Breeding Perennial Grain Crops
Based on Wheat

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ABSTRACT
Wild relatives offer a diversity of traits potentially useful in crop improvement. Identification of major genes for resistance to biotic and abiotic stresses, in conjunction with targeted introgression, has introduced some of these traits into cultivated varieties. The difficulty of transferring complex traits, and associated linkage drag, has limited the ability of breeding programs to fully use important genetic variation. Wheat (Triticum L.) is an example of an important crop with large secondary and tertiary genepools of species capable of producing viable offspring by cross pollination. Wheat is annual, but most of the related species in the Triticaceae Dumort tribe are perennials. An unknown number of genes control the perennial habit, although previous work has shown that it is a complex trait with quantitative expression. Lack of recombination between the parental genomes and complexity of the trait suggest another avenue for breeding: the addition of a genome. Multiple genera can hybridize with wheat, and some combinations produce stable amphiploids. The development of perennial grain and forage crops balancing the agronomics of wheat with the polycarpic habit of its relatives is promising. The approach is similar to Triticale (× Triticosecale Wittmack ex A. Camus), where new crops are developed combining the desirable qualities of the parents. Through prebreeding of the wild relatives, application of insight into meiosis, and strategic use of known genes, it should be more efficient to breed perennial grain crops adapted to diverse production systems.

All of the staple grain crops currently cultivated are annuals. Selection over thousands of years and directed breeding efforts in the last century have increased yields, altered quality traits, and changed the architecture of crop plants to facilitate harvest and, eventually, full mechanization. The potential benefits of perennial grain crops for diversifying agriculture and building soil have been well explored (Wagoner and Schaeffer, 1990; Cox et al., 2010). Most of the important grain crops have perennial relatives that are related enough to create hybrids, with varying degrees of difficulty (Cox et al., 2002).

This paper will review the history of projects that sought to develop perennial grain crops based on wheat, the genetics and biology of intergeneric hybrids, breeding strategies, and directions for future research.

HISTORY
Cytological efforts by Kihara (1965), Aase (1935, 1946), Sax and Sax (1924), Sax (1935), and others helped to define the relationships between the genomes of Triticum L. and other species in the Triticaceae Dumort tribe, which includes important grain and forage grasses (Dewey, 1984). In the early 1930s, the first crosses between Triticum species and their perennial relatives were attributed to N.V. Tzitzin (also transliterated Tsitsin and Cicin) in the Soviet Union (Armstrong, 1936). He claimed success in obtaining a new crop species grown on hundreds of thousands of hectares in the Soviet Union that exceeded either parent for yield and...
exhibited quality for both grain and forage (Tsitsin, 1965). Tzitzin made crosses of Agropyron Gaertn. and Elymus L. species with T. aesticum L. and T. durum Desf. (T. turgidum L. ssp. durum), obtaining hybrids with a number of combinations (Tsitsin and Lubimova, 1959). Subsequent taxonomic reorganization of species in the Triticeae makes it difficult to know exactly which species he used. Little of his material survives, and none equals the written claims.

The politics of the time prevented other researchers from accessing Tzitzin’s material, and breeding programs were initiated independently in multiple countries. Most of the literature available comes from American and Canadian researchers from the 1930s to 1960s who undertook a methodical investigation of the cytology, morphology, and agronomics of developing new perennial grain and forage crops by crossing Triticum species with perennial species in the Triticeae.

In Canada, the focus was on developing a large-seeded forage crop to improve establishment on the prairie (Armstrong, 1936). The American effort, headed by Coit Suneson at University of California, Davis, explored the crop for grain, forage, feed, and as a source of food for overwintering birds (Suneson et al., 1963). W.J. Sando, who worked for the USDA from the 1930s to 1960s, made numerous crosses between wheat and related species (Sando, 1960). His material remains an interesting collection of diversity and has been used by others looking to develop perennial grain crops (Schlehuber and Sebesta, 1959). Efforts dedicated to breeding wheat as a perennial crop disappeared for a time after the 1960s, and the bulk of the material has been lost.

In the 1990s, there was renewed interest in perennial grains for sustainable agricultural systems (Scheinost et al., 2001). Breeding programs with different methodologies working towards a common objective were initiated at Washington State University and the Land Institute, a nonprofit organization in Salina, KS. The ecological and agronomic potential of perennial grains has been well explored (Scheinost et al., 2001; Cox et al., 2006; DeHaan et al., 2007; Bell et al., 2010; Shi et al., 2011; Hayes et al., 2012; Larkin et al., 2014; Crews and DeHaan, 2015). Here, we focus on the genetic and practical aspects of developing perennial grain crops from wheat.

**WILD RELATIVES OF TRITICUM**

More than 75% of the species in the Triticeae tribe, which includes Triticum, are perennial (Dewey, 1984). There is not complete agreement, but cytogenetic investigations have shown there to be ~10 basic genomes in the perennial species of Triticeae on the basis of homology (Ceoloni et al., 2015). Combinations and repetitions of these basic genomes, with varying degrees of homoeology, make up the variation represented by these wild species, with ploidy ranging from diploid to dodecaploid. Most of the hybridization undertaken with related species has been for the purpose of introgressing simply inherited traits, such as resistance to pathogens, into Triticum species (Sharma and Gill, 1983).

There is continuing debate on the relative merits of a genomically based taxonomic system versus one that is focused on morphology (Dewey, 1984; Barkworth et al., 2009). Work to determine the relatedness of different species based on observation of meiosis in F1 hybrids has been helpful for breeders, who are more interested in genetic compatibility than morphology (Wang, 1989; Wei and Wang, 1995). Kellogg (1989) has made the observation that unwillingness to consider different placement of economically important members of the Triticeae, including Triticum, has kept the taxonomic treatment of the tribe in flux. A closer cooperation between breeders and taxonomists could be beneficial in expanding the number of combinations tested, benefiting both disciplines.

In their original presentation of the genepool concept, Harlan and de Wet (1971) offer the idea of classifying species in relation to each other on the basis of how easily they can produce offspring, the vigor and fertility of hybrids, and the degree of genetic recombination. The historical genus Agropyron, before being restructured, used to encompass many species in the secondary and tertiary gene pools of wheat that are agronomically important in their own right as forage grasses, and also as genetic resources for wheat improvement (Barkworth and Dewey, 1985). From our perspective, the wheatgrasses belong in the secondary gene pool of wheat given their ability to make crosses through controlled pollination, which leads to offspring with limited fertility, and limited recombination between genomes in those hybrids. A new understanding of genomic relationships among members of the Triticeae has helped develop phylogenies and has led to reorganization on the basis of molecular and cytogenetic characterization.

These changes in nomenclature regarding wheat’s perennial relatives can make determining the exact nature of combinations in previous studies difficult. The majority of work has been done with the current genus Thinopyrum Á. Löve, which contains several species that are genetically compatible enough with Triticum to produce offspring with limited fertility through cross pollination. When reviewing historic literature, reference to Agropyron elongatum (Host) P. Beauv. is especially prevalent and problematic. In the reorganization, A. elongatum became Th. elongatum (Host) D.R. Dewey and Th. ponticum (Podp.) Barkworth & D.R. Dewey, and within germplasm collections, those species can be listed as diploid, tetraploid, hexaploid, octoploid, or dodecaploid. This could be due to misclassification or application of morphological, rather than genomic, concepts for species delineation. Additionally, there are numerous genebank accessions for which no information on ploidy is presented.
The list of species in the Triticeae successfully hybridized with wheat to produce offspring, fertile or otherwise, is substantial and has continued to expand since reviewed by Sharma (1995). Whether two species can produce hybrid progeny through the application of extensive laboratory techniques is less relevant to the current discussion of breeding new crops than their ability to create viable, and ideally fertile, offspring by cross pollination. Thus, we focus here on the specifics of intergeneric hybrids between *Triticum* and *Thinopyrum* species because of their long history and genetic affinity, although this should not suggest that other avenues are not capable of success. The compatibility of the parents and their adaptation to environments of interest are the most important qualities.

Most of the *Thinopyrum* species are complex polyploids in their own right, all with the basic genomic number of 7 common to the Triticeae (Aase, 1935). Disagreement over genomic constitutions and their symbols in *Thinopyrum* (and other genera in the Triticeae) makes listing them here difficult and might contribute to the confusion. Conversation about how genomic and morphological data should be balanced for taxonomic placement and nomenclature continue to restructure the placement of species and boundaries of genera (Barkworth et al., 2009). We outline some general strategies and combinations for hybridization below, but a review of the literature relevant to species of interest is advised before developing a breeding program.

Zheng et al. (2014) surveyed the *Thinopyrum* accessions available from the USDA National Plant Germplasm System and found a range of ploidy levels among accessions listed as the same species and aneuploidy in some individuals. The practical applications of these findings are that unknown materials have to be verified cytologically, and the classification given by a genebank or other source cannot be accepted without verification.

Irregular transmission of chromosomes from the wheat-grass parent can lead to deviations from expected counts in F₁ progeny with wheat. Our own experience crossing *T. aestivum × Th. ponticum* has yielded mostly F₁ hybrids with the expected somatic number of 56, but also variants with 54. Beyond considerations of ploidy and genetic compatibility, the germplasm base available in genebanks is limited. Asay and Jensen (1996) reported that most cultivars of *Th. ponticum* from breeding programs could be traced back to a few accessions. Accurate information on the genomic constitution of accessions used in hybridization and a suitably diverse germplasm base are important for development of perennial grain crops from wheat.

**DEFINING THE PERENNIAL HABIT**

If perennation is considered a quantitative trait, with many genes acting in concert to regulate plant reaction to important phenological signals, breeding for the trait takes on the complexity of recognizing the qualities that define it (Thomas and Ougham, 2000). The availability of water, presence of pathogens, edaphic qualities, and climactic patterns will affect the fitness and longevity of individual plants. A plant that is perennial in one environment might not survive to reproductive maturity in another.

The robustness of the perennial phenotype—defined as the ability of the plant to persist across environments, genetic backgrounds, and disturbances—is a critical concept (Abley et al., 2016). Even plants that are characterized as perennial can die after a single season under unfavorable growth conditions, and an otherwise annual plant can behave as a biennial or weak perennial depending on its growing environment (Warnke et al., 2004). The wide adaptation of perennial grasses in the Triticeae should not be generalized to specific lines or plants taken out of their environmental context.

A perennial plant is one that exists for multiple years, but the more important distinction for a grain crop is the reproductive cycle of the plant. Plants can be classified as semelparous (monocarpic) or iteroparous (polycarpic) according to whether they set seed once and senesce or set seed multiple times, respectively (Amasino, 2009). Comparing the monocarpic model species *Arabidopsis thaliana* (L.) Heynh. with its polycarpic relative *Arabis alpina* L., Amasino (2009) notes examples where mutations in two genes convert *Arabidopsis thaliana* to polycarpic. This can be compared with the work of Lammer et al. (2004), where the addition of a single chromosome arm from *Th. elongatum* to *T. aestivum* altered the life history of the plant under some conditions. In this case, the plants continued to flower without returning to a vegetative state and seemed to lack, or be insensitive to, important genes for phenological signaling. In neither case did the simple disruption of an annual lifecycle result in a robust perennial.

The longevity of grasses can be considered at a range of scales. At the community scale, there is the sward, composed of individual crowns, which are made of tillers broken into phytomers. The phytomer is the basic unit of the grasses, and there is strong homology within the family. For perennial grasses, there are the additional considerations of the genet, a colony of clones, and the ramet, which is an individual clone from an original plant (Moore and Moser, 1995). In an ecosystem, a genet may survive while individual ramets perish, shifting the composition of the landscape. In an agroecosystem, a healthy sward must be maintained to suppress weeds, hold soil, and produce a crop.

Tillering is important for grass development and longevity. During vegetative growth, individual tillers will develop buds in the leaf axis that grow into new tillers. The persistence of the plant is limited by its ability to produce new vegetative growth (Jewiss, 1972). The rate of initiation of new buds contrasted with the senescence of
tillers effectively mediates the lifespan of the plant (Briske, 1991). Dormant buds act for an individual plant in the much same way that dormant seeds constitute a potential reserve of plants for the sward.

The concept of the bud bank relates to the number of immature buds that could initiate vegetative growth after disturbance or in the next growing season (Klimešová and Klimeš, 2007). Quantification of the bud bank could be one metric to assess the potential of a plant to survive to the next season after harvest. The number of buds, and their growth stage, could also be an indicator of a plant’s ability to compete with weeds. Within a mixed population, the bud bank of an individual will determine its genetic contribution to the sward over time.

Hendrickson and Briske (1997) investigated several species of perennial grass and found that axillary buds could remain viable or dormant for up to 24 mo after the death of the reproductive parental tiller. They also found that the tillers arising from newly formed axillary buds had the largest impact on persistence. Our observations of *T. aestivum × Th. ponticum* plants in the field seem to corroborate this. Plants that appear vigorous after harvest with a majority of tillers advancing towards flowering do not tend to survive even mild winters. Research on whether the number or growth stage of axillary buds after harvest in the fall is correlated with plant survival and vigor in the spring could help to inform selection and reduce cycle time.

Perennial grasses exhibit a range of growth habits based on the structures they use to store energy, which are categorized as caespitose, rhizomatous, or stoloniferous (Harrington, 1977). Caespitose grasses store energy in their crowns from season to season and spread slowly outwards as new tillers root and form buds. Rhizomes are thickened roots traveling underground, giving rise to new ramets, and stolons are their aboveground counterparts. Consideration of the parental habit is important in defining selection strategies when developing hybrids.

Even with species considered perennial in habit, complete survival within a population from season to season is not to be expected. Timing of flowering, necessary for a perennial grain crop, may affect the production of new vegetative tillers and the longevity of the stand. The ability of the sward to persist is a combination of surviving plants filling gaps and dropped seed giving rise to new plants (Jewiss, 1972). A population approach may be needed in terms of breeding and stand establishment. Plants also need to have the ability to return to a vegetative state of growth, as discussed below.

**Dormancy**

Definitions of dormancy coalesce around cessation or slowed growth of meristems (van der Schoot and Rinne, 2011), especially as a response to stress. Dormancy offers some protection to the plant, as rapidly dividing cells such as those found in floral meristems can be more susceptible to temperature shock (Single and Marcellos, 1974). The ability of the plant to return to a dormant, or at least vegetative, state after flowering and before periods of stress is important for survival in regions with harsh winters or long dry periods. Perennial grasses are known to go dormant according to environmental signals, including daylength, temperature, and availability of water and nutrients (Norton et al., 2009).

Environmental factors can be responsible for initiation, maintenance, and release from dormancy. The categories of para-, endo- and ecodormancy were applied by Lang (1987) to differentiate between signals originating from a part of the plant other than the one affected, from the affected structure, and from environmental effects that are nonspecific, respectively. A review of dormancy research by Horvat et al. (2003) suggests a complex relationship between dormancy, vernalization, and floral induction mediated by genetic and epigenetic actions. Temperate grasses vary in their response to temperature and moisture stress; choosing parents with adaptation for dormancy or avoidance of those stresses will be important in some environments (Norton et al., 2009).

**Return to Vegetative State**

Dormancy will be important for survival in some environments, but the ability to return to a vegetative state is needed to support not only survival, but also the perennial growth of the plant. Research on several monocot and dicot plant species suggests a connection between regulation of flowering and dormancy (Horvath, 2009). The difference between what some refer to as endodormancy and others might think of as reversion to vegetative growth may be one of degree, rather than distinction. In either case, it is not known what might signal or motivate the reversion from the flowering state in polycarpic plants, and there are likely differences based on environment (Rohde and Bhalerao, 2007). Being able to return to vegetative growth after flowering allows the plant to develop new structures to support further reproduction in subsequent seasons.

**Partitioning**

Investigations in perennial prairie grasses from several genera have not shown any interaction between sexual reproduction and development of vegetative buds (Ott and Hartnett, 2011). Additionally, an assessment of studies of related annual and perennial species across 28 genera found no relationship between seed production and lifespan (Vico et al., 2016). These results are encouraging, suggesting no intrinsic genetic link that prevents the development of a higher-yielding perennial grain crop.

**Considerations for Breeding**

The importance and balance of the factors outlined above for breeding will be dictated largely by the target
environments and habit of the perennial parent. The longevity desired from the crop will depend on the cropping system, the makeup of the population, the degree of management, and inputs. Consideration of the growth stage and habit of hybrids in the field multiple times during the season is helpful for selecting towards stable types that fit into the desired agroecosystem.

**DEVELOPING HYBRIDS**

The history of developing Triticale (× Triticosecale Wittmack ex A. Camus) offers key insights into what it might take to create perennial grain crops using wheat. Triticale remained a curiosity until enough resources were devoted to it to develop sufficient primary combinations that could then be intercrossed. Octoploid Triticales produced from crosses of *T. aestivum* × *Secale cereale* L. seemed to be a dead end but have proven valuable for crossing with hexaploid lines developed from *T. turgidum* × *S. cereale*. Developing lines for specific end uses such as baking, feed, or forage has also been effective in advancing the crop (Mergoum and Macpherson, 2004). An important lesson from Triticale is the beneficial nature of pursuing a range of types with different breeding objectives. In the early stages of developing amphiploids, variation, even if it appears undesirable, could be important for the future success of the perennial grain crops being developed. Rather than trying to meet current requirements for *Triticum*, it is best to think of these new species as completely new crops and be open to the possibilities.

Disruptions to meiosis and fertility in intergeneric hybrids make the development of mapping populations and calculation of broad-sense heritability for the perennial habit impractical (Nyquist and Baker, 1991). Developing efficient methods for producing diverse amphiploids stable at 42 or 56 chromosomes, a genome added to either tetraploid or hexaploid wheat or two to tetraploid wheat, should be the focus of breeding programs. Genetic studies can then be performed on successful combinations and populations with known structure to identify important genes.

**Defining an Ideotype**

Donald (1968) defined a crop ideotype as a breeding goal based on a vision for the combination of positive characteristics of the plant. This is in contrast with breeding away from perceived defects, such as shattering or lodging. The ideotype is based on the role that the crop needs to fill on the farm, in the environment, and in society.

Environment, cropping system, and desired end use are important factors shaping the ideal characteristics of perennial grain crops. Balancing vegetative forage production and grain yield, summer and winter dormancy, seed quality traits, and longevity are some considerations that will be specific to the agroecosystem where the crop is grown. In regions with available moisture and mild winters, continued growth for grazing or forage and weed suppression would be an advantage. Harsher environments with more severe winters or limited water might favor a plant that goes dormant rapidly after harvest to avoid drought and maximize cold tolerance. It is up to breeders, working with farmers, to determine how a perennial grain crop would fit into their regional agricultural system. The limits of both perennation and utility will be exposed as genetic material travels outside the range of adaptation.

The diversity of possible ideotypes for perennial grain crops based on wheat are united around several common traits. Polycarpic habit, threshability, and strong straw are all necessary to make the crop harvestable and agronomically viable. Qualities such as tillering or rhizomes, which can fill gaps left by senescence of neighboring plants, will have to be balanced against adaptation to high-density planting.

Synchronicity of flowering and height are important qualities for mechanical harvest. The grain crops currently under cultivation have been selected for these traits and so represent one end of the annual–perennial spectrum. Wild species tend to be more opportunistic in their pursuit of reproduction, and we have seen a range of habits from hybrids in the field. Vigorous growth is often manifested as continuous flowering, leading to a mixture of mature and unripe heads that, if in the same layer of the canopy, can significantly affect harvest.

**Direction of Cross**

In most cases, crosses have been made using the *Triticum* species as the female parent. There are several advantages to this approach: the wheat parent is easier to emasculate, the wild relatives tend to shed more pollen over a longer period, and successful hybrids strongly resemble the wild relative, making them easy to identify in a wheat background. Many researchers have attempted reciprocal crosses with largely or wholly negative results (Armstrong, 1936). Whether the cytoplasm donor of the hybrid is important in intergeneric combinations has been studied without conclusion and remains an open question for research (Bento et al., 2011). The reproductive biology of the perennial species being used in the cross should be considered, but using wheat as the female parent has been preferred for reasons stated above.

**Parent Selection**

Wheat varieties are usually developed as pure lines with minimal genetic variation between plants. In contrast, accessions of related species in genebanks often represent diverse populations. Both parents need to be tested in the target environments. The homozygous nature of inbred wheat lines makes selection by line reasonable, but selecting individual plants of the wild relative that express exceptional qualities is appropriate for making crosses, given the possibility of heterozygosity in populations.
There are genes influencing the crossability of wheat with other species (Riley and Chapman, 1967), and there appear to be similar factors affecting pollination and seed development acting in wild species as well (Sharma and Ohm, 1990). Investigation of the crossability of Triticale lines has shown variation in seed set from both parents, and the low crossability trait was partially dominant in some F₁s (Guedes-Pinto et al., 2001). The influence of crossability genes has implications for backcrossing and compatibility of advanced breeding material that have not been fully explored.

A review of 1400 bread wheat varieties found that only 76 had a crossability with rye >50%. A disproportionate number of these lines are landraces from Iran, East Siberia, China, and Japan or modern lines derived from them (Zeven, 1987). The lack of need for such genes in wheat breeding programs makes them susceptible to loss through genetic drift, requiring dedicated effort to bring them back into elite lines.

Mapping of crossability genes in bread wheat and developing markers would allow screening for them in a backcross program to develop adapted lines with increased seed set in wide crosses (Bertin et al., 2009; Manickavelu et al., 2009). A more cumbersome but effective method is to test progeny in each generation by crossing to rye, or with the species being used for hybridization, as a tester to confirm that the needed genes have been retained before making another backcross (Molnár-Láng et al., 1996).

It is not known how important crossability genes are for fertility in early generations of wide crosses or when intercrossing breeding lines. One potential benefit of having crossability genes as part of a breeding program should be increased seed set when crossing breeding lines back to the wild relative, allowing for more subtle manipulation of chromosome constitution (Molnár-Láng et al., 2010). Ploidy level of both parents may be important. Armstrong (1945) had much greater success crossing wheatgrasses with tetraploid species of *Triticum*.

Introgression of crossability genes into adapted varieties of wheat could increase the number of progeny from each hybridization (Molnár-Láng et al., 1996). This is especially important because many of the wild relatives exhibit outcrossing, with the associated heterozygosity allowing for many more possible combinations from one cross. Being able to sample a wider range of the possible combinations would be useful to develop populations expressing variation for perennation in a similar genetic background.

Hybrid necrosis has been observed in crosses of wheat with numerous related species (Chen et al., 1990; Franke et al., 1992). It can be expressed in the F₁ or subsequent generations, limiting the usefulness of some combinations (Bombbies and Weigel, 2007). Because the effect can manifest in many ways, observation of each combination will be necessary to determine how best to proceed. In such wide hybrids, there is the possibility of exposing genetic qualities that were otherwise not apparent, similar to the way in which different varieties of wheat react to another culture (Zhou and Konzak, 1992).

Local adaptation of both parents will likely play a role in the survival of hybrids in the field and in the response to environmental signals related to change of season. This extends beyond the ability to resist or tolerate biotic and abiotic stresses, requiring that the plant also be able to adjust its growth to the season. Both parents should be considered equally, as they both make significant genetic contributions to the crop.

### Pollen Transfer

Armstrong (1936) captured pollen from the wild species in glassine bags, bulked it in a Petri dish and applied it to the mature stigmas of emasculated wheat heads with a brush. In our crossing, we have found that heads harvested right before anthesis set in test tubes with water shed ample pollen over several days, also allowing pollen to be transferred between the field and greenhouse. Growth regulators can be applied to the female parent after emasculation to improve seed set, commonly 2,4-D and GA₃ (Jauhar and Peterson, 1996). A variety of mechanisms act to abort the embryo or endosperm after fertilization in wide crosses, but methods are available to intercede and grow the embryo to maturity (Sharma et al., 1996). At this point, depending on chromosome relationships between the two species, often the F₁ will be a polyhaploid with a single set of non-homologous chromosomes from each parent.

### Bridge Crossing

Bridge crossing is a way to circumvent incompatibility in combinations that cannot yield offspring through direct fertilization. In such cases, crossing one parent with an intermediary, often a different species, before hybridizing with the second desired parent can sometimes overcome obstacles (Mujeeb-Kazi and Hettel, 1995). This approach should be employed judiciously, as it greatly complicates crossing and the constitution of the resulting hybrids. Doubling the chromosome number of diploid species may also be effective in improving cross compatibility, as discussed below.

### Backcrossing

Chromosome loss and possible heterozygosity of the wild parent complicate backcrossing as a strategy for generation of amphiploids with the desired characteristics. The heterozygous nature of some perennial relatives means that there are a large number of possible combinations in the gametes, and that each F₁ plant is potentially distinct. Backcrossing to F₁ plants benefits from the ability to identify those with desired traits, which may not be possible until after flowering. In this case, it would be necessary to backcross to all progeny, advancing lines using post facto
information. With the broad range of possibilities, this quickly becomes unmanageable.

Investigating the meiotic behavior of wheat × rye hybrids, Thompson (1926) found that progeny from several BC₁F₁ plants crossed back to wheat had a higher chromosome number than either parent. Additionally, Gill and Sharma (1983) found that differences in crossability of the wheat parents persisted into the BC₁F₁ generation in wide crosses. Further investigation is needed to determine the potential of backcrossed progeny to stabilize in comparison with those from selfed or doubled F₁s.

**Prebreeding Wild Species**

With the exception of that done by Wagoner (1990) and at the Land Institute (DeHaan et al., 2013), very little work has been done to breed wild grasses to increase their utility as grain crops (Asay, 1992). Breeding of wild relatives can be a path towards domestication and, along the way, more agronomically suited plants can be used in hybridization. Domestication of wheat was influenced heavily by several key traits related to shattering and threshability (Kerber and Dyck, 1969; Kerber and Rowland, 1974). Perennial species in the Triticeae have not undergone much selection for these characters, and introduction of their genes in hybrids can lead to the expression of undesirable traits. Though there are some cases of partial self-fertility, Thinopyrum species are primarily outcrossing in nature (Asay and Jensen, 1996). This quality makes recurrent selection within developed populations a practical approach for improvement (Allard, 1960). Germplasm should be evaluated in the target environments and exceptional individuals then transplanted to a crossing block to intermate and produce seed for a new bulk population. Repeating the process allows for recombination of important characteristics and selection of plants more agronomically suited to grain production. Plants from each generation can be used to hybridize with wheat so both programs run concurrently. Individual plants are the unit of selection for hybridization, whereas diversity must be maintained in the population for continual adaptation.

The most rapid gains will be seen for traits that are easily selected phenotypically before flowering. In this case, individuals expressing undesirable traits, such as susceptibility to disease or lodging, can be rogued before cross pollination. Less heritable traits, such as seed yield, or traits that cannot be distinguished phenotypically, such as high crossability, will be more difficult. The structure and diversity of the initial population will be important factors in determining potential gains from selection. DeHaan and Van Tassel (2014) suggest developing subpopulations to expose deleterious alleles and focus selection on specific traits. Before a program begins, it is worth evaluating a range of wild species in the target environments to see what adaptation already exists and to evaluate the potential to improve agronomic performance (DeHaan et al., 2016).

**CHROMOSOMAL INHERITANCE**

The behavior of introduced chromosomes in a wheat background has been studied in a small subset of possible combinations, usually with the goal of introgressing a specific gene or genes with as little additional material as possible. Developing stable partial amphiploids (plants with the complete genomic constitution of one parent and a subset of another) or full amphiploids (where the new plant contains complete sets of both parental genomes) is not a regular practice in breeding crop plants. Chromosomes contain large numbers of genes, making study of their movement during hybridization more appropriate than finer-scale genetic work during the development of intergeneric hybrids.

In a review of genes in small grains that could potentially alter meiosis, Able et al. (2009) identified genes that could increase homoeologous pairing, unreduced gamete formation, and rates of recombination. Exploring the usefulness of these genes could help to increase seed set in the progeny of wide crosses and increase recombination between homoeologues. These characteristics could expand the possibility of backcrossing as well, increasing the range of variation from a given cross.

Mitotic instability in newly formed polyploids can lead to aneuploid cells and chimeric tissues (Comai, 2005). Brasileiro–Vidal et al. (2005) observed mitotic instability in *T. aestivum × Th. ponticum* hybrids but determined that this did not affect the transmission of chromosomes in meiosis. Bringing together key factors, identifying successful combinations, and applying appropriate selection pressures through generations requires an understanding of the paths that chromosomes travel during meiosis.

**Sterility**

Sterility in interspecific hybrids is seen across genera and can be related to the interaction of parental genes, chromosomal imbalance, cytoplasmic interactions, or unknown factors (Stebbins, 1958). Additionally, in the grasses there are self-incompatibility interactions that are not well understood, potentially complicating the creation of fertile intergeneric hybrids (Baumann et al., 2000). Work on a range of genera and their combinations has not revealed any general rules. It may be that there are a variety of mechanisms at work, making the manifestation of incompatibility–related sterility specific to the material being investigated (Matzk, 1980). Morphology of the flowers can offer some insight, and the direction of the cross has been shown to be important in some cases (Zenkteler and Nitzsche, 1984).

The heterozygotic and outcrossing nature of many grasses requires a different breeding approach for hybrids.
Behavior of Univalents
When individuals with different genomic constitutions are crossed, some chromosomes will lack homologues in the F₁ and be left as univalents during meiosis. In intergeneric crosses, in the absence of unreduced gametes, it is possible that all chromosomes will lack partners in the first generation after hybridization. In this case, as a simplification, the odds of obtaining a complete set of chromosomes in a gamete by random assortment are \( \frac{1}{2^n} \), where \( n \) is the number of univalents. The odds of obtaining a complete amphiploid by random chromosome segregation is that number squared, representing two such gametes coming together. Beginning with the 21 univalents from \( T. aestivum \) and adding any number from a wild relative makes this event extremely rare. Even if a complete amphiploid is not needed, breaking up the basic sets of seven chromosomes that make up wheat and its relatives might not be possible, which complicates matters (Sax and Sax, 1924).

Consideration of the ways in which unpaired chromosomes can act in meiosis suggests that a range of constitutions are possible. Precocious migration of univalents can give rise to noncompensating nulli-disomic gametes, where random loss and pairing of homoeologues leads to gametes disomic for one homoeologue and nullisomic for the other. These events greatly increase the number of possible combinations, even in gametes with an expected and balanced chromosome number (Oleszczuk and Lukaszewski, 2014).

Geng et al. (1979) developed a general probability model for random assortment of chromosomes, and analysis of aneuploid and interspecific hybrid datasets related to \( Triticum \) fit it well. In contrast, Sharma and Gill (1983) found an abnormally large number of BC₂ progeny with 49 chromosomes from crosses of \( T. aestivum \) with \( A. ciliare \) (Trin.) Franch. \( [Elymus ciliaris \ (\text{Trin.}) \ Tzvelev] \) and \( A. trachycanum \) (Link) Malte ex H.F. Lewis \( [Elymus trachycanulus \ (\text{Link}) \ Gould \ ex \ Shinners] \), suggesting that unpaired chromosomes may be transmitted as genomes more often than random chance would predict. The influence of additional chromosomes may alter this behavior both physically and genetically, but it suggests a basis for developing plans for breeding populations.

Work done with interspecific crosses of hexaploid and tetraploid wheat suggests that the chromosomal constitution of gametes in the F₂ and later generations deviate significantly from what would be expected if all possible combinations were viable. There is strong evidence to suggest that plants with 14 bivalents and any number of univalents tend toward the tetraploid number, and those with >14 bivalents will increase towards the hexaploid number over the course of generations (Jenkins and Thompson, 1930). In this study, with very few exceptions, plants did not have fewer bivalents than their parents, and those that had >14 bivalents had enough univalents to bring the overall number to 21.

An investigation by Thompson and Armstrong (1932) found that, in tetraploid × hexaploid wheat crosses, male gametes were produced in approximately theoretical proportions, but inviability of intermediate types altered chromosome counts in the progeny. If this is generally true, then it would act to favor numbers at the upper and lower limits of possibility, making selection in early generations important to reduce the contribution of the lower group to the overall population.

In terms of selecting desirable types, there are several complications related to the behavior of univalents in intergeneric hybrids of wheat with perennial relatives. Lack of homologs to pair with in early generations will affect recombination rates. Multivalent formation can disrupt linkage groups, leading to multiple or no doses of some genes. Overall chromosome constitution will change across generations, leading to potential change in dosage or loss of genes.

Unreduced Gametes
One path to polyploidization is through the production of unreduced gametes. The natural combination of unreduced gametes can lead to speciation and is thought to be the source of many useful crop plants, including wheat (Stebbins, 1959). Unreduced gametes can be the result of natural processes or induced, and their production can be dependent on the genes and chromosomal constitution of the plant.

The doubling of somatic chromosomes with colchicine is a standard practice for restoring fertility after wide crosses. Caffeine is another alternative for programs not set up to deal with something as hazardous as colchicine (Thomas et al., 1997). In the case where both the parents are polyploids, doubling of the F₁ can result in a large number of chromosomes, complicating meiosis even with a balanced constitution. A successfully doubled plant will have increased seed set and shed more viable pollen, increasing the options for further crossing. Encouragingly, Armstrong and McLennan (1944) found that chromosome numbers in
T. turgidum × A. glaucum (Desf. ex DC.) Roem. & Schult. [Th. intermedium (Host) Barkworth & D.R. Dewey] hybrids doubled with colchicine were largely stable between the F$_2$ and F$_3$ generations, and fertility of plants correlated with higher chromosome number.

An alternative path for producing unreduced gametes is the natural failure of one of the reductional divisions in meiosis, termed restitution. The stage of meiosis when restitution occurs has an impact on gamete formation and chromosome assortment (Ramanna and Jacobsen, 2003; Cai and Xu, 2007). Failure of the first meiotic division results in cells where sister chromatids fail to separate during meiosis I but assort and divide during meiosis II. Failure of the second division does not allow an opportunity for chromosome assortment and produces gametes with both sister chromatids. There are genetic controls in wheat for the formation of unreduced gametes, and incorporation of parents known to produce them in greater quantity could be beneficial in obtaining seed from interspecific crosses (Cai et al., 2010). Selection for this characteristic in other crop plants has shown reasonable heritability, making it an approachable target for breeding efforts if deemed useful (De Storme and Geelen, 2013).

The amphihaploid nature of F$_1$ hybrids between distantly related genera in Triticeae may be important for triggering processes leading to unreduced gametes (Silkova et al., 2011). Without homologs, the normal reductional division of meiosis is not possible. Pairing of homoeologous chromosomes in polyhaploids can interfere with the formation of unreduced gametes (Jauhar, 2007). The relative importance of these factors for breeding will depend on the species being used and the relationship between their genomes.

In addition to the production of unreduced gametes, functional aneuploid gametes produced by chromosome assortment have an important role in giving rise to the next generation. The union of an unreduced gamete with a functional aneuploid gamete will maintain the overall chromosome constitution for selection without necessarily leading to balance or stability. Developing wheat lines that regularly give rise to unreduced gametes may be useful for backcrossing and increasing fertility in selfed progeny.

**Ph1**

Pairing, which is necessary for recombination, among the homoeologous chromosomes that form the basic genomes of wheat is suppressed in the presence of the Ph1 gene (Sears, 1976). This function has been shown to extend to intergeneric hybrids with wheat as well, interfering with the pairing of wheat chromosomes with homoeologues in such hybrids (Riley et al., 1959). This regulation, along with the unknown genetic complexity of the perennial habit, makes introgression of all needed factors into Triticeum species an extremely unlikely possibility. This also keeps the favorable linkage groups from the wheat parent intact, aiding in the development of stable amphiploids.

It has been suggested by Cai and Jones (1997) that the presence of Th. ponticum chromosomes in the T. aestivum × Th. ponticum polyhaploid inhibits Ph1, allowing some homoeologous pairing, mostly among the Thinopyrum chromosomes. From the discussion above, it seems that this quality may interfere with haploidy-dependent pathways for forming unreduced gametes, but that needs further study. Overall, the role of Ph1 is central to the genome-based breeding approach by minimizing homoeologous recombination.

**Hybrid Genomes**

The frequency of pairing between homoeologous chromosomes within the respective genomes originating from each species in intergeneric hybrids has been studied in several cases (Cai and Jones, 1997; Cai et al., 2001; Chen et al., 2001). In general, results suggest low levels of pairing between wheat and Thinopyrum chromosomes and higher levels of homoeologous pairing among Thinopyrum genomes than among the wheat genomes.

Autosyndetic pairing of homoeologous Thinopyrum chromosomes in hybrids with Triticum may help prevent their loss without having to employ chromosome-doubling techniques. Recombination of homoeologues also adds layers of genetic complication during the formation of amphidiploids, as the additional genome or genomes can be hybrids of the parental Thinopyrum genomes (Fedak and Han, 2005). There are several examples of lines stable at 42 chromosomes where a portion of the T. aestivum complement has been substituted with Thinopyrum chromosomes (Fradkin et al., 2011; Burešová et al., 2014). These lines are stable in meiosis but have not been evaluated for perennial habit.

Fedak et al. (2000) used genomic in situ hybridization (GISH) to determine the genomic origin of eight stable partial amphiploids of T. aestivum with Th. ponticum or Th. intermedium having 56 chromosomes from different breeding programs. Their investigation revealed a range of 38 to 42 chromosomes from wheat, some with translocations, and 14 to 18 from Thinopyrum. Fluorescent visualization showed that the additional chromosomes in this material came from more than one Thinopyrum genome. Importantly, this shows the possibility of achieving stability without having four complete, or even distinct, genomes.

The combination of genomes in a new plant results in expression and interaction of the genes of both parents. Work on synthetic hexaploid wheat and Triticale has shown that there are losses of parental bands (measured by amplified fragment length polymorphism) from both parents in the hybrid and that losses are related to parental genome size (Bento et al., 2011). The potentially hybrid nature of additional genomes from wheatgrass parents...
in stable amphiploids may complicate intercrossing of advanced material, depending on the degree of rearrangement. This will be especially important when crossing materials developed by multiple breeding programs.

**PATHS FOR DEVELOPING TRITICUM × THINOPYRUM HYBRIDS**

The main crop species in *Triticum* are allohexaploid and allotetraploid, with the diploid species having a minor role in overall crop production. *Thinopyrum* species ranging from diploid to autoallodecaploid have been hybridized with wheat to produce sterile or partially fertile offspring. They have the added advantage of being important forage and conservation grasses, where some effort has been put into developing improved varieties with traits beneficial in hybrids with *Triticum* (Asay and Jensen, 1996). Even limiting combinations to this relatively well-studied set allows a range of breeding options for developing full or partial amphiploids with 42 or 56 chromosomes with early generations exhibiting phenotypes different from either parent (Fig. 1). Here, we will focus on the promising aspects of several species and ploidy levels based on previous work and desirable qualities.

**Triticum aestivum**

Crosses with *T. aestivum* take advantage of the resources, research, and breeding work that have gone into developing it as a crop, with genes relevant to developing leavened baked goods. Comparison of *T. aestivum × Th. ponticum* breeding lines with *T. aestivum* for bread baking quality at Washington State University showed that, although the breeding lines had less than optimal loaf volume, they were still capable of producing a leavened bread (Murphy et al., 2009).

**Triticum turgidum**

A major advantage of using *T. turgidum* in wide crosses is that the genetic barriers to intergeneric hybridization seem to be lower (Mujeeb-Kazi et al., 2007). Because of their lower ploidy level, hybrids with *T. turgidum* will have a proportionally higher total genomic content from the wild species with associated agronomic qualities. The lower ploidy also gives the opportunity to stabilize amphiploids with two additional genomes from the *Thinopyrum* species at 56 chromosomes.

**Diploid Thinopyrum Species**

Crosses with the diploid *Thinopyrum* species are the most analogous to Triticale breeding. Complete amphiploids can be developed by crossing with either species of *Triticum*, and then doubling the somatic set of the progeny or letting the F₁ self-pollinate and selecting on chromosome number in the progeny. Another possibility is to double the diploid *Thinopyrum* parent before crossing, then backcross the F₁s to *Triticum* and rely on autosyndetic pairing of the duplicated *Thinopyrum* genomes to achieve stability in the BC₁F₁.

This route offers the quickest path to stabilized amphiploids, which can then be evaluated in the field and intercrossed. It also offers the least diversity of any of the combinations. The F₁s from such crosses are haploid (all chromosomes lack homologs), so each doubled F₁ will yield only one genetic combination, without recombination. For this approach to work, the number of F₁ seeds produced must be large and the process for recovering amphiploids efficient.

**Tetraploid Thinopyrum Species**

There are several tetraploid species of *Thinopyrum* that could be used in crosses in the same manner as described above for artificially doubled *Th. elongatum* (Mujeeb-Kazi and Miranda, 1984). For species where the two genomes are similar enough to pair, amphiploids could potentially be developed by backcrossing the F₁ with the *Triticum* parent.

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Fig. 1. Heads from F₂ progeny of a *Triticum aestivum × Thinopyrum ponticum* cross with the wheat parent on the extreme left and the wheatgrass parent on the extreme right. × Tritipyrum.
The relatedness of the two Thinopyrum genomes will be an important factor, but the potential for recombination among them increases the potential diversity obtained from each F₁. This approach would be especially appealing in regions where these species of Thinopyrum are well adapted.

**Thinopyrum intermedium**

The strategy of the Land Institute to domesticate Th. intermedium is an example of what might be achieved with other Thinopyrum species before developing hybrids (DeHaan et al., 2013). Of the Thinopyrum species explored here, Th. intermedium has the most complex genomic constitution. This complexity means that the chromosomes constituting hybrid genomes in developed partial amphiploids may have less affinity when intercrossing advanced lines (Fedak et al., 2000). Lack of pairing can affect fertility and interfere with selection in the progeny, limiting overall combinations.

**Thinopyrum ponticum**

Thinopyrum ponticum offers a unique opportunity in hybrids with Triticum species because its chromosomes exhibit a higher level of autosyndetic pairing in such combinations than some other Thinopyrum species (Cai and Jones, 1997). The greater number of chromosomes contributed by Th. ponticum complicates meiosis in F₁ hybrids with Triticum but has at least three potential benefits. First, recombination among the Th. ponticum genomes increases the diversity from each cross. Second, newly recombined homoeologous chromosomes might allow multiple copies of important genes to be consolidated in the final set that is retained in a partial amphiploid. Third, the higher initial number of chromosomes means that more can be lost through random assortment while still achieving the goal of an additional, stable genome in crosses with Triticum species. Armstrong and Stevenson (1947) found that selecting on perenniality and fertility in T. aestivum × A. elongatum (Th. ponticum) crosses led to plants with few univalents and somatic chromosome numbers clustered around 56.

**Coordinated Efforts**

The variety of paths available means that some thought should be given to the relative advantages of a coordinated effort among independent programs that breed regionally. Coordinated breeding of Triticum species × Th. elongatum or Th. bessarabicum (Savul. & Rayss) A. Löve amphiploids, as suggested by Hayes et al. (2012), would develop a base of material that is genomically related for interbreeding. Some agreement on the germplasm base to be used would be necessary, since even the closely related Th. elongatum and Th. bessarabicum genomes do not pair regularly in crosses between amphiploids (Forster and Miller, 1989).

Most progress will be made by intercrossing stabilized hybrids to recombine their best qualities while maintaining a steady supply of new intergeneric hybrids. The persistent nature of the stable hybrids opens up several breeding opportunities that are not available with annual wheat. Promising plants and lines can be crossed to wheat, and then the F₁ progeny can be backcrossed to the original plant. This allows recombination of the wheat genomes while leaving the additional Thinopyrum genome intact.

Contributing to nomenclature will help to organize literature and facilitate communication among breeders (Curwen-McAdams et al., 2016). The taxonomic recognition of stabilized hybrids is an important aspect of crop development, affecting everything from nomenclature to how farmers will refer to it. Names are important, and cooperation among breeders, geneticists, and taxonomists is needed to develop ×Triticum and other perennial grain crops.

**SELECTION PRESSURES**

Determining a set of phenotypic and genetic characters to select on that will push breeding material in the appropriate direction is an unresolved question. In a paper discussing line selection in hybrids of T. aestivum × A. elongatum (Th. ponticum), Armstrong and Stevenson (1947) found that selection for wheat-like characters led to a reduction in the genomic contribution of the Thinopyrum parent in progeny over generations.

This is further complicated by findings that the addition of a single chromosome arm from Th. elongatum to the basic set of T. aestivum will cause the plant to display a polycarpic habit in some conditions (Lammer et al., 2004). If the expression of the perennial phenotype in hybrids of wheat and wild relatives is plastic, where identical genetics express different characteristics according to environmental conditions, gain from selection will be impaired (Snell-Rood et al., 2010). At least initially, selecting robust perennials will likely require leaving plants in the field for a minimum of two harvests to test their longevity.

**Bulk, Pedigree, and Line Selection**

Different strategies for managing the breeding project can be employed based on resources available and project goals. The extent of outcrossing will likely depend on the parental genes interacting with the environment but has been observed to be >6% in some cases with these kinds of hybrids (Marshall and Schmidt, 1954). For largely outcrossing species of Thinopyrum, a population-based approach is most appropriate (Jensen et al., 1990). Suneson and Pope (1946) felt that random outcrossing in populations they worked with had been beneficial to their program.

Bulk selection is the least resource intensive and allows the environment to alter the breeding population in conjunction with positive and negative selection by the breeder (Allard, 1960). There are a number of difficulties associated with urging populations towards perennation through bulk
breeding. Currently, the most obvious is cycle time, since the only way to know that a plant is capable of setting seed for multiple seasons is to observe it doing so. Even then, separating environmental from genetic effects is difficult at the scale of an individual plant (Yan, 2015). In addition to increasing the amount of time needed, failure of many plants to survive the winter can leave breeding nursery sparse. This environment does not well recreate how they will ultimately be cultivated, and weeds can become an issue. The main disadvantage is that there is no way to untangle a bulk population, and pedigree information that might help inform future crossing is lost.

Developing lines, or families, from individual plants that are selected and bulked through generations offers some benefits in partitioning genetic variance in traditional breeding programs. It is unclear if this is the case when a large amount of genetic change is attributable to chromosome loss and uncertain fate of univalents, rather than recombination and independent assortment. Examining the chromosomal constitution and perennial habit of the progeny from plants that fit the breeding ideotype will help to determine if the selections are being made appropriately.

Pedigree selection involves being able to trace the parentage of each plant through the breeding process. Large differences in fertility make pedigree selection advantageous by allowing the breeder to know which plants are contributing most of the seed for the next generation. Spacing individual plants so they can be followed for multiple years is one way to maintain identity and speed selection. This way, all plants with desirable traits are harvested, their seed is planted, and the survival of the parent plant can be related to the progeny. This strategy also works well with germinating seeds and then transplanting them so that even weak seeds are given an opportunity.

**Early-Generation Selection**

Early generations after intergeneric hybridization are subject to genetic reorganization beyond recombination. Loss of chromosomes, homoeologous pairing, dominance effects, and gamete inviability all skew expression of traits and complicate selection (Udall and Wendel, 2006; Bento et al., 2011). The trait of greatest importance is the perennial habit, characterized by the ability to continue growth after harvest and set seed for multiple years. Low levels of recombination between *Triticum* and *Thinopyrum* homoeologues means that obtaining the unknown number of factors that control the perennial habit as translocations in a true breeding line of *Triticum* is extremely unlikely.

In early generations, multiple copies of recessive factors can overcome dominant genes (Sax, 1922). The heritability of traits selected in early generations will be altered as chromosomes are lost, exposing dominant traits. Intergeneric hybrids of *T. aestivum* × *Th. ponticum* are a good example of how selection in early generations might be altered. Barring abnormalities in the parents, their F₁ hybrid has 56 somatic chromosomes, all lacking homoeologies. If not completely sterile, these haploid plants will produce a range of gametes in which both chromosome number and constitution will be altered significantly.

The heterozygous nature of the outcrossing *Thinopyrum* species means that the number of potentially unique F₁ progeny from a single cross is related to the number and combinations of chromosomes being contributed by the perennial parent. *Thinopyrum* species have many agronomically desirable traits in addition to the perennial habit, but depending on their genetic control, they might not be transmitted through the haploid gametes. Additionally, the heterotic effect of additional chromosomes in early generations may manifest as variation that has little relationship with eventual inbred lines (Bernardo, 2003).

It is possible to make clones from tillers of perennial plants to replicate them for more thorough evaluation, similar to what is done for forage grass breeding (Wilkins and Humphreys, 2003). The number of clones and environments will be determined by the scope of the breeding program, with the upfront investment of resources being offset by the elimination of undesirable types before backcrossing or other strategies are employed. This strategy can also be used to more rapidly increase seed from the best plants in the greenhouse or field.

Loss, duplication, and translocation of chromosomes in intergeneric hybrids add complexity to the already transitory nature of trait expression in early generations. Applying the appropriate selection pressures across generations becomes a function of a range of interacting factors. Two good examples are seed size and threshability. Our experience has shown that, given opportunity, even very small and deformed seeds can yield robust plants. It is easy to lose such small seeds in early generations due to poor threshability and weak germination if standard practices for harvesting and planting wheat are followed. Although threshability and increased seed size are reasonable goals for developing a perennial grain crop, in small populations of unstable material, direct or indirect selection for those qualities too early in the process could negatively affect other important traits.

**Advanced Generation Selection**

As plants, lines, or populations are advanced through generations, chromosome numbers should stabilize, making it possible to replicate individuals in multiple environments. Families that perform well can be tested more widely to determine the range of their adaptation and intercrossed to develop new lines. Direct phenotypic selection for perennial growth per se in intergeneric hybrids has not been successful in producing a plant that possesses a polycarpic life habit in combination with enough agronomic traits to make commercial cultivation economically possible in
any environment tested so far (Bell et al., 2008). This suggests that new selection criteria need to be developed, new target environments identified, or compromises made in the initial balance of agronomics and perennation.

Disease
The alteration from annual to perennial habit requires a shift in thinking about disease. Without tillage, it will be necessary to breed for resistance to pathogens normally controlled by crop rotation (Cox et al., 2007). Viruses are of increased importance because infected plants not killed can serve as hosts for multiple years. The benefit of having year-round vegetation is balanced with the additional challenge of managing a crop that serves as a green bridge.

Markers
Brasileiro-Vidal et al. (2003) determined that the position of 45S and 5S ribosomal DNA sites, along with the hybridization patterns produced by pAs1 and pSc119.2 probes, could differentiate individual Thinopyrum chromosomes. This experiment was only performed on one accession of Thinopyrum and it is unclear if it is universally applicable or how it might change in Triticum × Thinopyrum hybrids. Having robust markers for individual Thinopyrum chromosomes would open up a range of experimental possibilities towards identifying important genetic components of perennation.

Markers for genes specific to the perennial trait would help to cut generation time. Markers for Thinopyrum chromosomes to screen selections at the seedling stage would help to reduce population sizes in the field and simplify maintenance of field plots over multiple years, which is often complicated by the loss of individuals lacking persistence. Finding markers for specific chromosomes known to be important for postsexual cycle regrowth, such as 4E from Triticum elongatum, is a promising place to start (Lammer et al., 2004).

FUTURE DIRECTIONS AND RESEARCH
Advances in understanding of the genetics of Triticum and its wild relatives have laid a foundational knowledge for developing novel perennial grain crops. Characterization of germplasm in genebanks for important agronomic traits and crossability with wheat would lower the initial investment required for regional hybridization projects. Current investigation is appropriately focused at the level of the chromosome, and efforts should be dedicated to creating a wide range of hybrids to build a base of germplasm for interbreeding. This will set the stage for more precise genetic investigation of the perennial habit.

CONCLUSION
Much of the discourse around developing perennial grain crops from wheat has been focused on the ways they might contribute to the sustainability of agricultural land through soil conservation, ecosystem services, and input reduction (Cox et al., 2002, 2010; DeHaan et al., 2007; Glover et al., 2010; Shi et al., 2011; Culman et al., 2013). These are important goals to guide the long-term and philosophical development of the crops; however, they do not address the near-term, practical goals that must guide breeding through the initial stages of building a germplasm base. Any new crop is going to begin at the edges of agriculturally productive areas and require different adaptation from what will be needed as it evolves and becomes economically viable on more fertile ground.

The balance of economics, social acceptance, and access to appropriate processing equipment will have as much to do with the eventual success of perennial grain crops as the genetic, agronomic, and breeding work that must be undertaken for their inception.

If perennial grain crops are to be developed that fill some of the agricultural and functional roles of wheat, breeding programs are needed to develop a germplasm base. The perennial habit is quantitative, and determining its genetic basis is unlikely with the range of materials currently available. We are also not aware of an example of a crop where the details of a quantitative trait were worked out before breeding commenced. The nearest example is that of Triticale, and the effort required to establish it can be seen as an example of the path to come.

Conflict of Interest
The authors declare no conflict of interest.

References


