AN INTERDISCIPLINARY ANALYSIS OF A CORN-BASED SEED SAVING NETWORK

By

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An Interdisciplinary Analysis of a Corn-based Seed Saving Network

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Ryan Light

The practice of seed saving has the potential to play a critical role in enhancing the adaptive capacity of the U.S. agricultural system through the protection the crop genetic resource base. It is therefore of value to understand seed savers and the networks that connect them in order to assess their contributions to such a system. In this thesis I take an interdisciplinary approach at analyzing a network of corn (*Zea mays*) seed savers. Through interviews I explore the characteristics of and relationships among seed savers in the hopes of illuminating the strength of the network and its place in the larger agricultural system. Testing seeds for various seedborne fungal genes, I explore the possibility of interaction between seed savers' practices and the biology of their seeds. This study serves as a foundation for future research in seed saving network analysis and the interactions of social behavior on seed microbial communities.

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As we live in the wasteland of a materialistic culture, where amidst its images of abundance we have to search hard for fragments of meaning, the story of the seed tells of a regeneration in the darkness. If we can stay true to the sacred substance and sacred meaning of the seed, it will help us to be a place of rebirth: a place where the inner and outer worlds meet, where real nourishment can once again be born and flower. Working together with the Earth, with its wonder and mystery, we can help in its healing and regeneration.

Llewellyn Vaughan-Lee, "Sacred Seed"

Adapt or perish, now as ever, is Nature's inexorable imperative.

H.G. Wells, "The Mind at the End of its Tether"

1. INTRODUCTION

ADAPTABLE AGRICULTURE

Agriculture in the United States is approaching a turning point. However expansive, productive, and influential, U.S. agriculture is dominated by an industrial mentality that is, by nature, unsustainable. Pollution of air and water, soil mistreatment, fossil fuel reliance, and displacement of native landscapes for biologically barren monocultures are a few such unsustainable practices that not only harm human and environmental health (Horrigan et al. 2002), but also diminish the adaptive capacity of the agricultural system. A manufactured system that harms the ecological system on which it is built is left unsupported and therefore more vulnerable and less adaptable to change. We cannot afford a vulnerable agricultural system at this critical moment in history. According to a recent report from the USDA, "climate change presents an unprecedented challenge to the adaptive capacity of U.S. agriculture" (Walthall et al. 2012). The higher incidence of drought, more extreme temperatures, and changing precipitation patterns associated with climate change will all directly influence agricultural production (IPCC 2014). Yet the vulnerability of agriculture is "strongly dependent on the responses taken by humans to adapt" (Walthall et al.). With deliberate and well-intentioned actions on the part of the government, agribusinesses, farmers, and citizens, U.S. agriculture can be transformed into a sustainable, adaptable agricultural system.

The key to an adaptable agricultural system is diversity, the "raw material of evolution," in the words of a local seed saver. According to the FAO, plant genetic resources are the "most important raw material for farmers," and diversity in these resources "allows crops and varieties to adapt to ever-changing conditions and to overcome the constraints caused by pests, diseases and abiotic stresses" (FAO 2011). But this diversity has been threatened for some time by the systematic replacement of open-pollinated varieties with more productive yet genetically unstable hybrids. In 1973, the USDA warned, "the [genetic resource] situation is serious, potentially dangerous to the welfare of the nation, and appears to be getting worse rather than better" (Miller 1973). While there is no accurate approximation of the current state of genetic erosion, a recent study suggests there has been a "reduction in diversity due to the replacement of landraces by modern cultivars, but no further reduction after this replacement has been completed" (Van de Wouw 2010). Additionally, the FAO predicts at least 20% of landraces are currently threatened with extinction (FAO).

Indeed, the replacement of landraces by modern cultivars has been vast and thorough (Van de Wouw 2010). In the case of corn, the last century has seen an almost complete replacement of landraces by modern hybrids in the U.S., and over 50% replacement in its native region of Mexico (Smith et al. 2004, Van de Wouw et al. 2010). The genetic and varietal diversity is abandoned for a more productive crop (i.e. hybrids), the seed of which cannot be saved, for the next generation will be less vigorous and not true-to-type (Seed Savers

Exchange 2012). It is paramount that we halt and reverse this diminishing crop diversity by supporting open-pollinated varieties, in order to maintain and expand the genetic resources of the world's crops not only in our seed banks and vaults, but also in our fields (Smith et al.). Through active management and stewardship of these varieties, the adaptive capacity of our agricultural system is supported.

Increasing crop diversity can be accomplished many ways, a central one being the practice of seed saving. Through intentional selection and stewardship of open-pollinated seeds, seed savers help conserve varieties and genes, expand the genetic resource base, and ensure farmers control over their genetic resources. The USDA has identified the need of developing resilient crop systems "and the socio-economic and cultural/institutional structures needed to support them," as a means of enhancing the adaptive capacity of U.S. agriculture (Walthall et al. 2012). Seed saving and the networks that support them will be a significant and essential part of this solution.

SEED SAVING, STEWARDSHIP, AND NETWORKS

Seed saving - the practice of selecting, harvesting, processing, storing, and sharing seed - is one of the oldest of human traditions. Dating back to the dawn of agriculture about 12,000 years ago, human selection of the best and most nutritious wild plant seeds began a slow and continuous process of crop domestication that lives on today in the field and in the laboratory (Brown et al 2009). Without active and persistent stewardship of ancient crops and their seeds throughout the millennia, our ancestors would not have had the rice, wheat, and corn necessary for civilization, nor would any of us be here today. Those today that save seed from their crops are, consciously or not, continuing a pre-historical tradition of stewarding biological heirlooms.

Recently, the West has seen a resurgence in the practice of seed saving in response to the loss of crop biodiversity and the consolidation of the commercial seed supply (Howard 2009). Facilitated by the spike in enthusiasm surrounding food, farming, and going "back to the land," countless people across the country are attempting, successfully or not, to save their own seed. Whether or not part of a deliberate trend toward an adaptable agricultural system, this new cohort of seed savers is helping to preserve the crop biodiversity that remains and expand it by breeding new varieties. With the intention of facilitating crop varieties that can withstand droughts, floods, and pests - not to mention maintaining seed and food sovereignty - these seed savers could form the foundation of a system of agriculture that can adapt to climate change.

Vastly broad in interpretation and implementation, seed saving today is practiced by people around the world. Some are subsistence farmers in remote regions with no access to commercial seed, others for-profit farmers in the industrialized world making a living by selling seeds, and others are crop scientists in universities and laboratories working to breed varieties with greater biological fitness. As seed saving reaches the public eye, people of all walks of life are adopting the practice, seeking to preserve and expand the world's dwindling crop genetic diversity, keep alive the tradition of our ancestors, prepare for potential disaster, or maintain sovereignty in the face of corporate power. Those who commit fully to the practice may identify themselves as seed stewards, viewing it as an art and science that requires a combination of drive, experience, knowledge, and a desire to share both seeds and knowledge with others (The Seed Ambassadors Project 2010). These stewards take an active role in steering the trajectory of crop varieties in new and desirable directions. For these reasons, being an effective seed steward requires enormous quantities of time and attention, and many find it necessary to make it their livelihoods in order to survive financially (Adaptive Seeds, pers. comm., 24 Jan 2015).

Yet most people in the U.S. interested in seed saving have other jobs and responsibilities that limit the time they can devote to this practice. They learned about seed saving from Vandana Shiva or a flyer for a local seed swap, and they decided to add the practice to their gardening routine. These "amateur" seed savers bring great enthusiasm and sheer numbers to the seed saving community, yet, more often than not, they do not provide the same service to the genetic resource base as the "professional" seed stewards, who use their knowledge of genetics and breeding to improve these resources.

Regardless of experience level, seed savers constitute a diverse community, a network of individual seed savers and relationships connecting them. The contribution of seed saving to an adaptable agricultural system depends not only on the individuals, but also on the network as a whole; a strong and flexible, henceforth "resilient" network can adapt to external changes with more ease than can a fragile one. The resiliency of a network depends in part on the size of the network, the density of connections, the number of structural holes, the strength of the ties, and the distance between the ties (Kadushin 2012). Translated to a seed saving network, this means that the more seed savers, the more connections between seed savers, the more even distribution of connections, the stronger those connections, and the closer in proximity those connections, the stronger the seed saving network, and the more adaptable the network to future environmental changes. It is therefore worth looking at the existing seed saving networks for signs of these five factors, so that we may gain insight into the contributions of these networks to an adaptable agricultural system.

PACIFIC NORTHWEST

The present study is an analysis of a network of corn-seed savers based in the Pacific Northwest. I here define the Pacific Northwest as the Cascadia bioregion - identified by the World Wildlife Fund as the Pacific temperate rainforest - though with an emphasis on the United States territory. This region is arguably the center of the country's seed movement. Oregon, Washington, and Idaho are known for having "some of the best seed producing areas in the world," with 80% of the U.S. vegetable seed acreage lying within in these three states (OSA).

Farmers, gardeners, businesses, and organizations across the region are uniting to support the social, biological, and economic values of seed saving and sharing in this ideal growing location. Many farmers here are passionate about seed saving and either consume or produce organic seed. Businesses selling organic and open-pollinated seeds - such as Adaptive Seeds in Sweet Home, Uprising Seeds in Bellingham, and Siskiyou Seeds in Williams - are an asset to the regional network, providing enthusiastic gardeners across the region with carefully stewarded seeds. Seed swaps in the region are prolific and popular; there were 1200 people at the 2015 Eugene Spring Propagation Fair and Seed Swap, according to a key organizer, and 2000 packets worth of seeds were exchanged.

Organizations such as the Organic Seed Alliance (OSA), based out of Port Townsend, Washington, are key organizers and educators of producers and consumers of organic seed. OSA is currently the leading organic seed institution in the U.S., and the people running it are working to "build a robust and resilient seed system" and "advance the ethical development and stewardship of the genetic resources of agricultural seed" (OSA). In addition, OSA has been supporting and coordinating "participatory plant breeding, variety improvements, collaborative research with universities, on-farm trainings, organic seed production manuals, and biennial conferences" in the region for the past decade (OSA). The combination of prime agricultural land, motivated individuals, and productive organizations make the Pacific Northwest the ideal place to study resilient seed saving networks.

CORN

Corn (*Zea mays*) is another ideal candidate for such network analysis in regards to the species of seed that people save. For one, corn is native to the New World - specifically southwestern Mexico – and by the time of European colonization, corn was grown from Canada to Chile (Smith et al. 2004). This lends to it a history on this land that few other common crops share. Ranging 100 degrees in latitude and 10,000 ft in elevation, corn began with a remarkably diverse gene pool, assisted by the diversity of habitats (Evenson & Gollin 2002). Now, however, that diversity has been diminished.

Contemporary corn largely consists of modern hybrid varieties (MHV) that have replaced open-pollinated (OP) ones. In Latin America, MHVs now constitute more than half of corn grown (Van de Wouw et al. 2010); in the U.S. nearly all is hybrid (Smith et al. 2004). Beginning in the 1920s, after corn was first hybridized, corn acreage devoted to MHVs went from 1% in 1933, 78% in 1943, 90% in 1960, and close to 100% in 2001 (Smith et al.). During this time, yield increased from 26 bushels/acre to 138, not to mention the increased pest resistance, stalk and root quality, and abiotic stress tolerance (Smith et al.). Along the way, however, much of corn's genetic base was lost with the replaced varieties, including "specialty traits" now only found in OP varieties, such as stress tolerance, disease resistance, nutrient use efficiency, and grain quality (Kutka 2011). Also lost were the unique adaptations of corn to a farmer's climate, as well as the control farmers have over their seed (Navazio et al. 2011. The loss of - and need for – OP varieties is another reason to focus on corn.

Corn also possesses unique biological characteristics. One is that, due to a dependence on human influence, corn cannot sow its own seeds, instead relying on humans to remove and scatter them (Smith et al. 2004). Another is its cross-pollinating tendency; unlike wheat, rice, and other staple crops, corn will readily cross-pollinate with neighboring plants via wind pollination, exchanging genetic material (Evenson & Gollin 2002). This feature encourages genetic diversity, though it has also made it a focus for industrial hybrid research (Evenson & Gollin).

It cannot be excluded that corn is one of the U.S.'s, and the world's, most productive crops. The world's production of corn is nearly 3.5 billion bushels, with the U.S. producing roughly 40% of that (Smith et al. 2004). In fact, it takes "25 corn plants per person per day to support the American way of life" (Smith et al.). That is to say, we depend on corn, even though only 2.7% of that is for direct human consumption (USDA 2008). While the U.S. and other industrialized countries grow the most corn, many non-industrialized countries depend on corn in a far greater way, particularly those in Latin America. In these countries, corn is essential for food security, its genetic resource "of paramount importance for food security worldwide" (Ureta et al.

2012). For this reason, as well as the ones listed above, I here focus on a seed saving network that focuses on OP corn.

RESEARCH INTRODUCTION

To fully comprehend anything of great complexity, one must draw upon multiple perspectives to illuminate different aspects of the subject at hand. In the case of my research, I utilize techniques of the social and biological sciences to better understand the intersection of the social network of seed savers and the biological communities inside seeds. Working with my co-investigator, PhD student Lucas Nebert, I attempted to analyze the social and biological interactions in a corn-based seed saving network. I sought to understand the deeper layers involved in seed saving and how these layers manifest in a network. Others before me have thoroughly analyzed the social aspects of seed saving, but I am not aware of anyone besides Nebert to study both the social realm and its biological implications. Using an interdisciplinary approach, I hoped to understand the people who save seed, the interactions between seed savers' actions and perceptions and the biology of their seeds, as well as the contributions of seed saving networks to the adaptability of the larger-scale agricultural system.

My social approach aimed at understanding a network of seed savers: the demographics of seed savers, their experience and knowledge base, the relationships between savers, and the motivations behind the practice. By interviewing seed savers in Nebert's Community Research Network (CoRN), I expected to learn about these characteristics, the characteristics of a particular network, and also a qualitative understanding of the seed savers through their stories. I aimed to quantitatively assess the resiliency of the network by applying the data to the five attributes of a strong, flexible network detailed above: the size of the network; the number of connections between them; and the distribution, strength, and proximity of connections. To these attributes I added experience, knowledge, and motivations behind saving seed, as the quality of each could prove beneficial for the seed saving network.

I predicted to see in my sample network a moderately strong network. Nebert's CoRN currently includes 25 individuals, each of which, I expected, knew and shared seed with several others, who in turn shared seed with others. I therefore predicted the size to be fairly large, though ultimately indefinable, and the connections to be numerous. The distribution of connections, however, I thought to be unequal, due to the popularity of Adaptive Seeds in the network and the relative anonymity of all others. The strength of connections I expected to be high in the cases in which seed and knowledge were exchanged, which would usually be the case. At a glance I could tell that, while predominantly based in the Pacific Northwest, many people in the network lived elsewhere in the U.S. and one outside the country, so proximity was not in favor of a strong network. Balancing these factors with unknown levels of experience, knowledge, and motivation, I predicted an overall moderate strength, which would contribute a substantial but not excessive amount to the adaptability of U.S. agriculture.

My biological approach aimed at assessing the fungal composition in seeds in order to search for possible correlation between fungal content and the actions and perceptions of the people who save the seed. A seed, contrary to popular belief, consists of much more than plant cells; there is an entire community of microorganisms operating in symbiosis with the plant. Though hitherto unexplored, the idea that, in the intimate relationship between a steward and her seed, the practices and knowledge of the steward would impact this community inside the seed’s outer membrane as much as she influences the genetics is worth investigating. To test for such interactions, Nebert and I analyzed seeds from members of CoRN, testing them for fungal content, including that of total seedborne fungi, general *Fusarium* content, and fumonisin-producing *Fusarium verticillioides*. *Fusarium* is a genus of fungi that is abundant in soil, often absorbed into plants, and sometimes passed along from the plants into their seeds (Nebert, pers. comm.). While nothing is inherently bad about this fungus, some *Fusarium* species, including *F. verticillioides*, produce carcinogenic mycotoxins such as fumonisin. This fungus is understandably undesirable in seeds, yet it remains invisible for several generations before becoming noticeable. Hence even the most experienced seed savers may have this mycotoxin in their seeds but remain ignorant of the fact.

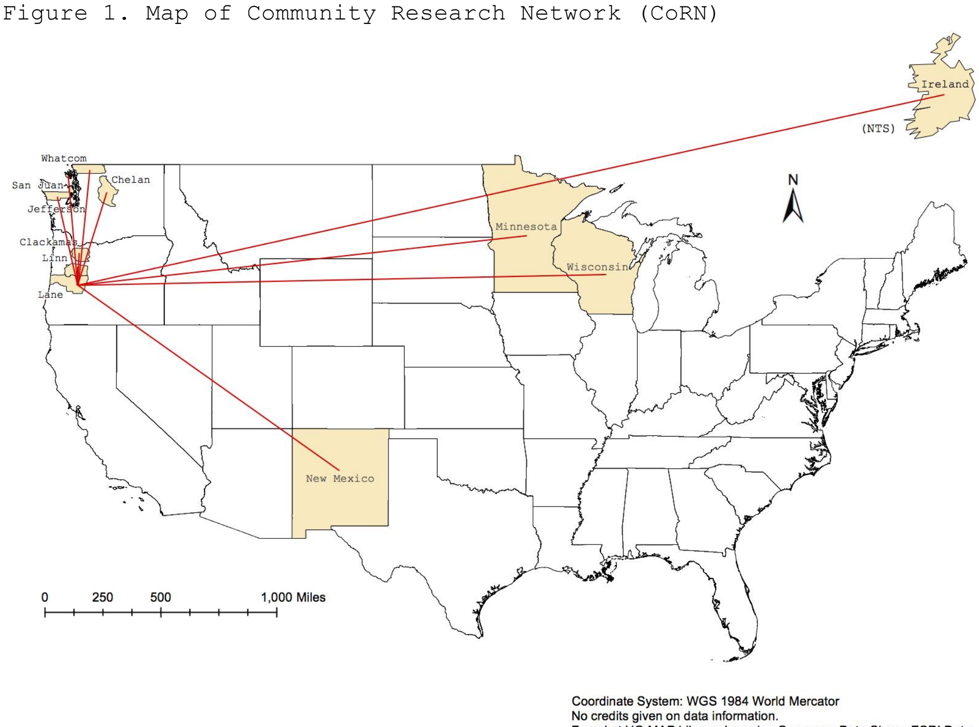
Given this information, I predicted to see no difference in savers seeds’ general *Fusarium* content, nor in their fumonisin-producing *F. verticillioides* content. However, I did expect to see a difference in total seedborne fungal content, for I would expect an organic, microbe-aware farmer to have more soil fungi, and in turn seed fungi than a non-organic farmer with low microbial content in her soil. I therefore expected to see correlations between total fungal content and agricultural practices and knowledge of the microbial communities in soil. If there are significant correlations between these factors, it can be assumed that there is some interaction between the social factors of seed saving networks and the biological factors of seeds.

2. METHODS

SOCIAL ANALYSIS

For my social analysis, I interviewed 16 seed savers - 11 in CoRN and 5 in a branched network connected through a CoRN member. The Community Research Network formed through voluntary subscription to Nebert' Microbial Inheritance project, either in person at conferences, through online registration via his website (www.microbialinheritance.org), or by another's referral. The geographic distribution of the network is widespread, with the greatest concentration being in the Pacific Northwest (Figure 1). After gaining permission from Nebert to contact those in his network, as well as exemption from the Institutional Review Board (IRB Protocol Number: 02162015.024), I proceeded to contact CoRN members via email. Of the 25 members, 14 responded, and of those 11 agreed to an interview. One of the central seed savers in the network connected me with 5 other seed savers, which became the branched sub-network.

Figure 1. Map of Community Research Network (CoRN)



Interviews consisted of 18 questions both quantitative and qualitative in nature (Appendix A). I developed these questions in order to prompt a discussion about seed savers' personal histories as agriculturalists, their land, agricultural practices, experience, knowledge of microorganisms, motivations, values, and more specific components of their seed saving practices. Ten of the interviews took place over the phone, the remaining six via email. Some of the returned email questionnaires lacked responses to certain questions, accounting for several incomplete results below. Typical phone interviews lasted 30 - 45 minutes and were recorded via the application AudioRecorderLite. At the start of the interviews, all interviewees were informed of the research and the reasons behind it. I asked if they were interested in participating in the study, if they had questions, and if they consented to being recorded. All information was kept confidential and secure, and interviewees were made aware of that. After the interviews, I transcribed them into an electronic document via the application InqScribe.

My social analysis of the network had two broad components, the quantitative and the qualitative. The former mode of analysis arose from responses to quantitative questions and the categories assigned to the qualitative responses based on observed trends. For example, a seed saver who told stories of the bonds seed sharing creates may see the social value of saving seed more than the seed saver who does not share seed, but rather saves seed to help adapt varieties to his local environment; this person may see the ecological value of seed saving more than the social. The quantitative analysis included frequency distributions and simple comparative bar charts. Names of interviewees were coded by their county if in the Pacific Northwest, by state if outside, and by country if outside the U.S. The qualitative analysis was two-fold; I created a word cloud from the interview transcriptions via the online application Wordle, and I selected memorable stories and quotes for a more sociological understanding of the seed savers and their relationships.

BIOLOGICAL ANALYSIS

The biological aspect of my methodology aimed at measuring gene copies of the three fungal variables in seeds: fumonisin-producing *F. verticillioides*, *Fusarium*, and total fungi. To do this I followed a procedure developed by Nebert that involved extracting DNA from seeds and using quantitative polymerase chain reactions (qPCR or real-time PCR) to quantify the amount of three particular genes in the DNA.

My fellow undergraduate lab assistants and I began by surface sterilizing seeds. We placed 20 seeds of each sample in sterile test tubes and washed them with 20ml of Tween 20 0.1% in sterile water (1 round), 3% NaClO (bleach) (2 rounds), and 95% ethanol (one round). Each round was separated by 5 inversions and 10 minutes in a mixer. Following the last round, we rinsed the seeds 3 times in sterile water. We then placed the seeds on sterile filter paper in sterile petri dishes, and left them to dry in the laminar flow cabinet (hood). This procedure was adapted by Nebert from Johnston-Monje et al. (2011).

Once dry, we ground the seeds with sterilized, ceramic-burr, manual coffee grinders (Porlex, Osaka, Japan). The grinders were taken apart and sterilized with Labtone detergent, MoBio LabCleaner (DNA/RNA cleanup solution; Santa Cruz, CA), and 95% ethanol. Non-plastic parts, including the ceramic burrs, were flame-sterilized between samples. We transferred approximately 1 ml ground seeds into 1.5 ml tubes and stored them in a -20 degree C freezer.

Next we extracted DNA from the seed samples using a protocol adapted by Nebert from the MoBio PowerPlant plant DNA extraction kit (MoBio), while working under a sterile flow hood. To more effectively lyse microbial cells, we added 0.5ml volume of 0.1 mm glass beads to bead-beating tubes that were provided by the kit and already contained 2 mm steel beads. With a flame-sterilized scupula, we added 50 mg +/- 10 mg of ground-up seeds from the previous step. We then added solutions of MoBio P1, P2, and RNAase A to the tubes and mixed in a vortex mixer, as outlined in the Mobio protocol. We then froze the tubes with liquid Nitrogen and thawed in a 65 C water bath for two cycles, aiming to break the cell walls of the plant tissue. Next, we placed the tubes in a high-speed bead-beater (FastPrep FP120, MP Biomedicals, Santa Ana, CA) at setting of 5.5. to break the seeds apart - repeating twice for 25 seconds each - before placing the tubes in the 65 C bath for 10 more minutes. We followed the MoBio PowerPlant kit protocol for the rest of the DNA extraction procedure. After the procedure, we quantified the DNA and stored at -20 C.

With the extracted DNA, we utilized quantitative PCR (qPCR) to assess the amount of particular genes starting in the seed sample. The three specific genes for which we monitored belonged to either total fungi, *Fusarium*, or fumonisin-producing *Fusarium verticillioides*. The qPCR procedure follows, written by Nebert:

> DNA was quantified using Qubit fluorometric quantitation (Life Technologies, Grand Island, NY. 1 ul of 10 ng of DNA was added to each qPCR reaction, which included 2x KAPA SYBR Fast qPCR buffer (Kapa Biosystems, Wilmington, MA), and primers at a final concentration of 200uM (Table 1). The qPCR thermocycler conditions were performed as follows: A

primary 95C denaturation step of 10 minutes was followed by 40 cycles of 95C for 15 sec., the annealing temperature for 30 sec, and extension at 72C. For ITS1 primers, the annealing temperate was 55C. For the IGS_Fus and FumVerti primers, the annealing temperature shifted between cycles according to a touchdown PCR protocol: for IGS_Fus it was 66C for 1 cycle, 64 for 2 cycles, 62 for 3 cycles, 60 for 4 cycles, and finally 58 for the remaining 30 cycles. Fumverti stepped from 72C down to 68C. At the end of each reaction there was a final extension step at 72C for 5 minutes. Fluorescence measurements were taken at each extension step. The qPCR run method was finalized with a melting curve analysis to assess the purity of amplified DNA. DNA was quantified by comparing sample Ct values to a *F. verticillioides* positive control with known copy numbers. LinReg PCR was used to correct for baselines, quantify qPCR reaction efficiencies and quantify copies of *F. verticillioides*. (Rutjer et al. 2009)

Table 1. qPCR primers

Primer specificity	**Gene region**	**Primer ID**	**Primer sequence**
Universal Fungi Forward primer	Internal Transcribed Spacer	ITS1_F_KYO2	TAGAGGAAGTAAAAGTCGTAA
Universal Fungi Reverse primer	Internal Transcribed Spacer	ITS1_KYO2	TTYRCTRCGTTCTTCATC
Fusarium specific Forward primer	Intergenic Spacer Region	IGS_FusF	CGCACGTATAGATGGACAAG
Fusarium specific Reverse primer	Intergenic Spacer Region	IGS_FusR	GGCGAAGGACGGCTTAC
Fumonisin producing F. verticillioides Forward Primer	Intergenic Spacer Region	VertFumF	GCGGGAATTCAAAAGTGGCC
Fumonisin producing F. verticillioides Reverse Primer	Intergenic Spacer Region	VertFumR	GAGGGCGCGAAACGGATCGG

I analyzed the data using SPSS statistical software (Version 20). Data were tested for normality, and log transformations of dependent variables were used to achieve normally distributed data. I used frequency distributions to model the quantities of fumonisin-producing *F. verticillioides*, *Fusarium*, and total fungi in the seeds. I also found the relative quantities of these genes in the 6 members of CoRN who sent in seed samples for analysis during the 2014 season. To compare the social and biological worlds, I used linear regression models to test the correlation of fungal gene content against years saving seed and years saving specifically corn seed. This test was to understand the relationship between experience and fungal content. I then used one-way ANOVA to compare gene content to agricultural practices, knowledge of microbes, quality of seed source, and motivations behind saving seed. These tests aimed at further investigating a possible correlation between social actions and perceptions and biological communities.

3. RESULTS

SOCIAL ANALYSIS:

The Community Research Network emerged diverse in demographic, practice, experience, knowledge, and motivation. Sixty-three percent of the 16 sampled CoRN members identify as gardeners, the rest as farmers. In terms of land on which seed is grown, 57% of the sample have less than one acre, 22% have 1-5 acres, 14% have 6-10, and 7% have more than 11 acres (Figure 2). Of the 16 seed savers sampled, 14 were 100% organic and 2 were mostly organic. Other notable practices included no-till – the absence of tilling to enhance long-term soil structure – and permaculture – the implementation of natural systems in a landscaping design (Figure 3). Several other practices were used by one or two people each. In regards to knowledge about microorganisms, nearly all seed savers showed an active interest, while only half considered themselves knowledgeable.

Figure 2. Frequency distribution of farm size

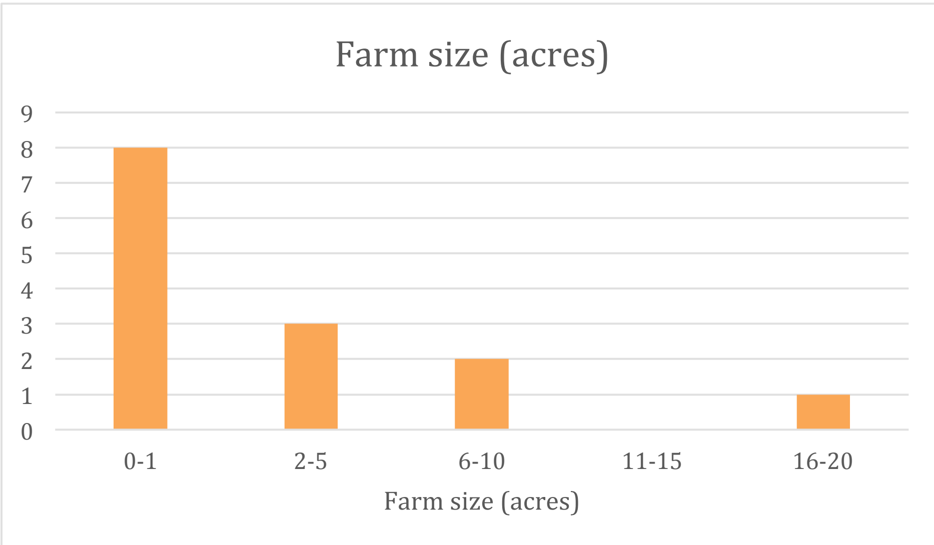
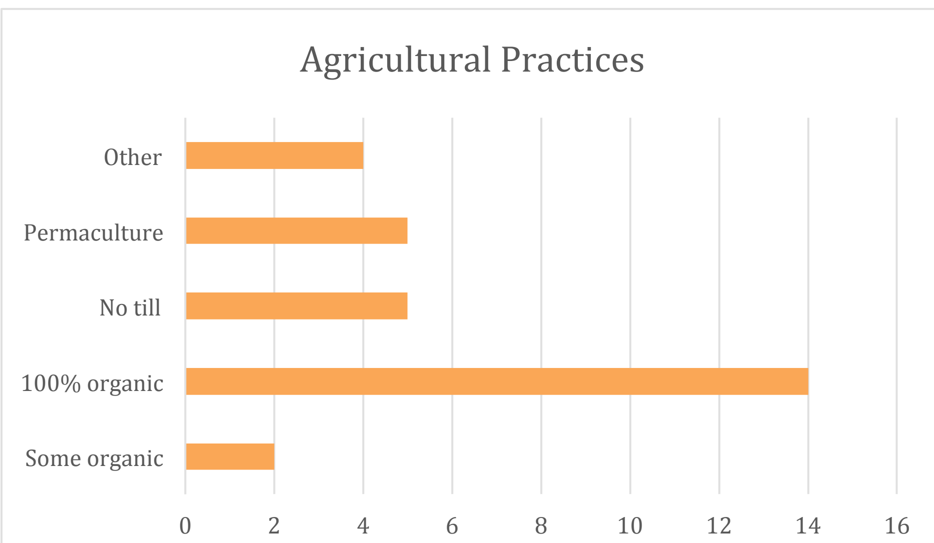


Figure 3. Frequency distribution of agricultural practices



There was an observed range of experience (Figure 4) and knowledge (Figure 5) in the network. While the highest number of individuals began saving seed in the last 5 years, there was an almost-even split between those with less than

10 years of experience and those with more than 10. Two seed savers had about 30 years of experience. In terms of seed saving related knowledge, six people had some formal training in a related field, such as botany or plant biology. Seven people were self-taught to some degree, four of who were confident in their level of knowledge. The remaining seed savers had gained their knowledge solely through their practice.

Figure 4. Frequency distribution of years saving seed

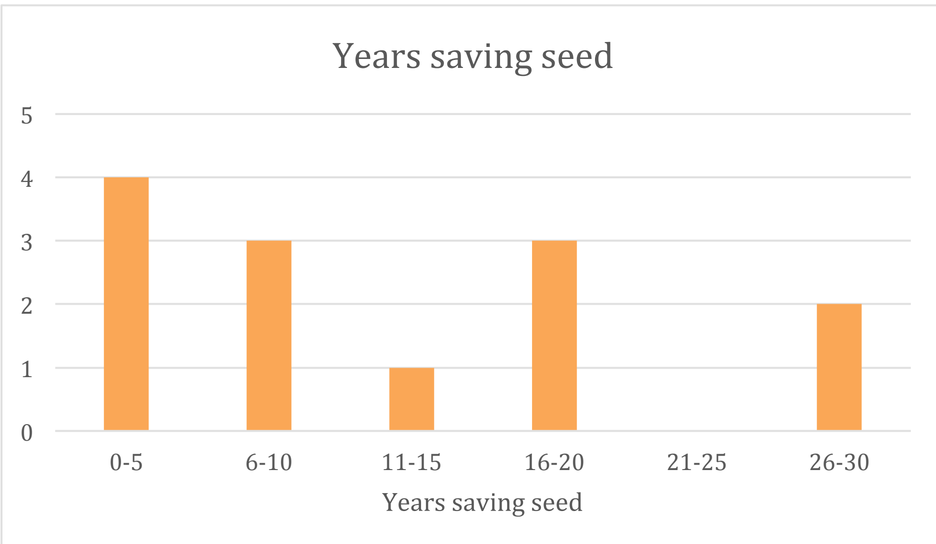
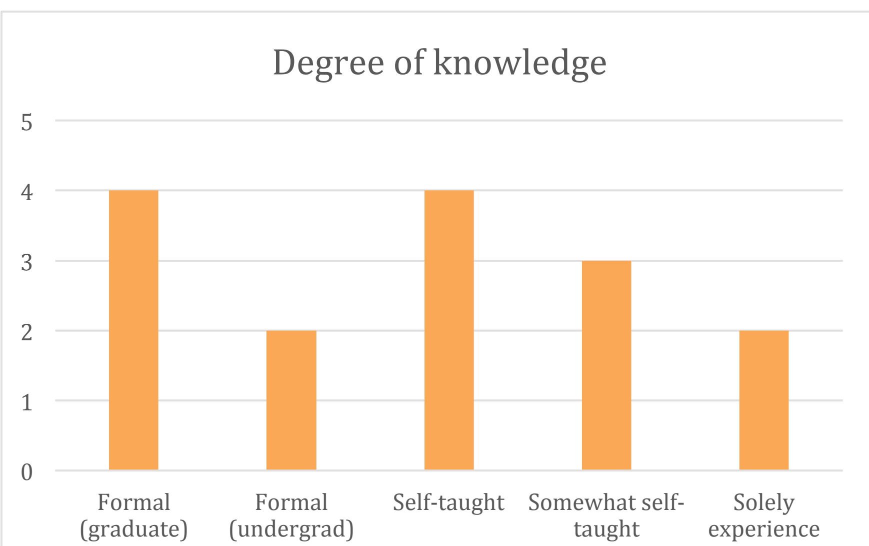


Figure 5. Frequency distribution of degree of knowledge



There were a plethora of motivations behind people's seed saving practice. There were five broad categories that I organized the seed savers into based on their responses to the question, “Why do you save seed?” The five major reasons people had for saving seed were: control/survival, adaptation/ecology, community/preserving heritage, fun, and economics (Figure 6). (Examples of statements that led to placement in a particular category can be found in Appendix B.) The first three of these were expressed by nine seed savers each, with some overlap between them. Six seed savers also saved seed because it was fun, and three did so for economic reasons – only one of these chose only economic.

When asked about the primary value of saving seed, 2/3 of the sampled network it as social, 1/3 as ecological, and none as economic, even those who saved seeds for economic reasons (Figure 7). Social values primarily involved the creation and strengthening of communities, the preservation of heritage, the culinary benefits to varied crops, and the implications for surviving some catastrophe (Appendix B). Ecological benefits involved adapting plants to local environments, expanding genetic diversity, and the implications of this diversity on ecosystems (Appendix B).

Figure 6. Frequency distribution of reasons for saving seed

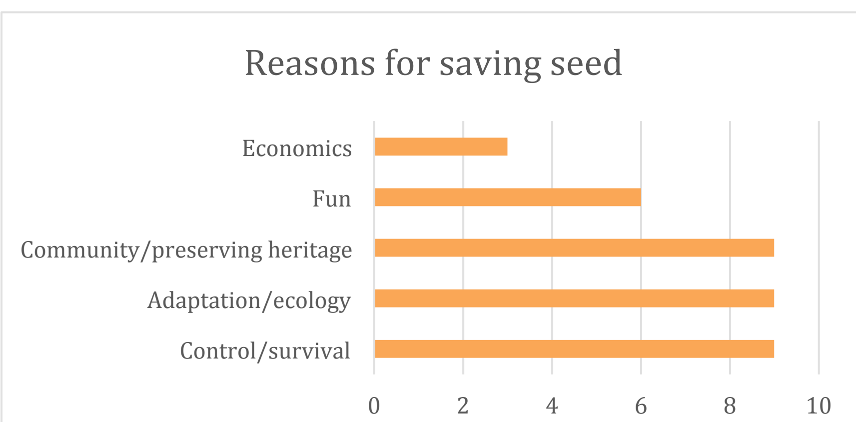
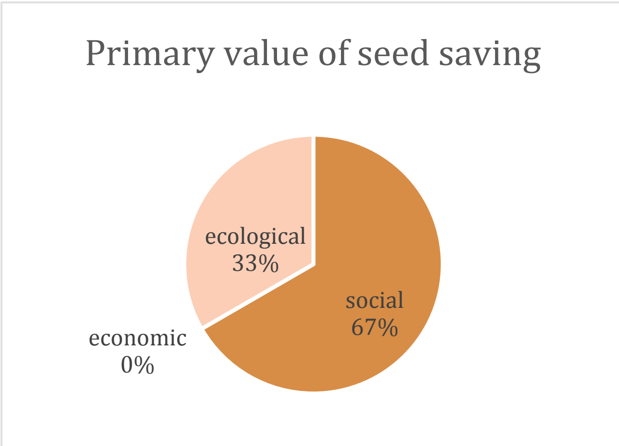
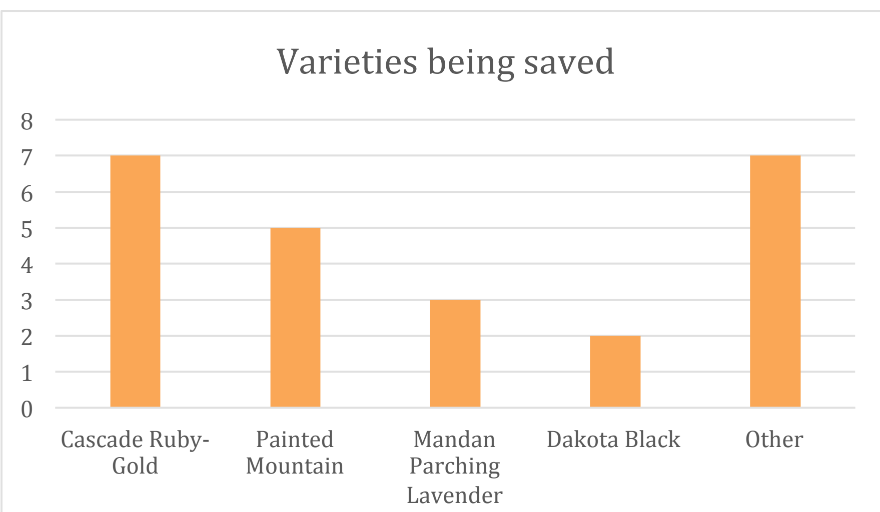


Figure 7. Primary value of seed saving



There were 16 corn varieties – not including sweet corn - being saved by 12 sampled CoRN members who are saving corn seed, plus 56 unidentified varieties that were saved by one individual, which may or may not include the other 16. Thirteen of the sixteen identified varieties were only being saved by one person each (Figure 8). Seven people were saving Cascade Ruby-Gold, five saving Painted Mountain, and three saving Mandan Parching Lavender. As varieties adapted to the Pacific Northwest's short summer, these varieties were expected to be some of the most popular.

Figure 8. Frequency distribution of varieties being saved



The majority of the seed savers got at least some of their seed supply from Adaptive Seeds or another small seed company, both professional sources. Others got their seeds from non-professional sources such as friends, seed swaps, the Seed Savers Exchange (SSE), conferences, Native Americans, or somewhere else (Figure 9). Most corn seed savers were actively selecting for traits, most commonly being adapted to the local environment, followed by size/vigor, color, diversity, maintaining traits, flintiness, and finally flavor/nutrition (Figure 10). Four people did not select for traits in their seed supply, either because of their lack of knowledge or lack of interest.

Figure 9. Frequency distribution of original seed source

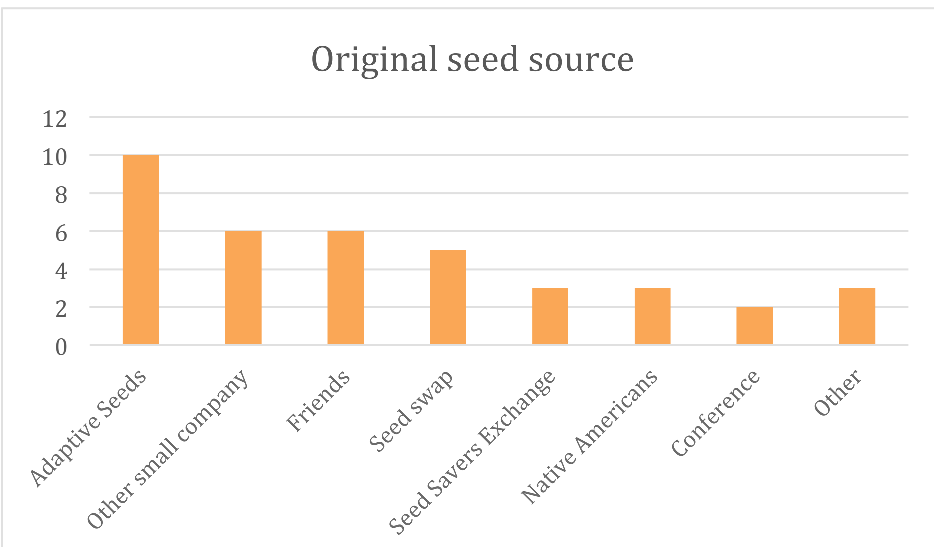
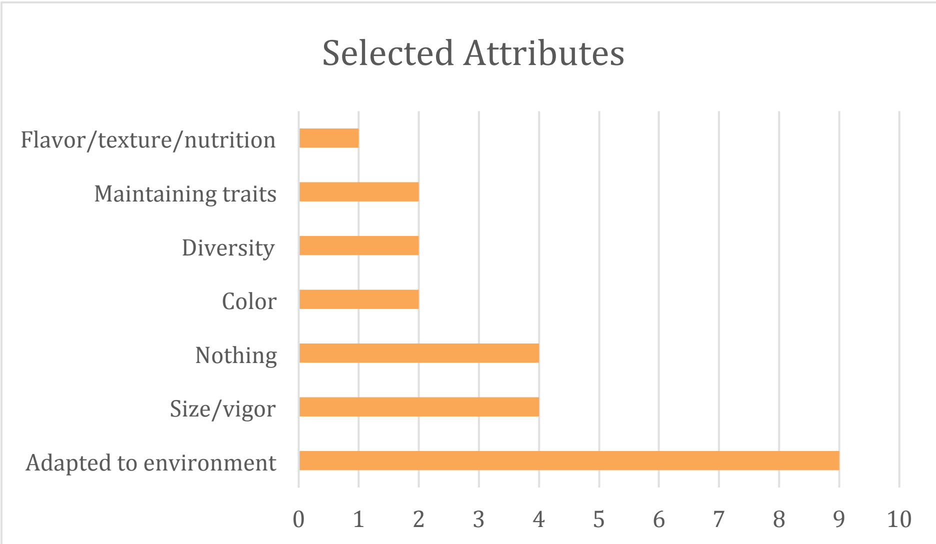


Figure 10. Frequency distribution of selected attributes



Most of the seed savers I interviewed share their seeds, as well as information about the seeds. While only two did not share seed with anyone, seven shared with their friends, five with people at local seed swaps, four with anyone, another four with Adaptive Seeds, two with children, and one with each family, seed libraries, farmers markets, stores, and website (Figure 11). There were many types of information shared with the seed, including, in order of popularity, history/stories, personal experience, genetic/breeding information, catalog information, growing tips, and resource links (Figure 12).

Figure 11. Frequency distribution of seed sharing

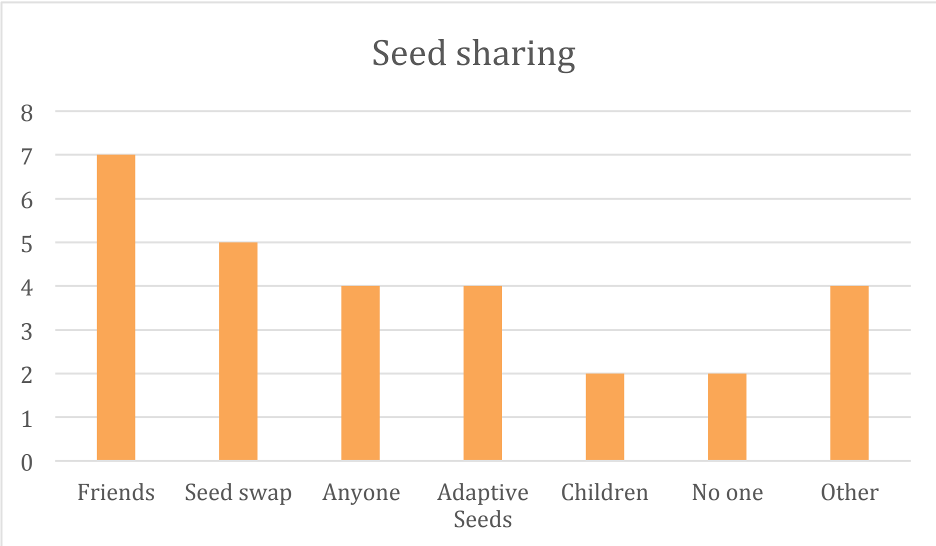
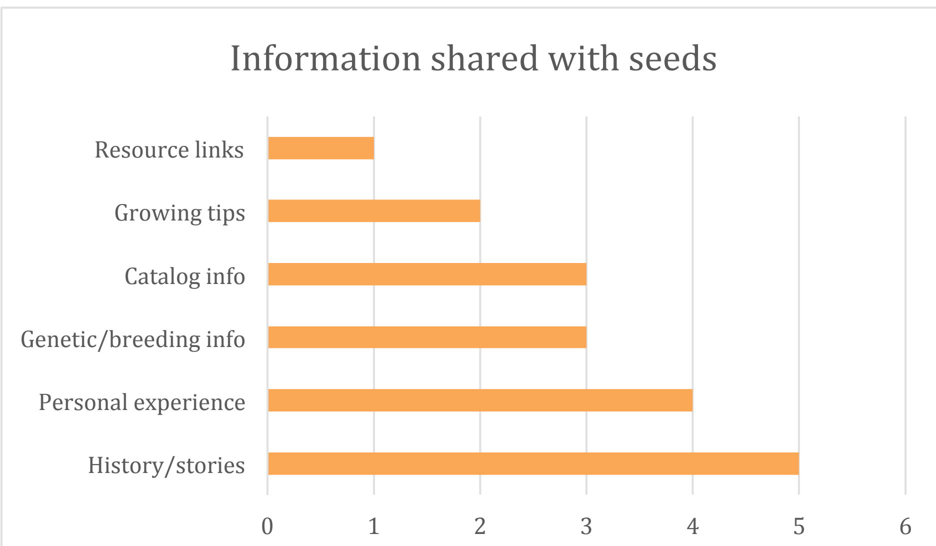


Figure 12. Frequency distribution of information shared with seeds



Moving from my quantitative to qualitative results, the stories told by seed savers were similarly illuminating. The Wordle word cloud of frequently used words in interviews illuminates the subjects that seed savers talk about (Figure 13). Notable of the most commonly used words were, in order of frequency: seed, corn, people, seeds, garden, grow, saving, soil, growing, food, and varieties. The frequency of the word "people" indicates the importance placed on people in the seed saving world. Also important is the soil that grows the plants, and the gardens to which people tend. Other words of note include: community, adapted, organic, microbes, share, swap, network, diversity, important, need, now, back, GMO, genetic, climate, control, past, adaptive, fair, better, together and happy. The words used by a group of people tell a story of people in a community sharing, saving, swapping seed together. They are aware of climate, genetics, and microbes, and they find the practice important for control, adaptation, and diversity.

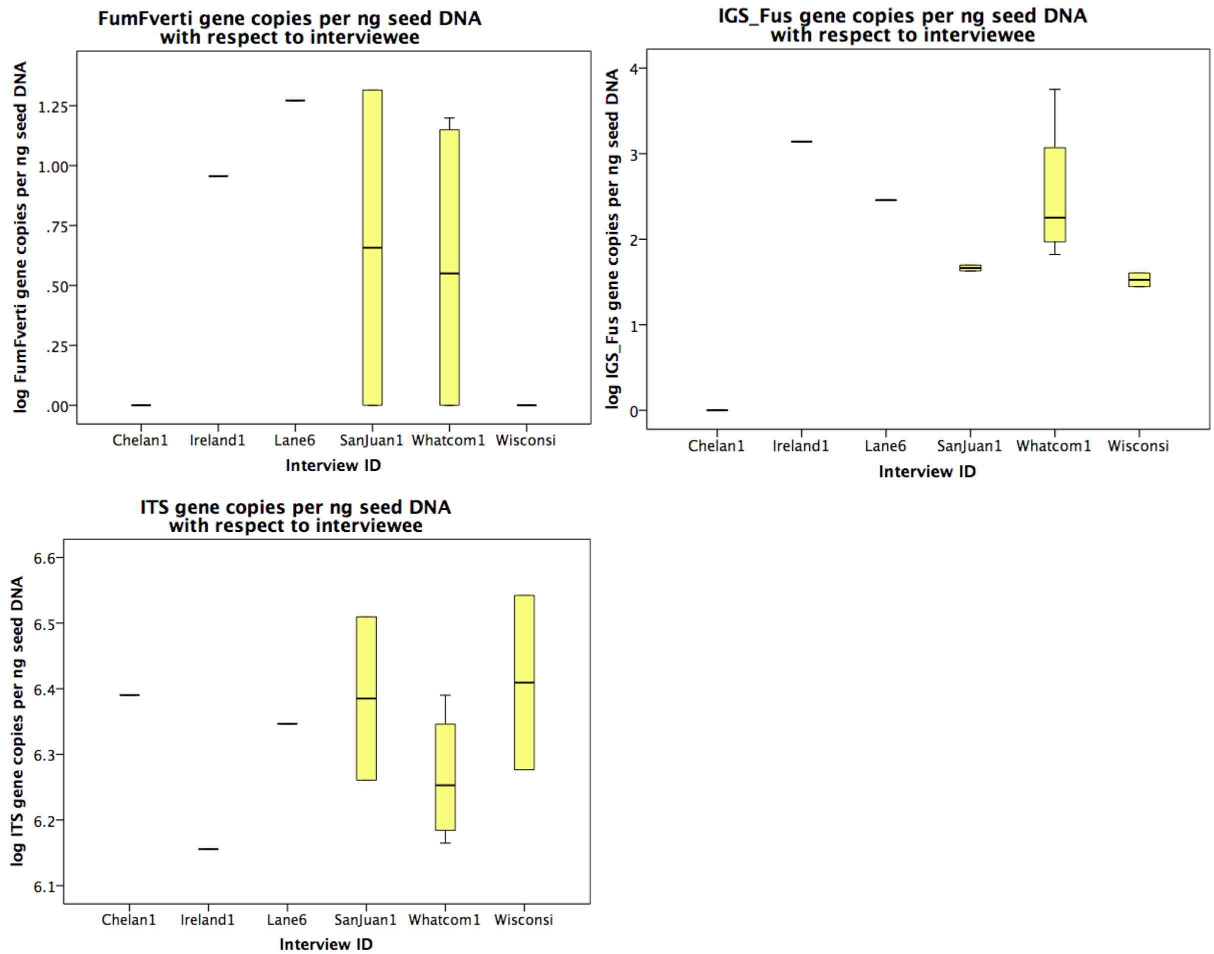
Figure 13. Word cloud

BIOLOGICAL ANALYSIS:

The qPCR procedure illuminated part of the sampled seed composition, including content of fumonisin-producing *Fusarium verticillioides* (FumFverti gene), *Fusarium* (IGS_Fus) and total fungal content (ITS). Of the seeds sampled, few held any FumFverti, yet those that did produced a substantial amount, up to 30% of all *Fusarium*. FumFverti varied from 0 - 31 gene copies per ng seed DNA (μ=4.51, SD=8.34), as compared to 0 - 8352 gene copies of *Fusarium*-specific IGS (μ=908, SD=1956) and approximately 1 - 4.5 million copies of ITS (μ=2.2 million, SD=0.8 million) (Appendix E). The frequency distribution of IGS_FUS and ITS had more normal distribution across the samples than FumFvert, so they were more amendable to standard statistical tests (Appendix D.2,3).

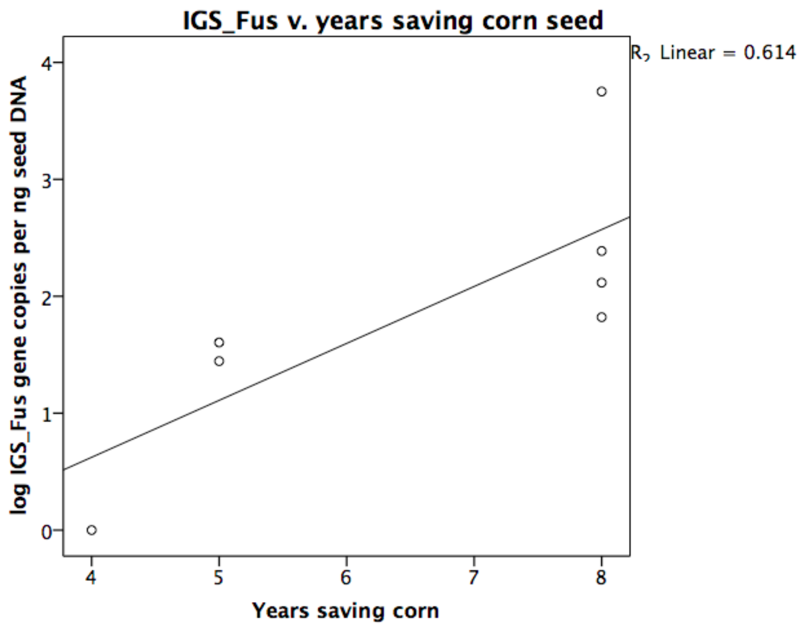
Each seed saver had differing content of fumonisin-producing *F. verticillioides*, *Fusarium*, and fungi per ng of total seed DNA. Neither Chelan1 or Wisconsin1 had any FumFvert in their seeds (Figure 14a). Ireland1 and Lane6, however, had relatively high quantities of this pathogenic *Fusarium*, and the other two, SanJuan1 and Whatcom1, had seeds varying from none to levels similar to the previous two. In terms of the *Fusarium*-specific IGS gene, Chelan1 were the only seeds with no *Fusarium* (Figure 14b). SanJuan1 and Wisconsin had moderate levels of *Fusarium*-specific IGS, Ireland1 and Lane6 had higher levels, and Whatcom1 once again had a range, from moderate to high. Total fungal content also varied amongst the samples: Ireland1 with the lowest, Chelan1 and Lane6 with moderate levels, Wisconsin1 with a range low to moderate, and the others with a moderate to high range (Figure 14c). *Fusarium* content was the only amplicon of the three that yielded significant results.

Figure 14. Gene copies per ng DNA of a) FumFverti, b) IGS_FUS, c) ITS with respect to interviewer



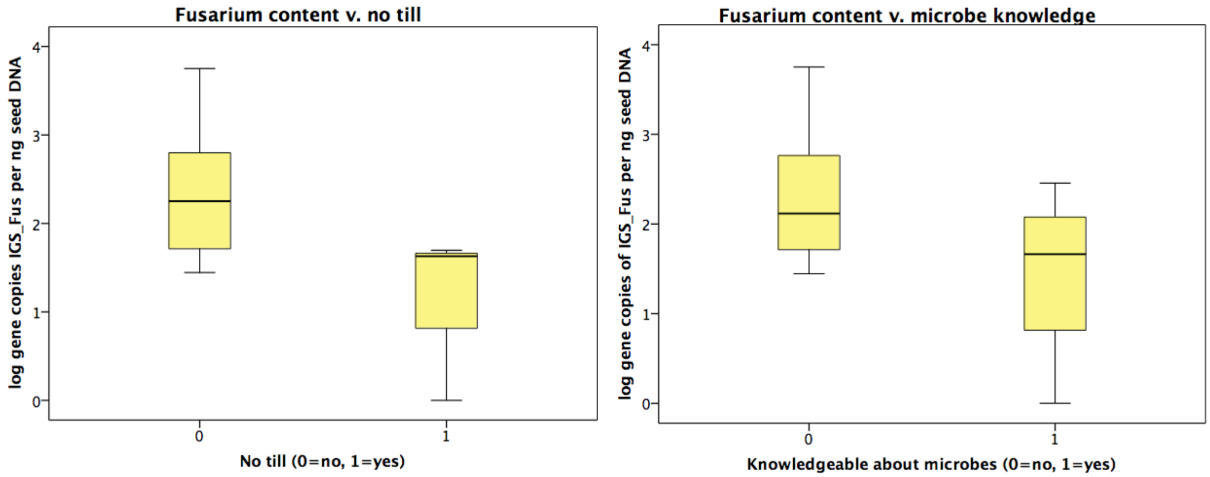
Linear regressions of gene copies by farm size, years farming, years saving seed, and years saving corn seed yielded only one significant trend, i.e., that between *Fusarium* content and years saving corn seed. This trend was a fairly strong positive correlation (df=1, R^2=0.61, F=7.95, P=0.037) (Figure 15). This indicated that the longer one saves seed, the more *Fusarium* can be expected in their seeds. Upon further investigation, however, it appeared only 3 of the 6 sampled seed savers answered this particular question about years saving corn seed, and more than half of the data points are replicates of the same seed saver. While there may still be a correlation, the true sample size skews the significance of this test. Comparing years saving corn seed and FumFvert or total fungal content did not yield any significant results, nor did years saving all types of seed with any of the three genes.

Figure 15. IGS_FUS v. years saving corn seed



In terms of agricultural practices used, the only near-significant result was that comparing *Fusarium* content and no till agriculture. In the sampled population, there was less *Fusarium* in seeds from untilled land (df=1, F=4.84, P=0.055) (Figure 16a). There was no such significance in the tests between Fumonisin-producing *Fusarium* nor total fungal content. Declared knowledge about microorganisms in a farm setting yielded an apparent difference in *Fusarium* content - the more knowledgeable the farmer the lower the *Fusarium* content - though the results were not significant (df=1, F=2.36, p=0.159) (Figure 16b).

Figure 16. IGS_FUS v. a) no till, b) knowledgeable about microbes, and c) professional seed source



4. DISCUSSION

I had several predictions entering into this research, some of which were supported, some of which were not. The first of my predictions was the moderate level of strength of the network. The data, as predicted, fail to show the size of the network, as there are nearly unbounded connections between seed savers, and I only interviewed 16 seed savers. Those that I did talk to, however, appeared to have multiple connections with others and shared seed with many people, including the 1200 people who attended the 2015 Spring Propagation Fair and Seed Swap. The connections, therefore, are numerous. I was not able to confirm the distributions of connections between seed savers, as I did not inquire about all of the interviewee's relationships. From the data, however, it is possible to deduce that the connections between seed savers are strong. Almost 90% of seed savers share seed with others, and most of these share some sort of knowledge along with the seed, one or more of six types of knowledge. History and stories of the seed is the main information shared with the seed, potentially indicating an intimacy or openness among those who share seed. Finally, as predicted, the proximity of seed savers was quite large, which does not indicate a strong network. Though ultimately unquantifiable, an estimate of this network's strength, as suggested by Kadushin (2012), would likely fall around moderate, taking into consideration the (unconfirmed) large size, numerous connections, (unconfirmed) distribution, strong connections, and far distances.

Adding experience, knowledge, and motivation elements into the equation, however, may increase this strength. Experience of seed savers was well distributed, from several to 30 years of saving seed. Knowledge was equally well distributed; most seed savers either had formal education related to saving seed or were to some degree self-taught. Motivations were another indicator of network strength, as each seed savers had a compelling reason for saving seed. Whether as the one person saving seed to survive financially or the nine people saving seed for control of their food source and a chance of surviving potential disaster, the seed savers with whom I spoke would not give up the practice easily, as each reason was one far bigger than the seed savers themselves. For example, the seed saver who witnessed an older native woman run out of her house overjoyed at the sight of seeds being returned to her that she hadn't seen since her grandfather grew them – that seed saver will not easily abandon a practice that "feels like the right thing to do."

The diversity of the network was an unforeseen component of the network. This network, its demographics, land size, location, and varieties – not to mention the characteristics described above – varied substantially. There were many gardeners and many farmers, with mostly small patches of land, though one with 20 acres. Many small patches of land in various microclimates may cause a higher genetic diversity in a given variety, as each strain will be adapting to a slightly different environment. Additionally, 25% of the network lives outside the Pacific Northwest, which adds to the diversity of environments. The diversity in corn varieties, also, is substantial, with up to 70 varieties represented in a group of 16 seed savers. This diversity, though not mentioned by Kadushin in his list of network characteristics, may increase the strength and flexibility of the network in the same way that a diversity of genetic resources contributes to adaptive capacity in agriculture. And, as mentioned, there is a correlation between the diversity of the seed saving network and the diversity of the gene pool. Therefore, if not for the strength of the network, the diversity adds to the strength of the agricultural system. All things considered, the network appears to be on the stronger end of moderate, though more quantitative data is needed to affirm this. With further assessment and this and other networks, the contributions of seed saving networks to an adaptable agricultural system can be further illuminated.

One aspect of the network not apparent from the data but mentioned by many people in their interviews is the lack of organization in the network. While there is communication between those who share seeds with each other, there isn't as much among those further away socially or geographically. One method used to reconcile this situation is the seed swap, a gathering of seed savers during which people share seed and stories with others in the area. At the Eugene Spring Propagation Fair, an annual event meant for just this purpose, there were 1200 people of all walks of life sharing and talking about seeds and scionwood - about 1% of Eugene's population! That said, one person I spoke to estimated that only 1% of the people there gave seed as well as taking. From what I saw, most of the seed that was at the swap was not labeled with the necessary information, which is a crucial part of sharing seed. Without information on the variety, parent plants, growing conditions, and date harvested, one cannot easily or accurately steward and care for the seed. Here comes into play the previously discussed tension between “amateurs” and “professionals” and the roles that each plays in a seed saving network. The professionals with whom I talked were frustrated by this absence of crucial information, for such negligence weakens the seed bank, the seed swap, and the ties that hold seed savers together. The amateurs, while bringing certain positive qualities to the network, such as numbers and enthusiasm, harm the network in other ways. Education may be a useful way to teach the amateurs if not to a professional level, then to a moderately experienced one. Therefore, not only organization but also education is a place in which this and likely other networks must improve in order to increase the strength of the network.

The biological component of this study also yielded unexpected results. My prediction was correct that there would be no trends observed in the case of fumonisin-producing *F. verticillioides*, yet there were also no trends in total seedborne fungus – contrary to my predictions - and the only near-significant trends were in the *Fumonisin* tests. There may have been a lack of significance in total fungal trends due to the wide variation of values spanning millions of copies of DNA, as opposed to the single-digits in the other two tests. While the only truly significant (i.e. $p<0.05$) trend observed was potentially skewed by the grouping of data, the data does suggest a positive correlation between experience saving corn seed and *Fusarium* content. This is a reasonable idea, given that a person with more experience saving corn seed has likely been growing out a strain of the same corn variety for generations, each year accumulating more of the fungus in the seeds. Fortunately, even with the skewed data, there was no such trend observed with fumonisin, which was predicted due to the random and unnoticeable presence of this mycotoxin – even an experienced seed saver would not be able to catch it until generations of the seed had passed. Though no trends were observed with fumonisin, I was able to determine its relative quantities in seed samples, which may be a useful strategy for the monitoring of seed savers seeds to prevent the proliferation of the mycotoxin.

The interaction between the social and biological realms yielded two nearly significant trends in the two areas previously predicted - agricultural practices and knowledge of microbial communities – though with *Fusarium*, not total fungi, and in the opposite direction as expected. Though neither was quite significant (p=0.055 and 0.159, respectively) nor far from being due to chance (F=4.84 and 2.91, respectively), they were the strongest of any other correlation with practices or perceptions. There were fewer copies of *Fusarium*-specific IGS gene copies per ng of seed DNA if a seed saver practiced no till farming and/or were knowledgeable about microbes. A possible explanation for this unexpected pattern could be that, though the total fungal composition in the soil was higher, less *Fusarium* was passed from the soil to the plant, and less was then passed from the plant to the seed. Further research is required, however, to affirm this trend and then to explore the possible reasons behind it.

There are certainly many areas of this research topic still unsupported and unexplored. This study should be taken as the foundation of such future research, which can explore new seed savers, new interactions, new fungal and bacterial genes, and even new networks. The strength and flexibility of this network, though still unquantified, point in the direction of resilience, which, if matched by other seed saving networks, is a positive sign for the adaptive captivity of the U.S. agricultural system.

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6. APPENDIX

APPENDIX A. QUESTIONNAIRE

1. What you do related to agriculture, and how did you get started?
2. Please describe your farm/plot.
3. What agricultural practices do you use?
4. What role does the microbial community play on your land?
5. How did you get into saving seed?
6. How long have you been saving seed? Corn seed?
7. Why do you save seed? Why corn?
8. Which corn varieties are you currently saving/stewarding?
9. Where did you get the original seeds?
10. How did you choose the varieties you chose?
11. Which attributes do you select for?
12. How much seed do you save?
13. If you share seed, with whom do you share, and what do they do with it?
14. What kinds of knowledge do you share with the seed?
15. How much do you know about the science behind seed saving (e.g. genetics, microbes, selection, evolution)?
    a. Where did you learn this?
16. Please describe the seed saving network of which you are a part.
17. What is the social, economic, ecological value of saving seed?
    a. Which, if any, do you consider the most important?
18. What role might seed saving/stewarding play in the future?

APPENDIX B. QUOTES AND CATEGORIES

ADAPTATION/ECOLOGY:

- The plants are adapted to the soil, microbes and amount of water I give them, while maintaining the characters I want them to have.

- Well just about one chapter when I talk about local adaptation--not just the biology of it, but the shared culture of it. Because, the cool thing about this whole process is that it isn't just genetic, it's this whole biological, genetic, cultural mish-mash.

- Because if something grew and thrived here (I'm not the most attentive, supportive gardener) and set seed, then I can have reasonable confidence that it will do so again and will also likely do so in my friends' gardens.

- Seed saving for me is getting things that are completely adapted to our conditions.

- Seed saving allows one to develop varieties that are acclimatized to the area.

- I am showing that one can get what they want out of the plant while maintaining ecosystem health.

- Imported foods are bad ecologically. Buying seed that is adapted to here, not some place warm and sunny. It needs to be adapted to the climate.

- Diversity helps, compared to monocultures, having a big, robust, complex ecosystem rather than giant fields sown just with one crop. Getting more and more people to grow a variety of plants, that makes good insect habitat, helps build the soil, creates self-control, especially if it's all organic, it's cleans groundwater, it clears the air, it's very positive.

- [Seed saving] may be literally life saving for humans and the planet’s ecosystems.

- When we have lost biodiversity, [seed saving] can provide a way to get at least some of it back.

COMMUNITY/PRESERVING HERITAGE:

- Seeds have always been the most precious, valuable cultural resource.

- Different people have different strengths, and we need to share with each other so we can get somewhere on this.

- Honestly, when I started saving seeds, it was kind of a survival mentality. That has changed pretty dramatically. Seed saving is this really cool way to socially participate in that more tribal behavior. Because, it encourages you to act as a community

- It takes a conscious effort to build community ties in our current culture but growing things seems to build them naturally

- I think socially, saving seed has the potential to create or deepen community ties.

- Creating seed saving community is right in the heart of recreating community.

- Saving seed preserves the heritage of a place.

- Passing on gardening heritage, varieties that have certain culinary potential, sharing that information about what grows well, what cooks well, what grows well together

- Sharing information around seeds and food preparation is at the heart of human culture.

- The seeds that I have grown and saved are a legacy of sorts, representing what I cared about in my life. How cool would it be to have my grandson grow corn derived from my seed? Or my grandson’s son? That would be sacred, that would mean a lot to me if there were a way to know it happened.

- A lot of things get lost in so many ways, and this is one thing I can help carry on.

CONTROL/SURVIVAL:

- It allows me to be in control of what kind of product I get.

- That's one of my motivations for doing this - to have a constant supply of viable seeds.

- It protects the population against widespread crop failure.

- It drives me nuts when seeds that I love are no longer on the market.

- I am learning a skill that could be very important for survival in future times.

- It may enable a few to survive while rebuilding human populations decimated by global warming or some other catastrophe. It may allow food to be grown when catastrophic climate change takes place as the seeds will have evolved with changing conditions over time.

- Being so far from basic, reliable functioning that it makes me nervous...I wish we had basic skills in reserve for survival.

- If times get tight, seed saving could become an invaluable tool in helping to feed a community.

- I really feel empowered by anything that creates less dependency.

ECONOMICS:

- I make a living from it.

- Because I'm cheap.

- Seed saving saves you money in the long run.

OTHER:

- The more people that are doing this, the more diversity we'll have, the more security we'll have, the more community we'll have, because we'll be talking to each other about it.

- I believe the more knowledge, food independence, and genetic variability to draw on the better.

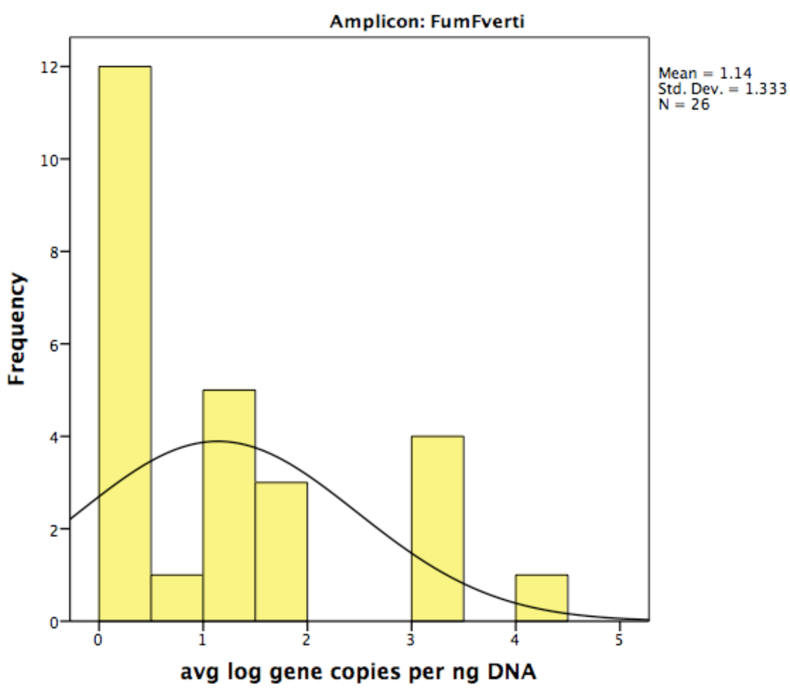
- When I eat corn that I have grown and prepare it as flour or parching corn, or polenta, it has power for me, not sure why, but it does.

- I’m curious about genetics.

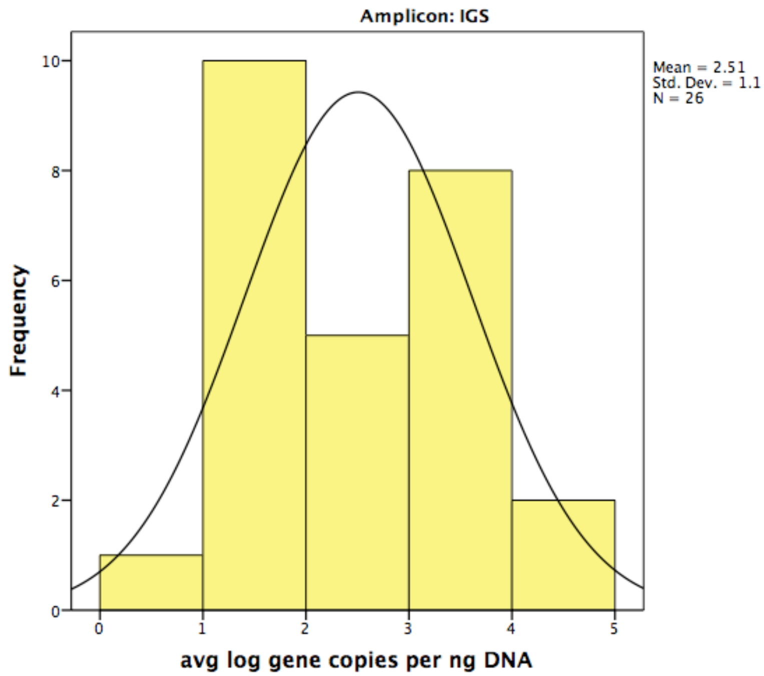
- That's one of the reason's I love growing seeds, is because it's a total metaphor for hope, the seed, like I'm working for something not against something.

- Resilience, independence and stewardship.

APPENDIX C.1. Frequency distribution of FumFverti gene



APPENDIX C.2. Frequency distribution of IGS_FUS gene



APPENDIX C.3. Frequency distribution of ITS gene