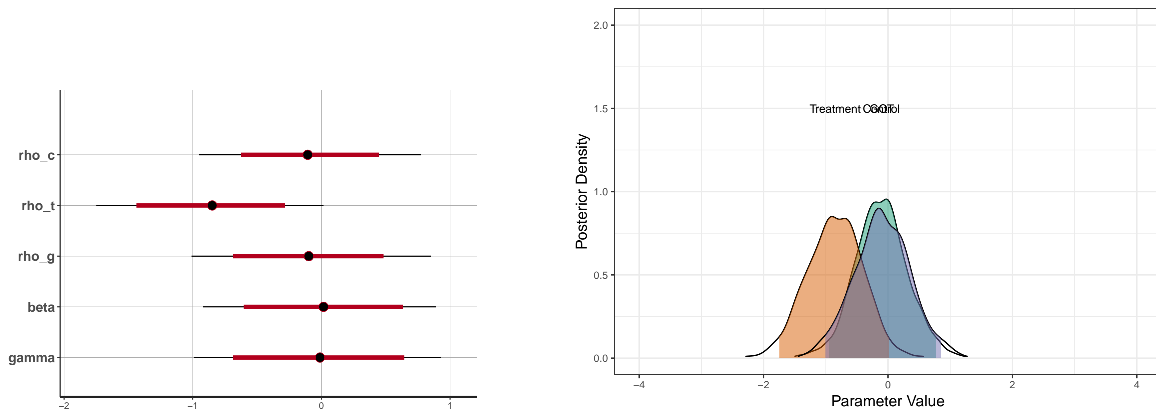

Figure A.9: Bayesian Analysis of response from baseline plating to treatment rho_x values indicate selection strength of each treatment. Right panel: Posterior distribution analysis of the control, treatment and grow out treatment (GOT) for the Malaysian strain.

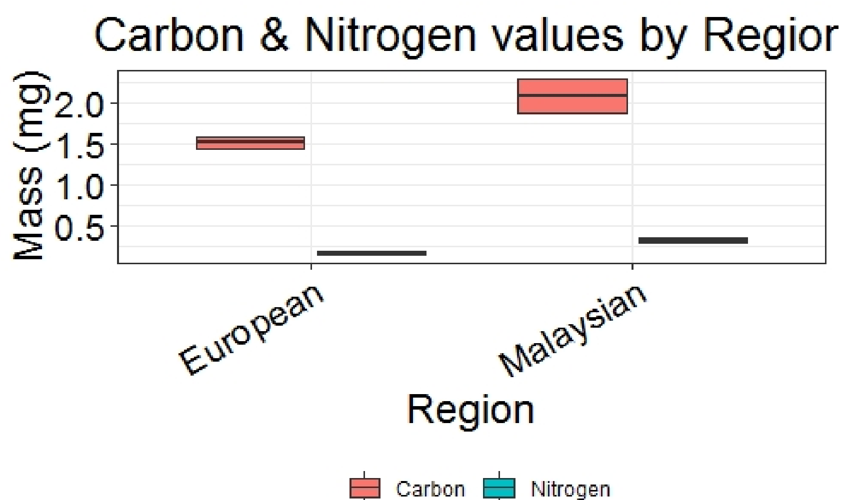

Figure A.10: Carbon-Nitrogen Analysis of aggregating and non-aggregating strains showing differences in total mass of Carbon and Nitrogen in two strain types.

Table A.4: Model analysis based on Akaike Information Criteria for both midpoint (2.5 days) and endpoint (5 days). AIC values indicated that random effects did not yield the best fitting model in comparision to a purely fixed effect model. AIC value analysis also prescribed that the lineage become a nested component within the region and not be treated independently.

Model	midpoint AIC	endpoint AIC
A- one random value	−889.1	−667.4
B- two random values	−883.9	−667.4
C- two random values	−885.9	−669.4
D- two random values	−887.9	−671.4
E- two random values	−884.5	−669.4
F- two random values	−886.2	−671.4
G- one random value	−888.2	−673.4
H- mostly random values	−349.2	−372.1
I- all fixed values	−919.1	−691.7
J- all fixed with nested lineage	−1057.6	−761.1

Table A.5: Complete Descriptive Statistics of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Sub-group	Lineages	% Spores	Variance
Ancestral	Total		62.59%	5.69%
Ancestral	European		2.96%	0.01%
Ancestral	West African		14.84%	<0.01%
Ancestral	North American		26.73%	0.71%
Ancestral	Japanese		9.29%	0.17%
Ancestral	Malaysian		20.50%	0.11%
Control	Total	1-20	15.79%	0.81%
Control	European	1,2,11,12	6.71%	0.10%
Control	West African	3,4,13,14	14.18%	0.02%
Control	North American	5,6,15,16	24.59%	2.00%
Control	Japanese	7,8,17,18	14.60%	0.27%
Control	Malaysian	9,10,19,20	18.86%	0.02%
Treatment	Total	1-20	14.86%	0.89%
Treatment	European	1,2,11,12	36.40%	0.70%
Treatment	West African	3,4,13,14	71.03%	0.07%
Treatment	North American	5,6,15,16	97.10%	0.03%
Treatment	Japanese	7,8,17,18	37.30%	0.70%
Treatment	Malaysian	9,10,19,20	71.12%	0.09%

Table A.6: Pairwise analysis of Ancestral strains. standard error= 0.0350, degrees of freedom =45.9

contrast	est	t-ratio	p-value
AM → JS	0.17447	4.983	0.0008
AM → MY	0.06231	1.780	0.8942
AM → WA	0.11899	3.398	0.0790
AM → WE	0.23771	6.789	< 0.0001
JS → MY	-0.11216	-3.203	0.1244
JS → WA	-0.05548	-1.585	0.9536
JS → WE	0.06324	1.806	0.8835
MY → WA	0.05668	1.619	0.9455
MY → WE	0.17540	5.009	0.0007
WA → WE	0.11872	3.391	0.0804

Table A.7: Pairwise of Treatment. standard error = 0.0338, degrees of freedom = 38.8

contrast	est	t-ratio	p-value
AM → JS	0.59802	17.704	< 0.0001
AM → MY	0.25982	7.692	< 0.0001
AM → WA	0.26065	7.716	< 0.0001
AM → WE	0.60697	17.969	< 0.0001
JS → MY	-0.33820	-10.012	< 0.0001
JS → WA	-0.33737	-9.988	< 0.0001
JS → WE	0.00895	0.265	1.0000
MY → WA	0.00083	0.024	1.0000
MY → WE	0.34715	10.277	< 0.0001
WA → WE	0.34632	10.253	< 0.0001

Table A.8: Pairwise of Treatment in European Wine region. degrees of freedom = 210.5. Tukey-adjusted p-values of $\alpha = 0.05$

contrast	est	S.E.	t-ratio	p-value
ancestral → control	-0.03743	0.0172	-2.170	0.6855
control → treatment	-0.29695	0.0160	-18.597	0 < .0001
ancestral → treatment	-0.33438	0.0172	-19.388	< 0.0001

Table A.9: Lineage Analysis European strains of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). Lineages are listed as mean of total for region (all four lineages) and then by individual lineage per group. P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Lineages	% Spores	Variance	p-value
Ancestral	1,2,11,12	2.96%	0.01%	–
Control	1,2,11,12	6.71%	0.10%	
Control	1	3.44%	< 0.01%	*
Control	2	4.90%	0.09%	**
Control	11	3.48%	0.01%	*
Control	12	8.68%	0.04%	***
Treatment	1,2,11,12	36.40%	0.70%	
Treatment	1	49.59%	< 0.01%	***
Treatment	2	27.92%	0.02%	***
Treatment	11	33.54%	0.01%	***
Treatment	12	34.65%	0.02%	***

Table A.10: Pairwise of Treatment in West African region. degrees of freedom =210.5. Tukey-adjusted p-values of $\alpha = 0.05$

contrast	est	S.E.	t-ratio	p-value
ancestral → control	0.00650	0.0172	0.377	1.0000
control → treatment	-0.56849	0.0160	-35.602	$0 < .0001$
ancestral → treatment	-0.56198	0.0172	-32.584	< 0.0001

Table A.11: Lineage Analysis West African strains of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). Lineages are listed as mean of total for region (all four lineages) and then by individual lineage per group. P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Lineages	% Spores	Variance	p-value
Ancestral	3,4,13,14	14.84%	< 0.01%	–
Control	3,4,13,14	14.18%	0.02%	
Control	3	13.68%	< 0.01%	*
Control	4	15.42%	0.02%	*
Control	13	14.32%	0.01%	
Control	14	13.42%	0.01%	*
Treatment	3,4,13,14	71.03%	0.07%	***
Treatment	3	68.98%	0.04%	***
Treatment	4	71.57%	0.05%	***
Treatment	13	74.21%	< 0.01%	***
Treatment	14	69.37%	0.02%	***

Table A.12: Pairwise of Treatment in North American region. degrees of freedom = 210.5. Tukey-adjusted p-values of $\alpha = 0.05$

contrast	est	S.E.	t-ratio	p-value
ancestral → control	0.02142	0.0172	1.242	0.9958
control → treatment	-0.72507	0.0160	-45.408	$0 < .0001$
ancestral → treatment	-0.70364	0.0172	-40.798	< 0.0001

Table A.13: Lineage Analysis North American strains of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). Lineages are listed as mean of total for region (all four lineages) and then by individual lineage per group. P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Lineages	% Spores	Variance	p-value
Ancestral	5,6,15,16	26.73%	0.71%	–
Control	5,6,15,16	24.59%	2.00%	*
Control	5	14.86%	0.02%	**
Control	6	17.48%	0.05%	**
Control	15	17.98%	0.07%	**
Control	16	48.08%	0.02%	***
Treatment	5,6,15,16	97.10%	0.03%	***
Treatment	5	97.64%	0.02%	***
Treatment	6	97.67%	0.03%	***
Treatment	15	97.66%	$< 0.01\%$	***
Treatment	16	95.47%	0.04%	***

Table A.14: Pairwise of Treatment in Japanese Sake region. degrees of freedom = 210.5. Tukey-adjusted p-values of $\alpha = 0.05$

contrast	est	S.E.	t-ratio	p-value
ancestral $\rightarrow$ control	-0.05313	0.0172	-3.080	0.1330
control $\rightarrow$ treatment	-0.22696	0.0160	-14.214	$0 < .0001$
ancestral $\rightarrow$ treatment	-0.28009	0.0172	-16.240	< 0.0001

Table A.15: Lineage Analysis Japanese strains of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). Lineages are listed as mean of total for region (all four lineages) and then by individual lineage per group. P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Lineages	% Spores	Variance	p-value
Ancestral	7,8,17,18	9.29%	0.17%	–
Control	7,8,17,18	14.60%	0.27%	**
Control	7	9.38%	0.02%	
Control	8	10.12%	0.01%	*
Control	17	18.80%	0.02%	***
Control	18	20.10%	2.54%	***
Treatment	7,8,17,18	37.30%	0.70%	***
Treatment	7	30.13%	0.08%	***
Treatment	8	28.67%	0.01%	***
Treatment	17	43.66%	$< 0.01\%$	***
Treatment	18	46.72%	$< 0.01\%$	***

Table A.16: Pairwise of Treatment in Malaysian region. degrees of freedom = 210.5. Tukey-adjusted p-values of $\alpha = 0.05$

contrast	est	S.E.	t-ratio	p-value
ancestral $\rightarrow$ control	0.01645	0.0172	0.954	0.9998
control $\rightarrow$ treatment	-0.52258	0.0160	-32.727	$0 < .0001$
ancestral $\rightarrow$ treatment	-0.50613	0.0172	-29.346	< 0.0001

Table A.17: Lineage Analysis Malaysian strains of LTEE. Group represents the three treatment groups assessed in the sporulation assay: Ancestral (frozen original strain sample), Control (evolved lineage not subjected to fly ingestion, but only to repeated serial transfer) and Treatment (evolved strain subjected to repeated fly ingestion and digestion). Lineages are listed as mean of total for region (all four lineages) and then by individual lineage per group. P-value indicates a significant difference from Ancestral in pairwise analysis.

Group	Lineages	% Spores	Variance	p-value
Ancestral	9,10,19,20	20.50%	0.11%	–
Control	9,10,19,20	18.86%	0.02%	
Control	9	19.24%	< 0.01%	
Control	10	17.87%	0.61%	*
Control	19	20.02%	0.01%	
Control	20	18.30%	0.02%	*
Treatment	9,10,19,20	71.12%	0.09%	***
Treatment	9	69.20%	0.03%	***
Treatment	10	69.90%	0.19%	***
Treatment	19	73.26%	0.05%	***
Treatment	20	72.11%	0.03%	***

Table A.18: Pairwise analysis of each lineage comparing individual lineages to the mean percent spore value at the endpoint of the sporulation assay.

Lineage	Estimate	Standard Error	t value	P-value	significance
1	0.02323	0.03139	0.74	0.4601	
2	-0.05275	0.03139	-1.68	0.0945	
3	0.16234	0.03139	5.17	$5.7X10^{-}7$	***
4	0.18116	0.03139	5.77	$3.0X10^{-}8$	***
5	0.28807	0.03139	9.18	$< 10^{-}16$	***
6	0.28968	0.03139	9.23	$< 10^{-}16$	***
7	-0.02162	0.03139	-0.69	0.4918	
8	-0.00626	0.03139	-0.20	0.8422	
9	0.19311	0.03139	6.15	$4.2X10^{-}9$	***
10	0.18990	0.03139	6.05	$7.1X10^{-}9$	***
11	-0.01016	0.03139	-0.32	0.7467	
12	-0.00859	0.03139	-0.27	0.7847	
13	0.18718	0.03139	5.96	$1.1X10^{-}8$	***
14	0.16310	0.03139	5.20	$5.0X10^{-}7$	***
15	0.34038	0.03139	10.84	$< 10^{-}16$	***
16	0.43595	0.03139	13.89	$< 10^{-}16$	***
17	0.08092	0.03139	2.58	0.0107	*
18	0.09552	0.03139	3.04	0.0027	**
19	0.22327	0.03139	7.11	$2.0X10^{-}11$	***
20	0.21851	0.03139	6.96	$4.8X10^{-}11$	***

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