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Comparative mapping of the Oregon Wolfe Barley using doubled haploid lines derived from female and male gametes

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Abstract The Oregon Wolfe Barley mapping population is a resource for genetics research and instruction. Prior reports are based on a population of doubled haploid (DH) lines developed by the *Hordeum bulbosum* (H.b.) method, which samples female gametes. We developed new DH lines from the same cross using anther culture (A.C.), which samples male gametes. Linkage maps were generated in each of the two subpopulations using the same 1,328 single nucleotide polymorphism markers. The linkage maps based on DH lines derived from the products of megasporogeneis and microsporogenesis revealed minor differences in terms of estimated recombination rates. There were no differences in locus ordering. There was greater segregation distortion in the A.C.-derived subpopulation than in the H.b.-derived subpopulation, but in the region showing the greatest distortion, the cause was more likely allelic variation at the *ZEO1* plant height locus rather than to DH production method. The effects of segregation distortion and pleiotropy had greater impacts on estimates of quantitative trait locus effect than population size for reproductive fitness traits assayed under greenhouse conditions. The Oregon Wolfe Barley (OWB) population and data are community resources. Seed is available from three distribution centers located in North America, Europe, and Asia. Details on ordering seed sets, as well as complete genotype and phenotype data files, are available at http://wheat.pw.usda.gov/ggpages/maps/OWB/.

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Introduction

Doubled haploid (DH) techniques, by accelerating the approach to homozygosity, are a useful tool for conventional and molecular plant breeding (Thomas et al. 2003; Forster et al. 2007). In the case of barley, DH populations have been widely used for constructing the linkage maps that underlie quantitative trait locus (QTL) mapping and marker-assisted selection (MAS) (Karakousis et al. 2003; Wenzl et al. 2006; Stein et al. 2007; Hearden et al. 2007; Varshney et al. 2007; Szücs et al. 2009; Close et al. 2009; http://wheat.pw.usda.gov/GG2/index.shtml).

Barley DH populations can be produced using female or male gametes. The former involves the interspecific crossing of an F_1 (as the female) with *Hordeum bulbosum*, followed by embryo rescue, plant regeneration, and artificial chromosome doubling (Kasha and Kao 1970). The latter involves using the F_1 (as the male) followed by

anther or microspore culture (Maluszynski et al. 2003). The potential efficiencies of androgenetic systems are much greater than those of gynogenetic systems because each inflorescence produces more pollen than eggs.

The principal issues related to the use of DH populations for linkage map construction relate to segregation and recombination. Segregation distortion—the deviation of observed genotypic frequencies from their expected values—complicates the application of genetic theory and analysis (Lu et al. 2002). The allele transmission and gamete survival frequencies that cause segregation distortion can be caused by exogenous factors such as temperature (Xu et al. 1997) and in vitro culture conditions (Graner et al. 1991; Foisset and Delourne 1996; Manninen 2000). Distortion can also be caused by genetic factors (Lambrides et al. 2004; Törjék et al. 2006) and may be more prevalent in some species than in others (Lu et al. 2002; Marshall et al. 2007). The implications of segregation distortion for genetic analyses and breeding are reviewed by Xian-Liang et al. (2006). To generalize—in the case of androgenetic systems in wheat, barley, and rice—it appears that selection for genes favoring microspore growth and development in culture media may not have negative effects on agronomic traits in the derived populations (Ma et al. 1999; Guzy-Wrobelska and Szarejko 2003; Cistué et al. 2005; Lapitan et al. 2009).

Representative linkage maps require "normal" rates of recombination between homologous chromosomes (Lenormand and Dutheil 2005). The levels of recombination observed in DH populations derived by the *H. bulbosum* technique (hereafter referred to as H.b.) and anther culture (hereafter referred to as A.C.) reveal the crossover frequencies in the megaspore and microspore mother cells. For barley, Devaux et al. (1995) found 1.047 and 0.912 recombination events per chromosome in A.C.- and H.b.-derived DH populations, respectively. Recent studies have demonstrated the effects of various factors on rates of recombination and the distribution of recombination breakpoints in plants (Li et al. 2007). Genome regions where recombination rates are significantly higher or lower than the genome average are termed recombination hot- and cold-spots, respectively (Mezard 2006), and in barley the relationship between genetic and physical maps was explored in depth by Kuenzel et al. (2000).

Of the many barley linkage mapping populations available (summarized in GrainGenes; http://wheat.pw.usda.gov/GG2/index.shtml), one of the most widely used is the Oregon Wolfe Barley (OWB). This population of DH lines was developed by the H.b. technique from the F_1 of the cross between the dominant and recessive morphological marker stocks developed by Wolfe (1972). The alternative alleles at the morphological traits loci determining the major germplasm groups of barley are represented in this population (Costa et al. 2001) and the sequential addition of new generations of markers (e.g. restriction fragment length polymorphisms (RFLP), amplified length fragment polymorphisms (AFLPs), simple sequence repeats (SSRs), diversity array technologies (DArTs), single nucleotide polymorphisms (SNPs), and restriction site-associated DNAs (RADs) has allowed the population to serve as a resource for linkage map and QTL integration. Szücs et al. (2009) integrated prior marker data with 1,472 SNPs represented in the three barley pilot oligonucleotide pooled assay (OPAs) (POPA1, 2, and 3) and Chutimanitsakun et al. (submitted) added over 450 RAD loci to the Szücs et al. (2009) map. Qualitative and quantitative loci determining morphological, phenological, and disease-resistance traits of importance to barley improvement have been mapped in the OWB (Börner et al. 2002; Costa et al. 2001; Jafary et al. 2008; Rostoks et al. 2005; Stein et al. 2007). However, as empirically demonstrated by Vales et al. (2005), limited population size can lead to underestimation of QTL number, overestimation of QTL effects, and failure to quantify QTL interactions.

The goal of this project was to increase the size of the OWB mapping population in order to improve it as a resource for genetic mapping and QTL detection. Since the A.C. technique was used to develop the new DH plants, we were able to compare linkage maps based on A.C.- and H.b.-derived subpopulations genotyped with the same high-throughput SNP assays. This allowed for direct comparisons of segregation distortion and linkage distance with the each of the two linkage maps and empirical assessment of improvements in QTL detection afforded by doubling the size of the mapping population.

Materials and methods

Plant materials

Complete information on the "Oregon Wolfe Barley" population can be found at http://barleyworld.org/oregonwolfe.php. Briefly, the mapping of the original set of 94 H.b.-derived plants was described by Costa et al. (2001), a population of 93 was mapped by Szücs et al. (2009) due to incomplete data on one line, and 82 were mapped by Chutimanitsakun et al. (submitted) after the discovery of 9 sets of identical DH lines. The reduction in population size has not affected estimates of locus order, distance, nor estimate of QTL number and effect (see "OWB population size" at (http://wheat.pw.usda.gov/ggpages/maps/OWB/). The discovery of the identical sets of lines was not made until after the research described in this paper was undertaken; as a consequence, this report was intended to compare maps based on 93 H.b.-derived

lines and 93 A.C.-derived lines. However, in order not to confound comparisons by the possible effects of identical genotypes, in this report, we compare the H.b. ($n = 82$), A.C. ($n = 93$) subpopulations, and combined populations ($n = 175$). The 93 A.C.-derived lines were produced from the F_1 of the cross between the recessive (OWB-R) and dominant (OWB-D) morphological marker spring barley genetic stocks (Wolfe 1972; Wolfe and Franckowiak 1991) as described by Cistué et al. (2003). This method includes a stress pre-treatment of the anthers with 0.7 M mannitol for 4 days at 24°C followed by 25 days on a modified FHG culture medium (Hunter 1988). Embryos were transplanted to FHG regeneration medium. DH lines were obtained by spontaneous doubling of the chromosome number of each haploid plant.

For measuring reproductive fitness traits (see "Phenotypes", below), the two sets of DH lines (H.b.-derived and A.C.-derived) were grown in separate greenhouse experiments at Oregon State University, Corvallis, OR, USA. For both experiments, greenhouse temperatures were 18 ± 1.5°C (day and night) with a photoperiod of 16 h day/8 h night provided by supplemental illumination from Sylvania Lumalux-Eco ET18 400 w lights suspended 1.5 m over the bench surface. Each DH line was replicated twice. The parents were replicated four times.

Markers

The process of developing the high confidence SNP markers has been described in detail by Close et al. (2009). Briefly, SNPs observed in ESTs and sequenced amplicons were used to design three Illumina 1536-plex pilot Oligonucleotide Pool Assays (pilot OPAs; POPA1, POPA2, POPA3). Based on the technical performance and other criteria, 3,072 SNPs were selected from three POPAs to generate two production barley OPAs (BOPA1 and BOPA2). The H.b.-derived OWB population was genotyped with three POPAs, whereas the A.C.-derived population was genotyped with two BOPAs. The common SNPs mapped on both H.b- and A.C-derived populations were used for analysis in this study. The SNP genotyping assays were conducted using the Illumina GoldenGate BeadArray SNP detection platform. The POPA assays were conducted at the Southern California Genotyping Consortium at the University of California, Los Angeles. Genomic DNA extractions for A.C.-derived population and BOPA SNP assays were performed at USDA-ARS Small Grains Genotyping Center in Fargo, ND. The naming convention for SNP loci appearing on the maps shown in this report is by the POPA numbers (e.g. 1_1311), where 1 = the POPA number (POPA1 in this case) and the subsequent four digits correspond to the SNP order in the corresponding POPA.

Mapping

JoinMap 4 (Van Ooijen 2006) was used for map construction. SNP-only maps were constructed for the 82 H.b.-derived DH lines and the 93 A.C.-derived DH lines. The two data sets were then merged and a combined map made based on 175 DH lines. For each of the data sets, at linkage LOD score 5, the 1,328 polymorphic markers formed seven linkage groups. The Monte Carlo maximum likelihood (ML) mapping algorithm was used to determine the marker order in each of the seven linkage groups. Recombination frequencies were converted to centiMorgans (cM) using Haldane's mapping function. The positions of ten morphological loci (*VRS1, ZEO1, WST1, ALM, HSH, SRH, ROB, GBSS1 (WX), NUD,* and *LKS2*) are shown on the A.C.-derived and combined map based on the flanking SNPs reported by Szücs et al. (2009).

Segregation distortion and recombination events

A full genome assessment of segregation distortion involves a series of chi-square tests for equality of allelic frequencies for every individual locus. This requires a multiple test correction of the level for assessing significance of each individual test. A very conservative Bonferroni correction would simply take the significance level for individual markers as the genome-wide level divided by the total number of polymorphic markers, (1,328 in this study) Thus, for 0.05, the required significance level for assessing significance of each test would be $\alpha_{BON} = 0.05/1328 \approx 0.00004$. However, this approach wrongly assumes independence of markers. Less conservative corrections make an effort to consider dependence between markers, as will be the case with markers within a linkage group. One such method estimates the effective number of independent tests across the genome by dividing the total map length in cM by 20–30 cM, which is an arbitrarily taken distance between any two marker pairs for relative independence (van Eeuwijk, personal communication). In this study the number of effective independent test would be approximately equal to 50, that is the ratio between 1,250 cM, reported roughly map length of barley, and 25 cM arbitrarily taken for relative independence. The significance level for individual tests would be equal to the genome-wide significance level divided by 50, the number of putative independent tests. This criterion would lead to an, $\alpha'_{0.05} = 0.001$, $\alpha'_{0.01} = 0.0002$, and $\alpha'_{0.001} = 0.00002$. Allelic frequencies giving rise to these significance levels are shown in Fig. 3 by differential dotted lines.

The number of apparent single crossovers (CO) was counted in each linkage group of each of the 175 DH. These values underestimate the actual number due to undetected CO in regions of monomorphic markers. An

analysis of variance was performed, using GenStat (Payne 2006), to test for significant differences between DH production method and between chromosomes.

Phenotypes

Phenology and reproductive fitness (yield component) traits were measured on each DH line as follows. Heading date (HD) was recorded as the number of days from seedling emergence until the first appearance of awns (or hoods). The number of fertile tillers per plant (spike number; SN) was counted and the spikes were harvested from each fertile tiller. Three fertile spikes were selected at random and used to determine: spike length (SL, cm), number of florets per spike (floret number, FN), number of grains per spike (grain number, GN), hundred grain weight (HGW, g), and plant height (PH, cm). Repeatability estimates for each of the phenotypic characters are shown in Supplemental Table 5.

QTL mapping

Quantitative trait locus analyses for each of the phenotypic characters were conducted for each of the data sets (82 DH-H.b., 93 DH-A.C., 175 DH-H.b. + A.C.) using the composite interval mapping (CIM) procedure (Zeng 1994) implemented in Windows QTL Cartographer 2.5 (Wang et al. 2001–2003). Skeleton maps—constructed from a set of 622 evenly distributed and non-co-segregating markers were used for QTL analysis (Supplemental Fig. 2). A forward-selection backward-elimination stepwise regression procedure was used to identify co-factors for CIM. The maximum number of cofactors used was seven. A 30-cM scan window was used for all analyses. Experiment-wise significance likelihood ratio (LR) test statistic thresholds ($P < 0.05$) for QTL identification were determined with 1,000 permutations and expressed as LOD (LOD = 0.217LR). For every significant QTL, we calculated individual R^2 (proportion of phenotypic variance explained by the individual QTL) and additive effect (expressed as one half of the difference between the two allelic classes). Negative values indicate that the parent line contributing the allele with the highest value was OWB-R. Epistatic interactions between QTL were tested by means of multiple interval mapping (MIM, Kao et al. 1999) using Windows QTL Cartographer 2.5 and a score statistic test with significance level of 0.05. Using MIM we also calculated for each trait the R^2 of the multilocus model that included the QTL detected in the CIM analysis (main and interaction effects). All genotype and phenotype data are available at http://wheat.pw.usda.gov/ggpages/maps/OWB/.

Results

The numbers of SNPs mapping to each chromosome are shown in Table 1. Of the 3,072 SNPs represented on BOPA1 and BOPA2, a total of 1,328 (43%) were mapped in the H.b.- and A.C.-derived sub-populations. The lengths (in Haldane cM) for five of the seven chromosomes (for the sake of brevity, the term "chromosome" will be considered synonymous with "linkage group") were longer for the A.C.-derived than they were for the H.b-derived population (Fig. 2 and Supplemental Fig. 1). However, these differences were only significant for 5H (Fig. 1). For this chromosome, the H.b. map is significantly longer. In the map, based on the combined data from the H.b.- and A.C.-derived populations (hereafter referred to as the

Table 1 Number of SNP loci used for mapping each of the seven chromosomes of barley in *Hordeum bulbosum*-derived (H.b.) and anther culture-derived (A.C.) doubled haploid (DH) populations of barley, as well as in the combined (H.b. + A.C.) population and the total length of each linkage group, and all linkage groups, in Haldane cM

Chrom.	Number of SNP	H.b. 82 DH	A.C. 93 DH	H.b. + A.C. 175 DH
1H	148	157.3	186.2	171.2
2H	199	188.8	193.8	190.4
3H	211	205.8	208.7	205.4
4H	187	127.4	156.9	141.9
5H	236	240.2	200.4	216.7
6H	182	159.1	146.3	151.0
7H	173	206.6	225.8	215.6
Total	1,328	1,285.2	1,318.1	1,292.2

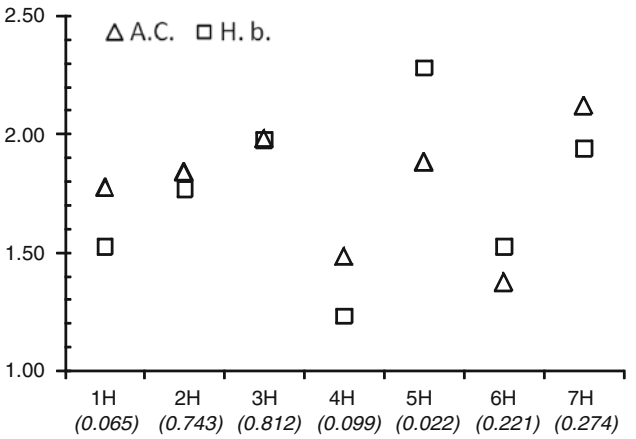


Fig. 1 Number of apparent crossovers for each of the seven chromosomes of barley in *Hordeum bulbosum*-derived (*H.b.*) and anther culture-derived (A.C.) doubled haploid populations of the Oregon Wolfe Barley. *Numbers* on the *X*-axis below chromosome numbers represent *p* values for the statistical contrasts between doubled haploid production methods, using square root-transformed data

combined population and/or combined map) cM values are intermediate between those for the two sub-populations. The average number of apparent crossovers (hereafter referred to as crossovers) per chromosome for each of the two subpopulations are shown graphically in Fig. 1; the significant differences between chromosomes (averaged across methods) are apparent, as are the significant interactions between method and chromosome (Supplemental Table 1).

Marker orders are consistent between the A.C. and the H.b. maps, although markers co-segregating in one map sometimes showed recombination in the other map. On average, there is one SNP/cM. However, there are regions of monomorphism (e.g. gaps) in both the H.b. and A.C. maps. The largest such gap is on the short arm of chromosome 2H (22.8 and 22.7 cM, respectively) and large gaps were also observed on 6HS and 7HL. These three gaps persist in the combined map. For the purposes of illustration, the chromosome 2H maps for the H.b., A.C., and combined populations are shown in Fig. 2. Linkage maps for the other six chromosomes are shown in Supplemental Fig. 1.

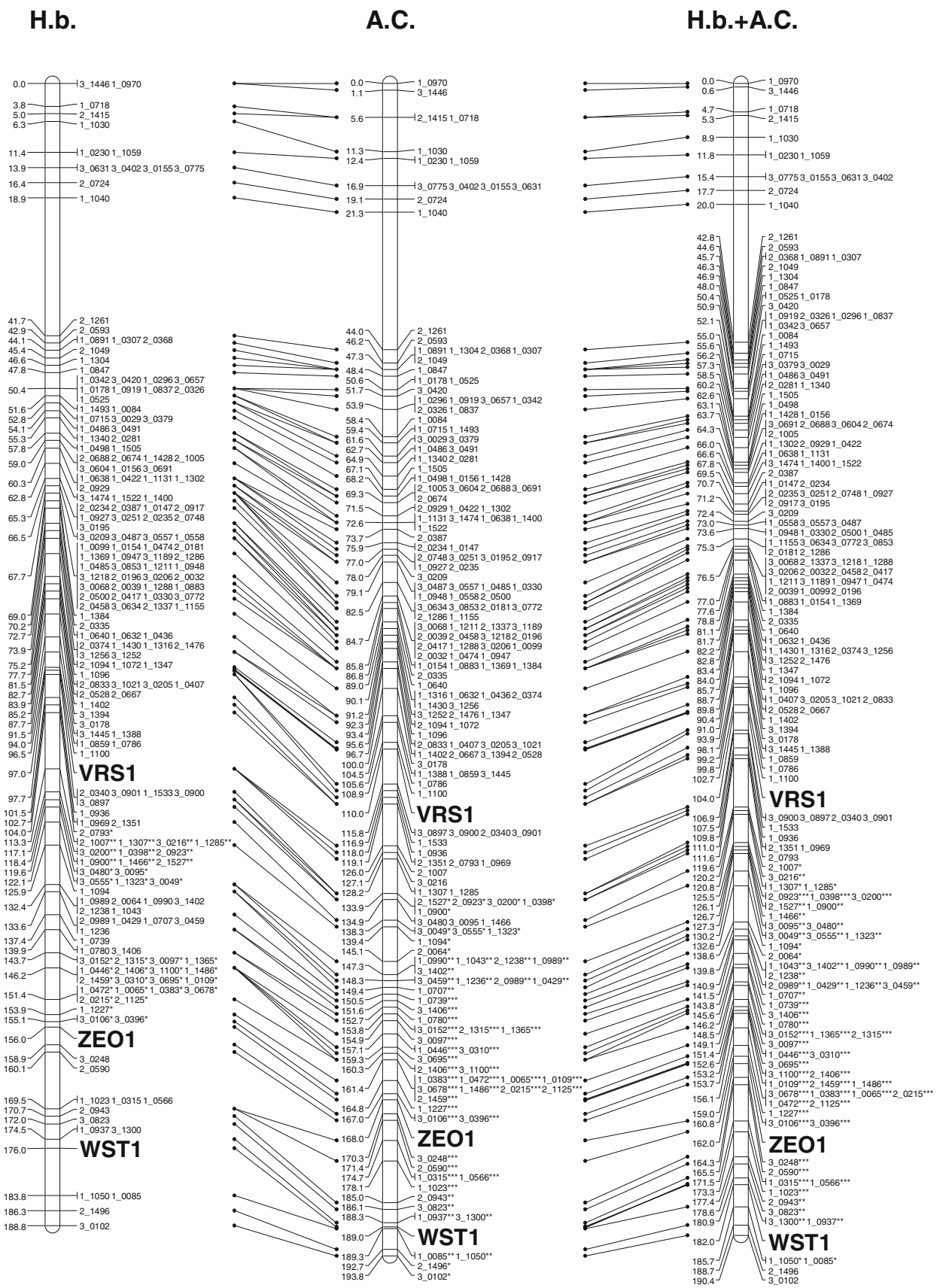
The long arm of chromosome 2H was associated with significant segregation distortion in the A.C. population and in the combined population (Fig. 3). The *ZEO-1* locus is associated with the most significant distortion in each case. For example, in the A.C. subpopulation, there were 25 and 68 plants in each of the allele classes. The region of significant distortion extends from *VRS-1* to *WST-1*. Genome-wide allele frequencies for OWB-D (dominant parent) alleles in the H.b.- and A.C.-derived populations are shown in the Fig. 3a, 3b. For the H.b. population, based on the significance threshold calculated by the modified Bonferroni system, there is significant distortion only on chromosome 3H. The distortion was in favor of the OWB-R (recessive parent) alleles. For the A.C. population, there was significant segregation distortion on 2H, 3H, 5H, 6H, and 7H. For 2H, 3H, 6H, and 7H, the distortion was in favor of the OWB-R alleles and on 5H it was in favor of the OWB-D allele. In the combined population (Fig. 3c), there was significant distortion on 2H, 3H, and 5H.

Considering all seven traits, 22 QTLs were detected in the H.b. subpopulation, 19 in the A.C. subpopulation and 22 in the combined data set (Table 2 and Supplemental Tables 2 and 3). Repeatability estimates were high or all characters (49–97%; Supplemental Table 5). QTLs for all traits, except for HD, were detected on 2H in the two subpopulations and in the combined population (Fig. 4a–c). Based on all three datasets, QTLs for SL and PH were coincident with *ZEO-1*. QTLs for GN, FN, HGW, and SN were coincident with *VRS-1* in the three populations. A QTL for HGW (coincident with *NUD*) on 7H was detected in the three populations. Six QTL (three for number of seeds on 1H, 2H, and 6H, one for number of florets on 1H and two for heading date on 4H and 6H) were detected in the H.b. and the combined populations but not in the A.C. population. Two QTL (one for number of florets on 3H and one plant height on 4H) were detected in the A.C. and combined populations but not in the H.b. population (Table 2, Supplemental Tables 2 and 3). Four QTL (one for number of seeds on 4H, two for number of florets on 4H and 6H and one for HGW on 6H) were detected only in the H.b. population (Supplemental Table 2). Five QTL (one for SL on 7H, one for HGW on 4H one for plant height on 7H, and two for heading date on 3H and 7H) were detected only in the A.C. population (Supplemental Table 3). Two QTL for SL (6H and 7H) were detected only in the combined population (Table 2). Twelve QTL, usually those with the highest effects, were detected for the seven traits in the three populations. For these, LOD scores were always highest in the combined population and lowest in the H.b. population (Table 2 and Supplemental Tables 2 and 3). Based on the score test, no significant epistatic interaction was detected at a significance level of 0.05.

Discussion

The OWB population provides a highly polymorphic and connective mapping resource (Costa et al. 2001; Rostoks et al. 2005; Stein et al. 2007; Szücs et al. 2009; Chutimanitsakun et al. submitted) and a unique genetic background for mapping determinants of certain phenotypes (Börner et al. 2002; Jafary et al. 2008). In order to further improve this resource for the genetics community, we increased the size of the mapping population. Larger populations allow for higher resolution linkage maps and better estimates of QTL number, location, effect, and interaction (Melchinger et al. 1998; Vales et al. 2005). One hundred and seventy-five DH lines (82 previously developed by the *H. bulbosum* technique and 93 by A.C.) are now available. The 175 lines are mapped with the same 1,328 SNPs.

The development of 93 lines by A.C. afforded an opportunity to empirically assess the effects of DH derivation method on estimates of recombination and linkage map quality. Devaux et al. (1995) reported recombination rates of 1.05 and 0.91 events per chromosome for A.C. and *H. bulbosum*-derived DH lines. Our estimates were 1.78 and 1.72, respectively, for the two techniques. The differences between the two reports may be due to the germplasm (Steptoe × Morex vs. Wolfe dominant marker stock × Wolfe recessive marker stock) and marker density (much higher in our case). In these two barley mapping populations, there are only slightly higher average rates of recombination in barley microsporogenesis versus


H.b.
A.C.
H.b.+A.C.
VRS1
ZEO1
WST1

◀ **Fig. 2** Chromosome 2H linkage maps from two subpopulations of doubled haploid lines (H.b. *Hordeum bulbosum*-derived and A.C. anther culture-derived) and the combined population (H.b. + A.C.) All maps were constructed using the same 199 SNPs. The map positions of three loci determining key morphological traits (*VRS1*, *ZEO1*, and *WST1*) are shown in *large font*. The single locus *p* values of the χ^2 test for segregation distortion are denoted by $*p < 0.05$; $**p < 0.01$; and $***p < 0.001$

megasporogeneis (Supplemental Tables 4 a–f). In contrast, Guzy-Wrobelska et al. (2007) compared recombination frequencies in wheat DH populations obtained via maize pollination and A.C. and reported that there was significantly higher recombination in pollen mother cells. Interestingly, we did find significant differences in recombination rates between chromosomes and an interaction of chromosome and method (Supplemental Table 1), but the actual differences are modest (Supplemental Tables 1–4). Even if map lengths differ, key issues in linkage map quality are locus order and segregation distortion.

There were no differences in locus orders between the A.C. and H.b. maps. We did observe more cases of significant segregation distortion in the A.C.-derived lines. The key question is whether this distortion is due to method or to germplasm. A lack of common markers between populations, and differences in anther/microspore culture protocols between labs, complicates the identification of genome regions associated with DH production capacity. The region of distortion we observed on 3H is coincident with a QTL for in vitro shoot regeneration on 3H (Manninen 2000) and the distorted region on 5H is coincident with a QTL for green plant regeneration (Muñoz-Amatriain et al. 2008). The distortion observed in the A.C.-derived subpopulation may be due to these QTLs. However, the region with the greatest segregation distortion (on chromosome 2H) was observed in the A.C.-derived and the combined populations and this is most likely due to the specific alleles segregating at the Zeocriton 1 (*ZEO1*) locus in the OWB. The dominant X-ray induced mutant allele at

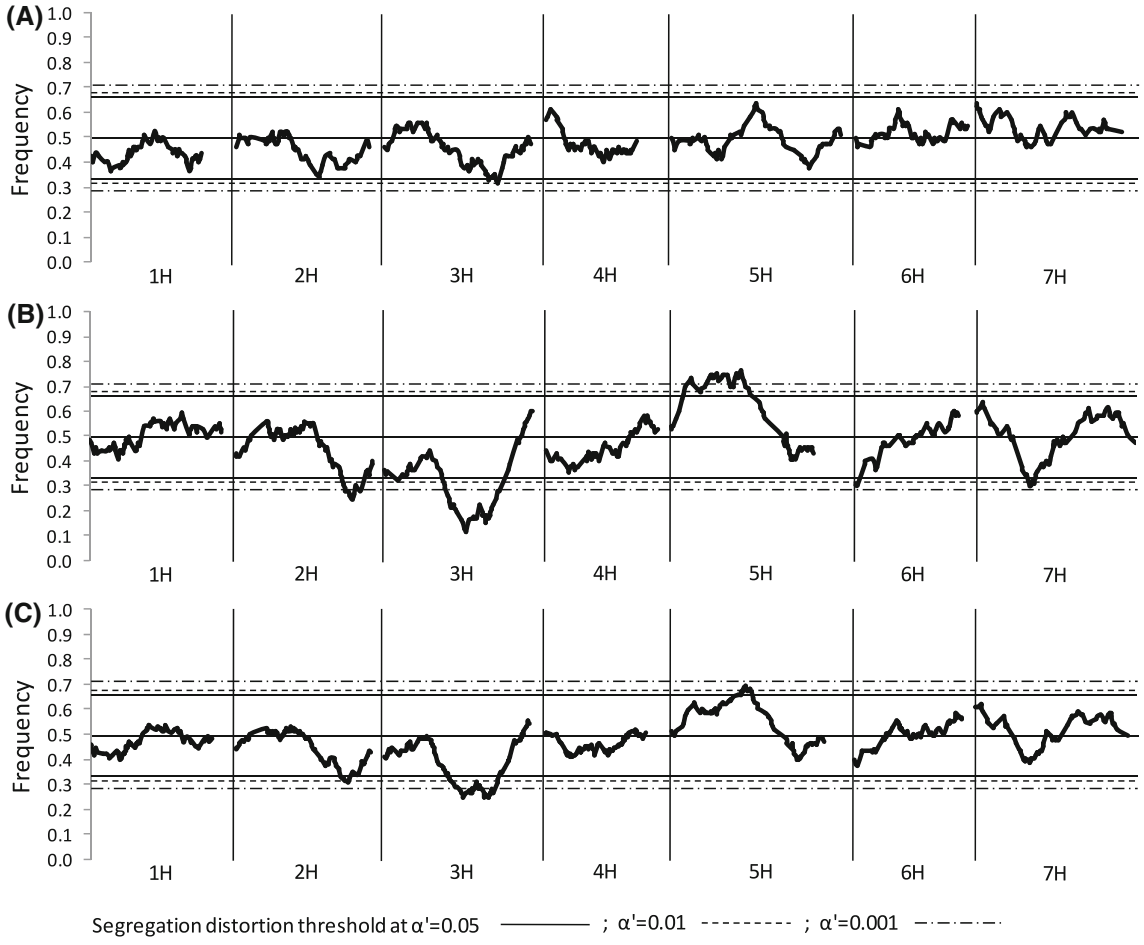


Fig. 3 Allelic frequencies for the OWB-dominant parental alleles across 1,328 loci sorted by map position. Genome-wide segregation distortion thresholds are calculated according to a modified Bonferroni correction, considering 50 effective independent tests. **a** Results based on 82 DH lines derived by the *Hordeum bulbosum* (H.b.) technique. **b** Results based on 93 DH lines derived by anther culture (A.C.). **c** Results based on 175 DH lines (82 H.b.-derived lines and 93 A.C-derived lines)

Table 2 Summary of reproductive fitness trait QTL detected in the Oregon Wolfe Barley mapping population (175 DH lines)

Trait	QTL No.	Chrom.	Peak Position (2-LOD conf. Interval)	Closest Marker	LOD	R^2	Additive effect	LOD Threshold	MIM R^2
Spike Length								3.0	0.90
	1	1H	170.7 (166.1–171.3)	2_0840	8.2	0.02	−0.48		
	2	2H	161.8 (160.6–163.3)	3_0396	93.4	0.83	−3.26		
	3	6H	92.2 (89.9–93.3)	3_0573	3.9	0.01	0.32		
	4	7H	124.6 (108.1–135.1)	2_1201	3.4	0.01	−0.32		
Grain Number								2.9	0.69
	1	1H	170.7 (103.4–106.3)	2_0840	4.0	0.03	−3.70		
	2	2H	104.8 (103.4–106.3)	1_1100	37.8	0.52	−14.61		
	3	2H	161.8 (154.0–168.8)	3_0396	5.6	0.05	−4.83		
	4	6H	82.8 (80.5–87.5)	2_0468	4.1	0.03	3.74		
Floret Number								3.0	0.89
	1	1H	169.0 (163.2–170.6)	1_0041	5.8	0.02	−3.24		
	2	2H	105.8 (104.1–106.8)	3_0897	87.3	0.81	−21.81		
	3	3H	62.4 (57.7–67.1)	3_0721	4.0	0.01	−2.64		
Hundred Grain Weight								3.0	0.67
	1	2H	104.8 (102.9–106.9)	1_1100	38.0	0.54	0.61		
	2	7H	117.6 (113.3–127.3)	2_0685	10.5	0.09	0.25		
Plant Height								3.1	0.75
	1	1H	79.3 (78.7–88.9)	2_0696	5.4	0.03	−4.25		
	2	2H	160.8 (159.8–162.8)	3_0396	53.4	0.64	−20.84		
	3	3H	57.7 (48.6–68.1)	2_1189	5.3	0.03	4.23		
	4	4H	138.1 (134.8–140.1)	3_1422	5.6	0.04	4.50		
	5	6H	86.9 (81.7–91)	2_0673	5.7	0.03	4.39		
Spike Number								3.0	0.29
	1	2H	105.8 (99.0–109.6)	3_0897	12.9	0.24	3.03		
Heading Date								2.9	0.30
	1	1H	170.7 (163.1–171.3)	2_0840	4.9	0.08	−2.41		

Table 2 continued

Trait	QTL No.	Chrom.	Peak Position (2-LOD conf. Interval)	Closest Marker	LOD	R^2	Additive effect	LOD Threshold	MIM R^2
	2	4H	134.1 (129.4–140.1)	2_0272	5.4	0.10	2.64		
	3	6H	88.5 (82.8–91)	2_0577	4.6	0.08	2.41		

− before the additive effect of the allele indicates that the larger value allele came from the OWB-recessive parent, + before the additive effect of the allele indicates that the larger value allele came from the OWB-Dominant parent, *MIM* multiple interval mapping

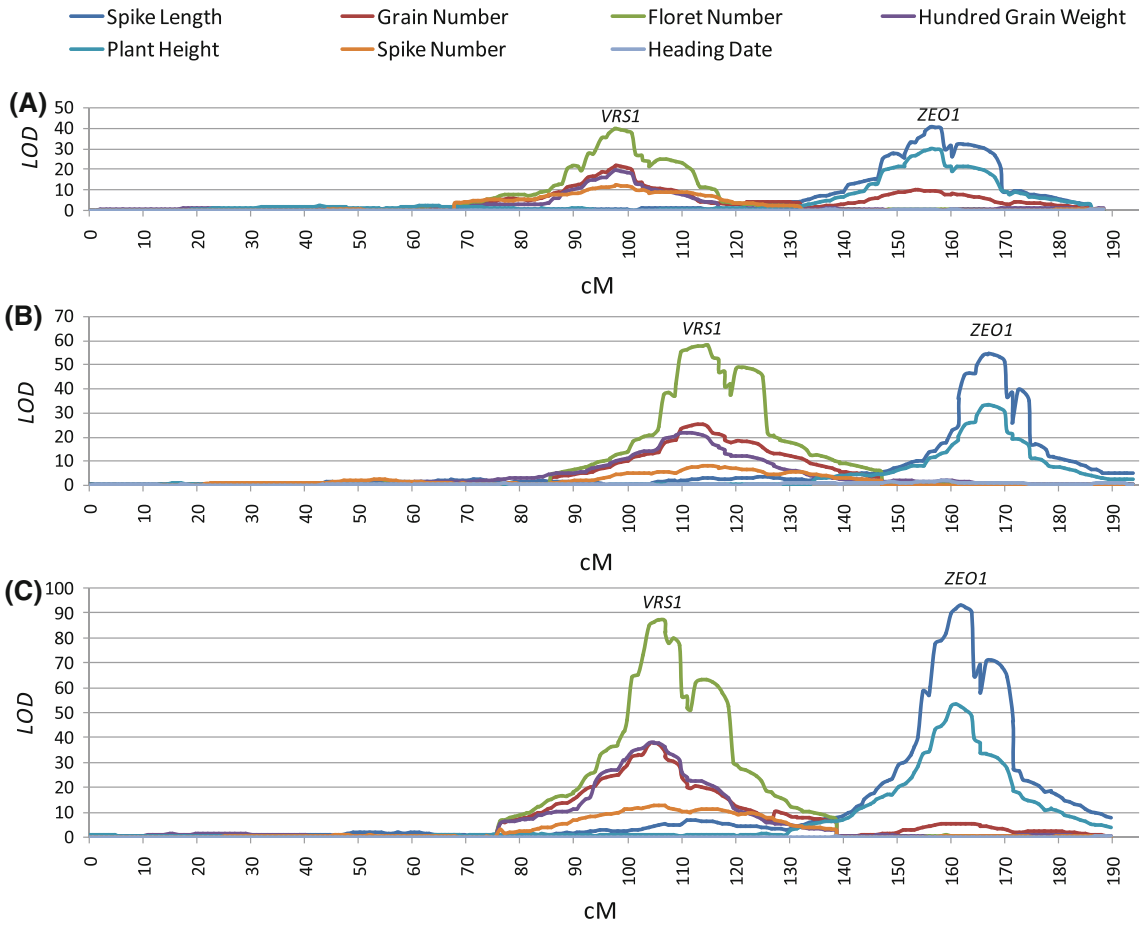


Fig. 4 LOD plots for reproductive fitness trait QTL on chromosome 2H mapped in the doubled haploid (DH) Oregon Wolfe Barley population. **a** Results based on 82 DH lines derived by the *Hordeum bulbosum* (H.b.) technique. **b** Results based on 93 DH lines derived by anther culture (A.C.). **c** Results based on 175 DH lines (82 H.b.-derived lines and 93 A.C-derived lines). Positions of two morphological trait loci—*VRS1* and *ZEO1* are shown

this locus causes extreme dwarfism (Lundqvist and Lundqvist 1998). We hypothesize that the *ZEO1* (dominant dwarfing) allele has a negative pleiotropic effect on in vitro growth and regeneration. A similar situation, related to negative effects on shoot regeneration in barley, was reported for the dwarfing allele at the *UZU* locus (Rikiishi et al. 2008). Androgenetic DH production systems are more efficient than gynogenetic methods (Maluszynski et al. 2003; Forster et al. 2007) and our results support that localized segregation distortion is a small price to pay for DH efficiency. Continued progress in techniques has reduced the incidence of albinism (Torp and Andersen 2009; Jacquard et al. 2009), led to the direct regeneration of well-developed embryos (Supena et al. 2008; Cistué et al. 2009), and the isolation of plants from the embryogenic phase rather than the callus phase (Maluszynski et al.

2003). Continued improvements in technique should further reduce the incidence of segregation distortion in A.C.-derived populations.

One of the objectives of developing linkage maps is to locate genes determining qualitative and quantitative phenotypes. One of the unique attributes of the OWB is that many of the genes determining the principal germplasm groups of barley are segregating in a single population. On the one hand, this allows for simultaneous mapping of these genes and multiple marker loci. On the other hand, some of these genes are likely to have pleiotropic effects on other phenotypes. This was certainly the case for the reproductive fitness traits measured under greenhouse conditions. Clearly, controlled environment conditions cannot reflect the complexity of conditions encountered under field conditions and it is not appropriate to equate yield component QTLs detected in an exotic cross such as the OWB under greenhouse conditions to agronomically relevant germplasm assayed under field conditions. This is certainly the case for *ZEO1*, which, as shown in Table 2, was coincident with highly significant QTLs for SL, GN and plant height (with LODs of 93.4, 5.6, and 53.4, respectively). Dwarfing genes, such as the *sdw1/denso* gene in barley (Jia et al. 2009) and the *Rht* genes of wheat (Febrer et al. 2009) are of tremendous agronomic value. In its current background, the dwarfing allele at *ZEO1* has too extreme an effect on plant height and negative pleiotropic effects on SL and GN to be of immediate agronomic interest. Furthermore, the pleiotropic effects of the *ZEO1* locus altered the expected pattern of favorable allele effects at the *VRS1* locus, which is the principal determinant of the two-row and six-row germplasm groups of barley.

Two-row and six-row refer to the number of fertile florets per rachis node. Although most barley breeders prefer to work within and inflorescence group type, crosses between groups are not uncommon and several important biparental QTL mapping populations have been derived from two-row × six-row crosses [e.g. Cali sib × Bowman (Chen et al. 1994), Gobernadora × CMB643 (Zhu et al. 1999), Harrington× Morex (Marquez-Cedillo et al. 2001), and Morex × Barke (Kota et al. 2001)]. Typically, two-row genotypes have fewer kernels/spike (lower GN), higher kernel weight and more spikes per plant. In the case of the OWBs, the pleiotropic effects of the *ZEO1* locus on SL reversed this pattern for GN. However, the effects in this exotic cross, under greenhouse conditions, were consistent with those reported from agronomically relevant crosses assessed under field conditions for HGW and spike number (Table 2). Although QTL mapping of reproductive fitness traits in this exotic cross under greenhouse conditions may be of limited practical utility, there is value in the OWB population as a model for QTL analysis and instruction.

Given the high repeatability of the phenotype data, the full set of 175 lines (although still a "small" population) should be useful for obtaining better estimates of QTL number, effect, and interaction than either of the smaller subpopulations. In the case of the traits reported herein, however, pleiotropy and segregation distortion—attributed to *ZEO1*—may be more responsible for the higher LODs observed for most traits on chromosome 2H in the $n = 175$ population versus the original population of $n = 82$ (Fig. 4). The same figure provides an illustrative example of two large-effect QTLs, both with candidate genes, which are on the same chromosome determining the same traits. We expected to optimize estimates of epistatic interaction with the larger population. However, no significant epistasis was detected.

In conclusion, the comparisons of linkage maps derived from DH population derived from the products of megasporogeneis and microsporogenesis revealed minor differences in terms of estimated recombination rates and were made possible by the very high quality and repeatability of the SNP data generated by the Illumina Golden Gate assay. For example, the OWB H.b. subpopulation was genotyped with three Pilot OPAS in one laboratory (Close et al. 2009) and the A.C. subpopulation was genotyped in another laboratory using Barley OPAS 1 and 2 (this report) and yet the two data sets were integrated seamlessly. There was greater segregation distortion in the A.C.-derived subpopulation than in the H.b.-derived population, but in the region showing the greatest distortion, the cause was more likely a unique allele at a plant height locus rather than an effect of the DH production method. The effects of segregation distortion and pleiotropy had greater impacts on estimates of QTL effect than population size for the traits studied. The OWB population and data are community resources. Seed is available from three distribution centers located in North America, Europe, and Asia. Details on ordering seed sets, as well as complete genotype and phenotype data files, are available at http://wheat.pw.usda.gov/ggpages/maps/OWB/.

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