The genetic archaeology of maize: reconstructing pedigrees to identify useful diversity for breeding

Rationale and Significance

Maize is a natural resource of fundamental national importance, vital for food, livestock feed, and fuel production. Maize is also the most valuable field crop in the United States, with production values at greater than $50 billion dollars every year since 2010 (?).

But while maize yields have increased over the last several decades, the rate of gain falls short of projected needs in the near future (?). Even under stable climatic conditions, current rates of maize improvement are insufficient to meet requirements of population growth over the next 30 years (?); in addition to requirements in terms of food and animal feed, worldwide ethanol use is projected to increase 40% within just the next decade (?).

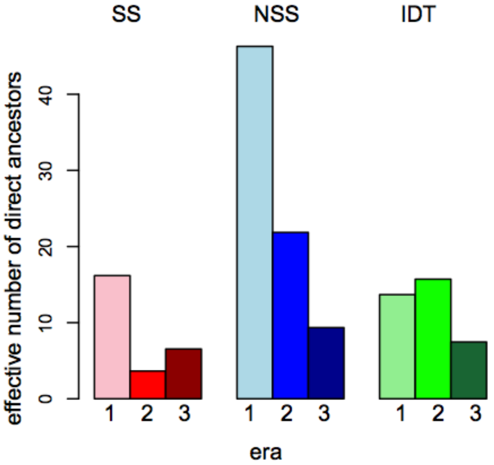
Changing climatic conditions, however, will likely further challenge our ability to meet needed yield gains. Historical analyses suggests that climate change over the last 30 years has already dramatically impacted maize yields worldwide, retarding gains from breeding and management (?). Moreover, predicted temperature increases will increase volatility in yield across the U.S. and may even decrease future yields (?), with some models suggesting a change of even 1°C could negatively impact yields by as much as 17% (?); more dire warnings suggest that U.S. maize yields could drop 30-46% below current levels by the end of the century (?). It is also predicted that these climate changes will interact with and change soil properties resulting in increased fertilizer inputs (?), and even further interact with pests and diseases (?). These different factors may give rise to completely novel environments far different from the ones that farmers and breeders had to contend with in the past. Substantial efforts will clearly be needed to preserve U.S. maize production and increase or maintain yields.

Much of the historical gains in maize yield can be directly attributed to breeding efforts (??), and **breeding must remain of central importance in order to meet increased yield demands**. Breeding is also of key importance in adapting maize to the challenges of changing climates (?), with recent models suggesting that efficient use of extant adaptive diversity in maize could significantly ameliorate the effects of climate change (?).

Diversity loss threatens breeding gains

Breeding, and adaptation in general, relies critically on the availability of genetic diversity. This is best represented mathematically by JL Lush's famous "breeder's equation":

Figure 1: Changes in genetic diversity (represented as the effective number of ancestors) of the three primary maize heterotic groups (SS: stiff-stalk; NSS: non-stiff-stalk; IDT: iodent). Inbreds are divided into eras representing different time periods: 1:1930-1950; 2:1950-1980; 3:1985-1992. Figure from **?**.



$$R = \frac{V_A}{V_P} s \tag{1}$$

showing that adaptation — the response to selection R — depends not only on the strength of selection s but also directly on the amount of heritable genetic variation V_A for the trait of interest (**?**). The advent of modern hybrid maize breeding, the development of distinct breeding pools and the winnowing of inbreds from individual breeding programs has led to a marked decrease in genetic diversity. Analyzing genotypes of more than 4000 public and recently released private lines, we recently showed that the diversity available to maize breeders in current germplasm is less than half what it was in before 1950 (Figure 1). With decreasing diversity available for breeding decreasing yield gains are a mathematical certainty.

While open-pollinated varieties and exotic inbred lines represent a viable source of new diversity, these have been only sparingly used in the private sector due to their poor agronomic performance, photoperiod sensitivity, and the necessary generations of backcrossing to adapted lines required to incorporate useful alleles into high-performing temperate germplasm (**?**).

In contrast, older U.S. inbred lines, though lower-yielding than their contemporaries, harbor novel genetic diversity of potential use for breeding (e.g. **??**), and are already adapted to the temperate U.S. Breeders have clearly been successful in advancing high-yielding germplasm and discarding ill-adapted material. This does not mean, however, that all discarded material has little genetic merit — beneficial alleles can be lost to genetic drift, particularly considering the polygenicity of agronomic traits and also because breeders are unable to select for all traits simultaneously. Our own population genetic analysis supports this notion; a number of underutilized older inbreds are enriched with favorable alleles (Figure 2).

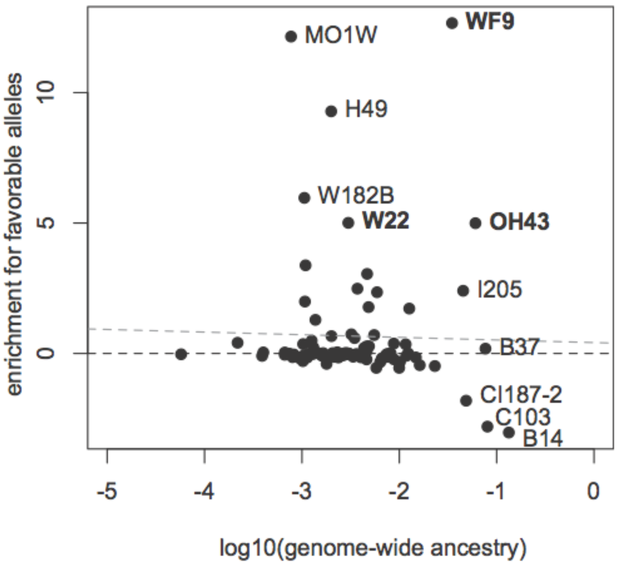


Figure 2: Enrichment (log probability ratio with respect to control loci) of favorable alleles in older (era 1) inbreds as a function of average ancestral contribution to modern (era 3) lines. The gray dotted line is a regression with slope -0.1). Inbred names are shown for lines with ratios higher than 4 or ancestry proportion above 0.03. Labels in boldface mark breeding lines of known historic popularity. Figure from **?**.

Lack of public resources limits use of diverse germplasm

Breeders will not blindly incorporate older material into their populations, however. Perhaps the most important piece of information required to effectively utilize older germplasm is pedigree. Pedigree data immediately gives a breeder information on crosses likely to produce higher yields due to heterosis, and often provides additional utility in identifying likely maturity (flowering time) and other agronomic characteristics. Combined with phenotype data, pedigrees can even be used to predict phenotype in the absence of genotype (**?**) and pedigree-based methods for identifying useful diversity have already been patented by industry (**?**).

While private industry may in many cases have detailed records of their own breeding material, the vast majority of private germplasm is founded on 20^{th} century public breeding lines (**?**).

Unfortunately, pedigree data for most U.S. maize inbreds is unavailable to most breeders. For example, of the over 45,000 worldwide inbreds in the USDA Germplasm Resources Information Network, only some 34,000 have some amount of ancestry determined, and much of this information is incomplete. If we look at available germplasm only, that number shrinks to 2931 accessions with any available ancestry information. While there are published compilations of germplasm, these are far from complete: **?**, for example, contains complete pedigree data for less than 20% of the germplasm in the USDA database. Moreover, what ancestry information that is available is not in electronic form nor easily accessible in any single resource. Instead, historical pedigree information exists primarily in the minutes of breeding committees, old breeding program books, and other hard-copy sources with limited distribution.

We propose to generate an open-source database of public maize pedigrees and use this resource to identify genotypic and phenotypic diversity of high utility in advancing maize breeding. Given the importance of maize to

U.S. agriculture, this proposal clearly aligns with the USDA AFRI program priorities of "Plant Breeding for Agricultural Production," particularly the "development and application of tools to predict phenotype from genotype to accelerate breeding of finished varieties". Our proposal will develop tools to accelerate breeding by allowing breeders to more quickly identify useful inbreds, and our application of population and quantitative genetic methods will identify specific genetic and phenotypic diversity of potential use for future breeding.

Introduction

Maize breeding

Maize has undergone dramatic phenotypic and genetic changes since its domestication and subsequent spread across the Americas (**??**). More recently, beginning in the mid-20^{th} century, the intensification of maize breeding efforts has lead to subtler but equally important changes including increasing yields and improved agronomic traits such as leaf angle and density tolerance (**?**).

Modern breeding programs (post-1960) take advantage of self-fertilization (or now double-haploid technology) to create homozygous inbred lines, which are maintained in separate breeding pools or heterotic groups. The most important heterotic groups among U.S. public germplasm are the "stiff stalk", "non-stiff stalk", and "iodent" (Figure 1). Inbred lines from separate breeding pools are then crossed to make hybrid progeny. These hybrids often display heterosis, meaning that yield and associated traits of the hybrid are superior to either inbred parent (**?**). Inbreds capable of producing high-yielding, heterotic offspring are said to have good "combining ability", and are recycled in their respective breeding pools. Inbreds that form less desirable combinations are usually discarded from the breeding pool. Useful inbreds within a group are crossed with each other, and their segregating progeny evaluated and self-fertilized to create new inbreds. Maintaining this system for propagation of inbred lines and hybridizing inbreds for evaluating production traits has worked well for many decades, but there is growing reason for concern that this method may need a genetic boost.

Decelerating yield gains and decreasing diversity

Maize yields have steadily increased since the advent of hybrid breeding in the 1930's. But linear increases necessarily mean a decrease in relative gain, and projections suggest that current trends are unlikely to meet future yield goals (**?**). Of even greater concern is the possibility that the rate of gain may actually be decreasing (Figure 3). While some of these yield trends are undoubtedly related to changing management practices, much of the change is indeed due to breeding (**?**), and current rates of yield gain are lower than historical trends even after correcting for nitrogen fertilizer inputs (data not shown).

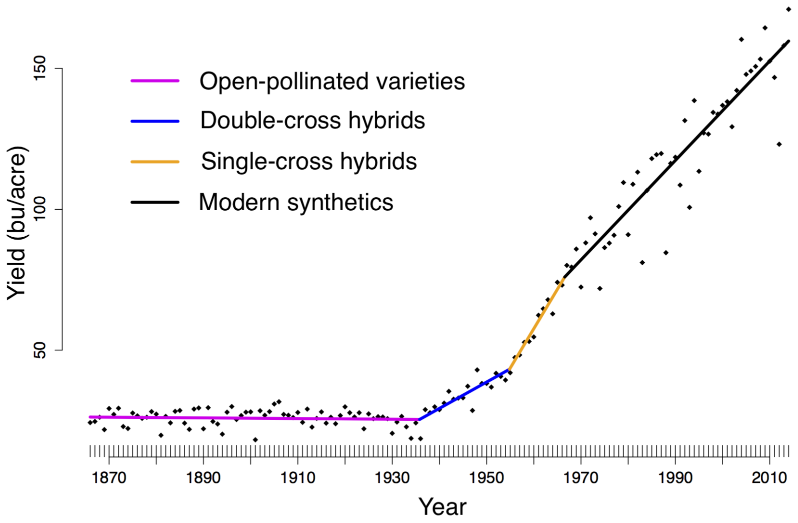


Figure 3: Piecewise regressions of time against total yield through the four eras of modern maize breeding. Colors represent distinct eras of maize breeding that roughly correspond to the eras referred to in Figure 1.

As selection progresses within a breeding program, inbred lines with poor performance are generally discarded, leading to a decreased effective population size (N_e) of the breeding pools. Response to selection is proportional to the additive genetic variance V_A (equation 1), and V_A, in turn, depends on N_e as well as the mutational variance per generation σ_m^2 (**?**):

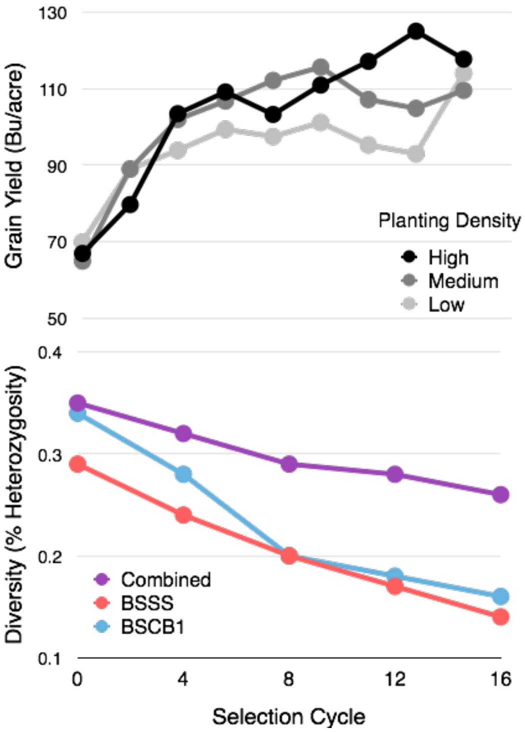
$$E[V_a] = 4N_e\sigma_m^2 \tag{2}$$

A reduction in N_e thus inherently leads to a reduced response to selection, leading to diminishing returns on yield unless new diversity is brought back into breeding pools. Evidence from the Iowa Reciprocal Recurrent Selection breeding program suggests these concerns are valid (Figure 4), as yield gains plateau over time (**?**) concomitant with continued declines in genetic diversity (**?**). Decreasing N_e (Figure 1) in U.S. germplasm risks our ability to maintain or increase rates of genetic gain in yield.

Useful diversity in old lines

As any breeder will attest, old lines have fallen out of use precisely because they showed inferior agronomic performance under a particular selective regime (usually combining ability). There are a number of reasons, however, why older lines may retain useful genetic diversity. Lines are often discarded or selected against because of poor performance for a particular trait, but this does not preclude that line's utility in breeding for other traits. Because of this, beneficial alleles for many traits of interest may remain in older germplasm, not having been advanced during breeding (Figure

Figure 4: Yield and genetic diversity in the Iowa Recriprocal Recurrent Selection Program across 16 cycles of selection. (Top) Grain yield at three planting densities; data are from ?. (Bottom) Genetic diversity of the BSSS and BSCB1 breeding pools separately and combined; data are from ?.



5b). For example, thanks to its combining ability, the U.S. inbred B73 quickly spread in popularity and now makes up a considerable portion of modern breeding material (?). Yet when evaluated for drought and heat stress, B73 significantly underperformed compared to less well-utilized lines such as B76 (?). Analysis of a suite of diseases also finds that many less well-utilized public inbreds show greater resistance than popular lines (?).

Most breeding programs consist of relatively small population sizes, and in these cases genetic drift can substantially impact the fate of even beneficial alleles (e.g. ?). The contribution of older lines to modern germplasm thus does not necessarily reflect their genetic potential: our population genetic analysis of maize ancestry, for example, found lines with more beneficial (as defined by a steady increase in allele frequency over time) alleles than expected given their contribution to modern germplasm (Figure 2).

A novel pedigree-based approach to incorporate new diversity

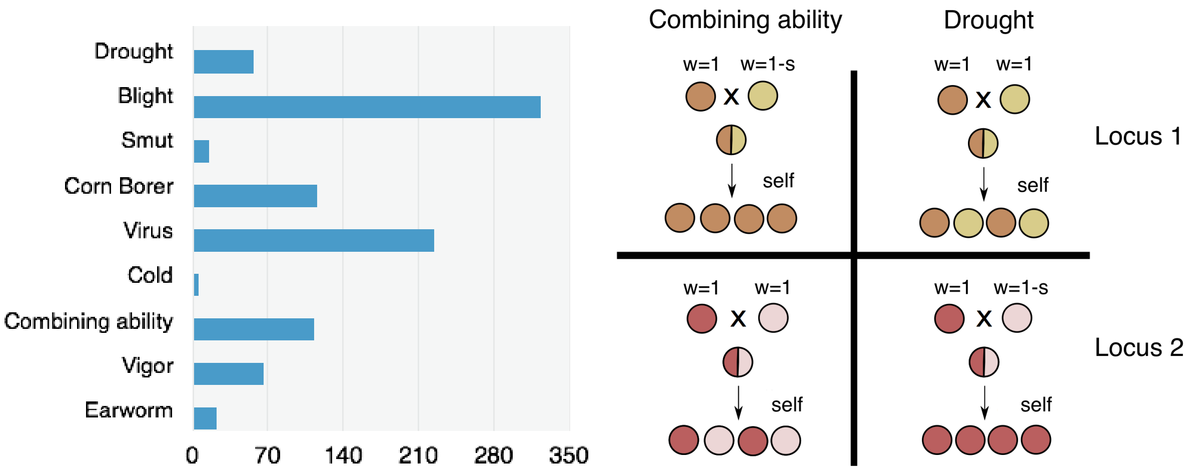
Incorporating novel diversity into contemporary modern maize lines is clearly an important goal necessary to maintain or accelerate breeding and rates of yield gain. While there are other public efforts that aim to achieve this goal, like the GEM (Germplasm Enhancement of Maize) program (?), these focus on incorporating exotic germplasm (e.g. CIMMYT lines, tropical hybrids, etc.) into U.S. materials by backcrossing to well-adapted U.S. cornbelt backgrounds. This complementary approach is good source of bringing in new diversity, but requires time-consuming back-crossing before it can generate lines that are of sufficient quality to incorporate into a breeding program.

We propose a different approach, using pedigree data to identify underutilized cornbelt germplasm. While this approach shares with projects like GEM the disadvantage that diverse material is often agronomically inferior, it has a number of advantages. First, in contrast to exotic germplasm, older U.S. germplasm is already reasonably well-adapted to the daylength and climate of the U.S., meaning it can be immediately incorporated into breeding programs without first requiring extensive back-crossing. Second, with sufficient information (see below), breeders may be able to learn a substantial amount about the potential utility of a line by predicting phenotype directly or understanding its relationship to known material. Finally, considerable information is available about many old breeding lines even without prediction (Figure 5a)

Advantages of pedigree approaches

Pedigree-based analysis offers a number of advantages for identifying useful diversity in older germplasm. First, most pedigree data contain not only parentage information, but a wealth of important phenotypic data and information about the breeding program inbreds were utilized in (e.g. Figure 5a). Pedigree approaches also allow the identification of beneficial allelic diversity (e.g. Figure 5b, see Aim 2). While population genetic approaches can achieve this latter goal to some extent (e.g. **?**), they have so far failed to identify many large-effect loci, though patterns of diversity within individual breeding programs suggest such loci are likely to exist (**?**). Pedigree approaches can identify strong selection within individual breeding programs (see Aim 2) without needing to average across many different programs. Pedigree approaches can also naturally deal with population structure, because expected relatedness is built into the pedigree itself. Finally, pedigrees allow prediction of phenotype even in the absence of genotype (**?**). While this is never a substitute for growing lines in the relevant environment for comparative purposes (see Aim 3), it doe save considerable time and expense and provides breeders an initial estimate of which germplasm could be most useful.

- observed vs. expected allow us to look allele specific “hard” breeder sweeps across the pedigree for combining ability
- but phenotypic information of each program contained in release sheets may also allow us to look at selective regimes within programs (while selection is relaxed in others).
- If alleles exist in other programs along with the specific diversity of that program, we can target those.



(a) (b)

Figure 5: a) Counts of a small set of germplasm descriptors from the USDA database for which we currently have genomic and ancestral data. b) Expected segregation of alleles at loci for two traits in different breeding programs. Selective regime for combining ability across two example breeding programs and loci. Fitness (w) of each allele indicated, with selection coefficient (1-s) for the allele being selected against in a given regime. Locus 2, though beneficial under drought conditions, risks loss due to drift in breeding programs focusing only on combining ability. *I think there's also the aspect that the alleles for drought are still preserved, because they are conditionally neutral in the combining program (breeder's selection is relaxed for drought - and yes, they can be lost to drift). These conditionally neutral alleles are preserved against a different genetic background. If we find lots of adaptive alleles in one environment, we might find them in another, but with a different background - introgressions can be planned in that way. I think of this more as local breeder's adaptation - when you cross lines from two or more populations/programs, you'll likely get a bunch of crap hybrids, but you may also get transgressive segregation of all beneficial alleles (subject to some stats - Pioneer can do crazy CTL things to avoid massive amounts of crosses) - this is exactly how heterotic groups work. But here, we're talking about creating lines from way more divergent crosses prior to losing these alleles to drift.*

The missing data challenge

Unfortunately, much of the pedigree information on older founder inbred maize lines sits in old volumes of Crop Science or the Agronomy Journal, experimental station technical bulletins, regional corn breeding meeting records, release sheets, or breeding books — unaviable and vulnerable to loss. The National Plant Germplasm Service (NPGS) database records ancestry information, but at least 25% of the accessions in the database have zero ancestry data, and another XXX have incomplete data. While private companies likely have their own pedigree databases, these data are private, patented, and not available to the public. **We will gather pedigree data from public sources (minutes of breeding committees, breeding program books, and other hard-copy-only sources) in order to preserve this historical in-**

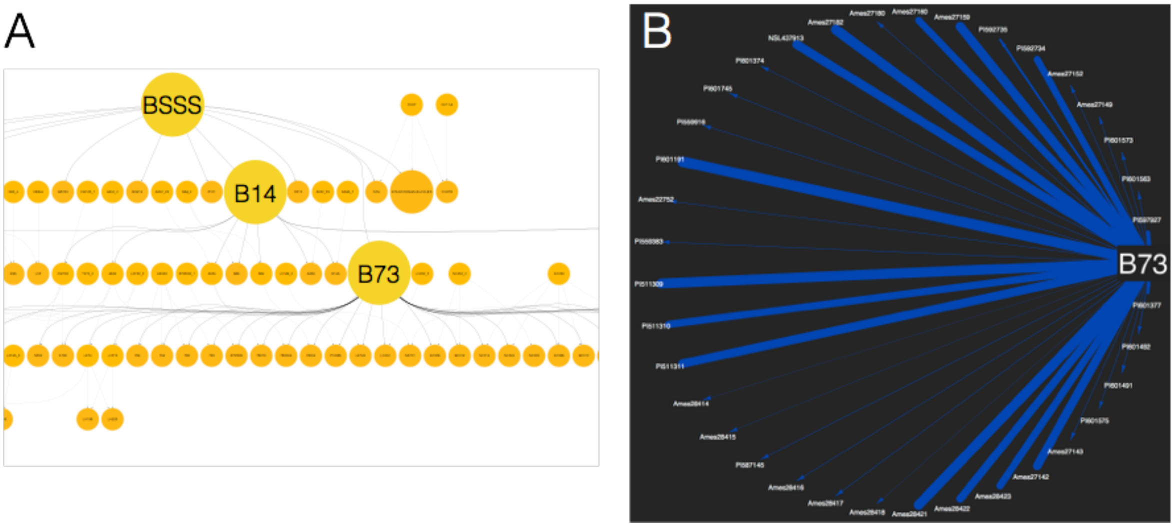


Figure 6: A) A small portion of our current pedigree, showing partial data for inbred B73. B) Pedigree of putative first-generation descendants of inbred B73. Blue arrows are weighted by the relatedness estimated from identy-by-state using GBS, clearly showing multiple errors in the putative pedigree.

formation and combine it with genotyping data to accelerate future public and private (see letter of support from Dupont Pioneer) maize breeding programs.

Preliminary work

To date, we (senior personnel Drs. Oscar Smith and Kate Crosby) have gathered parentage info for over 3193 lines from a variety of sources, including **?**, the NPGS, patent variety protection records, inbred release publications, and communication with breeders. We have standardized names across these datasets and stored these data in a SQL database, which can be queried by common name (e.g. "B73") as well as unique NPGS identifiers ("PI 550473" for B73). We have combined these data with genome-wide genotyping-by-sequencing (GBS) data (**?**), and built a large pedigree of ≈700 lines (Figure 6a) with complete parentage and genotype information. GBS data allows us to validate recorded pedigrees, identifying parental combinations that are impossible given the genotyping data (Figure 6b).

Approach

Our proposed research has three major aims in identifying and utilizing diversity to accelerate maize breeding.

- Aim 1: Digitize and curate pedigree and genotype information into a publicly available database.

- Aim 2: Identify useful diversity using a combination of pedigree and population genetic approaches.

- Aim 3: Field test individuals lines that feature heavily in the historical pedigree.

Aim 1: Public pedigree curation

Our first aim is stop the potential loss of hard copy pedigree data by gathering it from land grant institutions that previously hosted breeding research programs of maize inbred lines. Land grant schools of primary importance were identified by Co-PI Dr. William Tracy and Dr. Smith (senior personnel), who have between them have more than 70 years of breeding experience in maize. Dr. Tracy was senior author on the leading pedigree book of maize lines **?** and Dr. Smith spent 7 years working as maize breeder for the USDA (including working on the Iowa RRS population), follow by more than 20 years as a maize geneticist for Dupont Pioneer. The "high-priority" land grant schools they have identified include: the University of Wisconsin, Virginia Tech, the University of Georgia, the University of Florida, Pennsylvania State University, the University of Nebraska, North Carolina State University, and the USDA station at the University of Missouri.

Co-PI Tracy and Co-PI Holland's student will largely be responsible for visiting these schools in the first year to obtain and digitally scan this information with portable digital scanners. Co-PI Wisser's student will be responsible for visiting two schools starting in the second year of the grant. Co-PI Tracy will obtain information from the University of Wisconsin (host institution), the University of Florida, and the University of Nebraska. Co-PI Holland's student (in the first year) will be responsible for North Carolina State University (host institution), the University of Georgia. Dr. Sherry Flint-Garcia (senior personnel) has agreed to obtain and pass along records from the USDA station at the University of Missouri. Co-PI Wisser's student (to start in year two of the grant) will be responsible for the University of Pennsylvania and Virginia Tech. Drs. Tracy and Smith have extensive contacts with faculty past and present at these schools, so obtaining the information and scanning it at each school traveled to should take no more than a few days.

Optical character recognition software will be used where possible to automate extraction of information from scans, but we imagine a fair amount of hand-curation will be required. Dr. Kate Crosby (senior personnel), Dr. Taner Sen (support letter from maize GDB enclosed), senior personnel Dr. Oscar Smith, and Co-PI Dr. Bill Tracy will consult on an agreed upon standard format for recording data from scans. Standardized formatting will enable consistent mapping of current and future genomic data formats to pedigree information. Both the raw scans of pedigree records and our data sheets which will be made available publicly with permanent doi via services such as Github (`www.github.com`) or Figshare (`www.figshare.com`). We will translate current and new phenotypic data into standardized maize ontologies such as those supported by the Generation Challenge Program (http://www.cropontology.org/ontology/CO_322/Maize, ?)

We anticipate that we will identify some lines that feature heavily in the historical pedigree but that do not have any genotypic data available for them. To facilitate downstream applications (see Aims 2 and 3), we will use genotype-by-sequencing (GBS) (?) to obtain genome-wide genotype data. Germplasm will be requested from the relevant sources, and germination and DNA extraction will be performed at UC Davis. Genotyping will be outsourced to the Genomic Diversity Facility at Cornell University (www.biotech.cornell.edu/brc/genomic-diversity-facility), and sequence data processed following standard pipelines (?). We anticipate that no more than a few hundred inbred lines (2-4, 96-well plates) would need to be genotyped.

Dr. Crosby will be responsible for database generation and curation. The initial database will continue to be in SQL format, with entries for release dates, historical phenotype information, breeder notes, germplasm availability (or nearest neighbor germplasm availability), and GBS genotype. While other genotyping has been performed on a number of lines, GBS is the *de facto* standard (?) and merging data across platforms is beyond the scope of the work proposed here. GBS data will be stored in hdf5 format and made available via iPlant (www.http://iplantcollaborative.org). We will develop software to extract genotype data from specific genomic regions in subsets of lines and estimate allele sharing via identity by state (IBS) between sets of lines in specified genomic regions. Finally, we will implement simple phenotypic prediction (see Aim 2) functionality allowing users to estimate phenotypic values based on a set of loci from genome-wide association studies.

Starting in year two of the grant, the pedigree database will be made available publicly via MaizeGDB (www.maizegdb.org), the USDA-funded central resources for maize genomics (see letter of support from Dr. Taner Sen). We will update the database as new information arises, and provide functionality such that users can submit database updates (in the appropriate format) to MaizeGDB to ensure continued utility and availability of this resource.

Expected outcomes (Aim 1)

Our main deliverable from Aim 1 is an **open public pedigree database to be hosted on MaizeGDB (http://www.maizegdb.org/) for the long term future.** In addition to providing information on pedigree, this database will house both genotype and substantial phenotype information, a combination of critical importance for continued plant breeding efforts (?). Users will be able to contribute and revise incomplete information, with the goal of establishing a nearly complete public pedigree of maize inbred lines.

Eample usage might include a breeder querying the database for lines with descriptors suggesting drought and blight resistance, identifying the genotype of such lines at loci thought to be associated these traits from GWAS or other studies, and use the database to predict phenotype for related material with no descriptive data.

Ensuring data, code, and script are open-access or open-source (freely available to the public) is important for critique, debate, and dialogue in science. Drs. Ross-Ibarra and Crosby have a long track record of ensuring openness in science, data, and code. Throughout this project, both Dr. Ross-Ibarra and Dr. Crosby will endeavor to ensure that code and data are available via permanent, publicly accessible repositories such as github, figshare, maizeGDB, and iPlant.

Potential pitfalls & limitations (Aim 1)

¡¡¡¡¡¡¡ HEAD While we are very optimistic about the prospect of obtaining accurate pedigree information from these various institutions the nature of corn-breeding and record keeping of corn-breeding can be nebulous. Ideally, we would be able to accurately count the number of meioses in the total pedigree, in each breeding program, down to each line; thus, retracing the complete ancestry of a given contemporary inbred.

ok - but this is the potential pitfall liz lee was on about, and also howie? Thoughts?

Historical pedigree records and other information on old lines may be available, but the germplasm from these old lines may no longer be available. ======= While we are optimistic about the prospect of obtaining accurate pedigree information from these various institutions, we recognize that many records will be incomplete. In practice, while knowing the exact number of meioses between lines would be desirable, we expect we will recover only parentage information from a large number of lines. Parentage alone is sufficient for allele-dropping and prediction (see Aim 2), but not knowing the number of meioses makes it difficult to know the uncertainty associated with prediction or use pedigree data to understand recombination.

A second challenge is that while pedigree records and other information on old lines may be available, the germplasm from these old lines may no longer be available. ¿¿¿¿¿¿¿ 720c1b84f1fcaf658c28f86f2c67f73a625700b0 Approximately 12.9% of germplasm from NPGS for which we have parentage information is no longer available, and we expect similar or higher levels from less accessible sources. We note, however, that such lines remain useful in connecting available lines together in the pedigree, and pedigree alone can be used to predict phenotype. If we identify historically important germplasm that is no longer available, we will identify and genotype the nearest relatives for which seed are available.

Aim 2: Identify useful diversity

Mendelian segregation on a pedigree (Aim 2)

As different lines have been under different historical selective regimes or breeding programs, these phenotypes likely have an underlying genetic basis. Following the digitization of historical information, the Dr. Holland's student and the post-doc (Kate Crosby) will construct many small pedigrees of the different selection regimes and use the technique of **"allele-dropping"** to further identify breeder's selection and useful diversity.

"Allele-dropping" is a patented method (**?**), used in industry and works by identifying distorted segregation at all heterozygous loci within a single pedigree/breeding programs and across many pedigrees/breeding programs (Figure 7). As a simple Mendelian approach this technique identifies traces of selection by directly accounting for ancestry, and without the worry of having to account for shared population structure **?**, as can often be a problem or limitation in modern population genomics approaches - such as F_{ST} outlier scans from genome wide association studies (GWAS).

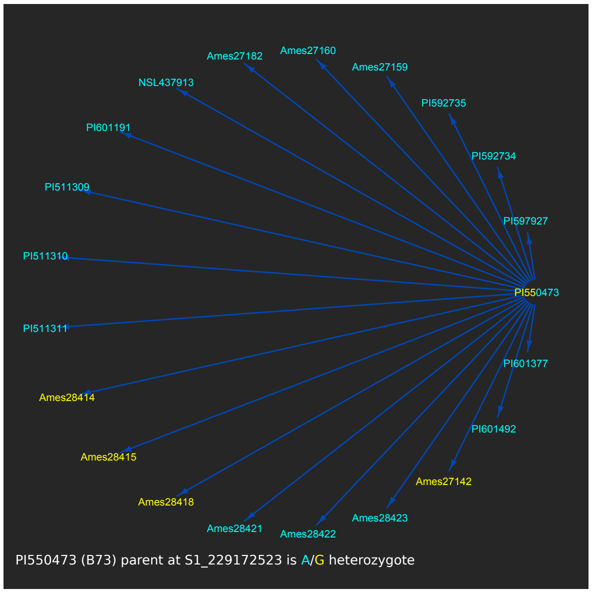


Figure 7: B73 (also known as PI550473 by NPGS and a number of its inbred progeny) is heterozygous at 379 loci out of over 954,000 loci with GBS markers). The blue labels represent which inbred progeny obtained the A allele, whereas the yellow labels indicate the progeny that have obtained the G allele at a particular locus. **NB: the other parent of these lines is not indicated for simplicity**.

With allele-dropping, if we observe the uni-directional shift of a given allele across many of these small pedigrees at a set of loci (or even within just one pedigree in a specific breeding program), this would tend to indicate selection. We would tend to predict that a consistent subset of alleles that are associated with combining ability would tend to increase in frequency across the pedigree. As an example, using Mendelian rules and starting with a mostly homozygous line (with some residual heterozygosity), we can examine single heterozygous locus (i.e. Aa). Because we know what our expectations (E) for number (N) of AA (or aa) homozygous inbred progeny at generation 6 (under no selection) should be (given by $E = 1/2N$), we can compare to the actual observed number of progeny. If our observations deviate from this expectation, we would tend to conclude breeder's selection for these alleles (especially if observed across a good portion of the entire pedigree).

Our current preliminary data set of 1696 accessions contains a fair amount of information on different selection regimes (Figure 5a). By applying allele-dropping to a number of these smaller breeding programs, and tracking unidirectional shifts, we (Dr. Holland's student and Dr. Crosby) will attempt isolate strong signals of breeders selection for a given selective agent (within a past breeding program). We may then look for these alleles across all other breeding programs where selection was presumably relaxed (Figure 5b), and if we can isolate these alleles, then they may be introgressed back in as useful diversity to other breeding programs.

Potential pitfalls & limitations (allele-dropping)

Genotypes when combined with a pedigree can be used to predict phenotypes (**???**). By using allele dropping we target individual loci within and across breeding programs, associating high frequency SNPs with their given selective regime (drought, disease, etc). However, there are two potential limitations with the allele-dropping approach. First, there may be a lack of statistical power in detecting selection on individual alleles, because there may be too few progeny of each line under a given selection regime. Secondly, allele dropping may not have the statistical power to detect a more diffuse polygenic signal of adaptation spread across many alleles in a genome (similar the drawbacks of using traditional QTL mapping and F_{ST} scans) **??**. However, this limitation of power is not an issue for the complementary population genetic approach we plan on using: the Q_x method described in **?** (see next section).

Predictive phenotyping (Q_x) (Aim 2)

here we show that if individual lines predictive phenotype is better than expected given pedigree/genomic background, then it's likely a good choice for breeding! *THIS SECTION NEEDS BANDAGING THE MOST - how to explain Qx simply - I attempted a figure*

Here we propose the first study to look for correlated allele shifts (thought to be associated with production traits) **through time** using a statistic in a recently described population genomics approach **?**: Q_x. Briefly, the approach Q_x uses SNP hits and their allelic effects (breeding values) across the genome from a GWAS analysis. The effects sizes of these breeding values can be summarized by comparing to a random set of SNPs in that population. Here, allelic effects α^2 (or breeding values), the same values that breeders can take advantage to get an estimate of V_A for any trait of interest. V_A can be estimated for one locus or summarized across all loci L in the genome (equation 3 below):

$$V_A = 2pq\alpha^2 = \sum_{l=1}^{L} \alpha_l^2 2p_l(1-p_l) \tag{3}$$

These hits are then projected to the expected breeding value based on the genotype of individuals in another environment (where the phenotype has not actually been observed).

If alleles are at high frequency in certain breeding programs and can also associated with a beneficial phenotype (e.g. blight resistance), they are potentially beneficial alleles that could be resurrected and introgressed back into a modern breeding program. By then searching the entire pedigree for these alleles and isolating those lines that have these SNPs, they may be targeted by breeders and public research programs. Further, the greater the V_A of a trait (the greater the allelic diversity of that trait, i.e. N_e), and thus, the greater R (response to selection) to any new selective program a breeder chooses to apply.

Expected outcomes (Aim 2)

The idea of applying these two complementary approaches is **the isolation of a consistent subset of SNPs within and across breeding programs**. These SNPs can then be tagged in the database as being important for yield-related traits under different selective pressures. In obtaining this set of SNPs, each of the masters students will be trained in separate but complementary population and quantitative genetics approaches, and provide them with a skill-set for applied industry or public research.

Aim 3: Field testing individual lines

A mass grow out and genotyping of all inbred lines in the total pedigree would be ideal, but realistically, we do not have the resources (either in terms of time, space, or available

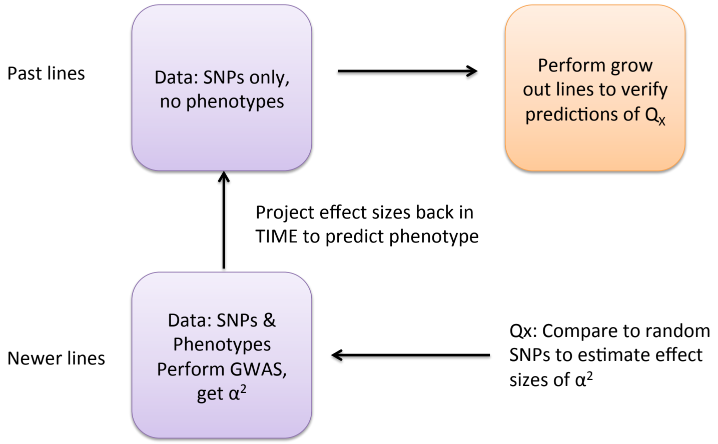

Past lines
Data: SNPs only, no phenotypes
Perform grow out lines to verify predictions of Q_X
Project effect sizes back in TIME to predict phenotype
Newer lines
Data: SNPs & Phenotypes Perform GWAS, get α²
Qx: Compare to random SNPs to estimate effect sizes of α²

Figure 8: Simple schematic of how the Q_x approach works

germplasm) to do this. However, we can target specific lines that have many progeny (thus, many alleles) or very few progeny (few alleles in the pedigree). These lines will have been identified with the allele-dropping method by Dr. Holland's student and Dr. Crosby. In the latter part of the second year, Dr. Wisser and his student will grow out several hundred of these lines, obtain tissue for GBS (for those with no genotypic information), and potentially also verify the phenotypic information provided on the release sheet (if the information is available).

In order to empirically (*right word?*) test and ground-truth the idea that inbred lines from the past express the predicted phenotypes from the Q_x approach Dr. Wisser and his student will grow out a subset of lines in the third year at the University of Delaware, and phenotype these. The subset of lines here will be based on those lines that show overdispersion for production and production associated traits from the Q_x approach described above, and predictably good agronomically performing lines from the allele-dropping method. We have allocated resources to grow out 50 of the predicted to be productive lines and 50 of the predicted "least" productive lines, with 2 rows of 10 each, and the middle 5 of each row will be phenotyped. We will include several contemporary ex patent-variety-protection (ex-PVP) lines to check the predictions given by Q_x.

Expected outcomes and potential pitfalls: Aim 3

This final aim of the project fills in any incomplete genotype and phenotype information for important lines in the pedigree. *NEED TO FIX THIS* If the "best old" lines show terrible agronomic performance relative to current ex-PVP inbred varieties, we will know the approach of integrating "old" diversity is an inefficient approach to adding diversity to the breeding pool for yield. *Slight comment - in fact, we won't really know until we do crosses - temporal ones, but we don't have the resources for this.*

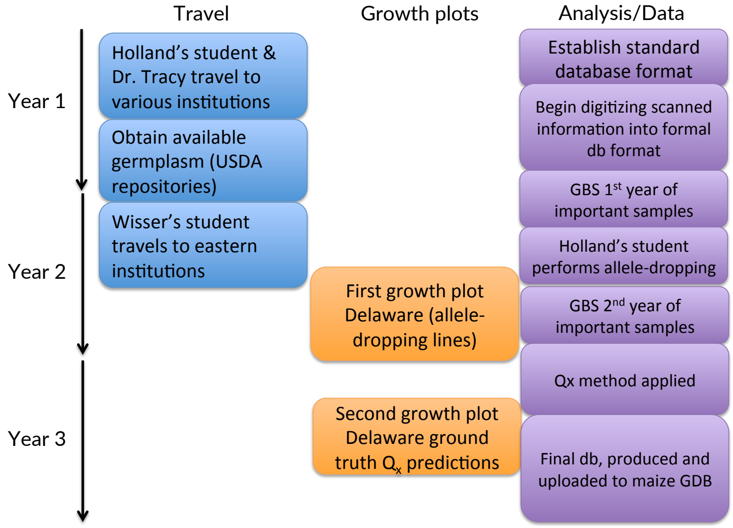

Travel
Growth plots
Analysis/Data
Year 1
Year 2
Year 3
Holland's student & Dr. Tracy travel to various institutions
Obtain available germplasm (USDA repositories)
Wisser's student travels to eastern institutions
First growth plot Delaware (allele-dropping lines)
Second growth plot Delaware ground truth Q_x predictions
Establish standard database format
Begin digitizing scanned information into formal db format
GBS 1st year of important samples
Holland's student performs allele-dropping
GBS 2nd year of important samples
Qx method applied
Final db, produced and uploaded to maize GDB

Figure 9: Graphical timeline of the major parts of our proposal.

Research Timeline

A graphical timeline of our three-year proposal is presented below (Figure 9). The first year will be spent traveling and gathering and digitizing pedigree records at land-grant institutions. Dr. Tracy and Dr. James Holland's masters student will be responsible for traveling to and scanning hard-copy records into digital format. We estimate that this should take no longer than a few weeks at each institution for Dr. Holland's student and Dr. Tracy.

The scanned digital records will then be translated into a usable data format for further analysis by Dr. Crosby. At worst, this will involve Holland's student and the postdoc (Crosby), physically reading and translating each of the scanned papers, and this could take several months to the entire first year depending on volume of records. Dr. Crosby will ensure that the database is up an running by the end of the first year, and act as database manager throughout the proposal to the end of the third year.

Dr. Smith will consult as needed with both students (see roles below) and Dr. Crosby throughout the grant as an expert on the naming and potential ancestry of lines.

At the end of the first year/start of the second year, any inbred line that is disproportionately present or absent in the pedigree with available germplasm, but that has no to little genetic information on it will be genotyped using the GBS platform - Dr. Crosby will be responsible for this aspect of the project, and Holland's student will receive training in GBS analysis from Dr. Crosby.

Dr. Holland's student will then begin to apply the technique of allele-dropping to various pedigrees, investigating which alleles undergo large directional shifts (in terms of allele frequency changes, generation-to-generation). He/she will also be responsible for obtaining available germplasm with the help of Dr. Flint-Garcia from various repositories for those lines that have no genetic information on them at present.

Dr. Randall Wisser's student will then start and visit the two land grant schools in the east (Virginia Tech and the University of Pennsylvania) to obtain the remaining records, and submit this phenotypic and pedigree information to the up-and-running database in the first 2-3 months.

Dr. Wisser's student will grow out the lines identified by Holland's student from the allele-dropping method and phenotype these lines for production related traits (flowering time, kernel size, kernel weight, etc), while at the same time (over a period of 2-3 months) learn the population genomics approach presented in (**?**). *Worth it to add in part about SFG phenotyping system? This is also something Sherry could consult on* In the final year, Dr. Wisser's student will apply the Q_x method, and choose the lines that show the greatest over-dispersion for production and production related traits of interest to grow out at Delaware.

As a final step, all phenotypic data, identified SNPs of related to various production traits, and ancestry information, will be transferred from Dr. Crosby's working database to maize GDB, and managed by administrators there for the long term future.

Broader Impacts

Training

Our proposal helps train two students in bioinformatics, population and quantitative genetics, plant-breeding, experimental design, statistics, and database management. All these applied skills that the students develop will help them in their future careers, be they academic or industry careers. They will both meet and work with some of the premier experts in maize breeding (Drs. Tracy & Smith). Both students will be expected and encouraged to attend the maize genetics conference, to present their results, and network with working professionals from academia, government, and industry.

Both students will work alongside the post-doc, Dr. Crosby as well as their respective adivsors to plan out details, troubleshoot, and advance project goals. Dr. Crosby, in turn, will gain valuable experience helping to mentor students along with their main advisors Drs. Wisser and Holland.

Jeff, say something about yourself?

Benefits to maize breeding and genomics

Novel diversity from old lines

With combined and interactive selective pressures of climate change, disease, drought, and pests many crops must quickly adjust to these new challenges. Our proposal seeks out novel diversity from old breeding programs; breeding programs where lines were generally exposed to one selective stress. It then identifies and tags beneficial alleles under a given stress, and allows breeders easy access to this information in other lines, so that they can make their own choices on crosses.

Public Pedigree database

Private companies such as Monsanto and Dupont Pioneer have their own pedigree databases and information that they can draw from in order to breed new hybrid commercial varieties. Breeders with less financial means (both in the United States and in countries abroad) do not have access to this information, nor do public researchers.

Our proposal aims to make this information accessible, and by hosting on maize GDB, will allow the information to be updated as public breeders themselves try out lines, and make their own observations, well beyond the timeline of this three-year project. Additionally, Dupont Pioneer (see letter of support) recognizes that much the historical information on maize inbred lines is disjointed, incomplete, scattered across many institutions, and vulnerable to loss. So, this information is a valuable contribution to private industrial databases too.

I could probably do the below, but it might be overkill

Open-source R package for database

The post-doc, Dr. Crosby while being responsible for database management and development, will also construct an open-source R-package. This package will allow users to access real-time pedigree information from the database with a suite of functions via an application program interface (API). The R-statistical programming language is open-source and one of the fastest growing languages in the United States and countries abroad for data analysis.

Application to other crop systems

Few other crops have attempted to make use of old diversity as novel diversity, in a comprehensive project, such as the one we propose here. Yet, useful diversity does exist in old lines (**?**).

We think a full examination of this old diversity in one crop system (maize) may act as a stimulus for other researchers in other crops to act quickly to preserve hard-copy records, and germplasm associated with these records too. Our proposal, is in essence, a litmus test of how old diversity could be useful in propagating new modern lines in many crops.