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ORIGINAL ARTICLE

Development of the first consensus genetic map of intermediate wheatgrass (*Thinopyrum intermedium*) using genotyping-by-sequencing

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Abstract

Key message **Development of the first consensus genetic map of intermediate wheatgrass gives insight into the genome and tools for molecular breeding.**

Abstract Intermediate wheatgrass (*Thinopyrum intermedium*) has been identified as a candidate for domestication and improvement as a perennial grain, forage, and biofuel crop and is actively being improved by several breeding programs. To accelerate this process using genomics-assisted breeding, efficient genotyping methods and genetic marker reference maps are needed. We present here the first consensus genetic map for intermediate wheatgrass (IWG), which confirms the species' allohexaploid nature ($2n = 6x = 42$) and homology to Triticeae genomes. Genotyping-by-sequencing was used to identify markers that fit expected segregation ratios and construct genetic maps for 13 heterogeneous parents of seven full-sib families. These maps were then integrated using a linear programming method to produce a consensus map with 21 linkage groups containing 10,029 markers, 3601 of which were present in at least two populations. Each of the 21 linkage groups contained between 237 and 683 markers, cumulatively covering 5061 cM (2891 cM—Kosambi) with an average distance of 0.5 cM between each pair of markers. Through mapping the sequence tags to the diploid ($2n = 2x = 14$) barley reference genome, we observed high colinearity and synteny between these genomes, with three homoeologous IWG chromosomes corresponding to each of the seven barley chromosomes, and mapped translocations that are known in the Triticeae. The consensus map is a valuable tool for wheat breeders to map important disease-resistance genes within intermediate wheatgrass. These genomic tools can help lead to rapid improvement of IWG and development of high-yielding cultivars of this perennial grain that would facilitate the sustainable intensification of agricultural systems.

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Introduction

Intermediate wheatgrass, *Thinopyrum intermedium*, is a perennial grass with a native distribution throughout the Mediterranean and Eastern Europe (Tsvelev 1983). Intermediate wheatgrass (IWG) was introduced into the United States from the Maikop region of Russian in 1932 for forage and erosion control, as it produces high quantities of biomass and is drought and frost tolerant (Vogel and Jensen 2001). *Th. intermedium* is an allohexaploid ($2n = 6x = 42$), and although its genomic constitution

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remains uncertain, three chromosome sets have been consistently identified and the most likely progenitors believed to be diploid species from *Thinopyrum* and *Pseudoregneria* (Chen et al. 1998; Tang et al. 2000). These chromosome sets were tentatively designated as diploid genomes similar to *Th. elongatum* and/or *Th. bessarabicum* (J); a modified *Th. elongatum* and/or *Th. bessarabicum* (J^s); and *Pseudoroegneria strigosa* (St) (Chen et al. 1998). A subsequent study demonstrated evidence of the potential contribution of other genera, including *Aegilops* (D), *Taeniatherum* (Ta), and *Dasypyrum* (V) (Mahelka et al. 2011). Most recently, genotypic data from EST-SSR markers suggested that more ancient putative genomes would more accurately describe the ancestral genomes of IWG (Wang et al. 2015). The authors suggested that the genomic designation of *Th. intermedium* should be $J^{vs}J^rSt$, with J^{vs} and J^r representing ancestral genomes of the present-day J^b of *Th. bessarabicum* and J^e of *Th. elongatum*, respectively, with J^{vs} being more ancient, and St being similar to genomes of the present-day diploid species of *Pseudoroegneria* from Eurasia (Wang et al. 2015). The genus *Thinopyrum* includes both autogamous (self-pollinated) and allogamous (cross-pollinated) species (Jensen et al. 1990). Although *Th. intermedium* is generally considered to be cross-pollinated, some individuals are self-fertile (Jensen et al. 1990).

IWG is important to agriculture for several reasons. As a highly productive cool-season forage grass (Robins 2010), it has increased the productivity of pasture and hay fields. As a long-lived perennial, IWG demonstrates resistance to many pests and diseases that affect wheat and other related cereal crops. IWG has been a valuable genetic resource for wheat improvement, due to the viability of interspecific crosses and homology with wheat genomes (Fedak and Han 2005; Friebe et al. 1996; Li and Wang 2009; Liu et al. 2011, 2013; Ohm and Anderson 2007; Sharma et al. 1995; Tang et al. 2000). During a search for new crops to enhance sustainability, it was also recognized for its relatively large seed size and its potential as a perennial grain crop (Wagoner 1990a). It was bred in the 1980s and 1990s for use as a perennial grain by the Rodale Institute and the USDA plant materials center at Big Flats, New York, USA (Wagoner and Jurgen 1990b). After more than a decade of evaluation, researchers at Rodale chose 14 genets with which to continue breeding for increased yield. Progeny from crosses between these individuals were used as the starting population of 1000 genets of IWG at The Land Institute in 2002. In each selection cycle, 50–70 genets with the most favorable phenotypes have been selected and intermated to produce the next generation of plants. Initial phenotypic evaluation spanned 2–3 years; however, phenotypes collected in the first year were representative of additional years of observation, allowing for annual rounds of selection. After just two cycles of selection, average seed size increased from a thousand kernel weight of 4.1–7.6 g (DeHaan et al. 2014).

In addition to improvements of seed size, breeders at The Land Institute have identified and selected for newly arisen 'domestication traits' that confer such drastic morphological changes that much of the population appears strikingly different from the wild accessions that were first brought to the USA. The leaves and stems of some plants are more than double the width of the widest measured leaf or stem in the original populations. Non-shattering and free-threshing have also been observed and selected for increased frequency. The cycle 3 population was evaluated during the Spring/Summer 2010 and one plant, C3-3471, was noted for its non-shattering and high yield of large, free-threshing seed and has been used in many crosses in The Land Institute breeding program. Three of the seven populations used for constructing the first consensus genetic map have C3-3471 as a parent or grandparent in its pedigree (Supplemental Figure 1).

Breeding programs to develop intermediate wheatgrass have since been initiated in Minnesota, USA and Manitoba, Canada (Runck et al. 2014). The program in Minnesota is part of the Forever Green Initiative, where new crops are being developed to enable sustainable intensification of production in the Upper Midwest, USA. A key portion of this effort has been new molecular marker technologies to enable rapid domestication of wild species (Zhang et al. 2016). Additional genetic resources, such as a dense genetic map, transcriptome, and reference genome, will help to accelerate breeding progress. The current work represents an initial investment in the development of those resources.

While traditional breeding via phenotypic screening and selection has resulted in clear improvements over the initial germplasm, individuals such as C3-3471 are rare and the free-threshing/non-shattering phenotype have been difficult to select through pedigree-based breeding. Modern genetic- and genomic-based approaches are needed to identify useful alleles for marker-assisted selection and to efficiently stack desirable traits. IWG, however, has an estimated 12.75 Gb genome (Vogel et al. 1999), no reference sequence, and minimal marker development. The large, understudied genome makes genetic studies and genomic-assisted breeding intractable. To address this, genotyping-by-sequencing (GBS) is an attractive approach for the development of genetic and genomic resources, because it combines molecular marker discovery and genotyping and has been successfully utilized in species with large, complex genomes, such as barley and wheat (Poland et al. 2012; Poland and Rife 2012).

To achieve our ultimate goal of accelerating the domestication and improvement of IWG using genomics-assisted breeding, reference genetic maps and genomics resources

Table 1 Summary of population data

Population	Parent 1	Parent 2	# Progeny	Location(s)
F253	C4-5353	C4-2856	142	The Land Institute, Salina, KS
F180/269	C4-5353/C4-8134	C4-8134/C4-5353	190	The Land Institute, Salina, KS
C3-3471xS	C3-3471	C3-3471	172	The Land Institute, Salina, KS
M26 × M35	M26	M35	376	The Land Institute, Salina, KS and USDA-ARS, Logan, UT
BA	WG116216	WG115302	130	University of MN, Saint Paul, MN
CA	WG117703	WG112027	131	University of MN, Saint Paul, MN
SS	C3-2331	C3-2595	176	University of MN, Saint Paul, MN

are needed. We utilized GBS to accomplish the following objectives: (1) identify thousands of SNPs for each of seven populations; (2) construct seven high-quality, marker-dense genetic linkage maps; (3) integrate the seven genetic maps using shared markers; and (4) investigate colinearity between the consensus genetic map and the physical map of barley.

Methods

Th. intermedium germplasm and genetic stocks

Six full-sib mapping populations and one self-derived family were chosen for marker development and genetic map construction. Some of the populations shared parents, giving overlap between the pedigrees of progeny. Pedigree and relationship information for all populations is depicted in Supplemental Figure 1 and a brief summary of population information is presented in Table 1. A more thorough description of each population follows.

Populations F253, F180/269, M26 × M35, and C3-3471xS were developed at The Land Institute (TLI) near Salina, KS. Population F253 consisted of an F_1 full-sib population of 142 individuals derived from a cross between two cycle 4 genets, C4-5353 and C4-2856. Population F180/269 consisted of an F_1 full-sib population of 190 individuals derived from two cycle 4 genets, C4-5353 and C4-8134.

C3-3471xS consisted of 172 individuals, self-derived from cycle 3 genet C3-3471. As a perennial plant with many stems and a large root system, portions of the crowns with new growth can be separated from the plant and propagated independently for further use. Plant C3-3471 was divided into 12 clones, and each clone was self-pollinated during Summer 2011 and Summer 2012. To prevent cross-pollination, heads from each plant were covered with a polypropylene monofilament 25-micron filter bag custom made to 12.7 × 50.8 cm with a drawstring (Baker Bags, Tamworth, NH). Additional clones were propagated and self-pollinated in Winter 2013. Due to suspicion of pollen contamination, paper baguette bread bags were used for subsequent self-fertilization efforts (WebstaurantStore, Lancaster, PA).

The M26 × M35 family is composed of 376 individuals descended from reciprocal crosses of two F_1 parental genets, M26 and M35. Vegetative propagules of the M26 × M35 population have been transplanted into replicated field evaluations at The Land Institute, near Salina, Kansas and the Utah Agriculture Experiment Station (UAES) in Providence, Utah. Source clones of the M26 × M35 genets are also maintained at the USDA ARS Forage and Range Research laboratory in Logan, UT.

Populations BA, CA, and SS were developed at a field site at the University of Minnesota, Saint Paul. Population BA consisted of an F_1 full-sib population with 130 individuals derived from a cross of genets WG116216 and WG115302. Population CA consisted of an F_1 full-sib population with 131 individuals derived from reciprocal crosses of genets WG117703 and WG112027. Population SS consisted of an F_1 full-sib population of 176 progeny, derived from reciprocal crosses of two cycle 3 genets, C3-2331 and C3-2595.

Genotyping

Young leaf tissue was collected and each sample was placed in a 96-well plate. The tissue was lyophilized for 3–4 days and ground with 3.9688 mm steel beads using a Retsch MM 400 tissue grinder. Genomic DNA was isolated using the Qiagen Biosprint 96 instrument and the associated Qiagen Biosprint DNA Plant kit. Genomic DNA was quantified with PicoGreen and 500 ng DNA was taken from each sample and normalized with a Qiagen QIAgility or Tecan Freedom EVO® robot so that each sample in the new 96-well microplate contained 25 µl ddH20 with a DNA concentration of 20 ng/ul.

Sequencing libraries were prepared using an adapted version of genotyping-by-sequencing (Elshire et al. 2011; Poland et al. 2012). The 384 ABCD barcodes set was used for all populations; however, several sequencing libraries were prepared with a mixture of two barcodes per sample

Table 2 Summary of GBS library preparation and sequencing information

Population	Barcodes mixture per sample	Plex-level	Sequencing platform	# Sequencing runs per sample	# Lanes of sequencing	Number (×1,000,000) barcoded reads per lane
F253	ABCD	95	Illumina HiSeq 2000	1	2	222.5 223.5
F180/269	ABCD	95	Illumina HiSeq 2000	1	2	218.6 225.8
C3-3471xS	AC	103	Illumina HiSeq 2000	1	1	176.4
	ABCD	80	Illumina HiSeq 2000	1[a]	1	214.8
	Ion-specific	80	Ion Proton	1[a]	1	72.7
M26 × M35	ABCD	95	Illumina HiSeq 2000	2 or 3[b]	11	207.2–245 Avg 221.1
SS	CD	47	Illumina HiSeq 2000	1	4	138.7–155.4 Avg 148.7
CA	CD	47	Illumina HiSeq 2500	1	2	278.9 279.9
BA	CD	95	Illumina HiSeq 2500	1	2	280.1 286.6

[a] These sequencing runs contained DNA from the same 80 individuals

[b] Some libraries from this population were sequenced twice, some three times

and others were prepared with a mixture of four barcodes per sample. The level of multiplexing per sequencing run ranged from 47 to 103 and each library was sequenced 1–3 times. A summary of this information per population, as well as sequencing platform per sequencing run, is included in Table 2.

The UNEAK bioinformatics pipeline (Lu et al. 2013) was used to filter reads and identify putative tag pairs for each group (Fig. 1). Default parameters were used to generate HapMap files; only the HapMap.hmc.txt file, which contains the allele counts for each genotype for each tag pair in each individual, was used for SNP calling.

To ensure marker quality for genetic map construction, filters were run using custom R (R Core Team 2016) scripts (Supplemental Document 1). First, tag pairs were tested for deviation from the expected 1:1 allele counts ratio for heterozygous genotypes; because IWG is an allohexaploid (functional diploid), the counts of each allele within a tag pair should be equal for all heterozygous genotype calls. For each marker, the counts of allele A and allele B were summed, respectively. The Chi square test was applied to test for deviation from the expected allele count ratio, with the null hypothesis that each marker fits the expected ratio. A threshold of p value <0.0001 on the Chi square test was used to discard markers. Second, populations were separated and markers with more than 40 % missing data in each population were discarded. Third, markers that were homozygous in both parents (or the one parent in the C3-3471xS population) were deleted. Fourth, a minimum of five allele counts were required to make a homozygous genotype call in cases where one allele was present and the other allele was not present. A heterozygous genotype was called when both alleles were present; however, for populations C3-3471xS, M26 × M35, F253, and F180/269, a heterozygous genotype was only called if the minor allele count accounted for greater than or equal to 10 % of the total counts for a given genotype. If the minor allele accounted for less than 10 % of the total counts (e.g., 20–1), the minor allele count was considered a likely genotyping error and the genotype call was homozygous for the major allele. For populations BA, CA, and SS, heterozygous genotypes were called when both alleles were present and the minor allele count was at least 2; if the minor allele count was 1 and the major allele count was over 20, the genotype was considered homozygous for the major allele. Genotype calls for all populations were made using a custom set of R scripts, included in Supplemental Document 1. The Chi square test in JoinMap 4.1 was applied to test for deviation from the expected genotype segregation ratios (Table 3).

Populations were then checked for unwanted self- and/or outcross contamination. We identified non-parental alleles in individuals using the R package related (Pew et al. 2015) and the software Cervus (Field Genetics Ltd.) (Kalinowski et al. 2007). Individuals found to not have the expected relationship to their parent(s) were not used in subsequent analyses.

Component maps construction

The resulting marker genotypes were coded according to three possible segregation types (<lmxll>, <hkxhk>, and <nnxnp>) for biallelic markers in the JoinMap 4.1 CP model for populations derived from a cross of two

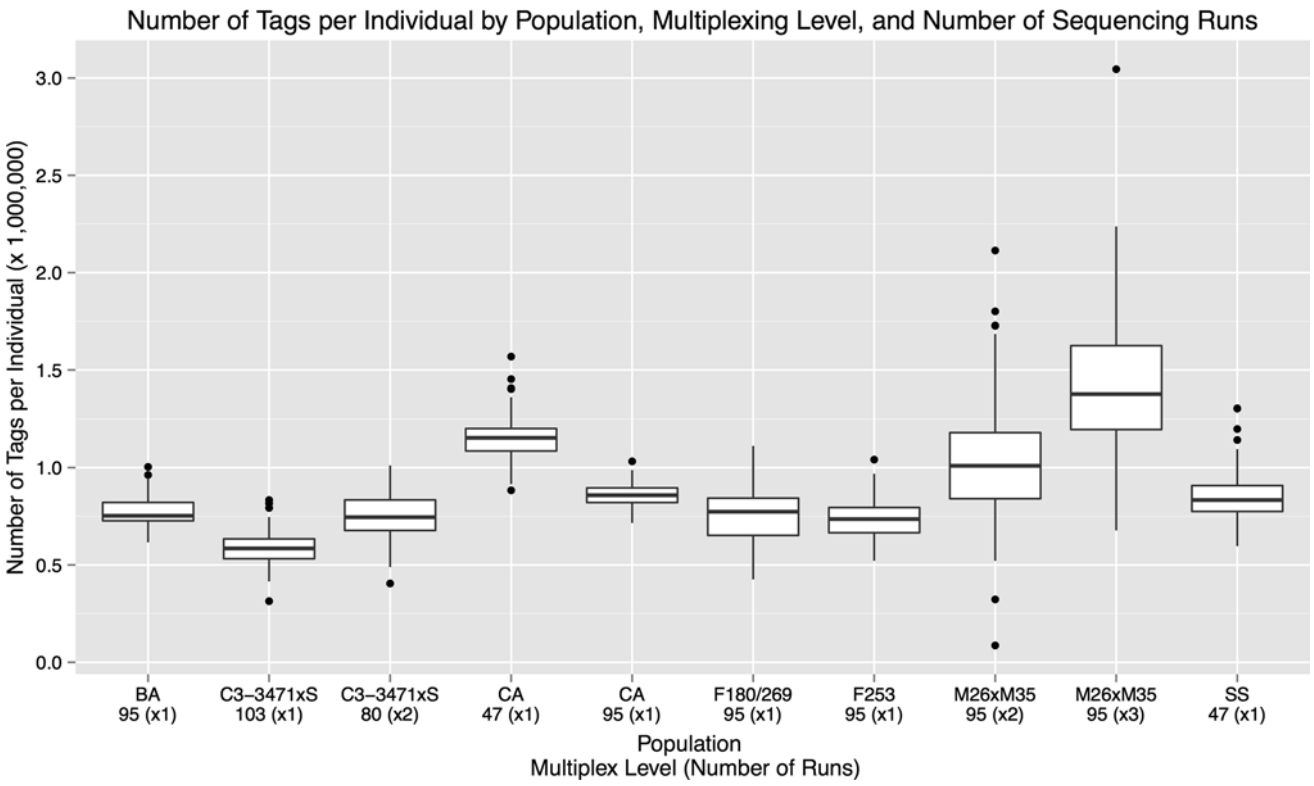

Fig. 1 Sequence read counts per population. Each *box* represents the mid-50 % distribution of tag counts per individual, grouped by DNA library; the *x*-axis labels provide the population, multi-plexing level, and how many times the library was sequenced

Table 3 Summary of markers included in the framework and final maps for each population

Population	Framework map—percent NA allowed per marker	Framework map—X2 significance threshold (segregation distortion)	Final map—percent NA allowed per marker	Final map—X2 significance threshold (segregation distortion)
F253	10	$p < 0.01$	20	$p < 0.01$
F180/269	12	$p < 0.01$	20	$p < 0.01$
C3-3471xS	12	No limit	25	No limit
M26 × M35	5	$p < 0.01$	25*	$p < 0.01$*
SS	3	$p < 0.01$	20	$p < 0.01$
CA	3	$p < 0.01$	10	$p < 0.01$
BA	3	$p < 0.01$	10	$p < 0.01$

* Allowed 29 markers that contained 25–40 % NA and/or a significance value beyond the threshold

heterogeneously heterozygous individuals (Van Ooijen 2006). Only markers with the segregation type <hkxhk> were used in Population C3-3471xS. JoinMap 4.1 determined the phases for markers in all populations.

We initially applied a threshold of less than 3 % missing data to construct a framework map for each population. However, this threshold was too strict for some populations with limited numbers of markers and we, therefore, relaxed the threshold for some populations (Table 3). The thresholds were chosen based on trial/error, using the minimum threshold necessary to achieve the expected number of linkage groups. For population C3-3471xS, it was necessary to allow markers with more missing data than listed to fill gaps in a few linkage groups of the framework map. For population M26 × M35, 29 markers that did not meet the missing data and segregation distortion thresholds were allowed to recover LG 6, including 11 markers that only had more missing data (between 25 and 40 %), 17 markers that only had higher Chi square significance values, and 1 marker that had more missing data and a higher Chi square significance value.

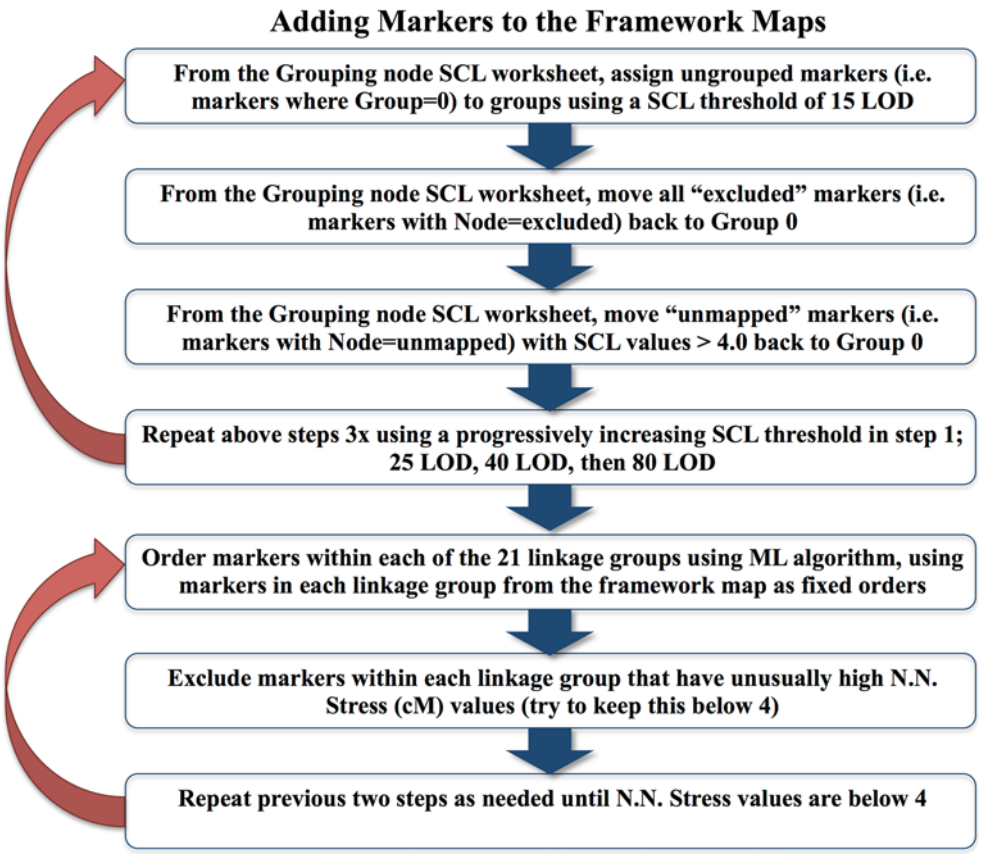

Fig. 2 Method used to add markers to each framework map

Genetic markers meeting the requirements listed in Table 3 were grouped in each population according to the independence LOD option, with a stepwise threshold of LOD 2-30, increasing by increments of LOD 1. At least 21 groups (the expected number of linkage groups) were chosen from the Groupings (tree) tab of the Population node. For a few populations, it was necessary to choose more than 21 linkage groups, as a few problem markers occasionally linked two linkage groups together at high LOD values. This became obvious when marker ordering was attempted for such a group. Smaller groups of markers were needed to identify and discard problem markers; these groups were then successfully combined to form a linkage group. The Maximum Likelihood algorithm (ML) was used for marker ordering and the Haldane function was used for map distance calculation. Within each linkage group, markers with the highest nearest neighbor stress (N.N. Stress) were deleted and the linkage group then re-ordered. This was repeated until no markers with a N.N. Stress level greater than 3 cM remained.

The framework maps were then compared with each other using custom R scripts to determine whether there were any major discrepancies in linkage group assignment and placement between shared markers. After resolving a few minor discrepancies, additional markers with more relaxed thresholds on missing data and segregation distortion were added to each map. To begin construction for each population's final map, a new grouping node was created using the final map nodes from each framework map's linkage groups. Within the grouping node, markers were added to the linkage groups according to the schematic diagram depicted in Fig. 2. Each new linkage group was ordered using the ML algorithm, with the markers from the framework map used as fixed orders, and the Haldane function was again used for map distance calculation.

Integrating linkage groups for a consensus map

Shared marker placement among linkage groups was compared across populations using custom R scripts so that each linkage group could be re-named to correspond to the same linkage group across populations. Marker order within the final seven maps was again compared using custom R scripts to determine whether there were inconsistencies between maps (Fig. 3). Each linkage group was then integrated using LPmerge (Endelman and Plomion 2014). LPmerge uses linear programming to minimize the mean absolute error between linkage maps from each population and the consensus map (Endelman and Plomion 2014). This minimization is accomplished with the use of linear inequality constraints that preserve the ordering of the markers across linkage groups. If marker

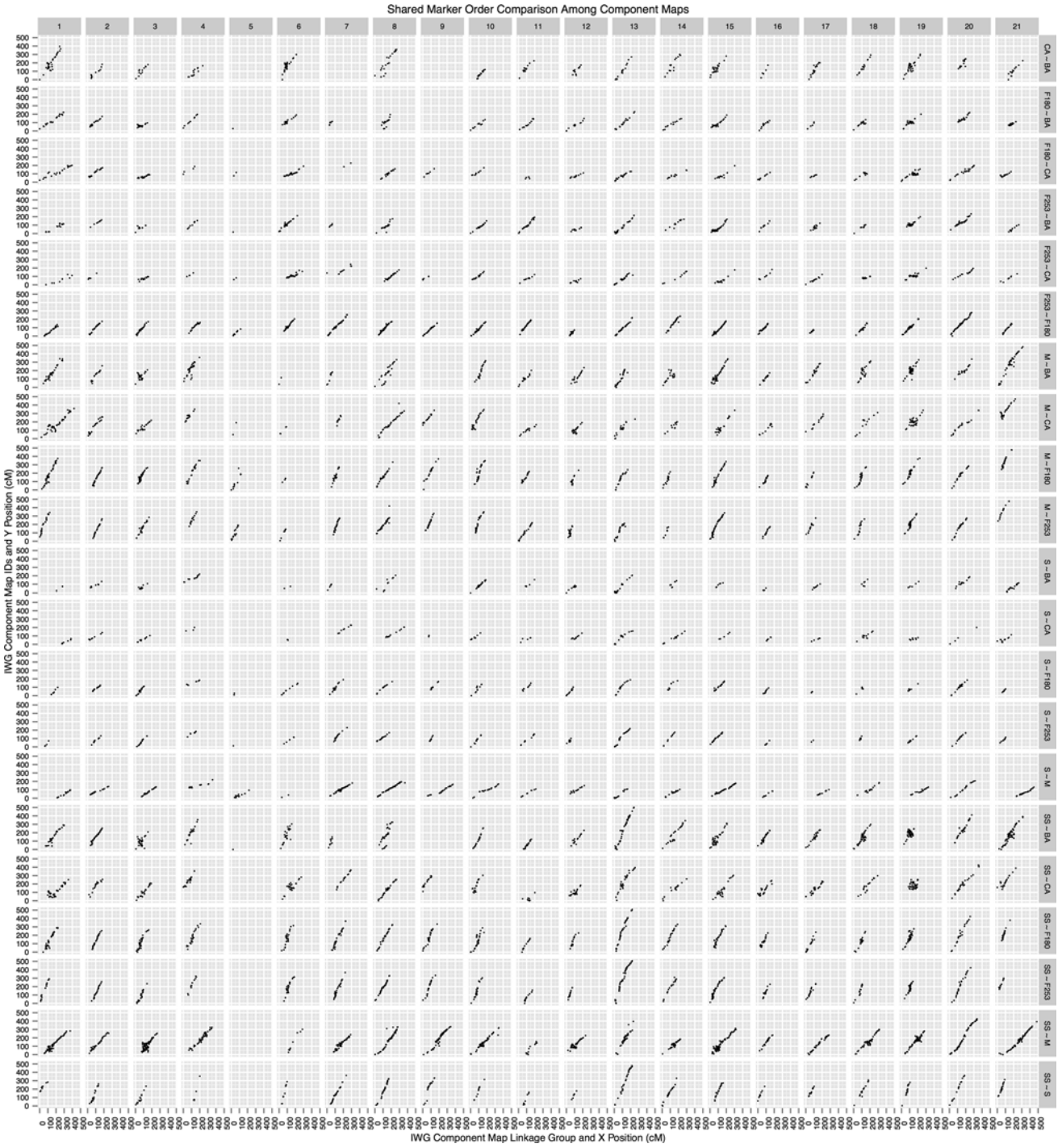

Fig. 3 Comparison of marker order among all IWG component genetic maps. Each row in the figure compares marker order between two maps. The *right y*-axis identifies the maps being compared, with the format MapY–MapX indicating that marker positions of MapY are on the *left y*-axis and MapX are on the *lower x*-axis. The *upper x*-axis identifies the linkage group being compared. *BA* map for BA population, *CA* map for CA population, *F180* map for F180/F269 population, *F253* map for F253 population, *M* map for M26 × M35 population, *S* map for C3-3471xS selfed population, and *SS* map for SS population

order is inconsistent between linkage groups, a minimum set of ordinal constraints is deleted to resolve the conflicts (Endelman and Plomion 2014). The maps from each population were weighted according to population size, as maps constructed with more individuals tend to be of higher quality (all other variables being equal) (Endelman

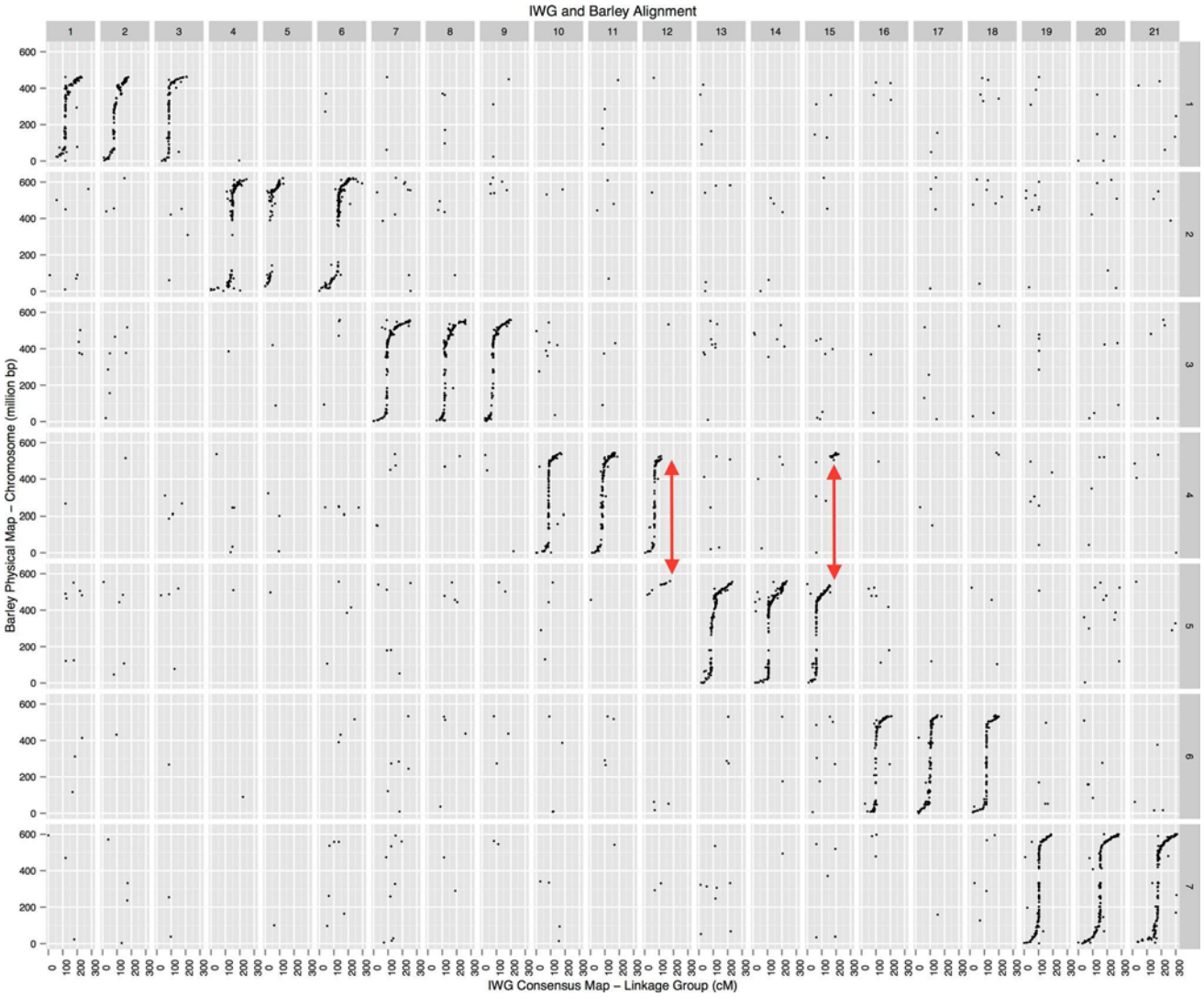

Fig. 4 Alignment of markers from the IWG consensus genetic map to the physical map of barley. Markers were aligned using BLASTN with a threshold of blast *e* value <1.00E−09. Chromosome translocations between group 4 and 5 are marked with *red arrows* (color figure online)

and Plomion 2014). Four consensus linkage groups were produced for each of the 21 linkage groups, one for each K value of $K = 1$–4. Most of the consensus linkage group selections minimized the root mean-squared error (RMSE), as recommended in the LPmerge manual. Occasionally, a consensus group was selected because of a smaller, more reasonable linkage group length, rather than the lowest RMSE, but only if the lowest RMSE was of a similar value to the selection's RMSE.

Comparing the IWG consensus map to barley's physical map

BLASTN was used to identify homologous sequences between the barley reference genome and GBS tags from IWG. Only markers with a blast e value <1E−09 were used for subsequent analyses. For multiple hits, only the best blast hits were considered. The locations of the hits within the barley genome were plotted against their locations within each of the 21 IWG linkage groups using custom R scripts (Fig. 4, Supplemental Document 1). Colinearity among syntenous markers in each IWG consensus linkage group and its homologous barley chromosome was measured as Spearman's rank correlation coefficient (ρ), where d_i is the difference in paired ranks and n is the number of cases:

$$\rho = 1 - \frac{6 \times \sum d_i^2}{n(n^2 - 1)}.$$

Table 4 Final number of individuals and markers included, as well as percent missing data allowed, per map

Population/ map	Number of individuals	Number of markers	% NA allowed (per marker)	% NA allowed (per individual)	Total map length (cM)	Number of LGs	Range of LG size (cM)	Range of number of markers per LG
F253	136	2123	20	25	3954	21	89–282	50–162
F180/269	184	2140	20	50	4238	21	118–276	44–163
C3-3471xS	170	1242	25	50	3474	21	97–243	31–109
M26 × M35	351	3856	25	40	7249	21	222–489	91–283
SS	172	3317	20	40	6545	21 (partial LG 5)	180–533 (LG 5 = 3 cM)	95–237 (LG 5 = 4 markers)
CA	115	1564	10	40	5366	21 (partial LG 5)	150–420 (LG 5 = 90 cM)	42–137 (LG 5 = 9 markers)
BA	131	1585	10	40	4273	20 (partial LG 5, no LG 9)	72–313 (LG 5 = 34 cM)	31–139 (LG 5 = 6 markers)

Results

Genotyping

DNA from seven populations was sequenced using GBS, and the raw data were processed with the UNEAK pipeline (Table 2; Supplemental Document 4). The multiplexing level and number of sequencing runs differed among populations, resulting in a wide range in the number of tags per individual (Fig. 2). Using the UNEAK pipeline, 741,828 unique tag pairs were identified across the populations.

Component maps

Quality filtering and discarding of uninformative markers for genetic map construction resulted in a range of ~2200–6700 SNPs with less than 25 % missing data per population. A lower level of multiplexing and/or an increased number of sequencing runs resulted in more markers for a given population. JoinMap v 4.1 was used to further filter individuals based on missing data and markers based on missing data, redundancy, and/or segregation distortion. After deleting additional markers with high N.N. Stress levels, the final number of markers remaining in the maps ranged from 1242 to 3856. Most of the component maps recovered the expected 21 linkage groups, with agreement in linkage group assignment for shared markers and only minor discrepancies in marker order within linkage groups (Fig. 3). For the BA population, only 20 linkage groups were identified; linkage group 9 was not recovered. Additionally, linkage group 5 was only partially recovered in populations BA, CA, and SS. Total map length varied across populations and ranged from 3474 to 7249 cM, with size increasing with the number of markers (Table 4).

Clustering of markers with significant genotypic segregation distortion was observed in all maps (Supplemental Figure 2). In particular, we identified chromosome regions where at least half of the markers had significant unphased genotypic segregation distortion and occurred within a 50 cM area of a linkage group. Within map C3-3471xS, clusters were observed in 11 linkage groups (LGs 1, 3, 4, 6, 7, 8, 10, 13, 14, 15, and 20). Map M26 × M35 also contained widespread segregation distortion, with clusters of distorted markers present in 10 linkage groups (LGs 6, 7, 8, 13, 14, 15, 17, 18, 19, and 20). Only two linkage groups (LGs 2 and 17) in map F253 each contained a cluster of distorted markers. Within map BA, one cluster was observed in linkage groups 2, 3, 5, and 13, with all six markers in LG 5 showing segregation distortion. In map CA, over 50 % of markers showed segregation distortion in LG 1 and LG 5 (only nine markers total), with one cluster in each LG 4 and LG 11. Within map SS, six linkage groups contained a cluster of distorted markers (LGs 1, 5, 6, 11, 13, 15, and 18).

Consensus map

The consensus map contained 10,029 markers in 21 linkage groups, matching the number of haploid chromosomes for IWG (Supplemental Document 2, Supplemental Figure 3). Of the 10,029 markers in the consensus map, 3601 markers were shared between at least two populations. The 21 linkage groups covered 5061 cM, with an average distance of 0.5 cM between each pair of adjacent markers. Linkage groups ranged in size from 144 to 324 cM and contained between 237 and 683 markers, with a linkage group containing on average 478 markers distributed over 241 cM (Table 5).

Table 5 Summary information for each consensus linkage group, including the number of markers, length, and *K* value of each linkage group selected for the consensus map

Linkage group	LPmerge *K* value	Number of markers	Length (cM)
1	3	521	278
2	1	396	185
3	1	375	229
4	1	448	299
5	4	237	144
6	1	537	318
7	1	586	277
8	3	559	288
9	1	451	219
10	1	497	190
11	1	398	192
12	1	313	185
13	1	579	245
14	1	547	256
15	1	546	220
16	1	336	228
17	1	372	246
18	1	451	222
19	1	583	220
20	2	683	298
21	1	612	324

BLASTN was used to identify 3620 markers that mapped to barley chromosomes with an e value <1.00E−09. Of these markers, 86.3 % were found to be syntenous, mapping to the expected homologous barley chromosome (Fig. 4). To add consistency, the IWG linkage groups were numbered to match chromosome order in barley. IWG linkage groups 1–3 are homoeologous to each other and homologous to barley chromosome 1; IWG linkage groups 4–6 are homoeologous to each other and homologous to barley chromosome 2, etc.

Using IWG and barley marker order information for the 3124 syntenous markers, colinearity between each IWG consensus linkage group and its homologous barley chromosome was measured with Spearman's rank correlation coefficient (ρ), as described in methods. Spearman's ρ averaged 0.9 across all linkage groups, ranging from 0.8 to 0.97 between each IWG linkage group and its homologous barley chromosome, with LG 3 as an outlier at 0.69 (Supplemental Table 1).

We observed a known translocation (King et al. 1994) between group four and five chromosomes in IWG relative to the barley genome (Fig. 4). This translocation corresponded to ~55 cM in the IWG consensus map and ~18 Mb in the barley genome.

Discussion

Component maps

Marker order among the component maps was relatively colinear, with some minor disagreements in marker order that could not be resolved due to imperfect data (Fig. 3). ll maps contained linkage groups with regions of segregation distortion, which is often detected in mapping populations (Liu et al. 2010; Lu et al. 2002; Luo and Xu 2003; Taylor and Ingvarsson 2003). Clustering of markers with segregation distortion was observed in 11 linkage groups in map C3-3471xS, 10 linkage groups in map M26 × M35, one linkage group in maps F253 and F180/269, seven linkage groups in map SS, and four linkage groups in maps BA and CA. Markers with significant segregation distortion were mostly biased toward heterozygous genotypes (all markers should segregate 1:2:1 or 1:1, as described in methods). There are two likely reasons for this bias, one technical and the other biological. First, distortion may be expected in markers with more missing data, as homozygous genotypes require more allele counts to call the genotype than a heterozygous genotype and will be underrepresented in markers with low allele counts. For this reason, if more than 50 % of the markers in a putative cluster had >15 % missing data, that putative cluster was ignored. Second, recessive deleterious alleles, reflecting the biological reality of genetic load, will be exposed in populations that are more inbred (Morgan 2001). The C3-3471xS population was inbred by definition, as it was derived from self-fertilization of one individual. The M26 × M35 population was also relatively inbred for an outcrossing species (expected $F = 0.125$; see Supplemental Figure 1 for the pedigree). In comparison, the F253 and F180/269 populations were derived from parents from distinct genetic pools and, subsequently, had less observed segregation distortion. Populations with deliberate inbreeding in the pedigree (populations C3-3471xS and M26 × M35) had on average three times the number of linkage groups with segregation distortion, lending support to the hypothesis that the segregation distortion is due at least in part to genetic load.

Segregation distortion is usually observed in regions where genes related to reproductive barriers are located (Armstead et al. 2008; Yang et al. 2012). The most severe and widespread segregation distortion occurred on linkage groups 1–6 (Fig. 5, Supplemental Figure 2), which are homologous to barley and chromosomes 1 and 2 (and wheat chromosome groups 1 and 2). This distortion may be due to incompatibilities of the well-documented self-incompatibility (SI) system in the grasses (Baumann et al. 2000; Cornish et al. 1980; Klaas et al. 2011). This SI system (S–Z) is gametophytically controlled and polyallelic at the S and Z loci, which are located toward the middle of chromosome

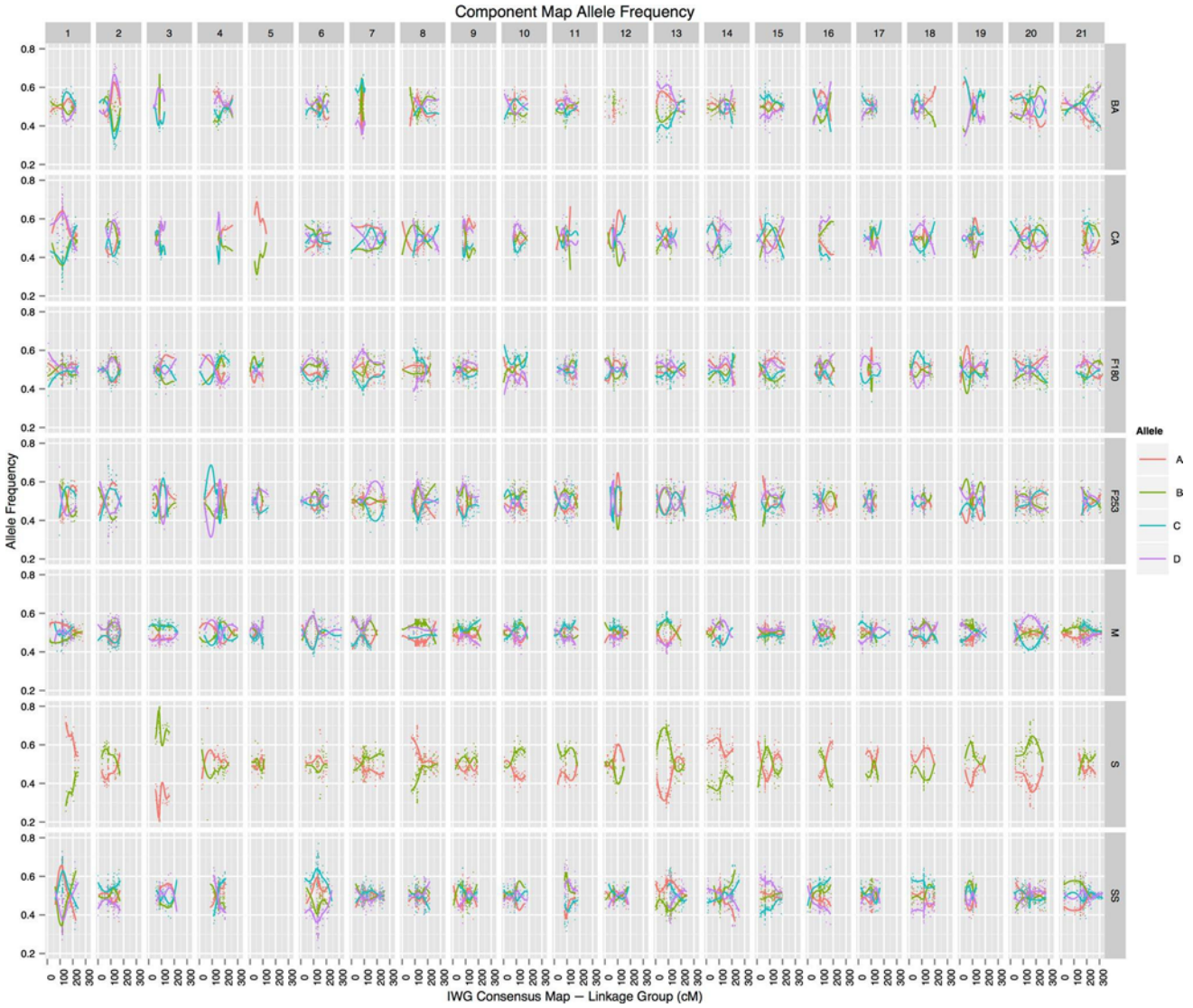

Fig. 5 Parental allele frequency distributions across component maps using the consensus map positions fit with a LOESS curve to visualize allele frequency trends. Alleles A and B are derived from parent 1 and alleles C and D are derived from parent 2. Expected allele frequency for all alleles is 0.5*. *BA* map BA, *CA* map CA, *F180* map F180/F269, *F253* map F253, *M* map M26 × M35, *S* map C3-3471xS, *SS* map SS. *Note: For segregation types <lm × ll> or <nn × np>, expected allele frequency is theoretically 0.25 for m or p alleles or 0.75 for l or n alleles; however, phase is unknown for the homozygous parent, so those alleles are excluded from this graph, bringing the expected allele frequencies to 0.5

1 and in the mid-lower region of chromosome 2, respectively, across the Triticeae (Thorogood et al. 2002); these regions showed extensive allelic (phased parental alleles) and genotypic (unphased) segregation distortion in several component maps (Fig. 5, Supplemental Figure 2, respectively). At least one of the S or Z alleles must be different between the pollen and pistil for a compatible pollination, which can lead to 0, 25, 50, or 100 % compatibility (Baumann et al. 2000). For example, segregation distortion in hybrids of two self-incompatible perennial wild rye species was apparently caused by epistatic interactions of different alleles of the S and Z loci on linkage groups 1a and 2a, respectively, manifested by co-segregation of markers on different chromosomes (Klaas et al. 2011).

Consensus map

This is the first consensus genetic map for IWG. The total map length, 5061 cM, reflects the average length of the seven component maps, weighted by population size. The map distances of the component maps, and therefore the consensus map, are inflated relative to other related species (Li et al. 2015; Zhou et al. 2015), which is likely due to missing data and genotyping errors, combined with the

large number of markers (10,029) and use of the ML algorithm and Haldane function (Buetow 1991; Hackett and Broadfoot 2002). To investigate the extent to which the number of markers and the use of the ML algorithm play in map distance inflation in our maps, 10 linkage groups from map M26 × M35 and 20 linkage groups from map F253 were re-made with even more strict thresholds than the framework maps so that linkage groups contained fewer than 100 markers and the Regression algorithm could be used to order markers. Distances were estimated with Kosambi's mapping function. These methods resulted in linkage groups that were 70–170 cM (LG lengths averaged 39 % of final lengths in M25xM35 and 66 % of final lengths in F253) and projected map lengths of 2600–2800 cM. This is close to the Kosambi distance of the consensus map (2891 cM) when the distance is calculated using the Haldane to Kosambi equation provided in the JoinMap manual. Additionally, the correlation between number of markers per map and total map length was $r = 0.874$, $p < 0.01$. These investigations support (1) the idea that the large number of markers and use of the ML algorithm are the primary causes for map distance inflation and (2) the integrity of the component maps and the resulting consensus map.

Overall, the homologous chromosomes of IWG and barley shared extensive conservation of synteny, supporting a high-quality consensus map. However, there were two inconsistencies between the IWG consensus map and barley's physical map. First, LG 12 and LG 15, which are homologous with barley chromosomes 4 and 5, respectively, showed evidence of a reciprocal translocation on their distal long arms. Second, there was a lack of markers mapping to the centromeric regions of some of the homologous barley chromosomes. Both these features have been observed in Triticeae. The chromosomes of the Triticeae are mostly colinear, with occasional chromosomal translocations (King et al. 1994). Bread wheat (*Triticum aestivum*) has a 4AL–5AL–7BS translocation that is shared with several closely related species (Hao et al. 2013). The 4AL–5AL portion of the translocation is between the terminal ends of the chromosomes and occurred at the diploid level, as it occurs in the diploid species *Triticum uratu* and *Triticum monococcum*. The translocation was proposed to be derived from a common ancestor, as similar translocations have been observed in other Triticeae species, including *Secale cereal* (Devos et al. 1993; King et al. 1994), and one of the two subgenomes of allotetraploid *Leymus cinereus* (Devos et al. 1995; King et al. 1994; Larson et al. 2012). Presumably, the distal portion of the chromosome 5A translocation on 4AL then exchanged with a terminal portion from chromosome 7BS in *T. turgidum*. The 4AL-5AL-7BS wheat translocation signature has been observed across all eight subspecies of *T. turgidum* (Hao et al. 2013).

As this reciprocal location has been identified in many species, presumably IWG linkage groups 12 and 15 belong to the same sub-genome; no evidence was found for another translocation within the linkage groups homologous to chromosomes 4 or 5. If this is the case, it is most likely that this subgenome is the speculated *Pseudoroegneria strigosa*, as the other two subgenomes are very closely related to each other and both would be expected to share any translocation present.

In most linkage groups, there are gaps near the centromeric region where few IWG genetic markers map to barley's physical map; these gaps are most pronounced in linkage groups homologous to barley chromosome 2 (Fig. 4). A lack of colinearity was also detected at the most centromeric regions when a high-density genetic map for wheat (*Triticum aestivum*) was compared with barley's physical map (Iehisa et al. 2014). IWG, like wheat, has a very large genome that likely shares features with the genomes of barley and wheat that could be responsible for this lack of colinearity in proximal chromosomal regions, which contain relatively large numbers of repetitive sequences and low gene density (Luo et al. 2013; The_International_Barley_Genome_Sequencing_Consortium 2012).

Conclusions

For genetic map construction for a highly outcrossing species, unless the goal is to identify regions associated with reproductive barriers, one should test for and use unrelated plants to make F1 populations. We found that it was difficult to determine which markers to discard and which to keep because it was biologically possible to have severe segregation distortion in inbred populations.

This study presents the first consensus genetic map of intermediate wheatgrass (*Thinopyrum intermedium*). It was produced using GBS data from seven mapping populations and included 10,029 SNPs distributed across 21 linkage groups, covering 5061 cM, with an average intermarker distance of 0.5 cM. This map is being used to map loci associated with agriculturally important phenotypes in *Th. intermedium* and provides a framework for future genetic studies for IWG, perennial wheat breeding (identifying IWG specific markers), and annual wheat breeding (identifying markers for disease resistance and introgressions of *Th. intermedium* in the wheat background). One of the component maps has already been used to study the potential for genomic selection in IWG (Zhang et al. 2016).

The spread of agriculture into marginal lands, climate disruption, and a growing global population contribute to the rising concern over food security. The cultivation of perennial species improved through targeted domestication

and selection has been proposed as an approach to facilitate the sustainable intensification of agriculture, conserving natural resources while producing food (Cattani 2014; FAO 2014; Glover 2014). The development of genetic and genomic resources for IWG has both short- and long-term benefits by providing resources to assist in both averting immediate food security by improving bread wheat with disease resistance and improving IWG as a sustainable source of grain, forage, and/or biofuel. The results from this study, in combination with future phenotyping and genotyping, will allow for the implementation of marker-assisted and genomic selection to rapidly fix beneficial alleles in the breeding population. These advances will not only improve the efficiency of the IWG breeding program, but also provide a case study for the domestication of other perennial species, moving our agriculture systems toward sustainable intensification.

Author contribution statement Designed and planned experiments: TK, SL, XZ, LD, JB, JA, JP; Developed genetic stocks: LD, XZ; Generated data: TK, XZ; Analyzed results: TK, SL, XZ, JP; Wrote manuscript: TK, XZ, JP. All authors reviewed and approved the final manuscript.

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Compliance with ethical standards

Ethical standard The authors declare that the experiments comply with the current laws in the United States of America.

Conflict of interest The authors declare that they have no conflict of interest.

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