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Resolving the Paradox of Polyploidy: Underexplored Facets of Polyploid Plants

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RESOLVING THE PARADOX OF POLYPLOIDY: UNDEREXPLORED FACETS OF POLYPLOID PLANTS

BY

BENJAMIN P. GERSTNER

B.Sc., Ecology & Evolutionary Biology, University of Rochester, 2017

DISSERTATION

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DEDICATION

To the estimated 100,000 lives that vanished from this world throughout the HIV/AIDS epidemic's first decade. Subsidized by ignorance, panic, and bigotry your lives became abridged. May this document stand as a testament to each of you; had you been allowed to live an unabridged life there is no telling all the impacts you would have had.

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I must first acknowledge and thank all the educators that have helped me build my mind and cultivate my thoughts. Through giving me countless opportunities and supporting me through them I have been privileged by exceedingly exceptional educators. Specifically, I must say thank you to all of those that have provided me grammatical and editorial feedback. It is through your efforts and behind the scenes work that I have learned to communicate my thoughts in an accessible form to others.

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My mother, Patricia Ann, who despite leaving this world many years ago still impacts this world. Her lessons in grit, tenacity and justice made me into someone who will stand up for the downtrodden and work against injustice. My sister, Ashleigh Mae, who selflessly stepped in and finished the job our mother started. We led one another from our sorrow-filled valley to find joy again.

My friends who have been a steadfast fixture in my life, DSM, MSD, MAM, CERM. You have all listened to me talk for hours and been there every time I have looked.

No part of this dissertation has been published elsewhere at the time of submission.

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ABSTRACT

Polyploidy, or whole genome duplication, is a common phenomenon in plants, but the establishment and persistence of mixed-ploidy populations remains a paradox. This dissertation explores factors that contribute to the persistence and establishment of mixed-ploidy populations in nature. The first chapter investigates the role of unreduced gametes in neopolyploid establishment and finds that variability in their formation rate can have a significant impact on polyploid establishment and persistence. The second chapter searches for evidence of soil microbes exhibiting ploidy-specificity, a pre-condition for microbe-mediated niche differentiation, a possible stabilizing mechanism contributing to ploidy coexistence. Finally, the third chapter tests for microbe-mediated niche differentiation in a mixed-ploidy population of *Larrea tridentata*. Using a plant-soil feedback experiment this chapter demonstrates that microbe-mediated niche differentiation can facilitate the coexistence of different ploidy levels. Overall, this dissertation demonstrates there are complex and interrelated factors that contribute to the persistence and establishment of mixed-ploidy populations in nature.

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Preface

This dissertation has been prepared as part of the fulfillment of the doctoral degree requirements set forth by the University of New Mexico and Department of Biology. Though the scholarship presented is of greatest interest to evolutionary plant ecologists and those that study polyploidy, I hope to make it accessible to a general audience. To aid in this I have chosen to include a summary of my personal journey to producing this dissertation. I then describe my interests and motivations in generating this scholarship and end with sharing two personal vows of where I will go from here.

I grew up in a rural community nestled on a ridge built by glacial till on the south side of Lake Ontario. I rarely thought of my childhood as any different than those around me. However, as I move further away from that time, it has become apparent that my upbringing has had a profound impact on the questions I ask in the world. Effectively raised by a single mother that slowly faded away before my eyes, I assumed responsibilities for survival that most would hire professionals to provide. My education was inadvertently guided by maturing at such a pace. After the curtain closed for the last time on my mother's life, I was left an adult that faced living through my teens. Education became a natural place for premature maturity to flourish. Educators embraced it and my peers ignored it. Finding myself three years into a pre-med track biology degree that I pursued to uphold a promise to my mother to become a doctor, I learned that was not the doctor I wanted to be.

I have always had a fascination with space exploration, perhaps a banal childhood interest or rooted in escapism from my reality or both, I cannot say. What I can say is that I came to ask my dissertation research questions because of this love. If we are ever going to leave this biosphere, we will need to be able to produce plant-derived foods. For many reasons we will have limited space to do such. Thus, I obsessed about how genetic diversity in small populations is maintained in nature. These interests brought me to applying to graduate school programs in evolutionary biology to study polyploidy. In simplest terms, polyploids can harbor more genetic diversity than a diploid counterpart which has impacts on population genetics. As with most pursuits the ideas are grand, and the research questions are seemingly mundane by comparison. But these are the questions that I ask in this dissertation and perhaps one day will be part of the grout to the larger foundations that support grander ideas.

Lost to history and unimaginable to name them all, I humbly bow my head in gratitude to the cultures, ancestors, and peoples of the lands I have occupied. Regardless of what I describe above, I benefit from innate privilege. My privileged existence is the product of colonization, centuries of implicit biases and institutions designed to benefit my race, my language, and my culture. This all weighs heavy on my mind. Thus, I vow to use the positions this document and title allows me, to work towards decolonization, challenging

implicit bias and listening to those who have historically had their voices ignored and silenced by my forefathers.

Lastly, over 20 years of formal education has awarded me literal years to hone my mind and gain expertise to secure my own financial security and societal respect – for this I am eternally grateful. However, for better or for worse many have descriptions of the past used as prescriptions for the future. I thank everyone that has helped me break from such a cycle and to pursue my own path in life. Though my past helps others understand me, it does not define me, and I vow to do all that I can to make this true for everyone.

Introduction

Polyploidy – the duplication of whole chromosome sets – is found across all kingdoms of life. In plants, polyploidy has been implicated in shaping species and ecological interactions; such as, escape and radiate coevolution between Brassicales and butterflies (Pieridae - Edger et al. 2015), range expansion in insect herbivores and shifted interactions with mutualistic fungi (Segraves and Anneberg 2016). Current evidence supports angiosperm diversity is, in part, due to multiple historic polyploidization events (Wood et al. 2009; Barker et al. 2016; Román-Palacios, Molina-Henao, and Barker 2019). Polyploidy directly leads to diversity through both short- and long-term phenotypic changes. It is perhaps best known for enlarged cell and organ size (e.g., gigas effect) which is often the target of artificial selection and has resulted in many of our cultivated crops and horticultural species (e.g., enlarged flowers in commercial rose production; Crespel, Ricci, and Gudin 2006). Instantaneous character differences can lead to wild polyploid species having an advantage (or disadvantage) in nature. Long-term phenotypic changes can result through neofunctionalization of the duplicated genes, leading to diversification of gene families and, ultimately, species.

Abbreviations & Definitions:

Unreduced Gametes – gametes that retain the complete parental chromosome complement (i.e., for a diploid parent, an unreduced gamete is diploid instead of haploid).

Cytotype – organisms of the same species with different karyotypes, specifically the number of genome copies.

MCE – Minority Cytotype Exclusion: because a newly arising cytotype is both infrequent compared to its progenitor cytotype and reproductively incompatible with it, the minority cytotype will have a low fitness due to gamete wastage and the formation of sterile hybrids and thus be excluded (Levin 1975).

FRUG – Formation Rate of Unreduced Gametes (u). This is analogous to mutation rate, μ, commonly used across evolutionary models.

OTU – Operational Taxonomic Unit: classification for closely related individuals (i.e., species, genus).

Specificity – probability that the plant belongs to that cytotype, given that the OTU has been found there.

Fidelity - probability of finding that OTU on that cytotype.

A large body of theoretical work has emerged on polyploidy, particularly surrounding polyploid plant formation and establishment. New polyploid individuals are most often formed through the union of unreduced gametes (an alternative path of somatic doubling that enters the germ line is rare, but possible). A generalized model describing barriers to polyploid formation and establishment was put forth in Levin (1975). Termed Minority Cytotype Exclusion (MCE), the theory generally posits that because a newly arising cytotype is both infrequent compared to its progenitor cytotype and reproductively incompatible with it, the minority cytotype will have a low

fitness due to gamete wastage and the formation of sterile hybrids and thus be excluded (Figure 1). Since Levin's 1975 work, more than a dozen models (Fowler and Levin 1984; Felber 1991; Bever and Felber 1992; Rodríguez 1996a,b; Felber and Bever 1997; Keeler 1998; Li, Xu, and Ridout 2004; Husband 2004; Yamauchi et al. 2004; Rausch and Morgan 2005; Oswald and Nuismer 2007; 2011; Suda and Herben 2013; Fowler and Levin 2016; Van Drunen and Husband 2019; Clo, Padilla-García, and Kolář 2022; Van Drunen and Friedman 2022) exploring minority cytotype exclusion have investigated conditions that allow for polyploid establishment. These models have identified many scenarios in which minority cytotype exclusion can be avoided. Here I highlight three scenarios: (1) the constant replenishment of the minority cytotype, (2) the minority cytotype having a fitness advantage over the majority cytotype, and (3) processes that limit gamete wastage and sterile hybrid formation.

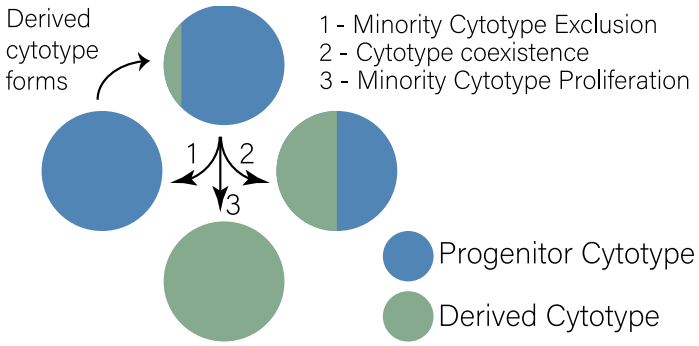

Figure 1: Possible outcomes when a new cytotype forms in a population.

In the first scenario, the constant replenishment of the minority cytotype happens by the continual production of unreduced gametes by the majority cytotype (Felber 1991; Felber and Bever 1997; Husband 2004; Li, Xu, and Ridout 2004; Rausch and Morgan 2005; Oswald and Nuismer 2007; Fowler and Levin 2016) and/or hybrids (Felber and Bever 1997; Yamauchi et al. 2004) of the majority and minority cytotypes. Prior models have found that when formation rates of unreduced gametes (FRUG) are high (e.g. 17%; Felber 1991; Rausch and Morgan 2005), the polyploid can establish a stable population frequency, minority cytotype exclusion is averted, and the two cytotypes may coexist. However, FRUG are typically low (0.5-2% in most estimates; Ramsey and Schemske 2002; Kreiner, Kron, and Husband 2017) and a constant low replenishment of the minority cytotype may occur, but minority cytotype exclusion will continue to remove them from the population. In the second scenario, given the chance event of polyploid formation having occurred, some models find that a higher relative fitness for the minority cytotype lowers the probability of minority cytotype exclusion and increases the likelihood of polyploid establishment. Minority cytotype fitness advantages

may arise through many means, for instance higher pathogen resistance in recently derived cytotypes (Oswald and Nuismer 2007) or adaptation to local habitat given varying degrees of spatial heterogeneity (Li, Xu, and Ridout 2004; Garmendia et al. 2018) and niche differentiation (Fowler and Levin 1984; Rodríguez 1996a; Fowler and Levin 2016; López-Jurado, Mateos-Naranjo, and Balao 2019). In the third scenario, prior models find that processes that limit gamete wastage and sterile hybrid formation severely decrease a major contributing component of minority cytotype exclusion, if not side-step them altogether, thus permitting establishment. These processes include assortative mating (Husband 2004; Li, Xu, and Ridout 2004; Münzbergová, Skuhrovec, and Maršík 2015), self-compatibility (Levin 1975; Felber 1991; Rodríguez 1996b; Yamauchi et al. 2004; Rausch and Morgan 2005; Oswald and Nuismer 2011; Fowler and Levin 2016) and asexual reproduction (Yamauchi et al. 2004). Cytotype specific shifts in flowering time (Husband 2000; Burton and Husband 2000), pollinator-mediated assortative mating (Husband and Sabara 2003; Laport, Minckley, and Pilson 2021), high fidelity for self-pollination, and local non-random seed dispersal (Fowler and Levin 1984; Li, Xu, and Ridout 2004), all limit the frequency of inter-cytotype pollinations and thus minimize gamete wastage and sterile hybrid formation. Asexual reproduction (Yamauchi et al. 2004; Karunarathne et al. 2018) (e.g. apomixis or parthenogenesis) avoids gamete wastage and sterile hybrid formation altogether and results in extending the persistence of the polyploid. In each of these scenarios the population dynamics equate to two competing species (Levin 1975; Felber 1991) and depend on the relative fitness of the cytotypes and/or the degree of cytotype niche separation to determine whether there will be ploidy coexistence in a mixed-ploidy population.

Empirical support for these three polyploid establishment modeling scenarios is limited and mixed (in support (Husband 2000; Burton and Husband 2000; Gross and Schiestl 2015; McIntyre and Strauss 2017); in opposition (Maceria, Jacquard, and Lumaret 1993; Burton and Husband 2000; Pavlíková et al. 2017; Castro et al. 2019)). Given the apparent strength of the minority cytotype exclusion barrier, the high frequency of historic and current polyploidy is thus paradoxical (Fowler and Levin 2016). In this dissertation I share evidence that helps reconcile theoretical predictions with empirical observations.

Chapter 1: Why so Many Polyploids? Accounting for Environmental Stochasticity in Unreduced Gamete Formation Lowers the Perceived Barriers to Polyploid Establishment

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Abstract

While polyploids are common in nature, existing models suggest that polyploid establishment should be difficult and rare. We explore this apparent paradox by focusing on the role of unreduced gametes, as their union is the main route for formation of neopolyploids. Production of such gametes is affected by genetic and environmental factors, resulting in variation in the formation rate of unreduced gametes (u). Once formed, neopolyploids face minority cytotype exclusion (MCE) due to a lack of viable mating opportunities. More than a dozen theoretical models have explored factors that could permit neopolyploids to overcome minority cytotype exclusion and become established. Until now, however, none have explored variability in u and its consequences for the rate of polyploid establishment. Here, we determine the distribution that best fits available empirical data on u. We perform a global sensitivity analysis exploring the consequences of using empirical distributions of u to investigate effects on polyploid establishment. We determined in many cases u is best fit by a log-normal distribution. We found environmental stochasticity in u dramatically impacts model predictions when compared to a static u. Our results help reconcile previous modeling results suggesting high barriers to polyploid establishment with the observation that polyploids are common in nature.

Introduction

Polyploidy, the occurrence and maintenance of more than two copies of a chromosome set within an organism, has intrigued researchers for nearly a century (Gates 1923). Polyploidy is present across multiple kingdoms of life, including animals, plants, and fungi (Rodgers 1973; Campbell et al. 2016). Plants have a long history of polyploidization (Landis et al. 2018). Between 319 and 192 million years ago, seed plants experienced two ancient polyploidization events before the origin and diversification of angiosperms (Jiao et al. 2011). More recently, 25-35% of all angiosperms have experienced polyploidization events thus a 3rd order or higher round of polyploidization (Wood et al. 2009; Barker et al. 2016). Real-time polyploidization events are also being recorded, as mixed-ploidy plant populations have been documented across the globe and across many genera (Baack 2004; Suda and Herben 2013; Blonder et al. 2019; Castro et al. 2019). These patterns beg the question of what mechanisms drive high levels of polyploid formation and/or maintenance.

Both the high frequency of polyploidization events inferred across the phylogenetic history of plants and the prevalence of mixed-ploidy populations are paradoxical to existing theoretical predictions concerning polyploid establishment. The main conceptual model describing the predicament of new polyploid establishment, Minority Cytotype Exclusion (MCE), posits that in a finite population because a newly arising cytotype is infrequent compared to its progenitor cytotype the newly arising cytotype will have fewer potential mates and produce fewer seeds per plant resulting in a lower probability of replacement in the next generation and be excluded (ultimate cause of minority cytotype exclusion; Levin 1975). The formation of sterile hybrids results in gamete wastage and increases the tempo of exclusion, by further lowering the probability of replacement in the next generation (proximate cause of minority cytotype exclusion). All else being equal between cytotypes, minority cytotype exclusion eliminates a novel cytotype from the population unless it starts at a frequency greater than 50% (Levin 1975).

Since Levin's 1975 work, over a dozen models have investigated parameter values and conditions that might allow polyploid establishment under the assumptions of minority cytotype exclusion. These include: inclusion of hybrid bridges or blocks between cytotypes

(Felber and Bever 1997; Ramsey and Schemske 1998; Husband 2004; Yamauchi et al. 2004; Fowler and Levin 2016); high degrees of assortative mating (Rodríguez 1996a; Li, Xu, and Ridout 2004; Oswald and Nuismer 2011); the minority cytotype having higher fitness than the progenitor cytotype (Fowler and Levin 1984; Felber 1991; Li, Xu, and Ridout 2004; Rausch and Morgan 2005; Oswald and Nuismer 2007); niche separation between cytotypes (Fowler and Levin 1984; Rodríguez 1996a; Fowler and Levin 2016); prezygotic isolation barriers (Husband and Sabara 2003); iteroparity (Rodríguez 1996b); and population spatial structuring (Griswold 2021). However, in each of these models, establishment typically occurs only under strict conditions, often requiring model parameters with biologically unrealistic magnitudes. For example, in explorations of cytotype fitness differences, fitness of the minority cytotype to permit overcoming minority cytotype exclusion needs to be 2-3 times that of the majority cytotype (Felber 1991; Li, Xu, and Ridout 2004; Rausch and Morgan 2005). Such an advantage is apparently rare; several studies have found the derived cytotype has *lower* fitness than the progenitor cytotype (Burton and Husband 2000 $W_{D/P} = 0.67$, where $W_{D/P}$ is the ratio of derived to progenitor fitnesses; Husband 2000 $W_{D/P} = 0.40\text{-}0.69$; Pavlíková et al. 2017 $W_{D/P} = 0.34\text{-}0.94$), and studies finding higher fitness only sometimes detect the 2-3x advantage needed (Gross & Schiestl 2015 $W_{D/P} = 2\text{-}3$, but see McIntyre and Strauss 2017 $W_{D/P} = 1.09\text{-}1.65$).

Absent a general polyploid advantage, it makes sense to investigate the rates at which new polyploid individuals are formed. The union of unreduced gametes is the main route by which new polyploid individuals are created and thus deserves particular attention (Harlan and De Wet 1975; Thompson and Lumaret 1992; Bretagnolle and Thompson 1995; Ramsey and Schemske 1998). All the above-mentioned models, whether explicitly present or implied, rely on the formation and joining of unreduced gametes for polyploid formation. Unreduced gametes are those that retain the complete parental chromosome complement (i.e., for diploid parent, an unreduced gamete is diploid instead of haploid). Aberrant meiosis can result in unreduced gametes and may occur following chromosomal non-reduction in meiosis I, chromosomal non-separation in meiosis II, or incomplete cytokinesis after either meiosis I or meiosis II (Brownfield and Köhler 2011). Higher formation rates of unreduced gametes (u) increase the numbers of novel polyploids being

formed, and thus increase the chances that a novel cytotype will escape minority cytotype exclusion and establish. Strikingly, however, the widely observed empirical mean *u* value for angiosperms is 0.5-2% (Ramsey and Schemske 2002; Kreiner, Kron, and Husband 2017), far lower than the value of 17% required to overcome minority cytotype exclusion predicted by models, all else equal (i.e. equal fitness across cytotypes, etc.) (Felber 1991; Rausch and Morgan 2005; Clo, Padilla-García, and Kolář 2022).

The disconnect between the biology and model outcomes suggests that we are not modelling *u* appropriately. Nearly all existing models have assumed a static *u*, despite evidence that *u* is dynamic. Both genetics and environment are general factors that can modify rates of meiotic errors and directly affect *u*. Two models that have included variability in gametic processes (Rodríguez 1996b; Li, Xu, and Ridout 2004) did not evaluate the specific effects the variability had on rates of polyploid establishment making it impossible to attribute the changes in rates of polyploid establishment to variability in gametic processes. A recent model from Clo, Padilla-García, and Kolář (2022) has included variability in gametic processes and investigated the specific effects it has on polyploid establishment while modeling *u* as an evolving quantitative trait. They investigate when *u* evolves by genetic drift and when that may result in polyploids fixing in a population. No model has examined environmental controls on *u* and how they may impact rates of polyploid establishment. Documentation of environmental controls on *u* supports an intuition that meiotic mistakes should be more frequent when the cell machinery is under duress. While experimental tests are scarce and sometimes have designs that limit their utility, there is evidence that stress can increase *u* in plants, including both temperature stress (Mason et al. 2011; De Storme, Copenhaver, and Geelen 2012; Laurent Crespel et al. 2015; Wang et al. 2017) and water stress (Giles 1939). As environments are often variable, variation in environmentally-induced stress could thus generate variation in *u*.

We thus propose that modelling *u* as a dynamic and stochastic parameter and evaluating its specific effects on the minority cytotype exclusion barrier may provide new insights into rates of polyploid establishment. Including environmental stochasticity has improved model fit in a number of evolutionary models, from models of male-biased dispersal

(Henry, Coulon, and Travis 2016) to those investigating the speed of evolution (Danino, Kessler, and Shnerb 2018). For simplification, we focus only on modelling the origination of autopolyploids. Here, we use variation in *u* observed in natural plant populations (24 Brassicaceae species; Kreiner, Kron, and Husband 2017) to perform a global sensitivity analysis of an minority cytotype exclusion model based on Rausch & Morgan (2005). We discuss the potential for our results to help reconcile theoretical predictions that polyploid establishment should be rare with empirical observations polyploid plants are common. This work has two main questions:

1) What distribution best fits the empirically observed formation rate of unreduced gametes (*u*)?
2) What effects, if any, does environmental stochasticity in *u* have on model predictions?

Methods

What distribution best fits the empirically observed formation rate of unreduced gametes (u)?

With flow cytometry, it is now possible to measure microgametophyte (pollen) *u* on a large scale. Kreiner et al. (2017) report estimates of pollen *u* from 60 populations of 24 different species within the plant family Brassicaceae. We used data from 59 populations (one population was excluded due to low sample size; 4 ind.), for a total of 1724 individuals. The distribution of unreduced gametes across all included individuals was positively skewed, with a range from 0% to 86.29% and a mean of 2.86% (SE = 0.15%; median = 1.47%).

We used the moment matching feature of the *FitDistrPlus* package in R (Delignette-Muller and Dutang 2015) to determine the best fit to the Kreiner et al. (2017) empirical data, exploring beta, gamma, log-normal, and Weibull distributions. To examine the generality of the fits, we first grouped the data in four different ways: by reproductive mode (predominately outcrossing n = 518, mixed mating n =517, predominately selfing n = 627 and predominantly asexual n = 66; following Kreiner et al. 2017), ploidy (2x, 4x, 6x, 8x,

12x, 20x, 24x, 30x), species (n = 24, see Kreiner et al. 2017 for complete list) and population (n = 59). For each group, we determined the best overall theoretical distribution using a strict AIC ranking, where the distribution with lowest AIC was deemed the best fit, regardless of the next closest AIC.

What effects, if any, does environmental stochasticity in u have on model predictions?

We performed a global sensitivity analysis using Sobol' indices (Sobol′ 1993) to assess how natural variation in *u* impacts model predictions and the importance of specific model parameters to overcoming theoretical barriers to polyploid establishment. Sobol' indices allow us to quantify how much of the uncertainty in the model output is due to each uncertain input parameter (Sobol′ 1993).

Table 1: Variables and parameters in the Rausch and Morgan (2005) model. Sampling distribution describe the distributions for each model parameter evaluated in the global sensitivity analysis.

Variables	**Symbol**	
Cytotype frequency at time t (diploid, tetraploid)	d_t, t_t	
Factor		
Scaling factor to ensure d_t and t_t sum to 1	k	
Parameters		**Sampling Distribution**
Formation rate of unreduced gametes	u	Lognormal($\mu = 0.026$, $\sigma^2 = 0.0016$)
Selfing rate (diploid, tetraploid)	s_d, s_t	$\mathcal{U}\{0,1\}$
Inbreeding depression (diploid, tetraploid)	δ_d, δ_t	$\mathcal{U}\{0,1\}$
Relative fitness of tetraploid to diploid	w	$\mathcal{U}\{0,2\}$

Base Model

We used the Rausch and Morgan (2005) model which is deterministic and seeks to ascertain how relative fitness, selfing, inbreeding depression and unreduced gamete production affect minority cytotype exclusion (MCE). The major findings from their analysis were that higher selfing and lower inbreeding depression of tetraploids (relative to diploids) are pathways to overcoming minority cytotype exclusion (Rausch and Morgan 2005).

The Rausch and Morgan (2005) model is a set of recursion equations (1-3) that model the frequency of diploids and tetraploids in a single, infinite-size population of annual hermaphroditic plants. Triploids are assumed to be inviable and are not included. See Table

1 for definitions of parameters and variables. Each time step *t* begins with mating of adults from the previous generation and ends before the mating of the next generation, *t + 1*. Micro- and megagametophytes are assumed to be produced at equal frequencies, with diploids producing either reduced haploid gametes (frequency 1- *u*) or unreduced diploid gametes (frequency *u*); tetraploids produce only reduced diploid gametes.

Equation 1: Growth of diploid population from Rausch & Morgan 2005

$$d_{t+1} = \{[d_t(1 - s_d)]^2 + d_t s_d(1 - \delta_d)\}(1 - u)^2/k$$

***Equation 2*:** Growth of tetraploid population from Rausch & Morgan 2005

$$t_{t+1} = \{[(1 - d_t)(1 - s_t) + d_t(1 - s_d)u]^2 + [(1 - d_t)s_t + d_t s_d u^2](1 - \delta_t)\}\frac{w}{k}$$

Equation 3: Scaling factor for total population from Rausch & Morgan 2005

$$k = \{[d_t(1 - s_d)]^2 + d_t s_d(1 - \delta_d)\}(1 - u)^2 + \{[(1 - d_t)(1 - s_t) + d_t(1 - s_d)u]^2 + [(1 - d_t)s_t + d_t s_d u^2](1 - \delta_t)\}w$$

Global sensitivity analysis and incorporation of environmental stochasticity in u

Global sensitivity analysis was performed using the *sobol_matrices*, *sobol_indices* and *sobol_dummy* functions from the *sensobol* R package (Puy et al. 2022). Sobol' indices were obtained for each uncertain model parameter and their interaction terms. Two important indices were recorded, first-order sobol (S_i) and total-order sobol (T_i). S_i measures the direct effect of each parameter on model outcome variance. If there are interaction effects, the sum of S_i values will be less than 1. T_i includes the sensitivity of both first-order effects as well as all higher order interactions between a given parameter and all other parameters. Sampling matrices used a quasi-random number sampling approach to select 7500 unique model input combinations for each model input parameter from bounded uniform distributions (Table 1). The quasi-random number approach was chosen to leave the smallest unexplored volume (Puy et al. 2022) and uniform distributions were chosen to maximize input variability. Azzini equations were used to calculate S_i and T_i as they have been shown to outperform other methods (Azzini, Mara, and Rosati 2020). Models were run for 250 generations and confidence intervals were calculated for S_i and T_i using 100

bootstrap replicates. A given input combination was deemed to have overcome minority cytotype exclusion if a tetraploid frequency above 50% was reached by 250 generations.

To evaluate the effect of environmental stochasticity in *u* we calculated S_i and T_i for two versions of the model. The first version held *u* constant at 0.02, which is the value used in prior models (Oswald and Nuismer 2011; Fowler and Levin 2016) described in the literature (Ramsey and Schemske 2002) and the mean in the population-level *u* dataset (Kreiner et al. 2017). From here forward we refer to this as the static model. The second version used a randomly drawn *u* value each generation from a population-level mean *u* distribution. From here forward we refer to this as the stochastic model. The population-level mean *u* distribution was determined by maximum-likelihood estimates for the moments of the empirical population-level mean *u* values from Kreiner et al. 2017 (Figure Appendix A: Figure S1). Estimates of the empirical *u* distribution moments were used to generate a log-normal distribution with matching moments (Table 2). To encapsulate the effect of stochasticity the same sampling matrix was evaluated 50 times (100 evaluations, SE = 0.0005 on Sobol' indices). The mean generation that minority cytotype exclusion was overcome for each input combination was used to calculate S_i and T_i. This approach allows for qualitative comparison of the S_i and T_i values between parameters in the static and stochastic evaluations.

Results

Table 2: Counts of best fits for *u* distributions from wild populations of 24 Brassicaceae species. The same data have been grouped in four different ways: by reproductive mode, ploidy, species, and population. N is the number of datasets analyzed under each grouping scenario. The remaining column headers correspond to the four distributions that were tested.

Grouping	*N*	*Weibull*	*Log-Normal*	*Gamma*	*Beta*
Reproductive mode	4	-	4	-	-
Ploidy	8	-	8	-	-
Species	24	1	21	2	-
Population	59	6	44	4	5

What distribution best fits the empirically observed formation rate of unreduced gametes (u)?

Regardless of whether the empirical data set was grouped by reproductive mode, ploidy, species or population, the majority of cases supported a log-normal distribution as the best fit (Table 2). For the analyses of populations (n=59), 44 supported a log-normal distribution as the best-fit, while 15 supported other distributions, with Weibull and beta as the next most common (Table 2, Appendix A: Table S1). 88% of population-mean *u* were above the distribution mean (0.02), supporting a strong skew in the data.

What effects, if any, does environmental stochasticity in u have on model predictions?

We investigated the importance of model parameters on overcoming minority cytotype exclusion, the percentage of times minority cytotype exclusion is predicted to be overcome and the mean generation that minority cytotype exclusion is overcome.

Overall, environmental stochasticity in *u* decreases the total-order importance of inbreeding depression and selfing rate, while increasing the total-order importance of the relative fitness relationship between cytotypes in determining whether minority cytotype exclusion is overcome (Figure 2). We found that the relationship between selfing and inbreeding values for both cytotypes changes from having a moderate impact on the percentage of times minority cytotype exclusion is overcome with a static *u* to having a lower impact with a stochastic *u* (Figure 3). Across parameters, we found the mean generation to overcome minority cytotype exclusion was reduced (approximately 30-40 generations) for most input combinations when a stochastic *u* is implemented compared to a static *u* (Figure 4C).

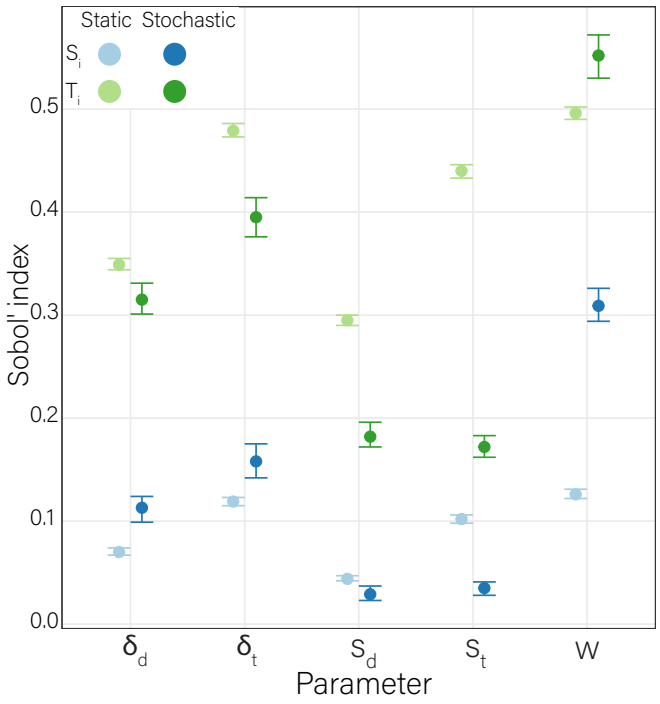

Figure 2: Sobol' indices resulting from static and stochastic evaluations of a model of minority cytotype exclusion. S_i is the first-order sobol index (blue points) and shows the direct effect each input parameter has on variation in model outcome. T_i is the total-order sobol index (green points) and shows the sum of first order and all interaction effects on model outcome. Color lightness corresponds to static (light) and stochastic (dark) evaluations. Parameter values are inbreeding depression of diploids (δ_d) and tetraploids (δ_t), selfing rate of diploids (s_d) and tetraploids (s_t), and the relative fitness of the tetraploid to the diploid (w). S_i values are higher for inbreeding depression and fitness under a stochastic model evaluation versus a static model evaluation. S_i and T_i values are decreased for selfing rate parameters under a stochastic model evaluation versus a static model evaluation, whereas they are increased for relative fitness of the tetraploid.

Inbreeding Depression

Overall, the inbreeding depression values relative relationship has a moderate impact on the likelihood of overcoming minority cytotype exclusion with a stochastic *u* (Figure 3). Including environmental stochasticity in *u* demonstrates the possibility to overcome minority cytotype exclusion where it is not possible with a static *u* (compare paucity of light blue points to their dark blue counterparts on the left of Figure 3). Under a static *u* the inbreeding depression values relative relationship has a strong association with the likelihood of overcoming minority cytotype exclusion: when the diploid has a much higher inbreeding depression value than the tetraploid, it becomes more likely than not that minority cytotype exclusion will be overcome (Figure 3).

We observe asymptotic behavior at the upper end of diploid inbreeding values (blue points in Figure 4A), where there is a plateauing of the mean generation minority cytotype exclusion is overcome starting around $\delta_d = 0.7$. Under a stochastic u, parameter interactions with δ_d primarily decrease to immeasurable levels, except $\delta_d S_d$, which has a slight increase (Appendix A: Table S3). Taken together these describe an exact biological parameter space corresponding to predominantly outcrossing species.

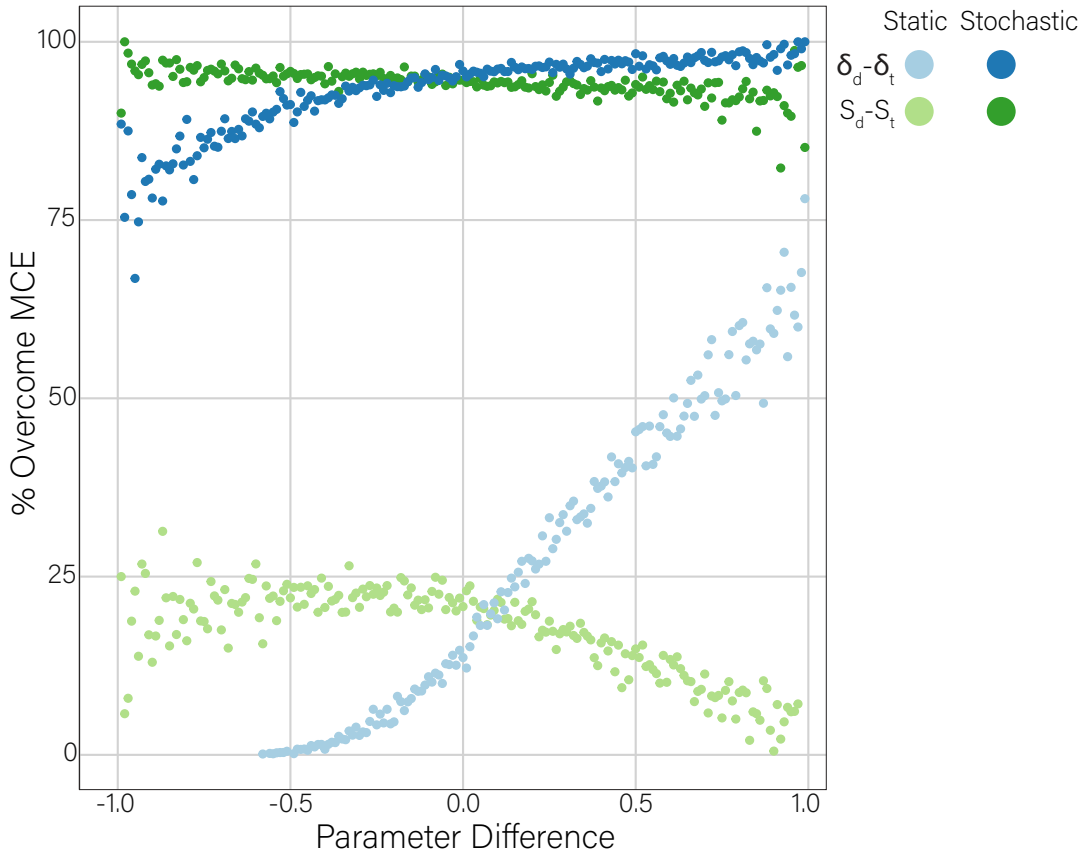

Figure 3: Percent of times minority cytotype exclusion is overcome in both static and stochastic evaluations for various relative relationships of cytotype inbreeding depression and selfing rates. Color lightness corresponds to static (light) and stochastic (dark) evaluations. Parameter values are inbreeding depression (blue points) of diploids (δ_d) and tetraploids (δ_t), selfing rate (green points) of diploids (s_d) and tetraploids (s_t). Thus δ_d-δ_t and $s_d - s_t$ represent the relative relationship of inbreeding depression and selfing rates faced by the two cytotypes, respectively. Stochastic evaluations have a higher likelihood of overcoming MCE for all relative relationships than do static evaluations. The inbreeding depression parameter relationship changes between a stochastic and static evaluation, from a near exponential relationship to a more linearized one.

Selfing Rate

We observe the first-order and total-order impacts are approximately equal for both cytotypes selfing rates ($S_{s_d} \cong S_{s_t}$ & $T_{s_d} \cong T_{s_t}$) with environmental stochasticity in u

(Figure 2, Appendix A: Table S2). Under a stochastic *u,* parameter interactions with $s_{d,t}$ have primarily negligible change or slight decreases to immeasurable levels (Appendix A: Table S3). When s_d and s_t values are more dissimilar from one another, under a static *u*, the times minority cytotype exclusion is overcome is lower than when they are similar (light green points in Figure 3). Under a stochastic *u* there is a more linear relationship for $s_{d,t}$ values to likelihood of overcoming minority cytotype exclusion, compared to a static *u*. When $s_t >> s_d$, minority cytotype exclusion is overcome more often than when the $s_d >> s_t$ for a stochastic *u,* though the change is minimal compared to a static *u* (dark green points in Figure 3).

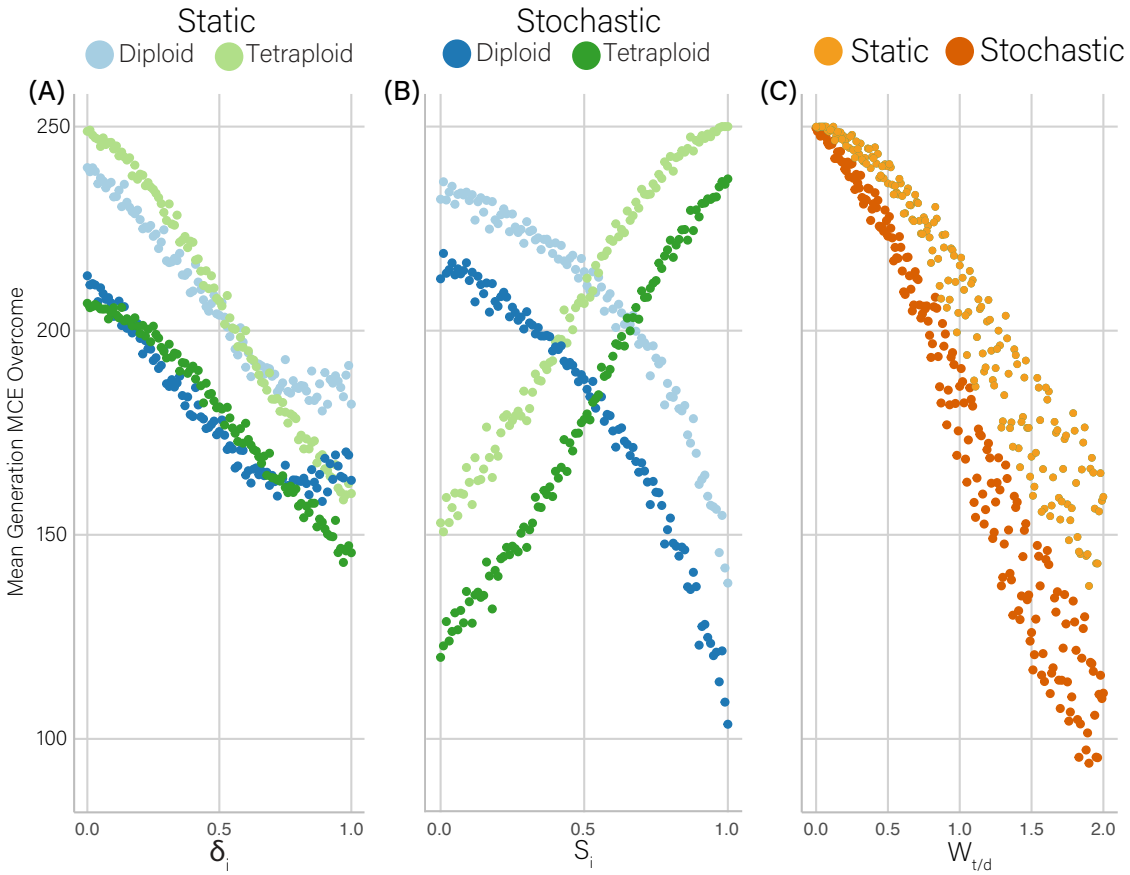

Figure 4: Mean generation minority cytotype exclusion (MCE) is overcome for exact values of each model input parameter for static and stochastic model evaluations. Color lightness corresponds to static (light) and stochastic (dark) evaluations. Parameter values are inbreeding depression (blue points) of diploids (δ_d) and tetraploids (δ_t), selfing rate (green points) of diploids (s_d) and tetraploids (s_t) and the relative fitness (orange points) of the tetraploid to the diploid ($w_{t/d}$). Stochastic model evaluations consistently have lower mean generation to overcoming MCE versus static evaluations for the same parameter input values.

Fitness

Under a stochastic *u,* the cytotype fitness relationship has the largest effect of all parameters on model outcome variance, with a large increase in the first-order impact and a small change for total-order impact (Figure 2). The mean generation minority cytotype exclusion is overcome has a similar linear relationship between static and stochastic evaluations (Figure 4C). At low w the mean generation to overcome minority cytotype exclusion is similar between static and stochastic evaluations, but more rapidly drops to lower generations under a stochastic evaluation with increasing w (negative slope of dark orange points compared to light orange points in Figure 4C).

Discussion

The key finding from this study is that accounting for environmental stochasticity in *u* lowers the perceived barrier posed by minority cytotype exclusion. Thus, including variation in *u* helps reconcile theoretical predictions that polyploid establishment should be difficult and rare with the observed prevalence of polyploidy in nature.

The distribution of u in natural populations

Variation in *u* has been largely ignored in previous models that consider processes to overcome minority cytotype exclusion. Only recently has it become possible to adequately quantify natural variation in *u* (Kreiner, Kron, and Husband 2017), thus permitting incorporation of this variation in models of polyploid establishment. Our analysis of the data from Kriener et al. (2017) found the underlying *u* distribution is often log-normal, which is not surprising. Unreduced gametes are the culmination of multiple possible meiotic errors, thus what we observe as *u* is the result of a multiplicative process. Multiplicative processes are generally expected to yield a log-normal distribution (Limpert, Stahel, and Abbt 2001). One reservation with this finding is that we only use *u* values from a subset of a single plant family, the Brassicaceae. It is possible there may be more or less variability in *u* in other clades due to underlying genetic architecture or biogeography. Fortunately, the model can easily be parameterized to more data, when available.

The importance of model parameters with environmental stochasticity in u

Including environmental stochasticity in u has nonuniform impacts on the effects of each model parameter. It increases the first-order effect on model outcome for inbreeding depression values (Figure 2, Appendix A: Table S2), whereas it both decreases and makes approximately equal the impact of selfing rates. The largest impact to first-order effects is for relative fitness, where the inclusion of stochasticity in u increases its impact nearly two-fold.

Inbreeding Depression

Theory predicts inbreeding depression should be higher in the progenitor cytotype than the derived cytotype (Bartlett and Haldane 1934)However, empirical evidence has found tetraploid inbreeding depression is close to the diploid value (Galloway and Etterson 2007) and overall is high in predominantly outcrossing species (Ozimec and Husband 2011). Inbreeding depression has been theorized to increase cytotype coexistence when cytotypes experience inbreeding depression asymmetrically , which has some empirical support (Ozimec and Husband 2011; Rosche et al. 2017; Siopa et al. 2020; Clo, Padilla-García, and Kolář 2022). Thus, predictions where inbreeding depression is equal or slightly higher in the diploid than tetraploid (Figure 3) are the most suitable to consider, given current understanding.

To reconcile theoretical and empirical observations requires more empirical measurements of inbreeding depression for progenitors and their neo-derived cytotypes. Inbreeding depression measurements need to be performed across time, as the values and their relationship to one another are known to change across time (see Siopa et al 2020). Regardless of these additional pursuits, we find environmental stochasticity in u significantly increases the likelihood of overcoming minority cytotype exclusion irrespective of the δ_d value (Figure 3).

Selfing Rates

Empirical work has found that when a neo-derived cytotype formed, self-incompatibility did not suddenly change from the progenitor, though variability in expression of self-incompatibility did increase (e.g., selfing rates can be similar in neo-derived cytotypes to their progenitor) (Siopa et al 2020). Thus, predictions where selfing rates are equal or slightly lower in the diploid than in the tetraploid (Figure 3) are the most suitable, given current understanding. When self-incompatibility is determined by S-alleles, there is evidence that neo-polyploidization directly results in increased capacity for self-compatibility suggesting a possible route for overcoming minority cytotype exclusion (Sutherland, Quarles, and Galloway 2018)

With selfing come the effects of inbreeding depression. Inbreeding depression decreases the benefit of selfing as the fitness of selfed offspring is reduced, thus potentially reinforcing the barriers of minority cytotype exclusion (Rausch and Morgan 2005). We observe this cost to selfing in our analysis when the mean generation to overcome minority cytotype exclusion increases with an increasing s_t value (Figure 4B). This is likely due to selfing events having an increasingly larger contribution to the tetraploid population in the next generation (e.g., t_{t+1}) than outcrossing events. The selfing events are scaled by inbreeding depression which ultimately lowers the per generation contributions to tetraploid population for selfing events, as compared to outcrossing events. A stochastic *u* does not change the overall relationship of s_i to a static *u* (same shape of green points in Figure 4B). However, a stochastic *u* does dramatically increase the likelihood that minority cytotype exclusion is overcome compared to a static *u* (Figure 3). Thus, we reason that environmental stochasticity in *u* decreases the overall cost of selfing, though does not change the tempo of overcoming minority cytotype exclusion.

Fitness

Finding that the cytotype fitness ratio is important comes as no surprise, as prior models have found the same (Yamauchi 2004, Rausch and Morgan 2005, Suda & Herben 2012).

However, the increase in the impact of the fitness parameter value on model outcome with incorporation of environmental stochasticity in *u* is noteworthy (Figure 2 & 4).

Comparisons with previous models of polyploid establishment that have included variation in the gametic process

Three prior models have incorporated stochastic variation in the gametic process (Rodríguez 1996b; Li, Xu, and Ridout 2004; Clo, Padilla-García, and Kolář 2022). Our approach differs from these previous models in that 1) our model variation originates directly from empirical *u* data, 2) we investigate the specific effects this variation has on model outcome and 3) we are examining effects of environmental stochasticity in *u* on polyploid establishment.

Rodríguez (1996b) included demographic stochasticity, through variation in the ovule number per diploid plant, based upon inferred ovule number variation in the pignut hickory tree (Sork, Bramble, and Sexton 1993). The variation in ovule number has a symmetric Poisson distribution, which is unlike the asymmetric log-normal distributions we use for *u* in the current study. Rodríguez (1996b) did not directly compare model outcomes with and without stochasticity in ovule number. The author posited that demographic stochasticity somewhat decreases the likelihood of polyploid establishment, which is the opposite of our results for environmental stochasticity in *u*.

Li, Xu, and Ridout (2004) included uniform random variation in gamete ploidy level. The model then simulates post-pollen-formation processes (pollen dispersal, pollination, and seed production), using draws from uniform random or half-Cauchy probability distributions. The stochasticity in pollen dispersal and pollination result in the inherent unreduced gamete frequency distribution approximating a log-normal shape. Thus, it is not surprising their results are similar to ours – stochasticity in the gametic process lowers the barrier to overcoming minority cytotype exclusion. However, their multi-step approach of simulating gamete ploidy, pollen dispersal, pollination and seed dispersal make it impossible to determine whether it is the stochasticity in gamete ploidy or post-pollen-

formation process that led to this result. Though the authors present a comparison of their stochastic modeling results to a deterministic model for some of the model characters (spatial heterogeneity, pollen, and seed dispersal), it is not clear the direct effect, if any, of including variation in unreduced gametes on model outcome.

Clo, Padilla-Garcia, Kolar (2022) focuses on genetic factors that modify rates of *u*, while our approach focuses on environmental factors. They similarly found that self-fertilization was less important than previously thought, which suggests stochasticity in *u*, regardless of its origin, is important to include in any future models examining minority cytotype exclusion. However, they find that *u* needs to be at least 15% for polyploidy to fix, in initially diploid populations, which is like previous studies (17%) and is much higher than what is found in nature (~2%). This is a major difference between our findings. Our approach using population-level *u* variation supports that observed levels of *u* in nature permit overcoming minority cytotype exclusion. The Clo, Padilla-Garcia, Kolar (2022) model is an important contribution to the field, as it should motivate estimates for the number of loci contributing to *u* and their heritability. Having accurate estimations of these values is critical to begin understanding the gene-environment interactions that contribute to observed variation in *u*.

Conclusions and future directions

Here, we have shown that accounting for natural variation in *u* lowers the perceived barrier posed by minority cytotype exclusion, permitting polyploid establishment at naturally observed population-level mean *u* values. However, we have only a nascent understanding of variation in *u* in nature. More studies of empirical *u* variation are needed to better understand its role in polyploid establishment in different clades and gene-environment interactions. Kron and Husband (2015) outline methods suitable for investigating the temporal, spatial and environment-induced (i.e., temperature and water stress) *u* variation in both natural populations and under controlled greenhouse conditions. Further, understanding whether and how *u* distributions change intra- and inter-annually, and whether *u* can be temporally autocorrelated due to environment or heritability, could further help resolve the paradox of polyploidy. Temporal variation in *u* could contribute to

successful or failed recruitment events for either cytotype, which in turn may either contribute to their long-term coexistence or hasten minority cytotype exclusion and result in a loss of the neopolyploid.

Renewed efforts towards understanding and quantifying female unreduced gamete formation are required. Much of the work on unreduced gametes assumes male and female gamete dynamics are the same, despite evidence to the contrary (Brownfield and Köhler 2011); a set of paired experiments with *Dactylis glomerata* L. (De Haan et al. 1992; Maceira et al. 1992) found the average unreduced female and male gametes at 0.49% and 0.98% frequencies, respectively. Across taxa, if female *u* were discovered to be lower than male *u* (as suggested in *Dactylis*), the perceived barriers to polyploid formation would increase. In this case, more male unreduced gametes will result in ineffective matings. The rate of production of female unreduced gametes would then be the key parameter to measure as it would likely determine the model outcome. Alternatively, if female *u* were discovered to be greater than male *u*, the perceived barriers to polyploid formation would be lowered, as the occurrence of effective matings from unreduced gametes should be higher.

Our finding that environmental stochasticity in *u* eases the barrier to overcoming minority cytotype exclusion contrasts with models that have found demographic stochasticity can increase the barrier (autopolyploids: Rodríguez 1996b, allopolyploids: Fowler and Levin 2016) and aligns with others that find it decreases barriers (Li, Xu, and Ridout 2004; Rausch and Morgan 2005; Clo, Padilla-García, and Kolář 2022). It would be non-trivial to incorporate both environmental and demographic stochasticity into a single model to determine how the effects of environmental stochasticity interact with demographic stochasticity. Further, existing work (Li, Xu, and Ridout 2004, Griswold 2020) has found and others have theorized (Burton and Husband 2000; Levin 1975) the crucial role spatial relations between individuals can have towards dramatically lowering the barriers to minority cytotype exclusion. Thus, future work may include individual plant *u* variation in an agent-based spatially explicit model to move our understanding forward to answering why there are so many polyploids.

Chapter 2: Differentiation of Root-Associated Fungal Assemblages by Host Ploidy Level in Mixed-Ploidy *Larrea tridentata* Populations

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Abstract

Polyploidy—whole genome duplication—is common in plants. Studies over the last several decades have documented numerous mixed-ploidy populations. Whether arising via recurrent whole genome duplication events within a population, or from secondary contact, the persistence of mixed-ploidy populations is possible by niche differentiation. Specifically, one mechanism facilitating ploidy co-occurrence is microbially-mediated niche differentiation (MMND), wherein cytotypes occupy different niches via interactions with different sets of microbes. Inherently cryptic, MMND is underexplored in polyploid plant populations. Here, we search for evidence of MMND in creosotebush (*Larrea tridentata*), a dominant desert shrub of the southwestern U.S. and northern Mexico. We sequenced root-associated fungal taxa in soils of diploid, autotetraploid, and autohexaploid plants growing in two naturally-occurring mixed-ploidy populations. Within populations, we found substantial fungal assemblage overlap across host plant cytotypes. However, using indicator species analysis, we identified some fungi that are differentiated by host plant cytotype, satisfying a necessary condition for MMND. Future study is needed to determine the degree of niche differentiation conferred, if any, and whether the identified fungi play a role in the long-term persistence of multiple cytotypes within populations.

Introduction

Angiosperms have a long history of polyploidization (Landis et al. 2018), the origination and maintenance of more than two complete chromosome sets within an organism. Within a single species, polyploid complexes can form via recurrent polyploidization events within a population or through migration between populations, resulting in multiple cytotypes (e.g., diploid, tetraploid, etc.) occurring at the same location. Over the last several decades, there has been a renewed interest in understanding the population-level processes driving cytotype co-occurrence and patterns of biodiversity (Coyne and Orr 2004; Ramsey and Ramsey 2014; Segraves and Anneberg 2016; Laport and Ng 2017).

Niche differentiation is one mechanism that permits cytotype coexistence within populations (Fowler and Levin 2016). Niche differentiation among cytotypes has been documented for many species and is linked to both abiotic niche factors (e.g., substrate, elevation, temperature, moisture; (Laport et al. 2013; López-Jurado, Mateos-Naranjo, and Balao 2019; Wan et al. 2019; Decanter et al. 2020) and biotic niche factors (e.g., herbivores, pollinators; (Münzbergová, Skuhrovec, and Maršík 2015; Laport, Minckley, and Ramsey 2016; Muñoz-Pajares et al. 2018; Čertner et al. 2019; O'Connor, Laport, and Whiteman 2019). Even slight differences in traits between diploids and polyploids may facilitate successful coexistence (Husband 2000) by easing direct ecological competition and promoting assortative mating. Niche differences may arise upon formation of new cytotypes, or through a period of post-polyploidization isolation and adaptation to novel ecological conditions prior to secondary contact. The diversity of phenotypic differences that have been documented between diploids and polyploids (Levin 1983; Segraves and Thompson 1999; Husband et al. 2008; Maherali, Walden, and Husband 2009; López-Jurado, Mateos-Naranjo, and Balao 2019) may help explain why so many present-day plant communities contain polyploids co-occurring with their close diploid relatives (Gaynor et al. 2018).

Microbially-mediated niche differentiation (MMND) represents a possible cryptic and underexplored mechanism of niche differentiation for polyploid complexes. In MMND, microbes can help plants acquire nutrients and thus expand or shift their niche dimensions.

For example, derived cytotypes (e.g., tetraploids, hexaploids, etc.) can have different quantities of root exudates (Wu et al. 2019), which may allow them to recruit distinctive microbial communities (Segraves 2017). Prior research on mixed-ploidy populations of orchids in the *Gymnadenia conopsea* group have found cytotype-specific root-associated fungal assemblages (Těšitelová et al. 2013). This observation held for both field-collected adults and seedlings and was most pronounced within a site with closely sympatric adults (within $1m^2$). In close proximity, diploid *G. conopsea* shared only one occurrence of the same fungal OTU with tetraploid *G. conopsea* (Těšitelová et al. 2013). This compositional difference in root-associated fungal assemblages may contribute to different niche occupation (*sensu* MacArthur 1958) and allow for coexistence of these orchid cytotypes. In contrast, observations from *Aster amellus* and *Centaurea stoebe* indicate no significant differences in arbuscular mycorrhizal fungi between diploids and tetraploids, suggesting root mycorrhizal associations may not be strong contributors to niche differentiation in all polyploid species (Sudová et al. 2014; Sudová et al. 2018). Thus, it remains unclear if MMND is common among taxa comprising multiple cytotypes.

The North American creosote bush [*Larrea tridentata* (DC.) Coville; Zygophyllaceae] is an autopolyploid complex distributed across the southwestern U.S. and northern Mexico (Mabry, Hunziker, and Difeo, Jr. 1977). The complex comprises three distinct cytotypes (diploid, $2n = 2x = 26$; tetraploid, $2n = 4x = 52$; and hexaploid, $2n = 6x = 78$) with distributions approximately corresponding to the three warm deserts of North America (Chihuahuan Desert, Sonoran Desert and Mojave Desert, respectively) in which they are dominant shrubs (Mabry, Hunziker, and Difeo 1977). Prior work has mapped the cytogeography of the complex, identifying multiple natural contact zones (Hunter et al. 2001; Laport, Minckley, and Ramsey 2012; Laport and Ramsey 2015). The current distributions with relatively narrow areas of $2x$-$4x$ contact near the boundary between the Chihuahuan and Sonoran Deserts, and broad overlap of the $4x$ and $6x$ cytotypes in the Sonoran Desert, likely represent secondary contact after complex biogeographic histories involving migration and adaptation during glacial and post glacial periods (Hunter et al. 2001; Laport, Minckley, and Ramsey 2016), though polyploid cytotypes may be recurrently formed and could represent instances of primary contact (Laport, Minckley,

and Ramsey 2016). Although the cytotypes appear to be at least partially ecologically differentiated along several niche axes (e.g., climatic, vegetation communities, herbivore specificity, pollinator visitation; Laport et al. 2013; Laport, Minckley, and Ramsey 2016; O'Connor, Laport, and Whiteman 2019; Laport, Minckley, and Pilson 2021) it remains unclear whether such differences are sufficient to maintain sympatry or whether MMND may interact with other niche differences to facilitate coexistence at natural areas of contact.

Here, we investigate whether host-cytotype specific microbial associations are present, a necessary pre-condition of MMND. We hypothesize that in mixed-ploidy populations, 1) cytotypes have largely dissimilar root-associated fungal assemblages and 2) exhibit host-cytotype root-associated fungal specialization.

Methods

Study system

Larrea tridentata is a long-lived perennial evergreen shrub that reproduces via seed, but may also propagate clonally (Mabry, Hunziker, and Difeo, Jr. 1977). The three cytotypes have relatively well-defined distributions, likely maintained by abiotic environmental variation, but also potentially determined by pollinator-mediated assortative mating or galling midge interactions at cytotype contact zones (O'Connor, Laport, and Whiteman 2019; Laport, Minckley, and Pilson 2021). In mixed-ploidy populations, 4*x L. tridentata* tend to be found in denser vegetation associations than 2*x* or 6*x* plants, which tend to be found at higher elevations, in more species-rich communities and on coarser soils (Laport, Minckley, and Ramsey 2016). Tetraploids tend to flower earlier and produce more flowers than 2*x* or 6*x* plants (Laport, Minckley, and Ramsey 2016). The size of morphological structures tends to increase with ploidy (e.g., larger diameter pollen grains, longer stamens and pistils, and longer, wider petals and leaves) though 4*x* plants tend to be taller than either 2*x* or 6*x* plants (Laport, Minckley, and Ramsey 2016). In mixed-ploidy 2*x*-4*x* populations, 4*x* plants have a significantly higher rate of bee visitation, but 2*x* pollen is over-represented on native bees, which may contribute to assortative mating and the maintenance of cytotype

coexistence (Laport, Minckley, and Pilson 2021). The distributions of specialist herbivore species have also been documented to be concordant with 2*x* and 4*x* *L. tridentata*, potentially resulting in cytotype-specific fitness differences that may also enable narrow zones of cytotype co-occurrence (O'Connor, Laport, and Whiteman 2019).

Field collections and sample preparation

Prior research leveraging flow-cytometric analyses to infer DNA content has identified multiple mixed-ploidy *L. tridentata* populations comprising permanently marked plants (Laport, Minckley, and Ramsey 2012, Laport and Ramsey 2015). In the current work, we sampled root-associated soils under plants of known ploidy from one 2*x*-4*x* population (San Pedro 3; 32.60°, -110.54°) and two 4*x*-6*x* populations (Algodones N4; 33.00°, -115.07° and Algodones S3; 32.81°, -114.87°; Laport and Ramsey 2015; see Figure 5). Because the mixed-ploidy sites are asymmetrically mixed, we combined the two 4*x*-6*x* populations in our analyses (and hereafter refer to them as a single population - Algodones) to balance the numbers for each co-occurring ploidy. Population sampling was limited due to their remote locations and the physical demands of sample collection. In April 2021, root-associated soils and fine roots were collected from multiple holes dug inward near the shrub base until fine roots were observed (minimum of 30cm

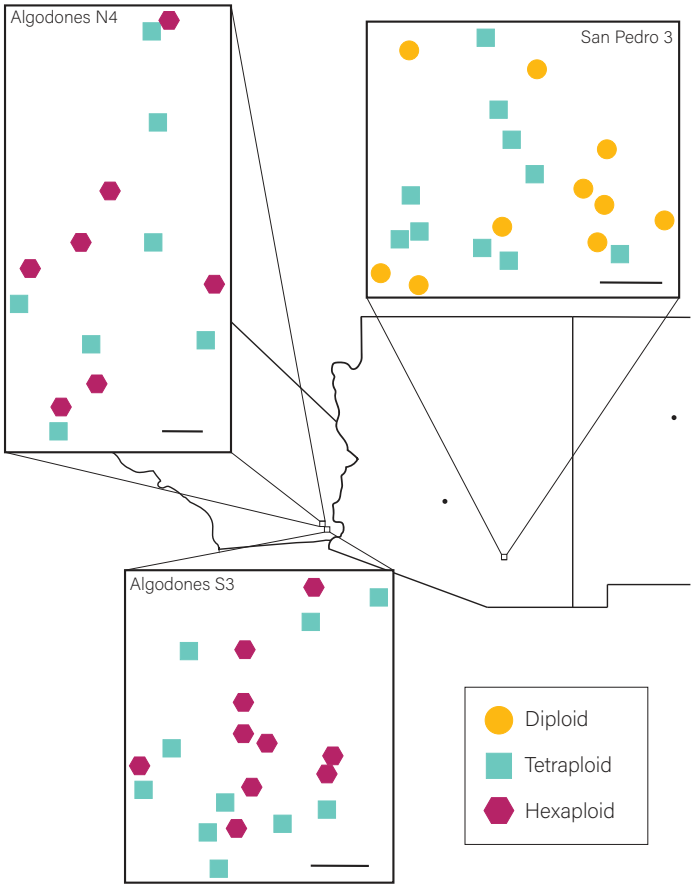

Figure 5: Localities for 54 rhizosphere collections of diploid (2*x*; circles), tetraploid (4*x*; squares) and hexaploid (6*x*; hexagons) *Larrea tridentata* from southeastern California and southern Arizona. A 100 m scale is represented within the insets for each collection site (black bar) to show spatial proximity of co-occurring plants.

deep) and pooled (to obtain a minimum of ~10mL of soil). In total 10 diploid, 27 tetraploid and 17 hexaploid root-associated soil samples were collected.

Soil samples were stored on ice and refrigerated for up to 72 h prior to performing DNA extractions. DNA extractions were performed in randomized batches with Qiagen PowerSoil kits and completed within a two-day period at the University of New Mexico. Extraction quality and DNA yield was assessed using a Qubit 3.0 Fluorometer.

Molecular and bioinformatic work

Fungal DNA in each soil extract was amplified with fungal-specific primers (ITS3-FUN and ITS4-FUN) spanning the ITS2 region (Taylor et al. 2016). Each reaction used the following PCR incubation conditions using Phusion polymerase: First, an initial denaturation at 98°C for 30 seconds, followed by 27 cycles of annealing at 58°C for 10s, extension for 60°C for 4 minutes, and then concluded with a final extension at 60°C for 20 minutes. A second PCR was conducted to add the Nextera adaptors following the same conditions but for 7 cycles. Each sample was pooled at equal concentration and sequenced on 2 x 300 cycle Illumina MiSeq runs using a single lane and library. The forward and reverse reads were merged using USEARCH9 (Edgar 2010). The primer regions were removed using Cutadapt 3.5 (Martin 2011). The sequences were then filtered to include less than one expected error, and then clustered into OTUs at 97% similarity with UPARSE (Edgar 2013). The taxonomy for each OTU was determined by running SINTAX (Edgar 2016) from USEARCH and using Utax 8.2 (Abarenkov et al. 2021) as the reference dataset. The OTU table was created by mapping reads to OTUs using the *usearch_global* function in USEARCH. The non-fungal OTUs were removed from the OTU table and then fungal OTUs were rarefied to 8000 reads in R 4.1 (R Core Team 2022) using *EcoUtils* package (Appendix B: Figure S1, Salazar 2022).

Statistical analyses

We tested for differences in fungal assemblage composition using Bray-Curtis dissimilarities and a PERMANOVA model (composition ~ ploidy, 10,000 permutations)

via the *adonis2* function in the R 4.1 package *vegan* (Oksanen et al. 2020). We ran separate PERMANOVA models for San Pedro and Algodones populations. To visualize potentially discrete fungal assemblages, we utilized non-metric multidimensional scaling (NMDS) via the *amp_ordinate* function from *ampvis2* (Andersen et al. 2018) and visualized using *ggplot2* (Wickham 2016).

To investigate whether individual root-associated fungal OTUs were associated with different co-occurring cytotypes we performed indicator analyses. Indicator species analysis was performed on the OTU relative abundance dataset using the *indicspecies* package (v 1.7.12; Cáceres and Legendre 2009) in R, with the root-associated soil samples from each plant designated as originating from different 'sites' and plant cytotype as the 'type' of site. Given this coding, each mixed-ploidy population represents a metacommunity, with the root-associated fungal OTUs being linked by potential dispersal between the root-associated communities under separate plants. This analysis resulted in indicator value indices, which can be parsed to two conditional probabilities, specificity and fidelity (Dufrêne and Legendre 1997; Cáceres and Legendre 2009). Specificity is the probability that a plant belongs to a given cytotype, given an association with a particular fungal OTU. Fidelity is the probability of finding a given fungal OTU in association with a plant of a particular cytotype. Specificity and fidelity values aid in determining that specialization may be present, and whether specialization is genuine and not simply because fungal OTUs are rare or present in the dataset due to sequencing artifacts. To reduce false positives, we filtered out all doubleton and singleton OTUs and employed a stringent ≥ 0.5 cutoff for both specificity and fidelity regardless of whether OTUs with specificity and fidelity values < 0.5 were statistically significant.

Differences of normalized read counts for each fungal OTU between cytotypes in mixed-ploidy populations were tested using paired t-tests with the Benjamini-Hochberg procedure of p-value adjustment for multiple comparisons (Appendix B: Figure S4). All reported p-values for indicator species analysis (Tables 3 & 4) have been corrected for multiple comparisons using the Šidák method built in to the *indicspecies* package (v 1.7.12). Confidence intervals were calculated using the *strassoc* function, which uses the simple

percentile bootstrapping method (10,000 replicates; Manly 2013)). Putative functional assignments for OTUs were made by querying the FungalTraits V1.2 database (Põlme et al. 2020). All R code used the *tidyverse* package (Wickham et al. 2019).

Results

In total we detected 2177 OTUs (1552 in San Pedro 3, 1122 in Algodones; 1011, 1635, 854 in 2*x*, 4*x* and 6*x* root-associated soils, respectively, Appendix B: Table S1). In mixed-ploidy populations, root-associated fungal assemblages exhibited considerable overlap in functional group assignments and did not significantly differ between cytotypes (Figures 6A, 6B; San Pedro $R^2 = 0.043$, $p = 0.8221$; Algodones $R^2 = 0.036$, $p = 0.5867$). Comparisons were made of normalized read counts for all fungal OTUs, none significantly differed between cytotypes (Appendix B: Figure S4).

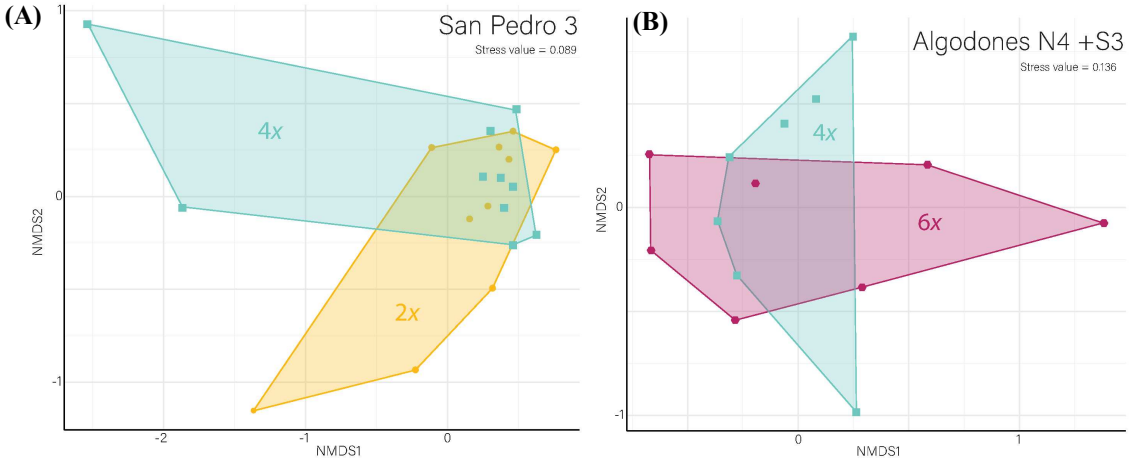

Figure 6: NMDS ordination for fungal assemblage composition as a function of ploidy for 2*x*-4*x* (**A,** 2*x* = yellow, 4*x* = blue) and 4*x*-6*x* (**B,** 4*x* = blue, 6*x* = red) *Larrea tridentata* populations. Each point represents the fungal assemblage composition for a single plant rhizosphere. Rhizosphere fungal assemblages exhibit some overlap between ploidies for both populations, but the fungal assemblages are not concordant.

Indicator species analyses revealed differences between cytotypes in rarer fungal OTUs that were not identified in the assemblage-level analyses. Despite the degree of overlap in fungal OTUs between co-occurring cytotypes, our analyses suggested the cytotypes in both mixed-ploidy populations had at least some unique fungal associations (Tables 3, 4 & Appendix B: Table S2). Eight fugal OTUs were identified in the 2*x*-4*x* population (San Pedro; three indicating 2*x*, and five indicating 4*x*), while five fungal OTUs were identified

in the combined 4*x*-6*x* populations (Algodones; three indicating 4*x*, and two indicating 6*x*) associated with a particular cytotype. We were unable to make specific functional assignments from the FungalTraits database for one of the OTUs associated with 2*x L. tridentata*, but those that could be assigned were soil saprotrophs (Table 3). We were unable to make functional assignments for some OTUs associated with 4*x* plants, but those that could be determined are mostly soil saprotrophs (Tables 3 & 4). We were unable to make functional assignments for fungal OTUs associated with 6*x* plants (Table 4).

Table 3: Indicator OTUs, by ploidy, for the San Pedro 2x-4x *Larrea tridentata* population (San Pedro 3) based on presence/absence data. Ploidy is either diploid (2x) or tetraploid (4x) and is the cytotype for which the OTU is an indicator. Specificity is the probability that the plant belongs to that cytotype, given that the OTU has been found there. Fidelity is the probability of finding that OTU on that cytotype. Values in parentheses are 95% confidence intervals based on 10,000 bootstrap replicates. P-values have been corrected for multiple comparisons using the Šidák method. Bolded levels of classification came from searches on NCBI BLAST, all others are from SINTAX/USEARCH results. Primary/Secondary Lifestyle are from FungalTraits 1.2V.

Ploidy	Specificity	Fidelity	p-value	OTU	Phylum; Class; Order	Family; *Genus species* Primary/Secondary Lifestyle
2*x*	0.97 (0.75-1)	0.56 (0.13-0.89)	0.039	369	**Chytridiomycota**; **Chytridiomycetes**; **Chytridiomycetales**	-; Unable to determine
2*x*	0.95 (0.72-1)	0.56 (0.22-0.89)	0.026	439	**Ascomycota;** **Sordariomycetes;** **Hypocreales**	**Stachybotryaceae;** ***Albifimbria sp.*** Soil saprotroph/mycoparasite
2*x*	0.86 (0.68-0.96)	0.78 (0.43-0.83)	0.013	566	**Basidiomycota;** **Tremellomycetes;** **Tremellales**	**Bulleribasidiaceae;** ***Vishniacozyma dimennae*** Soil saprotroph/extremophile
4*x*	1 (1-1)	0.6 (0.29-0.88)	0.015	1777	Ascomycota; Dothideomycetes; Pleosporales	Pleosporaceae; *Curvularia sp.* Plant pathogen/Litter saprotroph
4*x*	1 (1-1)	0.5 (0.2-0.83)	0.023	52	Ascomycota; **Sordariomycetes;** **Sordariales**	**Lasiosphaeriaceae;** ***Ramophialophora sp.*** Soil saprotroph
4*x*	0.97 (0.83-1)	0.6 (0.27-0.89)	0.035	250	Ascomycota; Eurotiomycetes; Chaetothyriales	-; - Unable to determine
4*x*	1 (1-1)	0.5 (0.18-0.83)	0.023	536	Ascomycota; **Dothideomycetes**; **Pleosporales**	**Sporomiaceae**; ***Preussia sp.*** Dung saprotroph
4*x*	1 (1-1)	0.5 (0.18-0.8)	0.038	806	**Chytridiomycota**; **Chytridiomycetes**;	-; - Unable to determine

Table 4: Indicator OTUs, by ploidy, for the Algodones 4x-6x *Larrea tridentata* population (Algodones N4 + S3) based on presence/absence data. Ploidy is either tetraploid (4x) or hexaploid (6x) and is the cytotype for which the OTU is an indicator. Specificity is the probability that the plant belongs to that cytotype, given that the OTU has been found there. Fidelity is the probability of finding that OTU on that cytotype. Values in parentheses are 95% confidence intervals based on 10,000 bootstrap replicates. P-values have been corrected for multiple comparisons using the Šidák method. Bolded levels of classification came from searches on NCBI BLAST, all others are from SINTAX/USEARCH results. Primary/Secondary Lifestyle are from FungalTraits 1.2V.

Ploidy	Specificity	Fidelity	p-value	OTU	Phylum; Class; Order;	Family; *Genus species* Primary/Secondary Lifestyle
4*x*	0.95 (0.76-1)	0.5 (0.2-0.8)	0.014	1030	Basidiomycota; Cystobasidiomycetes; Cystobasidiales	Cystobasidiaceae; *Cystobasidium pallidum* Mycoparasite
4*x*	0.98 (0.67-1)	0.58 (0.27-0.88)	0.032	438	**Mortierellomycota;** **Mortierellomycetes;** **Mortierellales**	**Mortierellaceae**; ***Mortierella sp.*** Unable to determine
4*x*	0.94 (0.77-1)	0.5 (0.2-0.82)	0.016	847	Ascomycota; Eurotiomycetes; Chaetothyriales	**Trichocomaceae;** ***Knufia sp.*** Soil saprotroph
6*x*	0.95 (0.85-0.99)	0.86 (0.67-1)	0.011	47	Ascomycota; Sordariomycetes; **Xylariales**	-; - Unable to determine
6*x*	0.99 (0.95-1)	0.57 (0.3-0.81)	0.009	1566	Ascomycota; Sordariomycetes; -	-; - Unable to determine

Discussion

Microbially-mediated niche differentiation (MMND) is a hypothesized cryptic means of niche differentiation for polyploids through the differential associations of microbes recruited to plants of different ploidy. Here we found that naturally co-occurring diploid, tetraploid, and hexaploid cytotypes of *L. tridentata* exhibited broad overlap in root-associated fungal associates. Yet, we also found support of a necessary condition for microbially-mediated niche differentiation in both 2*x*-4*x* and 4*x*-6*x* mixed-ploidy populations as diploids, tetraploids, and hexaploids each had some unique fungal associates.

Understanding rhizosphere fungal assemblage overlap and host cytotype specialization

We hypothesized that cytotypes from mixed-ploidy populations would have differentiated root-associated fungal assemblages but found that total root-associated fungal assemblages were similar for co-occurring cytotypes (Figures 6A, 6B, Appendix B: Figures S2 & S3). Ordination of the sampled fungal OTUs suggested very little differentiation among fungal assemblages on co-occurring diploids and tetraploids (Figure 6A) or co-occurring tetraploids and hexaploids (Figure 6B), and there was no support for assemblage differences in either PERMANOVA. Thus, plants within a population had very similar total root-associated assemblage regardless of cytotype.

We further hypothesized that there would be root-associated fungal specialization to host cytotype. We found that cytotypes from both mixed-ploidy populations were associated with at least some distinctive fungal OTUs (Tables 3 & 4), consistent with host-cytotype specific microbial associations that represents a necessary condition for MMND.

At first, observing no major differences between root-associated fungal assemblages among cytotypes seems incongruent with finding strong support for host cytotype specialization. One possible explanation for these observations is how the same data are analyzed by PERMANOVA vs. indicator species analyses. PERMANOVA results are driven by abundant species (as measured by normalized read counts), whereas indicator

species analysis is driven by unique species occurrences. The fungal OTU data suggests there is a 'core' root-associated fungal assemblage of abundant taxa associated with *L. tridentata* regardless of ploidy (Appendix B: Figure S4). Yet, there also appears to be a group of rarer root-associated fungal taxa that exhibit cytotype-specific associations (Tables 3, 4 & Appendix B: Table S2). Though not clear from our descriptive analysis alone, it is possible ploidy-specific fungal OTU associations may result in cytotype-specific niche exploitation; these differences should be experimentally investigated.

When using non-identity metrics, prior studies failed to find evidence of cytotype-specific root-associated fungal differences between ploidies. In *Aster amellus* and *Centaurea stoebe* (Sudová et al. 2014; Sudová et al. 2018) there was no significant difference between ploidies in arbuscular mycorrhizal root colonization or extraradical mycelium length. Regardless of finding no cytotype-specific differences, without using identity metrics it is not possible to ascertain whether rare taxa form cytotype-specific associations (a necessary condition for MMND) in these two systems. A different study that used fungal identities has documented differences in root-fungal associations between co-occurring diploid and tetraploid *Gymnadenia conopsea* orchids (Těšitelová et al. 2013). Interestingly, cytotype-specific OTUs with the highest relative abundances predominately belonged to the Tulasnellaceae, which commonly form endomycorrhizal associations with orchids. Five of these cytotype-specific OTUs showed evidence of being distinct from *Tulasnella* reference species in GenBank and may be most closely related to a known wood saprotroph, *Gleotulasnella cystidiophora*. These findings are consistent with a necessary condition for cytotype-specific differences for MMND.

Our work differs from some prior studies (Sudová et al. 2014; Sudová et al. 2018) in our use of fungal identity-based measures and (Těšitelová et al. 2013) in our use of root-associated soil samples containing fine roots. Sequencing root-associated soils where fine root pieces are present means we are identifying a more complete root-associated fungal assemblage (both endo- and ectomycorrhizal associations), whereas prior studies have only evaluated endomycorrhizal associations (Těšitelová et al. 2013, Sudová et al. 2014; Sudová

et al. 2018). Similar to the results of Těšitelová et al. (2013), we find support for a necessary condition for MMND using fungal identity-based metrics.

Potential functional differences derived from host-specialized OTUs

Attributing function to host-specialized OTUs allows us to hypothesize about how root-associated microbial interactions might contribute to microbially-mediated niche differentiation (MMND) between *L. tridentata* cytotypes. Primary and secondary lifestyle assignment of identified fungal OTUs come from the FungalTraits V1.2 database (Tables 3 & 4) and are specific to the OTU genus. These assignments are useful to consider as there are a wide variety of possible interactions between the root-associated fungi with each other and with their hosts. For example, the tetraploid associated OTUs in both mixed-ploidy populations (Tables 3 & 4) show different saprotrophic specializations (*Ramophialophora* – soil*, Preussia* – dung*, Knufia* – soil *& Curvularia* – litter*)* with moderate fidelity and perfect specificity values. These fidelity and specificity values mean the OTUs are not associated with every tetraploid, in their respective populations, but they are only present in soil associated with those tetraploids. Saprotrophs feed on organic material, thus when present in root-associated soils, they make nutrients accessible that would otherwise be inaccessible to the host plant (Boddy and Hiscox 2016). As such, tetraploid *L. tridentata* may access nutrients liberated by these saprotrophs that are not accessible to the other cytotype in the population, possibly resulting in niche separation between the co-occurring cytotypes.

It is also possible that microbe-microbe interactions differ between *L. tridentata* ploidies. Some fungal genera are known to produce secondary metabolites that harm other fungal genera. For example, tetraploid-associated *Preussia* spp. (Table 3) can produce secondary metabolites that act as antifungal agents against plant pathogen taxa in the genera, *Sordaria* and *Ascobolus* (Sarrocco 2016). These antifungal functions could conceivably affect microbial competition and help tetraploid plants gain a fitness advantage due to a lower pathogen load. We thus hypothesize that soil fungi and other microbial functions—and their interactions—could play an important role in MMND.

Caveats

A major assumption of our study is that contemporary plant-fungal interactions are informative about past processes that have contributed to cytotype co-occurrence. Post-polyploidization evolutionary change and adaptation are likely to have occurred since the formation of tetraploid and hexaploid *L. tridentata* (Walters and Freeman 1983; Laport and Ramsey 2015), which may have also influenced cytotype-specific fungal associations. Thus, the fungal associate differences we documented here, and any potential MMND resulting from such differences, does not necessarily reflect the interspecific interactions that were important historically during the formation and establishment of the tetraploid and hexaploid cytotypes. Further, characterizing the population dynamics of long-lived perennials is challenging. The timeframe over which *L. tridentata* plants live makes it difficult to infer the fitness and niche divergence consequences of fungal associate differences when typical population dynamics may unfold over centuries or millennia (Cody 2000).

In addition to uncertainty over whether contemporary fungal associate differences contributed to historical cytotype niche differentiation, it is also unclear whether seasonal dynamics may influence the population dynamics of root-associated soil fungi. We collected root-associated soils at a single point in time (spring), and thus these samples represent a narrow window into potentially complex root-associated fungal assemblage dynamics. The strong and varied seasonality of rainfall patterns in the Chihuahuan, Sonoran, and Mojave Deserts suggest the possibility of substantial fungal assemblage turnover over time (Clark, Rillig, and Nowak 2009). Relic DNA in soil has also been shown to hinder the detection of temporal dynamics for soil microbial communities and may complicate estimates of soil fungal and other microbial diversity (Carini et al. 2017; 2020). For example, one recent study has documented seasonal turnover in rhizosphere fungal communities on diploid and tetraploid *Salicornia* (Gonçalves, Pena, and Albach 2022), which could play an important role in facilitating polyploid population dynamics.

Future directions

Theoretical predictions indicate mixed-ploidy populations should be short-lived due to Minority Cytotype Exclusion (MCE). MCE posits that because a newly arising cytotype is both rare compared to its progenitor cytotype and reproductively incompatible with it, the newly arising cytotype will have low fitness and will typically be excluded – a form of frequency-dependent selection (Levin 1975; Husband 2000). Cytotype-specific soil microbiome differences may be important determinants of cytotype-specific relative fitness in such mixed-ploidy populations. Although we found that some of the root-associated fungal OTUs differed between diploid, tetraploid, and hexaploid *L. tridentata,* we also found that the overall root-associated assemblages were similar. As with other studies of polyploid soil microbiomes, it therefore remains unclear how important soil fungal associate differences might be in contributing to MMND and facilitating cytotype co-occurrence without additional experimental investigations.

Manipulative experiments employing a plant-soil feedback design (Smith-Ramesh and Reynolds 2017) focused on co-occurring intra-specific cytotypes have the potential to reveal the strength of MMND. Such experiments may also prove useful for predicting long-term population dynamics in polyploid species by revealing otherwise cryptic inter-specific ecological interactions that have only been accounted for indirectly in other studies of polyploid species. For example, accounting for microbially-mediated niche differences would help predict whether one cytotype is likely to competitively exclude a co-occurring cytotype in the population or whether cytotype coexistence is likely.

Coupled with root-associated microbe community sequencing, fungal OTU functional group characterizations could also aid investigations into how whole genome duplication alters plant traits and patterns of biodiversity (Segraves 2017; Laport and Ng 2017). For example, our finding that soil fungal OTU assemblages differ between the 2*x*-4*x* mixed site and the 4*x*-6*x* mixed site suggests landscape-level processes are important for determining soil fungal assemblages at large scales (Appendix B: Figure S5, Kovacs 2014) . Yet, it also appears that different fungal OTUs may be involved in unique interactions among diploids and tetraploids than those observed for tetraploids and hexaploids at small scales

(Appendix B: Figure S4). These differences need additional study, as such knowledge may help reveal ways in which community-level biotic structure and interactions are susceptible to increasing ecosystem disturbance, and how non-native species introductions and climate change may affect the persistence of native species (Segraves and Anneberg 2016). What is clear is that soil fungal associated assemblages of polyploid species may be more complex and consequential than previously thought, and additional investigations of soil microbiomes and their interactions with polyploid species are needed to better quantify their effects on mediating community dynamics.

Chapter 3: Evidence of Distance-Dependent Plant-Soil Microbe Feedback Effects in a Tetraploid-Hexaploid *Larrea tridentata* Population

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Abstract

Theory predicts that mixed-ploidy populations should be short-lived due to a reduced probability of replacement and establishment for the ploidy at lower frequency. However, mixed-ploidy populations are common, suggesting that frequency-dependent mating success limitations can be countered. We investigated whether stabilizing mechanisms facilitated through soil microbes are operating in a *Larrea tridentata* tetraploid-hexaploid population in the Mojave Desert. Prior work has documented ploidy-specific root-associated microbes in this population, a necessary pre-condition for microbe-mediated niche differentiation. We used a plant-soil feedback experiment to examine whether microbe-mediated niche differentiation may facilitate tetraploid and hexaploid coexistence in this mixed-ploidy population. Microbe-mediated niche differentiation functions as a stabilizing mechanism when it results in intra-ploidy competition being higher than inter-ploidy competition (e.g., negative frequency-dependence) that can counter the frequency-dependent mating success limitations present in mixed-ploidy populations. Further, we examined whether the stabilizing effects changed with distance from a conditioning plant (distance-dependence component of Janzen-Connell hypothesis). Across the distance transect, we detected microbe-mediated niche differentiation and observed that it changed from stabilizing near the conditioning plant to destabilizing further away. This supports the idea that soil microbes play a role in contributing to ploidy coexistence in mixed-ploidy populations and likely to plant spacing in *L. tridentata* populations, generally. For the first time, microbe-mediated niche differentiation is demonstrated as a possible mechanism contributing to ploidy coexistence in a mixed-ploidy population.

Introduction

Polyploidy is the presence of more than two haploid-genome copies within an organism. Found in all kingdoms of life, it is most common in plants (Brownfield and Kohler 2011; Campbell et al. 2016; Baduel et al. 2018). Variation in ploidy exists widely across species ranges, including within a population (Sudová et al. 2014; Plue, Kimberley, and Slotte 2018; Muñoz-Pajares et al. 2018; Kiedrzyński et al. 2021). Theory predicts that due to minority cytotype exclusion (Levin 1975), mixed-ploidy populations should be short-lived (Anneberg et al. 2023). Minority cytotype exclusion has two major components: (1) frequency-dependent mating success limitations result in a lower probability of replacement for the ploidy at lower frequency in the population and (2) the formation of sterile hybrids between ploidies results in gamete wastage that decreases the minority cytotype fitness more so than the majority cytotype fitness. Numerous models have investigated how the frequency- and density- dependent factors of minority cytotype exclusion may be eased or eliminated by niche and fitness differences between the ploidies (Felber 1991; Van Dijk and Bijlsma 1994; Li, Xu, and Ridout 2004; Oswald and Nuismer 2011). Empirical tests of model postulates are few (Husband 2000; Chrtek et al. 2017), in part due to the challenges of estimating the necessary model parameters.

Niche differences work to promote ploidy coexistence in these models when they act as stabilizing mechanisms (modern coexistence theory - Chesson 2000). Stabilizing mechanisms promote species coexistence by increasing the strength of intraspecific competition relative to interspecific competition (e.g., resulting in negative frequency-dependence). Mixed-ploidy populations have interploidal and intraploidal competition in place of interspecific and intraspecific competition. Thus, applying a modern coexistence theory framework allows us to quantify the niche differences between ploidies. The resulting negative frequency dependence may work to counter the frequency-dependent mating success limitations present in mixed-ploidy populations (e.g., minority cytotype exclusion) and result in ploidy coexistence or exclusion of progenitor altogether.

Plant-soil feedback (PSF) experiments have been used for decades (Bever, Westover, and Antonovics 1997; Chung and Rudgers 2016) to understand patterns of species coexistence

and quantify stabilizing and, more recently, equalizing mechanisms (Kandlikar et al. 2019). The simplest PSF metric, Bever's Interaction Coefficient (I_s), computes the net result from feedback that occurs when plants induce changes to their soil microbe communities that then have effects back on the those plants with the effect that the same microbe community has on a different plant species (or in this case, ploidy) (Bever 1994; Bever, Westover, and Antonovics 1997). We applied a PSF design to identify potential stabilizing mechanisms due to the soil microbe communities in a mixed-ploidy population. Previous work in this mixed-ploidy population has found root-associated fungi specificity with host-plant (Gerstner et al., in review; Chapter 2), which is a necessary condition for microbe-mediated niche differentiation (Těšitelová et al. 2013). However, it remains unclear whether ploidy-specific soil microbes could result in feedback consistent with microbe-mediated niche differentiation (pathogenic or mutualistic) and ease the impacts of minority cytotype exclusion (e.g., negative frequency dependence).

Larrea tridentata is a long-lived perennial evergreen shrub comprising a polyploid complex with diploid, tetraploid, and hexaploid cytotypes. Cytotype distributions roughly align with the Chihuahuan (diploid), Sonoran (tetraploid) and Mojave (hexaploid) deserts of North America (Laport, Minckley, and Ramsey 2012). Both single-ploidy and mixed-ploidy *L. tridentata* populations have weakly uniform spacing between plants (Phillips and MacMahon 1981). Mixed-ploidy populations show different patterns of spatial clustering by cytotype (significant in 2*x*-4*x*, non-significant in 4*x*-6*x*; Laport and Ramsey 2015), with none exhibiting cytotype clines. Such ploidal spatial heterogeneity may be the result of distance-dependent stabilizing effects (i.e., distance-dependence component of Janzen-Connell host-specific pathogens hypothesis) strengthening the negative frequency-dependence effects at closer distances to a conditioning plant. Evidence from the tree species, *Prunus serotina*, supports that host-specific pathogens can function as a distance-dependent stabilizing mechanism through PSF (Packer and Clay 2000). Motivated by these observations and calls to understand PSF context-dependency along abiotic gradients, we examined whether similar distance-dependent effects were present in a mixed-ploidy population of *L. tridentata* (Smith-Ramesh and Reynolds 2017).

In short, we tested whether microbe-mediated niche differentiation acts as stabilizing mechanism and further whether the stabilizing effects increase with distance from a conditioning plant (i.e., negative frequency dependence decreases), consistent with the distance-dependent Janzen-Connell host-specific pathogen hypothesis. We ask and answer these two questions in this work:

(1) Is there evidence of plant-soil microbe feedback operating between tetraploids and hexaploids in a mixed-ploidy *Larrea tridentata* population?

(2) Do the stabilizing effects of microbe-mediated niche differentiation increase with distance from a conditioning plant in a mixed-ploidy *Larrea tridentata* population?

Methods

Study system

Larrea tridentata is a long-lived perennial evergreen shrub that reproduces via seed and clonally (Mabry, Hunziker, and Difeo, Jr. 1977). The three cytotypes have relatively well-defined distributions, likely maintained by abiotic environmental variation, but also potentially determined by pollinator-mediated assortative mating and galling midge interactions at cytotype contact zones (Laport, Minckley, and Pilson 2021; O'Connor, Laport, and Whiteman 2019). In mixed-ploidy populations, 4*x L. tridentata* tend to be found in denser vegetation associations than 6*x* plants, which tend to be found at higher elevations, in more species-rich communities and on coarser soils (Laport, Minckley, and Ramsey 2016). Tetraploids tend to flower earlier and produce more flowers than 6*x* plants (Laport, Minckley, and Ramsey 2016). The size of morphological structures tends to increase with ploidy (e.g., larger diameter pollen grains, longer stamens and pistils, and longer, wider petals and leaves) though 4*x* plants tend to be taller than 6*x* plants (Laport, Minckley, and Ramsey 2016).

Field collections and sample preparation

Prior research using flow-cytometric analyses to infer DNA content has identified multiple mixed-ploidy *L. tridentata* populations comprising permanently marked plants (Laport, Minckley, and Ramsey 2012, Laport and Ramsey 2015). In April 2021, we collected soils at eight distances along a transect from plants of known ploidy from two 4*x*-6*x* sites

(Algodones N4; 33.00°, -115.07° and Algodones S3; 32.81°, -114.87°; Laport and Ramsey 2015; see Figure 7). We combined the two 4*x*-6*x* sites in our analyses (and hereafter refer to them as a single population). Sampling distances were standardized with the shrub dripline as 0m, then sampled at -0.1m (under shrub canopy) and at 0m, 0.25m, 0.5m, 0.75m, 1.0m, 1.5m and 2.0m. We set transects in directions that minimized obstacles (i.e., avoided other plants). Soil collection was done by twisting two 50ml sterile tubes into the soil (approximately 11.5cm deep) at each sampling distance, inverting the tube and capping. When the soil surface layer prevented the tube from entering, we brushed aside the top layer (e.g., pebbles) using a soil knife. We used this method with the intention being to collect soil microbes that a seedling would first experience in nature. We sterilized all sampling equipment between samples with a 10% bleach solution and allowed each to dry completely. In total we collected 288 soil samples, 16 each from nine soil-conditioning tetraploids and nine soil-conditioning hexaploids.

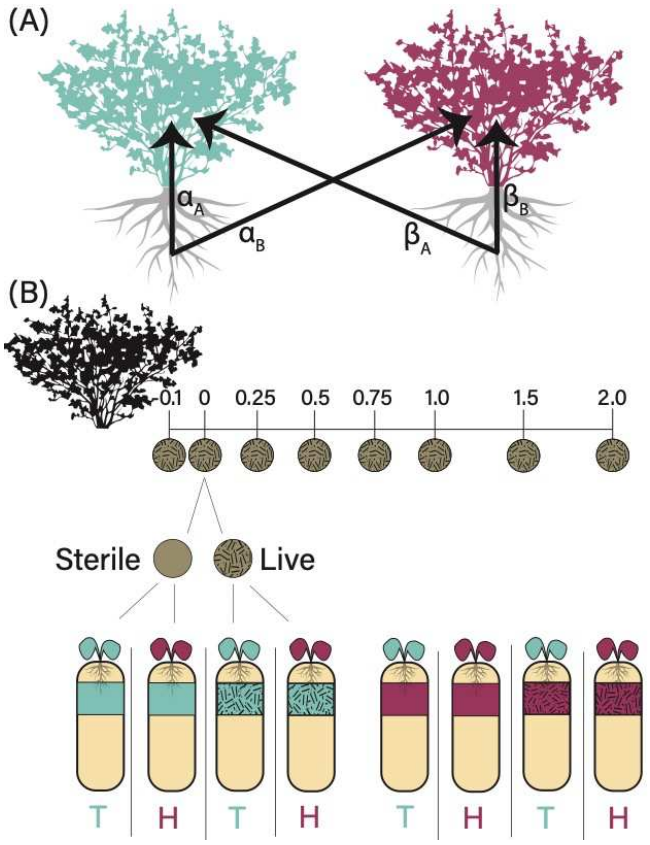


Figure 7: Simplified plant-soil microbe feedback experimental design and sampling scheme. **(A)** Depiction of plant-soil microbe feedback values for tetraploid (teal) and hexaploid (maroon) *L. tridentata*. α_A represents the direct effect tetraploid soil microbes have on tetraploid plants, α_B the indirect effect tetraploid soil microbes have on hexaploid plants, β_A the indirect effect hexaploid soil microbes have on tetraploid plants and β_B the direct effect hexaploid soil microbes have on hexaploid plants. **(B)** Simplified sampling scheme and greenhouse experimental setup. Each brown circle represents a field-collected soil sample at one of 8 locations along a 2.1m transect; the plant silhouette is the conditioning plant. 0m on the transect was set at the shrub dripline, to standardize the sampling across plants with varying crown sizes. Sterile and live refer to soil inoculum used in the greenhouse experiment setup. Soil inocula were sterilized by autoclaving and field-collected soils were left live. Eight unique pots were used for each distance and each conditioning pair. The plant was either a tetraploid (teal) or hexaploid (maroon), the band under the plant is the soil inoculum which is either sterile (solid color) or live (cross hatched), and the inoculum ploidy is assigned to match the ploidy of the conditioning plant the transect originated on.

Soil samples were stored on ice in coolers for up to 72hrs before being refrigerated at the University of New Mexico. The two tubes from each distance were combined, the total volume was divided in half; one half remained under refrigeration for later use as live inoculum and the other half was sterilized for use as sterile inoculum. All sterilizations were done in an autoclave on a gravity cycle with a 180min sterilization and 60min dry period. We collected fruits directly from plants in May 2021 in zones of known single-ploidy plants (according to Laport and Ramsey 2015). Tetraploid seeds were collected from CA-O (32.91°, -115.27°) and hexaploid seeds were collected from CA-S (33.11°, -114.90°).

Experimental design

Plant-soil microbe feedback experiments depend on comparisons (Figure 8); thus, a paired design was used with each pair containing one tetraploid and one hexaploid plant from the field. The resulting nine conditioning pairs had eight cone-tainers representing either the tetraploid or hexaploid soil conditioner, live or sterile inoculum, and seed ploidy for each of the eight distances (Figure 7B). The full experimental design comprised 576 cone-tainers representing nine tetraploid-hexaploid pairs. Cone-tainer position was randomized for inoculum, distance, conditioning plant ploidy, and seed ploidy across 16 racks in the greenhouse.

We ran a pilot test to examine the extent of vertical transfer of microbes from *L. tridentata* seeds using culturing. We selected 25 seeds at random of each ploidy, sliced them in half and placed them on agar plates. After multiple weeks there was no fungal growth observed on any of the plates, leading us to conclude there were no culturable fungi present and thus we did not perform any seed sterilization.

Greenhouse work

We sterilized cone-tainers (SC10R, Stuewe & Sons, Inc.) in a 12hr 10% bleach bath soak and allowed them to dry in bleach-sterilized tubs. In the greenhouse, we positioned between 24-48 sterilized cone-tainers per cone-tainer rack (RL98, Stuewe & Sons, Inc.), leaving an

empty space on all sides. We then added an autoclaved-sterile cotton ball to each. We filled each with an autoclave-sterilized 30/70 soil/sand mixture (approx. 90% of total volume) and added a plant tag indicating the sterile or live inoculum (approx. 10% of cone-tainer volume).

We removed seeds from their fruit capsules and placed them on moistened paper towels in plastic clamshells and incubated in the dark (room temperature) for 12-24 hrs until the radicle began to emerge. We then used sterile forceps to place a single germinated seed on the inoculum surface and capped with an autoclave-sterilized sand layer (~1-2mm). We immediately misted cone-tainers until saturation (water dripped from cone-tainer bottom).

In total, we evenly spaced 16 racks across a single greenhouse bench under grow lights (Spydr 600, BML Horticulture) that were set to a 14/10 day/night cycle for the experiment duration. Every other day for the first four weeks we hand-watered the cone-tainers from above until saturation, we then switched to an automatic mister system that watered for 5-mins every three days for the experiment duration. After four days, if no seedling sprouted, we removed and replaced the germinated seed, recapping with autoclave-sterilized sand. We repeated this up to three times for each cone-tainer, after which no additional planting took place for the experiment duration. We uprooted and discarded any additional seedlings that emerged (e.g. *L. tridentata* from the soil seed bank in live-inoculum pots).

We measured plant height and counted leaves every two weeks for the first 14 weeks. Height was measured as the distance from the substrate surface to the highest open leaf tip, and the total number of emerged green leaves was recorded. After six months of growth, leaf discoloration and leaf drop spiked, which we attributed to soil nutrient depletion. We trialed nutrient application to non-experimental plants and observed leaves remained on the plant and returned to green. Thus, we applied once-monthly fertilization treatments of 5mL general-purpose fertilizer at 250ppm (20-20-20 Peters Professional) directly to each cone-tainer for the experiment duration.

We harvested plants after approximately 52 growing weeks. To harvest, we cut the plant off at the substrate level, dropping the above-ground material into an individual paper envelope. We then inverted the cone-tainer onto a sieve, washed the soil through and recorded the mass of all the retained root material. We haphazardly removed roots from across the root system (approximately 20 1-inch portions) preserving them in tissue culture holders in 70% ethanol. We recorded a second mass of the root system mass to be able to calculate the contribution of root material we removed for root scoring and then placed the root material into an individual paper envelope. We dried all envelopes containing above-ground and below-ground plant material at 60ºC for at least 72hrs before recording dry biomass (all on the same Mettler Toledo PL303 balance). We stained the reserved root material for fungal hyphae (Vierheilig et al. 1998) and made permanent slides. All slides were scored under 200x magnification, recording observed hyphae for at least 80 (max 100) fields of view on each slide (McGonigle et al. 1990).

Analyses

Dataset

We assessed roots from all harvested plants for root colonization by fungal hyphae (i.e., endomycorrhizal fungi). From this full dataset we created two data subsets for analysis: 1) plants grown in live inoculum with root colonization and 2) all plants grown in live inoculum. The first dataset captures the effect of known endomycorrhizal fungi and bacteria whereas the second dataset captures a more general effect of the entire soil microbiome (i.e., soil fungi and bacteria). Due to the 52-week growth period duration there was ample opportunity for environmental contamination within the greenhouse. We approximated the effect of environmental contamination by examining the plants grown in sterile inoculum that had root colonization observed.

Microbe-mediated niche differences

We calculated Bevers Interaction Coefficient value (I_s) to examine plant-soil microbe feedback relationships at each transect distance on all datasets. I_s summarizes the net effect of plant-soil microbe feedback as either stabilizing or destabilizing and is useful to predict

whether the ploidies will coexist through cyclical stability (Bever, Westover, and Antonovics 1997). Negative I_s indicates a stabilizing effect, meaning plant-soil microbe feedback may contribute to cytotype coexistence through niche differentiation (i.e., mycorrhizal differences). A positive I_s indicates a destabilizing effect, meaning plant-soil microbe feedbacks may not contribute to niche differentiation (i.e., similar mycorrhizae). I_s is calculated from four values ($\alpha_{A,}$ $\alpha_{B,}$ β_A and $\beta_{B,}$ same as in Figure 7A). Each represents a different treatment: tetraploid plants growing in soils conditioned by tetraploid plants ($\alpha_{A,}$), tetraploid plants growing in soils conditioned by hexaploid plants (α_B), hexaploid plants growing in soils conditioned by tetraploid plants (β_A) and hexaploid plants growing in soils conditioned by hexaploid plants (β_B). G(A) and G(B) are the total dry biomass of plant A and B, respectively (in grams). α and β are the soils conditioned by plant A and B, respectively.

Equation 4: *Direct effect of α microbes on plant A*

$$\alpha_A = G(A)_\alpha - G(A)_0$$

Equation 5: *Indirect effect of β microbes on plant A*

$$\beta_A = G(A)_\beta - G(A)_0$$

Equation 6: *Indirect effect of α microbes on plant B*

$$\alpha_B = G(B)_\alpha - G(B)_0$$

Equation 7: *Direct effect of β microbes on plant B*

$$\beta_B = G(B)_\beta - G(B)_0$$

The relation of these four values determines I_s:

Equation 8: Bever's Interaction Coefficient

$$I_s = \alpha_A - \beta_A - \alpha_B + \beta_B$$

Substituting in ***Equations 4-7***

$$I_s = [G(A)_\alpha - G(A)_0] - [G(A)_\beta - G(A)_0] - [G(B)_\alpha - G(B)_0] + [G(B)_\beta - G(B)_0]$$

Simplification

$$I_s = G(A)_\alpha - G(A)_\beta - G(B)_\alpha + G(B)_\beta$$

To test for distance-dependent effects we examined the relationship of I_s across the 2.1m transect with linear regression (I_s ~ distance) using total dry biomass for G(A) and G(B) (Figure 8).

Results

In total 76% of plants survived to the final harvest (438 of 576) and 52% of the surviving plants exhibited the expected effects of applied treatment (150 live inoculum with colonization; 79 sterile inoculum with no colonization). Environmental contamination was found in 66% (151) of surviving plants grown in sterile inoculum (Appendix C: Figure S1). Root colonization was approximately the same for plants grown in live and sterile inoculum with root colonization (Appendix C: Figure S2).

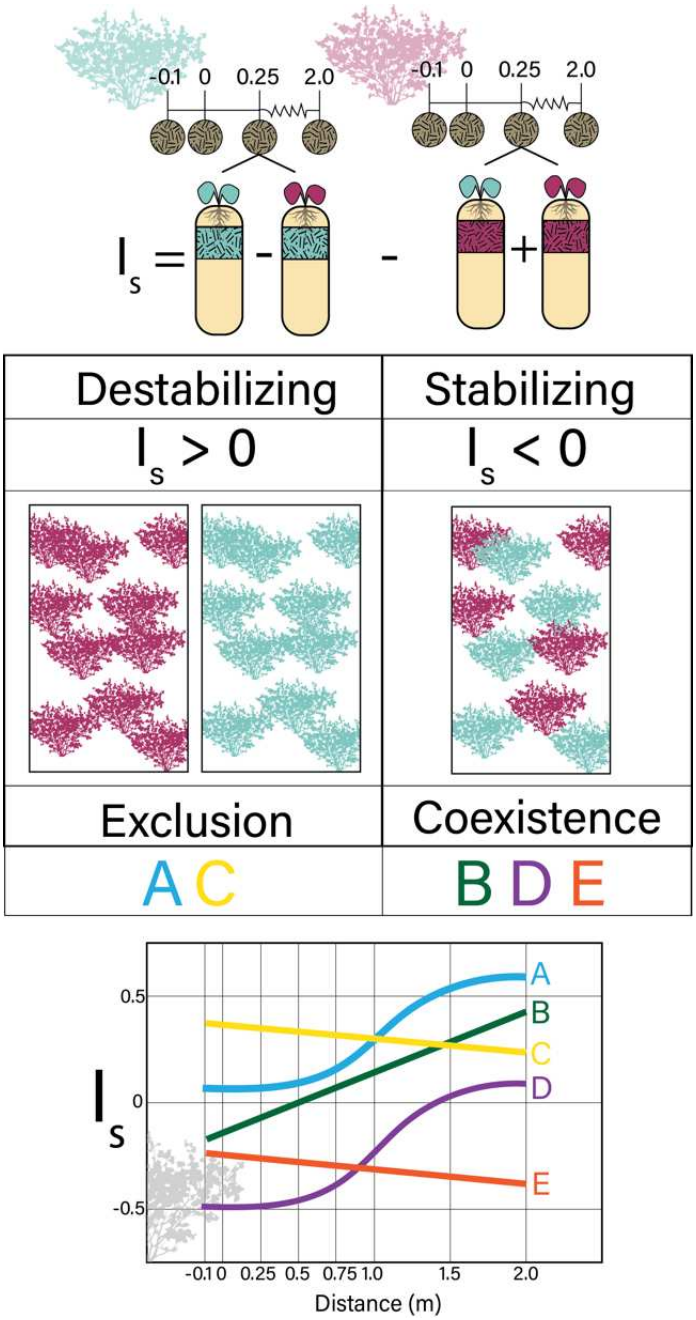


Figure 8: Conceptual framework linking plant-soil feedback with possible outcomes for minority cytotype exclusion. The top portion identifies the treatments necessary to calculate I_s, namely a pair of conditioning plants must be used to calculate each value. The middle panel identifies the primary interpretation for I_s, that when the sign is positive; plant-soil feedback will result in effects that allows minority cytotype exclusion to operate and lead to exclusion. When I_s sign is negative, plant-soil feedback will result in net stabilizing effects that may work against minority cytotype exclusion to promote ploidy coexistence. The bottom panel shows possible results for the change in I_s with distance. At a single distance the sign of I_s predicts exclusion ($I_s > 0$) and coexistence ($I_s < 0$), the slope predicts whether there is a distance-dependent effect. If the line crosses $I_s = 0$, that suggests that the distance-dependent effect of plant-soil feedback could result in positive/negative feedback through space that can result in ploidy coexistence.

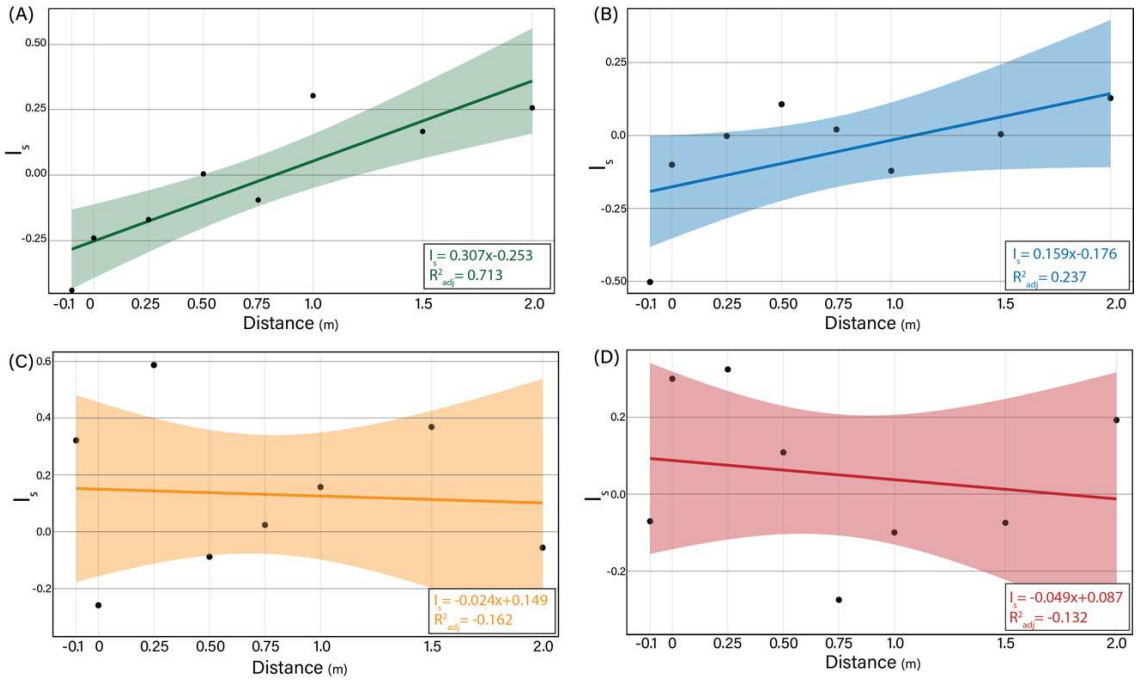


Figure 9*:* Average Bever's Interaction Coefficient (I_s) across the distance transect. Bever's Interaction Coefficient provides the net effect of plant-soil microbes. **(A) Live inoculum with colonization:** A significant positive relationship observed across the transect, (I_s ~ distance, green line; 95% CI shaded region, intercept = -0.253 (SE ± 0.072, p-value =0.013), slope = 0.307 (SE ± 0.071, p-value = 0.005), R^2_{adj} = 0.713), demonstrating an increase in I_s with increasing distance from the conditioning plant. **(B) All live inoculum:** A non-significant positive relationship fit by the linear model (I_s ~ distance, blue line; 95% CI shaded region, intercept = -0.176 (SE ±0.090, p-value =0.099), slope = 0.159 (SE ± 0.089, p-value = 0.125), R^2_{adj} = 0.237). **(C) Sterile inoculum without colonization:** A non-significant weak negative relationship observed across the transect (I_s ~ distance, yellow line; 95% CI shaded region, intercept = 0.149, (SE ± 0.156, p-value = 0.374), slope = -0.024, (SE ± 0.155, p-value = 0.881), R^2_{adj} = -0.162). **(D) Sterile inoculum with colonization:** A non-significant weak negative relationship observed across the transect (I_s ~ distance, red line; 95% CI shaded region, intercept = 0.087, (SE ±0.118, p-value = 0.488), slope = -0.049, (SE ±0.117, p-value = 0.683) R^2_{adj} = -0.132).

There is a positive association between distance from conditioning plant and the sign of the plant-soil feedback. Regardless of whether fungal hyphae were present, plants grown in live inoculum exhibit an increasing I_s with distance from the conditioning plant (live inoculum with colonization, I_s = 0.307x-0.253, R^2_{adj} = 0.713, p-value = 0.00515; live inoculum with and without colonization, I_s = 0.159x-0.176, R^2_{adj} = 0.237, p-value = 0.125). Distances nearest the conditioning plant (-0.1m to 0.75m) exhibited negative feedback that increases toward zero to slight positive feedback further away (Figure 9A & B). There is no support for environmental contamination (slope and intercept are not significantly different than 0; Figure 9D) nor underlying distance-dependent effects in sterilized inoculum (slope and intercept are not significantly different than 0; Figure 9C) driving the relationship detected.

Discussion

Mixed-ploidy populations are predicted to be short-lived due to frequency-dependent processes that negatively impact the ploidy at lower frequency in the population, yet mixed-ploidy populations are commonly observed. Plant-soil feedback is a cryptic mechanism of niche differentiation that may serve to minimize and potentially overcome the negative frequency-dependent effects (i.e., minority cytotype exclusion). Our findings suggest distance-dependent plant-soil feedback is present and could contribute to mixed-ploidy population longevity through differential destabilizing/stabilizing effects across a 2.1m transect. This is the first time, to our knowledge, that evidence of distance-dependent plant-soil feedback has been described in a mixed-ploidy population.

Microbe-mediated niche differences

Microbe-mediated niche differentiation is a biotically driven process that has the potential to be widespread given the known ubiquity of plant-soil feedback. Ploidy-specific microbes are a necessary, but not sufficient condition for microbe-mediated niche differentiation. Ploidy-specific microbial associates must additionally impact the plant performance in particular ways. Evidence of ploidy-specific microbial communities has been reported in a handful of polyploid complexes (*Gymnadenia conopsea* - Těšitelová et al. 2013; *Triticum spp.* – Wipf and Coleman-Derr 2021; *Arabidopsis thaliana* – Ponsford et al. 2022; *Salicornia europea, S. procumbens* – Gonçalves, Pena, and Albach 2022; *Larrea tridentata* – Chapter 2). However, none have examined whether and how these differences in microbial associates may impact ploidy coexistence.

Studies in non-tree species have quantified spatial variation of plant-soil microbes between 0 to 2 meters, with the greatest changes in microbe community composition occurring within 0.5m of the conditioning plant (reviewed in Chung 2023). Most like the *L. tridentata* system is the greasewood shrub (*Adenostoma fasciculatum*), a common shrub in the Californian chaparral ecosystem (Klironomos, Rillig, and Allen 1999). Researchers examining the spatial scale of plant-soil microbe interactions found bacterial, root and fungal biomasses peaked at 0.1m, 0.3m and 0.5m, respectively, from the conditioning

plant. There was variability in all three out to 1.2m (the extent of their sampling). Their primary conclusion being that knowledge of soil spatial heterogeneity can be used to reduce total sample sizes and increase statistical power. We incorporated this with our sample pooling and intensive spatial sampling.

Our measures of I_s are similar to those found in prior research on long-lived species (0 to -0.5, McCarthy-Neumann and Ibáñez 2013), in arid environments and two-species models (0.25 to -0.5, Reinhart 2012; Chung, Collins, and Rudgers 2019). Prior tests of comparable distance-dependent plant-soil feedbacks have focused on single tree species. Generally, they have found similar patterns of negative plant-soil feedback near the conditioning plant that weakens as distance from conditioning plant increases (*Prunus serotina* – Packer and Clay 2000; Reinhart and Clay 2009, *Ormosia spp.* – Liu et al. 2012; 2015). The scales at which the shift from negative to positive occurs in tree species is larger (2-30m) than our finding of a change around 0.8-1m (Figure 9A).

Consequences of distance-dependent plant-soil feedbacks in a L. tridentata population

There are two scales to consider when examining I_s in our experiment, first as a single point estimate at each distance and second as the slope of the average I_s line across the distance transect. The sign of I_s at any single distance predicts whether exclusion ($I_s > 0$) or coexistence ($I_s < 0$) is likely due to plant-soil feedback. However, minority cytotype exclusion is a population-level process and thus it is necessary to consider how I_s varies across distance to understand its effects on the population. Our results show that at distances near the conditioning plant (-0.1 to 0.75m, Figure 9A & B), coexistence is predicted and from 1-2m from a conditioning plant exclusion is predicted. Most importantly though, I_s not only increases with distance from the conditioning plant, but the sign changes from negative to positive across that distance (e.g., from coexistence to exclusion). The latter point is crucial as it suggests plant-soil feedbacks operate to maintain coexistence in mixed-ploidy *L. tridentata* populations through Janzen-Connell dynamics (e.g., negative distance dependence). The negative distance dependence means a hexaploid seed has greater success under and near a tetraploid conditioning plant, than a hexaploid

conditioning plant (and *vice-versa*). That success could result in a greater chance of hexaploid establishment in a tetraploid population and counter the frequency dependent factors of minority cytotype exclusion. This could be a crucial component in the initial formation of *L. tridentata* mixed-ploidy populations. Beyond approximately 1m from a hexaploid conditioning plant, plant-soil feedback changes to positive meaning that a hexaploid has lower success compared to a tetraploid at certain places in the population. The combination of these differences suggests distance-dependent plant-soil feedbacks may function to maintain mixed-ploidy *L. tridentata* populations.

Plant spacing within *L. tridentata* populations has long been posited to result from allelopathy, water, and nutrient availability (Knipe and Herbel 1966; Boyd and Brum 1983; Brisson and Reynolds 1994; Miller and Huenneke 2000). While such ecological interactions likely contribute to population structuring, our findings suggest another possible contributing factor – soil microbe interactions. The negative distance dependence (e.g., Janzen-Connell dynamics) would result in spatial effects in both single- and mixed-ploidy *L. tridentata* populations. In single-ploidy populations the decreased performance in the soils near a conditioning plant could result in plants spacing out beyond that negative impact zone (beyond 1m). In mixed-ploidy populations the same effects on plant spacing may occur while also resulting in ploidy spatial heterogeneity.

General implications to mixed-ploidy plant populations

Previous work has focused on apparent abiotic and biotic differences between ploidies in mixed-ploidy populations. Our finding that the nature of plant-soil microbe feedbacks change with distance supports that it is necessary to consider ploidy spatial heterogeneity in mixed-ploidy populations as a component of coexistence. The fitness-density covariance component of coexistence theory and the density-dependent factors of minority cytotype exclusion further emphasize this necessity. For example, if a primary process dictating mixed-ploidy populations are host-specific pathogens (i.e., Janzen-Connell hypothesis) a single ploidy may never be in high enough density that spatial homogeneity could have an impact on population dynamics. However, if enemy release is a primary process, then we could expect to see greater ploidy spatial homogeneity. In either case, any considerable

degree of ploidy spatial sorting requires greater consideration in how fitness-density covariance could impact the population dynamics and predictions of ploidy coexistence.

Caveats

Plant biomass as a response metric is reasonable for a one-year growth of a woody shrub (Younginger et al. 2017), yet it is complicated by ploidy. The gigas effect could contribute to ploidy-specific biomass differences that are due to the ploidy condition and not the effect of microbes, *per se* (Segraves 2017). Further, it is unclear how ploidy-specific biomass differences would play out over long time periods where above-ground biomass may increase and decrease in response to environment (e.g., drought, wind damage, etc.). Our data, however, does not support there being a significant association between biomass and ploidy in *L. tridentata* seedlings ($W = 24349$, p-value = 0.767), but this may not be the case in all systems.

We observed a considerable degree of environmental contamination which we identified from sterile-inoculated pots that showed root colonization. Given our pilot test results of no culturable fungi present within the seeds, we consider the most probable source for contamination was airborne spores. Our greenhouse takes in outside air and conditions temperature but does not filter out fine particulates. It is possible that airborne spores colonized initially-sterile pots with greater success than field-inoculated pots because there were no competitors present. Without sequencing soils and roots, it is impossible to determine the exact source of root colonization appearing in sterile treatments. However, when analyzing the sterile pots with colonization and calculating I_s we see no relationship between I_s and distance (Figure 8D). This is encouraging, because it suggests that environmental contamination, which could also have affected live-inoculum pots, is not likely the underlying source of the significant relationship between I_s and distance we observed (Figures 8A & B).

Conclusions and future directions

Absent apparent abiotic and biotic differences driving niche differences between ploidies, mixed-ploidy populations face a near certain fate – minority cytotype exclusion. However, given the prevalence of mixed-ploidy populations it stands to reason that there are processes at work which permit their continued existence. Microbe-mediated niche differentiation is inherently cryptic and up until now had been untested as an important contributor to mixed-ploidy population stability.

Here, we have reported evidence for plant-soil microbe feedbacks contributing to niche differentiation that can function to counter minority cytotype exclusion. Given plant-soil microbe feedback ubiquity, it would be surprising if similar results were not found for other polyploid complexes. In follow-up work to test this idea it would be prudent to include plant-plant competition amongst the treatments (Thompson, Husband, and Maherali 2015). This would allow for the use of standardized frameworks of measuring niche and fitness differences due to microbes (Kandlikar et al. 2019; Ke and Wan 2020; Yan, Levine, and Kandlikar 2022). Such standardization would permit comparisons of microbe-mediated niche and fitness differences between polyploid complexes. Future work should also include sequencing the microbes from field-collected soils, greenhouse soils and plant roots. Comparisons of microbe communities between these three environments could help expand our limited knowledge of the key players driving plant-soil microbe feedbacks and how they may change across abiotic and biotic environments.

Conclusion

This dissertation attempts to resolve the paradox of polyploidy by refining our knowledge of polyploid biology. In the first chapter, I investigate the consequence of environmental stochasticity in the formation rate of unreduced gametes on a generalized model of autopolyploid formation. I have found that including environmental stochasticity in FRUG decreases the importance of selfing and inbreeding depression and increases the importance of the relative fitness relationship between ploidy levels on model outcome. In the second chapter, I identify root-associated fungal community differences by cytotype in two mixed-ploidy populations of the *Larrea tridentata* polyploid complex. These differences are a likely precondition for the occurrence of microbially-mediated niche and fitness differences, which is the impetus for the third chapter. Using a greenhouse study, I ask whether cytotype-specific root-associated fungal communities in the *Larrea tridentata* polyploid complex mediate niche differentiation, thus reducing the effects of minority cytotype exclusion. I found that not only are there microbe driven niche, but that they change with distance from a focal plant. Each of these projects refines our understanding of how random processes (stochastic variation in unreduced gametes – Chp 1) and microbes (microbe-mediated niche differentiation – Chp 2,3) ease the perceived barriers to polyploid formation and establishment. These are two previously under explored facets in the field. The findings presented here make for better parameter estimates that align model predictions with the ubiquity of polyploids in nature. Overall, this dissertation has implications to our understanding of the patterns, distributions, and origins of biodiversity beyond polyploid plants.

Appendices

Appendix A: Chapter 1 Supplemental Information

Appendix B: Chapter 2 Supplemental Information

Appendix C: Chapter 3 Supplemental Information

Appendix A: Chapter 1 Supplemental Information

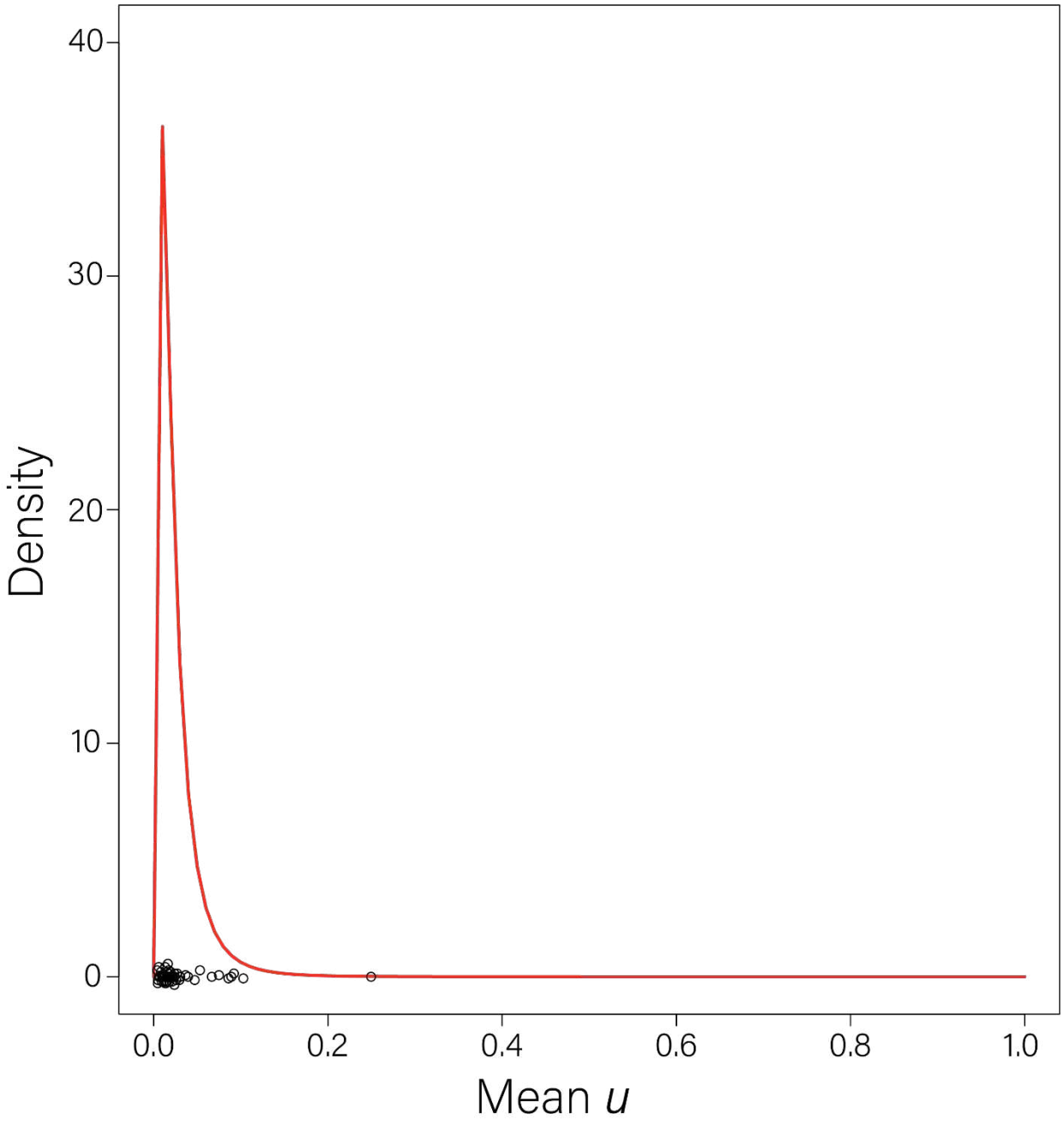


Figure S1: Population mean formation rate of unreduced gametes data for 59 populations of 24 species of Brassicaceae (black open circles), with the best fit log-normal distribution (log mean = -3.99, SE = 0.109; log standard deviation = 0.83, SE = 0.077, red line).

Table S1: AIC values for distribution types fit to population-level *u* distributions for 24 Brassicaceae species. Lowest population-level AIC is in bold.

Population	Genus	Species	Location	weibull.aic	lognorm.aic	gamma.aic	beta.aic
A.ER	Alliaria	petiolata	ER	-188.67	**-196.90**	-194.14	-194.00
A.LT	Alliaria	petiolata	LT	**-93.93**	-91.83	-93.57	-93.64
A.PP	Alliaria	petiolata	PP	-623.16	**-641.03**	-637.90	-637.62
A.SRR	Alliaria	petiolata	SRR	-209.94	**-214.72**	-213.80	-213.75
A.V&C	Alliaria	petiolata	V&C	-211.61	**-215.13**	-214.74	-214.72
A.VCRK	Alliaria	petiolata	VCRK	-177.46	**-189.04**	-186.37	-186.14
A.WTRRD	Alliaria	petiolata	WTRRD	-110.44	**-122.32**	-114.45	-113.61
Barb.BIOD	Barbarea	vulgaris	BIOD	-323.03	**-325.77**	-325.03	-325.02
Barb.VICT	Barbarea	vulgaris	VICT	-158.12	**-168.48**	-161.91	-161.75
Barb.ARKLL	Barbarea	vulgaris	ARKLL	-157.53	**-163.44**	-161.41	-161.34
Bras.SLVRCRK	Brassica	napus	SLVRCRK	-197.66	-197.83	-198.17	**-198.18**
Bras.Vict	Brassica	napus	VICT	-350.68	-352.88	**-353.33**	-353.32
Bras.WDLWN	Brassica	napus	WDLWN	-159.37	**-166.49**	-163.63	-163.51
Capsella.BIOD	Capsella	bursa-pastoris	BIOD	**-147.13**	-143.54	-145.35	-145.42
Capsella.WLGNTN	Capsella	bursa-pastoris	WLGNTN	-108.99	-108.44	-109.17	**-109.19**
CardCon.LT	Cardamine	concatenata	LT	-117.82	**-129.95**	-115.27	-104.16
CardCon.RARE	Cardamine	concatenata	RARE	-160.15	**-171.60**	-157.66	-155.17
CardCon.STKY	Cardamine	concatenata	STKY	-24.51	**-27.15**	-23.65	-26.53
CB.BRDRN	Cardamine	bulbosa	BRDRN	**-150.81**	-149.58	-150.54	-150.57
CB.RARE	Cardamine	bulbosa	RARE	-284.68	**-306.28**	-298.64	-298.30
CP.ARB	Cardamine	pensylvanica	ARB	-117.23	-117.09	-117.75	**-117.79**
CP.PP	Cardamine	pensylvanica	PP	-243.35	**-263.74**	-249.12	-248.33
Desc.A&N	Descurainia	sophia	A&N	-70.56	-70.21	**-70.92**	-71.00
Desc.JP	Descurainia	sophia	JP	**-80.39**	-74.46	-78.60	-79.32
Diphylla.LT	Cardamine	diphylla	LT	-139.08	**-141.50**	-141.36	-141.29
Diphylla.STKY	Cardamine	diphylla	STKY	-83.84	**-87.32**	-86.07	-85.91
Diplo.GJ	Diplotaxis	tenuifolia	GJ	-305.48	**-315.16**	-307.86	-307.66
Diplo.HB	Diplotaxis	tenuifolia	HB	-190.39	**-202.41**	-196.44	-196.31
Diplo.WB	Diplotaxis	tenuifolia	WB	-181.82	**-190.60**	-184.83	-184.72
EH.Cobalt	Erysimum	hieraciifolium	Cobalt	-166.31	-167.84	**-167.85**	-167.84
EH.E&W	Erysimum	hieraciifolium	E&W	-127.68	**-130.48**	-129.72	-129.69
EH.JP	Erysimum	hieraciifolium	JP	-205.84	**-211.98**	-210.65	-210.62
Erucastrum.ASH	Erucastrum	gallicum	ASH	-246.97	**-255.92**	-254.01	-253.99
Erucastrum.CP	Erucastrum	gallicum	CP	-340.91	**-357.30**	-346.64	-346.50
Erucastrum.YD	Erucastrum	gallicum	YD	**-164.98**	-163.60	-164.59	-164.60
EryCheir.GOGAMA	Erysimum	cheiranthoides	GOGAMA	-181.67	**-186.32**	-185.00	-184.97
ErysCheir.CP	Erysimum	cheiranthoides	CP	-260.15	-261.28	**-261.47**	-261.44
ErysCheir.GJ	Erysimum	cheiranthoides	GJ	-150.76	**-155.88**	-154.36	-154.32
Hesp.ER	Hesperis	matronalis	ER	-184.24	**-187.05**	-186.75	-186.74
Hesp.LT	Hesperis	matronalis	LT	-179.48	**-187.24**	-184.93	-184.86
Hesp.UoG	Hesperis	matronalis	UoG	-304.20	**-355.88**	-331.28	-329.69
Lepid.A&N	Lepidium	campestra	A&N	-103.12	**-108.21**	-104.85	-104.64
Lepid.A&V	Lepidium	campestra	A&V	-509.08	**-533.12**	-524.48	-524.19
NM.3L	Nasturtium	microphyllum	3L	-114.06	**-120.43**	-117.77	-117.56
NM.ARB	Nasturtium	microphyllum	ARB	-229.62	**-236.17**	-234.57	-234.43
NM.YORK	Nasturtium	microphyllum	YORK	-119.61	**-126.35**	-123.05	-122.81
NO.ASH	Nasturtium	officinale	ASH	-123.36	**-128.51**	-126.14	-126.06
Sinapis.ARB	Sinapis	arvensis	ARB	-172.26	**-174.53**	-174.32	-174.30
Sinapis.CP	Sinapis	arvensis	CP	-472.29	**-496.77**	-478.10	-477.83
Sinapis.WLGTN	Sinapis	arvensis	WLGTN	-176.11	**-178.19**	-177.65	-177.64
SisAlt.JP	Sisymrium	altissimum	JP	-160.09	**-166.44**	-163.26	-162.96
SisLo.ARKL	Sisymrium	loeselii	ARKL	-134.47	-114.13	**-134.49**	-134.08
SisLo.JP	Sisymrium	loeselii	JP	-206.48	**-211.24**	-210.26	-210.25
SisLo.VIC	Sisymrium	loeselii	VICT	-200.29	-201.43	-201.79	**-201.80**
SisOff.RHR	Sisymrium	officinale	RHR	-145.38	**-156.10**	-149.26	-149.04
Sisoff.WB	Sisymrium	officinale	WB	-238.13	**-256.38**	-246.89	-246.43
Thlaspi.A&V	Thlaspi	arvense	A&V	-155.93	**-159.87**	-158.58	-158.54
Thlaspi.CP	Thlaspi	arvense	CP	**-66.99**	-65.89	-66.85	-66.98
Thlaspi.NSGWY	Thlaspi	arvense	NSGWY	-157.23	**-168.89**	-163.03	-162.83

Table S2: Sobol' indices for global sensitivity analysis by *u* sampling technique. Parameter is one of the five input parameters: $\delta_d, \delta_t, s_d, s_t, w$. Value identifies the lower confidence interval (L) and upper confidence interval (U), found through bootstrapping of the original estimated (O) Sobol' index. S_i is the first-order Sobol' index, T_i is the total-order Sobol' index.

		Static		Stochastic	
Parameter	Value	S_i	T_i	S_i	T_i
δ_d	L	0.062	0.332	0.098	0.31
	O	0.072	0.349	0.11	0.324
	U	0.084	0.368	0.121	0.335
δ_t	L	0.101	0.453	0.14	0.393
	O	0.113	0.475	0.152	0.411
	U	0.128	0.495	0.163	0.43
s_d	L	0.038	0.282	0.029	0.186
	O	0.045	0.294	0.034	0.2
	U	0.054	0.31	0.042	0.211
s_t	L	0.089	0.422	0.039	0.19
	O	0.1	0.439	0.045	0.2
	U	0.113	0.455	0.053	0.21
w	L	0.12	0.483	0.256	0.523
	O	0.132	0.502	0.27	0.539
	U	0.147	0.522	0.284	0.553

Table S3: Sobol' indices for global sensitivity analysis by u sampling technique and parameter interactions. Parameter interaction identifies which of the five ($\delta_d, \delta_t, s_d, s_t, w$) input parameters are interacting. The lower confidence interval (L) and upper confidence interval (U) are found through bootstrapping of the original estimated (O) Sobol' index. Sobol' indices for interaction terms are small in comparison to the first-order and total-order indices.

Static			Parameter Interaction	Stochastic		
L	O	U		L	O	U
0	0.005	0.018	$s_d s_t$	0	0	0.006
0.029	0.042	0.055	$s_d \delta_d$	0.043	0.052	0.062
0	0.014	0.027	$s_d \delta_t$	0.001	0.012	0.023
0	0.007	0.019	$s_d\, w$	0.001	0.01	0.017
0	0.013	0.029	$s_t \delta_d$	0	0.004	0.011
0.028	0.047	0.062	$s_t \delta_t$	0.02	0.029	0.039
0.025	0.042	0.053	$s_t w$	0.01	0.018	0.029
0	0.013	0.028	$\delta_d \delta_t$	0.001	0.015	0.028
0	0.014	0.027	$\delta_d w$	0.011	0.027	0.037
0.041	0.057	0.071	$\delta_t \delta_t$	0.045	0.064	0.08
0	0.008	0.024	$s_d s_t \delta_d$	0	0	0.009
0	0.009	0.023	$s_d s_t \delta_t$	0	0.005	0.014
0	0.012	0.027	$s_d s_t w$	0	0	0.008
0	0.005	0.021	$s_d \delta_d \delta_t$	0	0	0.012
0	0.018	0.038	$s_d \delta_d w$	0	0.008	0.021
0	0.003	0.024	$s_d \delta_t w$	0	0.006	0.021
0	0.01	0.032	$s_t \delta_d \delta_t$	0	0.001	0.012
0	0.015	0.035	$s_t \delta_d w$	0	0	0.013
0.012	0.034	0.048	$s_t \delta_t\, w$	0.012	0.025	0.038
0	0.017	0.033	$\delta_d \delta_t\, w$	0.001	0.015	0.034

Appendix B: Chapter 2 Supplemental Information

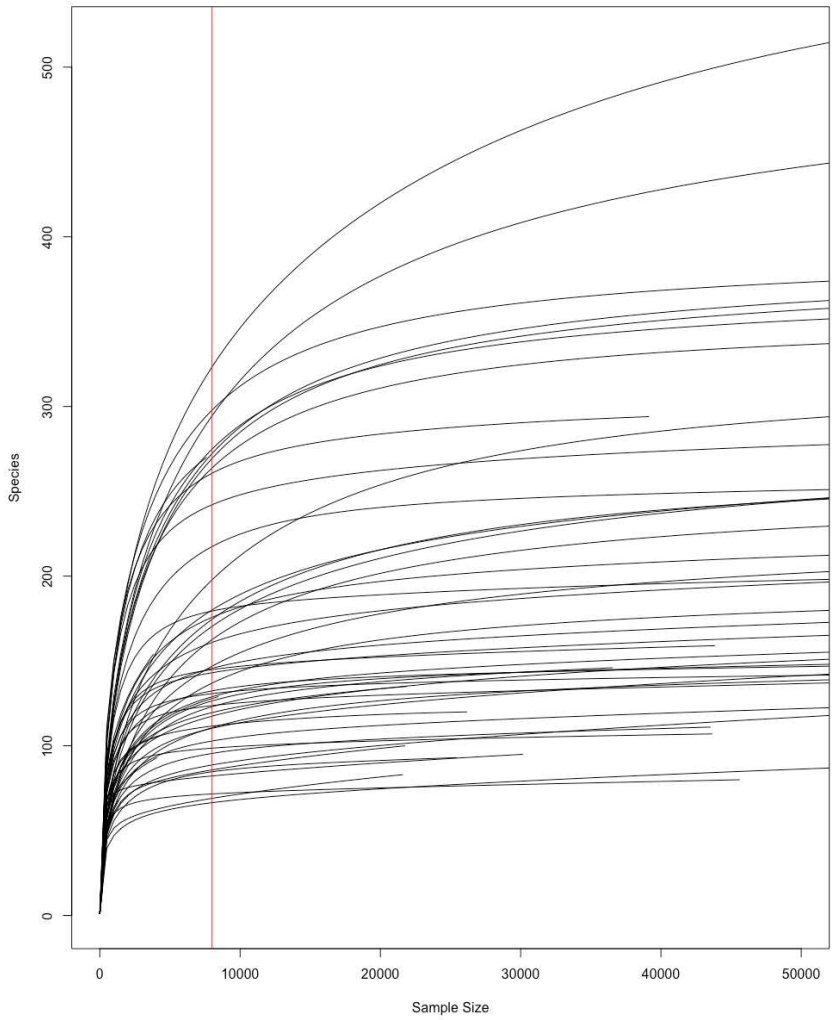


Figure S1: Rarefaction curves for fungal OTU sequencing reads. Created using *rarecurve* function in *vegan* v2.6-4 in R 4.1. Each line is a rarefaction line for one row of input data. The vertical red line marks 8,000 reads, the threshold value used for filtering total sequencing reads.

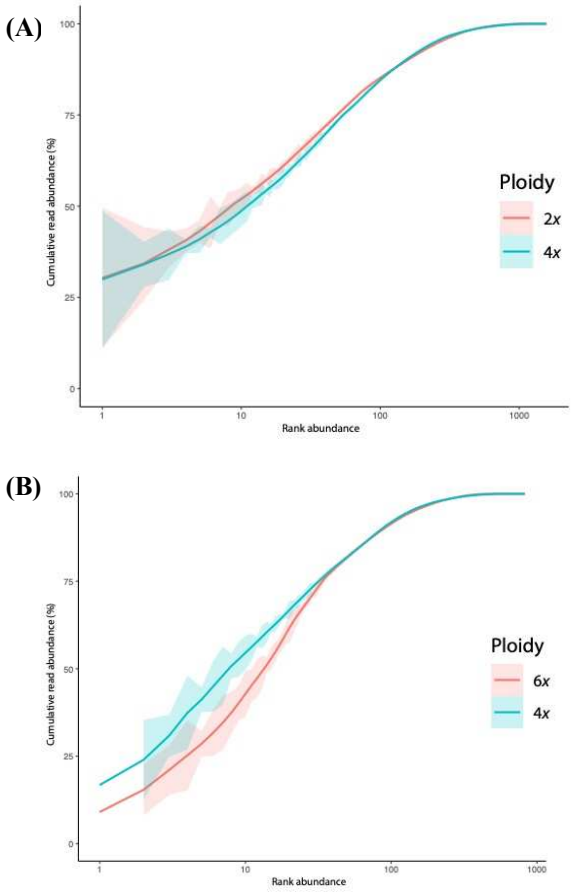
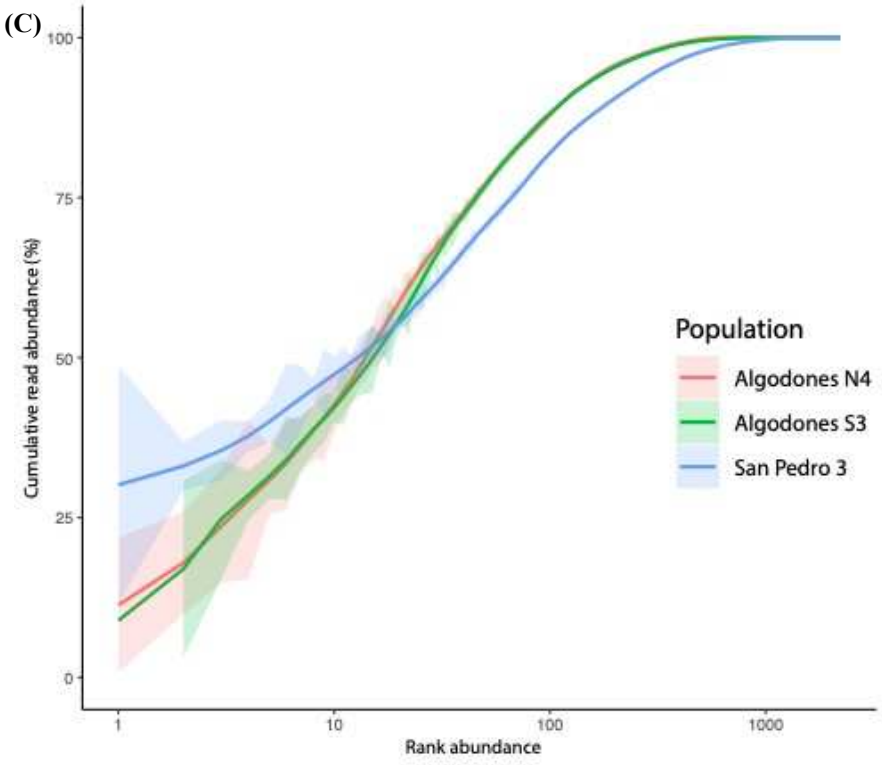


Figure S2: Cumulative read abundance by OTU rank abundance for **(A)** 2*x*-4*x*, **(B)** 4*x*-6*x* and **(C)** Population Identity. **(A)** shows read abundance does not differ between 2*x* and 4*x* cytotypes in the 2*x*-4*x* population (San Pedro 3). **(B)** shows 4*x* had a faster climb in read abundance for lower rank abundance values, meaning 4*x* had fewer OTUs making up their total reads (i.e., lower richness) in the 4*x*-6*x* populations. **(C)** shows read abundance differs between the San Pedro 3 population (2*x*-4*x*) and the Algodones N4 and Algodones S3 populations (4*x*-6*x*), which had approximately the same change in read abundance, meaning that the rhizospheres of the 2*x*-4*x* population had fewer OTUs making up their total reads (i.e., lower richness) than 4*x*-6*x* populations.



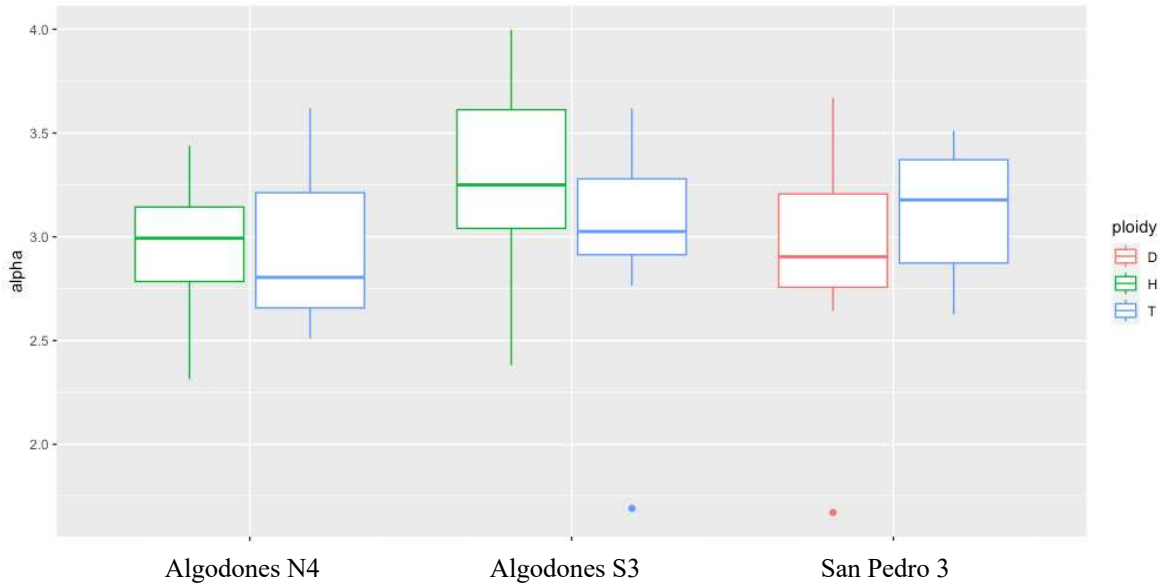


Figure S3: Alpha diversity of root-associated fungal communities (Shannon) for each ploidy in each population. Neither ANOVA model (alpha ~ population + ploidy + population*ploidy; p-values: population = 0.0843, ploidy = 0.9166, population*ploidy = 0.7134) nor (alpha ~ population; p-value: population = 0.0711) indicate significant differences in any terms.

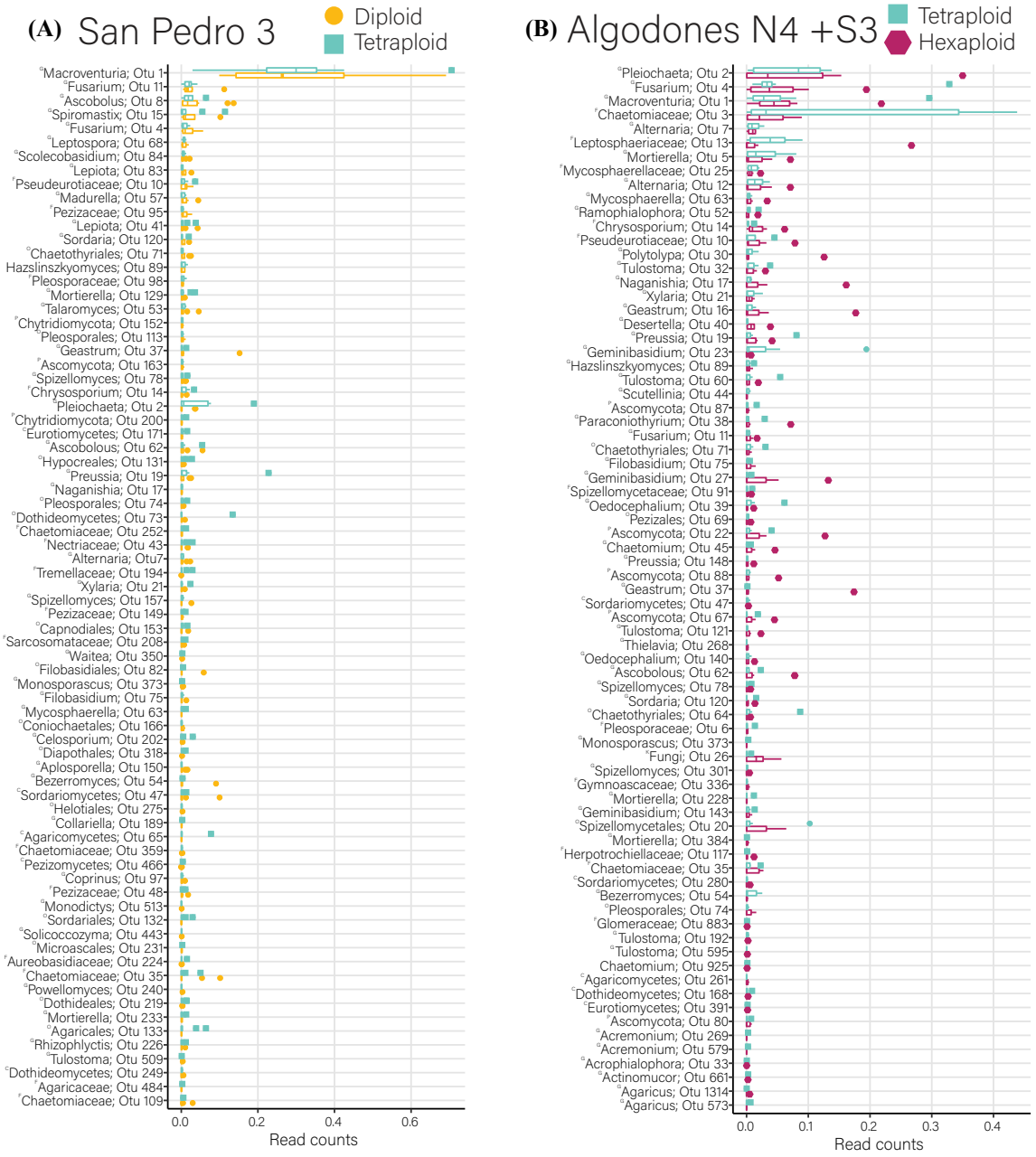


Figure S4: Top 75 fungal OTUs, by normalized read counts, for 2*x*-4*x* (**A,** 2*x* = yellow, 4*x* = blue) and 4*x*-6*x* (**B,** 4*x* = blue, 6*x* = red) populations. Boxplots show the median and interquartile range with outliers shown as dots. Taxa are ordered by highest median normalized read count to lowest. The lowest degree of biological classification is named for each OTU, and the leading superscripts identify the classification level, (^{K} – Kingdom, ^{P} – Phylum, ^{C} – Class, ^{O} – Order, ^{F} – Family, ^{G} – Genus). None of the normalized read counts are significantly different between ploidies.

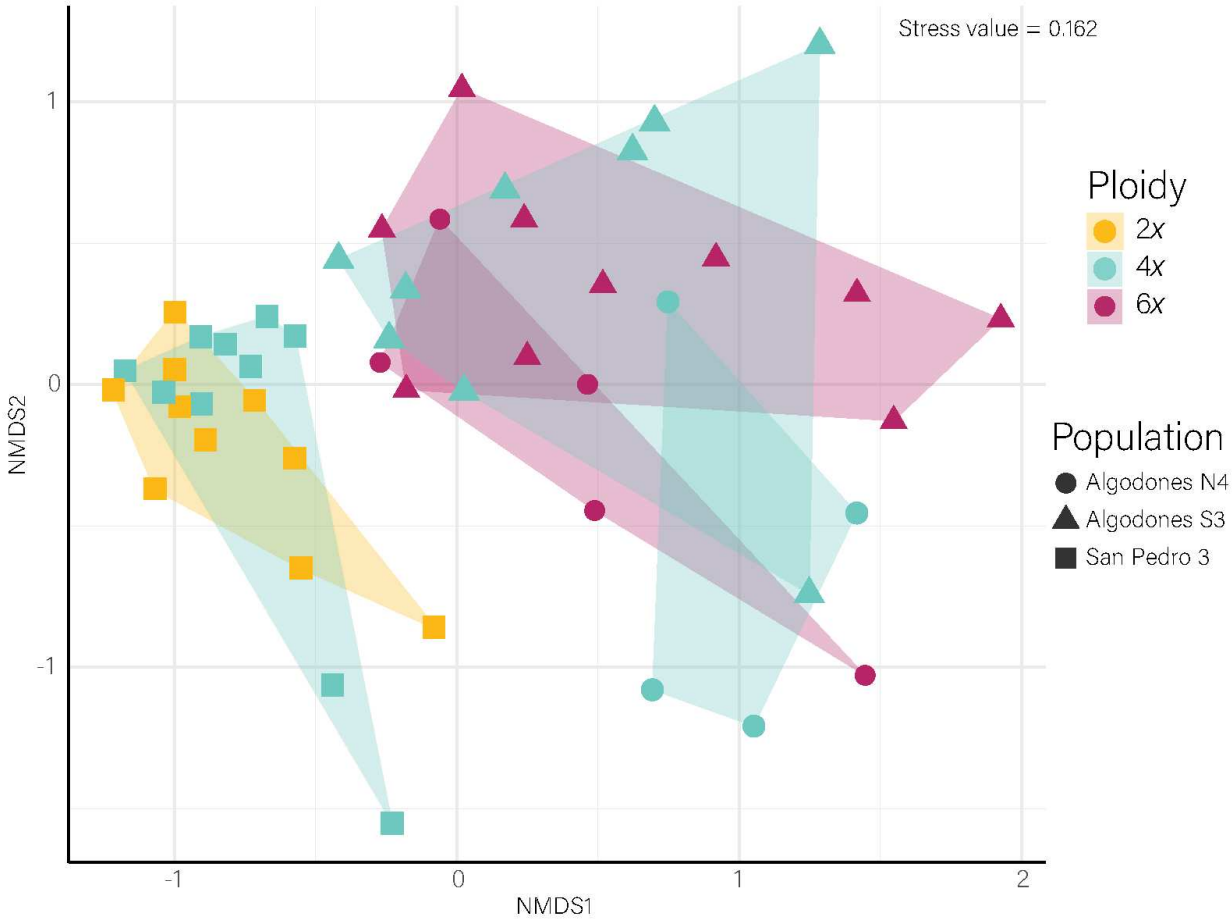


Figure S5: NMDS ordination for rhizosphere fungal assemblage composition of *Larrea tridentata* cytotypes in mixed-ploidy populations. There is minimal overlap in rhizosphere fungal assemblage composition between 4*x* at Algodones N4 and Algodones S3. Otherwise cytotypes with the same population identity have the greatest overlap in rhizosphere fungal assemblage composition.

Table S1: Total read abundance for each soil rhizosphere DNA extraction. Ploidy is diploid (D), tetraploid (T) or hexaploid (H). Site is San Pedro (2*x*-4*x*) or Algodones N4 + S3 (4*x*-6*x*).

Extraction	Ploidy	Site	Total Read Abundance	Extraction	Ploidy	Site	Total Read Abundance
LR26	T	4*x*-6*x*	19	LR18	T	2*x*-4*x*	75066
LR31	T	4*x*-6*x*	1292	LR9	T	2*x*-4*x*	76618
LR40	T	4*x*-6*x*	3827	LR54	T	4*x*-6*x*	76626
LR28	H	4*x*-6*x*	4078	LR24	H	4*x*-6*x*	78325
LR29	T	4*x*-6*x*	4239	LR4	D	2*x*-4*x*	79085
LR17	D	2*x*-4*x*	7614	LR51	T	4*x*-6*x*	86720
LR30	T	4*x*-6*x*	8461	LR16	D	2*x*-4*x*	94370
LR22	T	4*x*-6*x*	21613	LR2	D	2*x*-4*x*	95349
LR43	H	4*x*-6*x*	21771	LR47	H	4*x*-6*x*	100020
LR45	T	4*x*-6*x*	21904	LR35	T	4*x*-6*x*	116009
LR49	H	4*x*-6*x*	25460	LR15	D	2*x*-4*x*	117240
LR13	D	2*x*-4*x*	26182	LR8	T	2*x*-4*x*	117903
LR10	T	2*x*-4*x*	30156	LR37	T	4*x*-6*x*	118853
LR21	H	4*x*-6*x*	36569	LR38	H	4*x*-6*x*	119245
LR6	D	2*x*-4*x*	39163	LR53	T	4*x*-6*x*	121480
LR33	T	4*x*-6*x*	43540	LR42	H	4*x*-6*x*	125360
LR52	H	4*x*-6*x*	43655	LR23	H	4*x*-6*x*	128878
LR11	D	2*x*-4*x*	43853	LR20	T	2*x*-4*x*	134028
LR12	T	2*x*-4*x*	45629	LR7	T	2*x*-4*x*	135991
LR19	T	2*x*-4*x*	52337	LR5	D	2*x*-4*x*	141291
LR14	T	2*x*-4*x*	56824	LR36	H	4*x*-6*x*	143422
LR50	H	4*x*-6*x*	57392	LR44	T	4*x*-6*x*	144826
LR34	H	4*x*-6*x*	58353	LR39	H	4*x*-6*x*	166105
LR41	T	4*x*-6*x*	61002	LR1	D	2*x*-4*x*	168646
LR3	T	2*x*-4*x*	66327	LR48	H	4*x*-6*x*	188995
LR46	T	4*x*-6*x*	71218				

Table S2: Total read abundances for OTUs identified in Tables 3 & 4. OTUs present in Tables 3 & 4, as single distinctive OTUs for cytotype. Both OTUs with low total abundances (i.e., rare occurrence) and those with high total abundances contribute to distinctive associations.

OTU	Total Abundance
1777	44
1030	168
806	230
847	290
536	303
566	501
439	681
369	1030
1566	1108
438	1484
250	1908
52	10333
47	26077

Appendix C: Chapter 3 Supplemental Information

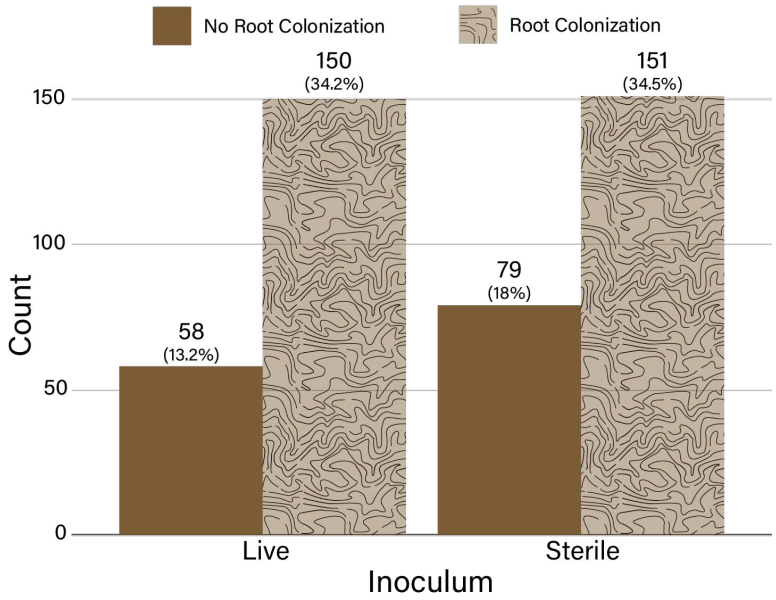


Figure S1: Final harvest root colonization counts by inoculum treatment. Darker bars indicate no root colonization was observed from root slide scoring. Lighter bars indicate root colonization was observed from root slide scoring. Above each column are counts and the percentage of total from all harvest. Live without colonization were not included in any analysis. Chi-squared = 82.3, df = 1, p = 0 for model percent colonization ~ inoculum.

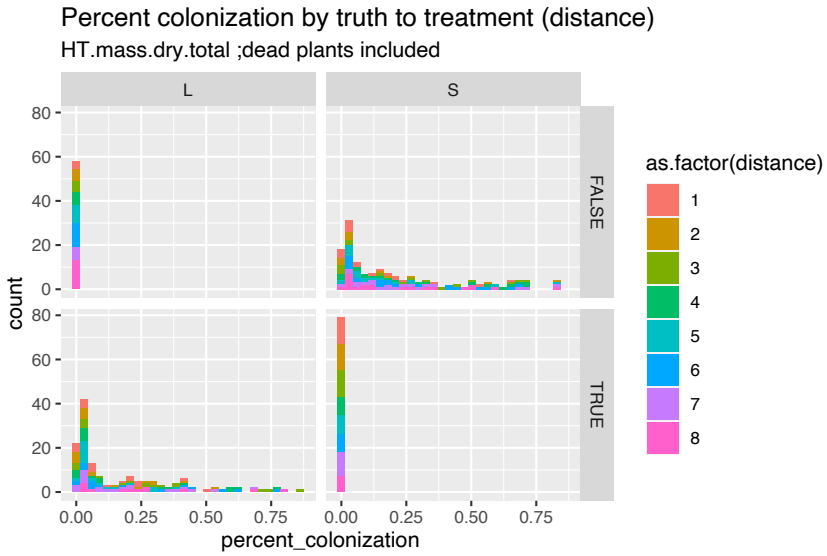


Figure S2: Percent colonization for live and sterile inoculum with root colonization or not. L and S are live and sterile inoculum, false and true are no root colonization and root colonization, respectively. Overall, the percent colonization between the live and sterile have similar distributions.

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