

An Improved Consensus Linkage Map of Barley Based on Flow-Sorted Chromosomes and Single Nucleotide Polymorphism Markers

María Muñoz-Amatriaín, Matthew J. Moscou, Prasanna R. Bhat, Jan T. Svensson, Jan Bartoš, Pavla Suchánková, Hana Šimková, Takashi R. Endo, Raymond D. Fenton, Stefano Lonardi, Ana M. Castillo, Shiaoan Chao, Luis Cistué, Alfonso Cuesta-Marcos, Kerrie L. Forrest, Matthew J. Hayden, Patrick M. Hayes, Richard D. Horsley, Kihara Makoto, David Moody, Kazuhiro Sato, María P. Vallés, Brande B.H. Wulff, Gary J. Muehlbauer, Jaroslav Doležel, and Timothy J. Close*

Abstract

Recent advances in high-throughput genotyping have made it easier to combine information from different mapping populations into consensus genetic maps, which provide increased marker density and genome coverage compared to individual maps. Previously, a single nucleotide polymorphism (SNP)-based genotyping platform was developed and used to genotype 373 individuals in four barley (*Hordeum vulgare* L.) mapping populations. This led to a 2943 SNP consensus genetic map with 975 unique positions. In this work, we add data from six additional populations and more individuals from one of the original populations to develop an improved consensus map from 1133 individuals. A stringent and systematic analysis of each of the 10 populations was performed to achieve uniformity. This involved reexamination of the four populations included in the previous map. As a consequence, we present a robust consensus genetic map that contains 2994 SNP loci mapped to 1163 unique positions. The map spans 1137.3 cM with an average density of one marker bin per 0.99 cM. A novel application of the genotyping platform for gene detection allowed the assignment of 2930 genes to flow-sorted chromosomes or arms, confirmed the position of 2545 SNP-mapped loci, added chromosome or arm allocations to an additional 370 SNP loci, and delineated pericentromeric regions for chromosomes 2H to 7H. Marker order has been improved and map resolution has been increased by almost 20%. These increased precision outcomes enable more optimized SNP selection for marker-assisted breeding and support association genetic analysis and map-based cloning. It will also improve the anchoring of DNA sequence scaffolds and the barley physical map to the genetic map.

Published in *The Plant Genome* 4:238–249. Published 21 Nov. 2011.
doi: 10.3835/plantgenome2011.08.0023
© Crop Science Society of America
5585 Guilford Rd., Madison, WI 53711 USA
An open-access publication

All rights reserved. No part of this periodical may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Permission for printing and for reprinting the material contained herein has been obtained by the publisher.

M. Muñoz-Amatriaín and G.J. Muehlbauer, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108; M.J. Moscou and B.B.H. Wulff, The Sainsbury Lab., Norwich Research Park, Norwich, NR4 7UH, UK; P.R. Bhat, J.T. Svensson, R.D. Fenton, and T.J. Close, Dep. of Botany and Plant Sciences, Univ. of California, Riverside, CA 92521; J.T. Svensson, Dep. of Plant Biology, Univ. of Copenhagen, DK-1871 Frederiksberg C, Denmark; J. Bartoš, P. Suchánková, H. Šimková, and J. Doležel, Centre of the Region Haná for Biotechnological and Agricultural Research, Institute of Experimental Botany, Sokolovská 6, CZ-77200 Olomouc, Czech Republic; T.R. Endo, Lab. of Plant Genetics, Graduate School of Agriculture, Kyoto Univ., Kyoto, Japan; S. Lonardi, Dep. of Computer Science and Engineering, Univ. of California, Riverside, CA 92521; A.M. Castillo, L. Cistué, and M.P. Vallés, Departamento de Genética y Producción Vegetal, Estación Experimental Aula Dei, CSIC, 50059, Zaragoza, Spain; S. Chao, USDA-ARS, Biosciences Research Lab., Fargo, ND, 58105-5674; A. Cuesta-Marcos, P.M. Hayes, and L. Cistué, Dep. of Crop and Soil Science, Oregon State Univ., Corvallis, OR 97331; K. L. Forrest and M.J. Hayden, Dep. of Primary Industries Victoria, Victorian AgriBiosciences Centre, La Trobe R&D Park, Bundoora, VIC 3083, Australia; R.D. Horsley, Dep. of Plant Sciences, North Dakota State Univ., Fargo, ND 58108; K. Makoto, Bioresources Research and Development Dep., Sapporo Breweries Ltd., 37-1, Nittakizaki, Ota, Gunma 370-0393, Japan; D. Moody, InterGrain Pty Ltd, Kensington, WA 6100, Australia; K. Sato, Institute of Plant Science and Resources, Okayama Univ., Kurashiki, 710-0046, Japan. M. Muñoz-Amatriaín and M.J. Moscou contributed equally to this work. Received 5 Aug. 2011.
*Corresponding author (timothy.close@ucr.edu).

Abbreviations: BOPA, Barley oligonucleotide pool assay; DH, doubled haploid; EDTA, ethylenediaminetetraacetic acid; FC, Foster × Clho 4196; HA, Haruna Nijo × Akashinriki; HO, Haruna Nijo × OHU602; ID, Igri × Dobra; LG, linkage group; MB, Morex × Barke; MH, Mikamo Golden × Harrington; OPA, oligonucleotide pool assay; OWB, Oregon Wolfe Barley; OWB_{A.C.}, anther culture-derived Oregon Wolfe Barley; OWB_{H.b.}, Oregon Wolfe Barley created with the *Hordeum bulbosum*-based approach; OPA, pilot oligonucleotide pool assay; RIL, recombinant inbred line; SI, signal intensity; SM, Steptoe × Morex; SNP, single nucleotide polymorphism; VB, Vlamingh × Buloke; WB_{TAL}, wheat–barley ditelosomic addition line; WGS, whole-genome shotgun.

BARLEY (*Hordeum vulgare* L.) is cultivated worldwide due to its adaptation to cold, drought, salinity, and alkaline conditions. It ranks fourth among cereals in terms of total production and area of cultivation (FAO-STAT, 2011) and its main uses are animal feed and in the malting and brewing industry, although benefits for human health have reignited interest in barley as a food (Baik and Ullrich, 2008). Since barley is a true diploid, it represents an attractive genomic model for other *Triticaceae* species such as wheat (*Triticum aestivum* L.). The importance of barley in agriculture and its position as a model species for genetic studies led the barley research community to form the International Barley Sequencing Consortium with the goal of sequencing the >5000 Mb highly repetitive barley genome (Schulte et al., 2009; International Barley Sequencing Consortium, 2011). A combination of map-based sequencing and whole-genome shotgun (WGS) sequencing strategies are being followed to unveil the barley genome and, although significant achievements have been accomplished (Mayer et al., 2011; Sato et al., 2011b; Schulte et al., 2011), more effort and possibly new technologies will be required to overcome challenges associated with this large genome.

Genetic linkage maps are crucial for a variety of studies including quantitative trait loci identification, marker-assisted selection, association mapping, comparative genomics, and map-based cloning. At the same time, high-fidelity and dense genetic maps help in the genetic anchoring of physical maps and in the ordering and orientation of WGS scaffolds. Recently, high-throughput, robust molecular marker technologies have been developed that have resulted in more densely populated genetic maps. As a result, these dense genetic maps have increased occurrence of common markers between individual maps. This has allowed the integration of individual linkage maps into consensus genetic maps, which enable the mapping of an increased number of loci and facilitate the use of markers across different germplasm. Several consensus maps of barley have been published combining information of a minimum of three to a maximum of seven mapping populations and different types of markers (restriction fragment length polymorphism, simple sequence repeat, single nucleotide polymorphism [SNP], diversity array technology, and amplified fragment length polymorphism) (Marcel et al., 2007; Rostoks et al., 2005; Stein et al., 2007; Varshney et al., 2007; Wenzl et al., 2006).

The high-throughput SNP genotyping platform developed by Close et al. (2009) was a major step forward in the development of high-density genetic maps for barley. This platform consists of three pilot oligonucleotide pool assays (POPA1, POPA2, and POPA3) containing 4596 SNPs, which resulted in two final Barley oligonucleotide pool assays (BOPA1 and BOPA2) of 3072 SNPs. In the same work, the authors constructed a consensus genetic map with SNP data from genotyping four doubled haploid (DH) populations that involved a total of 373 lines. The resulting SNP consensus map contained 2943 loci grouped in 975 marker bins and covered a

distance of 1099 cM (Close et al., 2009). Since its release, the barley SNP platform has been extensively used by the barley community to characterize germplasm collections (e.g., Cockram et al., 2010; Comadran et al., 2011; Hamblin et al., 2010) and for association mapping studies (e.g., Cuesta-Marcos et al., 2010; Massman et al., 2011; Ramsay et al., 2011; Roy et al., 2010). The premise of the present work was that incorporation of SNP genotyping data from additional populations would considerably improve the resolution and accuracy of the consensus map.

Historically, cytogenetic resources developed during the 20th century have provided a complementary approach to genetic mapping. The recent development of chromosome arm sorting (Suchánková et al., 2006) coupled with whole genome amplification (Šimková et al., 2008) has made the application of these resources to high-throughput genomics technologies possible. An example of this was the analysis of wheat–barley disomic chromosome addition lines (Islam, 1983) using the Affymetrix Barley1 GeneChip (Bilgic et al., 2007; Cho et al., 2006) for transcriptome analyses. A total of 1787 transcribed genes were mapped to chromosome 2H to 7H of barley (chromosome 1H wheat–barley disomic chromosome addition line is not available) (Cho et al., 2006) and later, 1257 were mapped to chromosome arms using a similar approach (Bilgic et al., 2007). As sufficient DNA from individual chromosomes and arms can now be isolated, it is feasible to apply these cytogenetic resources to the same high-throughput genotyping technologies used in genetic mapping. This approach has many strengths, such as not depending on gene expression and the ability to map genes with little or no sequence variation. It also provides complementary mapping information to genetic map-based mapping and allows for the definition of pericentromeric regions if wheat–barley ditelosomic addition lines (WB-TALs) are used.

Here, we reexamined the SNP genotyping calls from the original four populations included in the previous SNP consensus map (Close et al., 2009) and added data from another six mapping populations and additional individuals from one of the original populations. For most of the markers two independent lines of direct evidence, genetic mapping and flow sorting, supported the map positions. Cumulatively, we present a robust SNP-based consensus genetic map that incorporates marker data from 1133 individuals. Due to both its higher number of bins and improved marker order, the consensus map developed in this study constitutes a significant achievement in support of SNP selection for marker-assisted breeding, association genetic analysis, map-based cloning, and anchoring DNA sequence scaffolds and a physical map to the genetic map.

MATERIALS AND METHODS

Mapping Populations

A total of 10 mapping populations were used in this study, including nine segregating populations of DH lines and one recombinant inbred line (RIL) population. Four of

those DH populations (Oregon Wolfe Barley [OWB] created with the *Hordeum bulbosum* L.-based approach [OWB_{H,b}], Steptoe × Morex [SM] 1 [SM1], Morex × Barke [MB], and Haruna Nijo × OHU602 [HO]) were used previously to construct a SNP consensus map (Close et al., 2009). The six additional populations were anther culture-derived OWB (OWB_{A,C}), Haruna Nijo × Akashinriki (HA), Mikamo Golden × Harrington (MH), Vlamingh × Buloke (VB), and Igri × Dobra (ID) DH populations and a Foster × CIho 4196 (FC) RIL population. These six populations had been previously developed in the context of other studies and some of them have been published (Cistué et al., 2011; Horsley et al., 2006; Sato et al., 2011a; Sato and Takeda, 2009). An additional 57 individuals from the SM population were also used and, together with the SM1 lines, made up population SM2. More details on the 10 populations and the additional individuals included in the SM population SM2 are given in Table 1.

Single Nucleotide Polymorphism Genotyping and Data Analysis

The high-throughput SNP-genotyping platform developed by Close et al. (2009) was used to genotype all the populations included in this study. Three of the populations (OWB_{H,b}, SM1, and MB) were genotyped with the three pilot Illumina (San Diego, CA) GoldenGate oligonucleotide pool assays (POPA1, POPA2, and POPA3), which involved 4596 SNPs (Table 1). Additional individuals of the SM population were genotyped only with POPA1 and POPA2 (3060 markers) and were considered, together with SM1, as an individual population for mapping purposes (SM2). Foster × CIho 4196 was genotyped only with the 1524 SNPs represented on POPA1. Highly informative SNPs represented in these three POPAs were used to generate two BOPAs (BOPA1 and BOPA2), which were used to genotype the OWB_{A,C} population, with a total of 3072 assayed markers (Table 1). The rest of the populations (HO, HA, MH, VB, and ID) were genotyped only with BOPA1 (1536 SNPs).

Visualization and analysis of SNP data was performed using BeadStudio software (Illumina, 2008). Every SNP data cluster for each population was manually inspected to apply an accurate and consistent clustering method. Uniform criteria for inclusion or exclusion of SNPs were applied to all the populations and, as a consequence, some cases of apparent polymorphism were not used. Typical reasons for exclusion of an apparently polymorphic data clustering pattern included (i) homozygote clusters that are insufficiently separated (theta compressed) to readily distinguish heterozygotes from homozygotes in different germplasm, (ii) clusters that are vertically but not horizontally separated, which we found from the use of flow sorted chromosomes usually to be attributed to polymorphism in a locus different from the targeted SNP, and (iii) excessive dispersion of subclusters within an apparent homozygous cluster, which often manifested as segregation distortion but could be explained by signal interference from a different locus. In cases of minor doubts about

the reliability of a SNP, we took advantage of barley–rice (*Oryza sativa* L.) synteny, viewed using HarvEST:Barley (HarvEST, 2011), to decide whether or not the cluster settings would cause the marker to map to a locus that seemed sensible in the context of synteny. Once this initial BeadStudio analysis was performed, genotyping data from each population were exported from the software for subsequent data processing.

Construction of the Individual Maps

Marker data were curated to identify and remove identical individuals and to exclude monomorphic or highly segregation-distorted markers. Individuals with a high number of heterozygous SNP loci and/or producing “No Calls” at an excessive number of SNPs were also detected and removed from the analysis. In general, these types of issues can be attributed to poor quality DNA samples, cross contamination between DNA samples, or intercrossing between lines. Individuals carrying nonparental alleles were also discarded; such individuals must represent errors in propagation, outcrossing, or DNA sample preparation. Command-line MST_{MAP} v4.3 (Wu et al., 2008; Wu, 2008a), which efficiently builds genetic maps by computing the minimum spanning tree of a graph associated with the genotyping data, was used to generate individual genetic maps for the 11 populations, using a cut off *p*-value of 0.000001, maximum distance between markers at 15.0 cM, no estimation before clustering, and the COUNT objective function and with genetic distances estimated using the Kosambi function (Kosambi, 1943).

Linkage groups (LGs) were assigned to chromosomes based on the previous Close et al. (2009) map. As a fairly stringent *p*-value cut off was used, several maps have two or more LGs that assign to the same chromosome. In this case, LGs were merged based on the ordering from the Close et al. (2009) map and confirmed for order and orientation based on two-point linkage analysis from MadMapper (Kozik, 2006; West et al., 2006). The MadMapper software was also helpful to visualize and validate all 11 genetic maps as well as to identify double recombinants. All double recombination events were inspected and only those supported by several markers or that were preceded and/or succeeded by long genetic distances were considered as “real,” while double recombinants for singleton markers not involving large genetic distances were called as missing data.

Pilot oligonucleotide pool assay names were used to designate SNP loci in the maps (e.g., 1_0894), where the first number corresponds to the POPA number and the next four digits indicate the SNP order in the corresponding POPA. A cross reference between alternative marker names is included in Supplemental Table S1.

Construction of the Consensus Map

All 11 genetic maps were used to generate a consensus genetic map using MergeMap v1.2 (Wu et al., 2011; Wu, 2008b), a software based on graph theory wherein individual maps are converted into directed acyclic graphs that

Table 1. Information on the individual mapping population data used for consensus map construction.

Population	Abbreviation	Growth habit ¹	Type ²	No. of lines (before curation)	No. of lines (after curation)	No. of SNPs ³ assayed	No. and percentage of polymorphic SNPs	Reference
Oregon Wolfe Barley created with the <i>Hordeum bulbosum</i> L.-based approach	OWB _{H.b.}	S × S	DH	93	82	4596	1469 (32%)	Costa et al., 2001; BarleyWorld. 2006
Steptoe × Morex 1	SM1 ⁴	S × S	DH	93	92	4596	1215 (26%)	Kleinhofs et al., 1993; USDA-ARS, 2011b
Morex × Barke	MB	S × S	DH	94	93	4596	1574 (34%)	N. Stein, personal communication, 2010
Haruna Nijo × OHU602	HO	S × W	DH	100	94	1536	759 (49%)	Sato and Takeda, 2009
Steptoe × Morex 2	SM2	S × S	DH	150	146	3060	835 (27%)	Kleinhofs et al., 1993; USDA-ARS, 2011b
Anther culture-derived Oregon Wolfe Barley	OWB _{A.C.}	S × S	DH	94	93	3072	1271 (41%)	Cistué et al., 2011
Haruna Nijo × Akashinriki	HA	S × S	DH	68	54	1536	734 (48%)	Sato et al., 2011a
Mikamo Golden × Harrington	MH	S × S	DH	95	91	1536	491 (32%)	Zhou et al., 2011
Vlamingh × Buloke	VB	S × S	DH	347	289	1536	440 (29%)	D. Moody, unpublished data, 2010
Foster × Clho 4196	FC	S × S	F ₈₋₉ RIL	94	89	1524	409 (27%)	Horsley et al., 2006
Igri × Dobla	ID	W × F	DH	106	102	1536	446 (29%)	M.P. Vallés, unpublished data, 2010
Total				1250	1133			

¹Growth habit of the parental genotypes used for the cross (S, spring barley; W, winter barley; F, facultative).

²DH, doubled haploid; RIL, recombinant inbred line.

³SNP, single nucleotide polymorphism.

⁴SM1 is subset of SM2.

are then merged into a consensus graph on the basis of their shared vertices (Jackson et al., 2005; Jackson et al., 2008; Yap et al., 2003). Equal weight was given to all genetic maps (weight = 1.0). MergeMap implements an efficient algorithm for resolving conflicts in the marker order among individual maps by deleting the smallest set of marker occurrences (Wu et al., 2011). In the case of equal probability of deletion among maps, we manually inspected the quality of each marker in conflict and assigned a higher weight to the most reliable maps. This only occurred once on chromosome 5H, where SM2 was given priority over SM1 due to the greater number of lines in SM2 (54 additional lines).

The current implementation of MergeMap (Wu et al., 2011; Wu, 2008b) inflates genetic distances between markers in the consensus genetic map. Previously, Close et al. (2009) used the arithmetic mean of individual LGs to determine an appropriate scaling factor for each LG. Here, we compared the genetic distances between consecutive markers in individual genetic maps to the same genetic distance as estimated in the consensus genetic map. The most stable estimate was found by dividing the arithmetic mean of these genetic distances in individual genetic maps by that of the consensus genetic map, with a scaling factor of 0.612 ± 0.062 .

Plant Material for Flow-Sorted Chromosomes

Seeds of WBTAL (21" + t") carrying chromosome arms 2HS, 2HL, 3HS, 3HL, 4HS, 4HL, 5HS, 5HL, 6HS, 6HL, 7HS and 7HL were obtained from the collection maintained at Kyoto University, Japan.

Preparation of Material for Chromosome Sorting

Chromosome preparation and sorting was performed according to Suchánková et al. (2006). Briefly, metaphase

cells were accumulated by treatment of root tips with 2 mM hydroxyurea (18 h), recovery in hydroxyurea-free medium (6.5 h), treatment with 2.5 μ M amiprophos-methyl (2 h), and overnight incubation in ice cold water. Chromosomes were released by mechanical homogenization after mild formaldehyde fixation (for details see Vrána et al. [2000]). Chromosome suspensions were stained by 2 μ g ml⁻¹ DAPI (4',6-diamidino-2-phenylindole) and analyzed using a FACS Vantage SE flow cytometer (Becton Dickinson, San Jose, CA). Preparation of material for chromosome 1H was reported previously by Šimková et al. (2008). Chromosome arms were sorted from corresponding WBTAL at a quantity of 25,000 each and placed into 20 μ L of double distilled H₂O in a 0.5 mL polymerase chain reaction tube.

Amplification of Chromosomal Arm DNA

Flow-sorted arms were processed and amplified according to Šimková et al. (2008). Chromosome arms were treated with proteinase K (3 μ g per 25,000 arms) for 36 h at 50°C in 70 μ L (chromosomes) or 90 μ L (arms) of buffer consisting of 2.5 mM Tris (pH 8.0), 1.25 mM ethylenediaminetetraacetic acid (EDTA) (pH 8.0), and 0.125% (w/v) sodium dodecyl sulfate. Half of the original amount of proteinase K was added after 20 h. Proteinase K was removed using a Microcon YM-100 column (Millipore Corporation, Bedford, MA) in four rounds of centrifugation (for details see Šimková et al. [2008]). Chromosomal DNA was amplified using an illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Chalfont St. Giles, UK) in 20 μ L reaction for 90 min according to manufacturer's instructions. Amplified DNA was lyophilized and subsequently diluted to a final volume of 100 μ L by 10 mM Tris-HCl and 0.1 mM EDTA (pH 8.0).

50 μL were then purified using MicroSpin G50 columns (GE Healthcare).

Assignment of Genes to Chromosomes and Arms using Flow-Sorted Material

Flow-sorted chromosome 1H or arms, following amplification and purification by gel filtration, were applied to BOPA1 and BOPA2 to determine the location of each gene. Two independently prepared samples were used as replicates for all samples except 2HS, 3HL, and 5HS, for which only a single sample of each was applied to BOPA1. The location of each gene was determined by comparing the signal intensities (SIs) from all flow-sorted samples to the SIs from barley genomic DNA samples as positive controls (Morex, Betzes, and Akcent) and to negative controls, either salmon (*Oncorhynchus keta*) sperm DNA for BOPA1 or *Escherichia coli* DNA for BOPA2. The proportion of flow-sorted chromosomes or arms in each sample was adjusted by mixing with negative control DNA to achieve two to three times the relative concentration as would be in complete barley genome DNA. The final total DNA concentration was $80 \text{ ng } \mu\text{L}^{-1}$, of which 250 ng was applied to the GoldenGate assay. The BeadStudio software (Illumina, 2008) for BOPA1 (data generated fall 2007) and Genome Studio (Illumina, 2010) for BOPA2 (data generated winter 2010) were used to cluster the data points. In general the data could be partitioned into signals that clustered with the positive or negative controls (Supplemental Fig. S1). In nearly all cases the data clusters required manual adjustment because the default clustering algorithm is intended to first seek heterozygotes and then identify homozygotes whereas in our case the distinction was simply gene presence or absence in the DNA sample. For some SNPs the data could not be adequately partitioned into gene-negative or gene-positive clusters, and in these cases the data were not used for further analysis. The SNP locus was assigned to a chromosome or arm if all replicate samples for that arm or chromosome provided the same interpretation; otherwise the SNP locus was considered to be unassigned.

RESULTS AND DISCUSSION

Data Analysis and Curation

To achieve uniformity in the analysis of each individual population contributing to the consensus map, we reexamined the previous genotyping data corresponding to the $\text{OWB}_{\text{H.b.}}$, SM1, MB, and HO populations. Due to the more stringent criteria for SNP inclusion in the present work (see Materials and Methods), fewer polymorphic SNPs were considered for individual map construction compared to Close et al. (2009) (Table 1). In particular, 5.9, 4.3, and 4.7% of the polymorphic markers included in the previous $\text{OWB}_{\text{H.b.}}$, SM1, and MB genetic maps, respectively, were not included in the present study. In contrast, for the HO population, the number of SNPs considered was increased by 3.4% with respect to the previous study,

due to the addition of genotypic data from five more lines of this population (Table 1). The removal of less-reliable markers from these populations was intended to help reduce conflicts in the marker order among component maps and therefore assist in the construction of a consensus map (Jackson et al., 2008). The loss of markers in some individual maps was sometimes compensated by their presence in other maps such that in total, 116 markers that were included in the Close et al. (2009) consensus map were not included in the new consensus map produced in this work. A list of those 116 markers, along with their consensus map LG positions and neighboring markers, is provided in Supplemental Table S2.

The same criteria were followed for inspecting the SNP data corresponding to the six additional populations included in this work and the 54 additional lines from the Steptoe \times Morex population that had been genotyped with a subset of POPA markers. Although this cannot result in a higher number of mapped markers, their inclusion increased the number of recombination events in the SM population and hence the marker resolution. We were also able to use a new OWB population of 94 lines developed by anther culture ($\text{OWB}_{\text{A.C.}}$) (Cistué et al., 2011), which, as expected due to its high degree of phenotypic variation (Costa et al., 2001), contributed a high number of polymorphic SNPs (1215; Table 1). A high percentage of polymorphism (49%; Table 1) was also found in the Japanese HA population developed by Sato et al. (2011a) from crossing the malting cv. Haruna Nijo with the food landrace Akashinriki. The fact that both parents were expressed sequence tag donors from which oligonucleotide pool assay (OPA)-SNPs were identified increased the likelihood of the platform to detect polymorphisms (Sato et al., 2011a). The other four new mapping populations, which included parents from Japan (Mikamo Golden), Australia (Vlamingh and Buloke), the United States (Harrington, Foster, and Cihó 4196), and Europe (Igri and Dobra), had lower numbers of polymorphic SNPs (Table 1). Their lower polymorphism rate was probably due to the similarity of the parental genotypes or perhaps their absence from the SNP discovery panel (Close et al., 2009; Moragues et al., 2010).

Single nucleotide polymorphism data were examined afterward to identify identical individuals as well as problematic lines. With the development of high-throughput genotyping technologies, it is becoming easier to detect identical lines in mapping populations, resulting in removal of redundant genotyping information that can cause bias in the linkage analysis. To identify duplicate lines, we compared the genotype calls between all pairs of individuals. The presence of 11 and 14 duplicated individuals had been observed previously in the $\text{OWB}_{\text{H.b.}}$ and HA mapping populations, respectively (Chutimanitsakun et al., 2011; Sato et al., 2011a). In addition, we found and removed one duplicated individual from the $\text{OWB}_{\text{A.C.}}$, SM1, and ID populations, two duplicated lines from SM2, MH, and FC, five duplicated individuals from HO, and 42 duplicated lines from VB. Lines with an excessive number

of heterozygous SNP calls and/or “No Calls” were also identified and removed from the data set, in particular one line from both MB and HO populations, two lines from the SM2 and MH populations, three individuals from FC and ID, and 16 lines from the VB population. Table 1 shows the final numbers of lines from each population that were considered for further analysis while the specific lines removed from each population can be found in Supplemental Table S3.

Generation of Individual Linkage Maps

After curation of the SNP data, we constructed component maps from the 11 high-quality datasets. We chose the software tool MST_{MAP} (Wu et al., 2008; Wu, 2008a) to develop all the individual genetic maps due to its good performance compared to other available tools, especially in the speed and accuracy of map construction (Cheema and Dicks, 2009). The resulting linkage maps were also compared with those produced by JoinMap 4.0 (Van Ooijen, 2006). Maps generated by both programs were identical in marker order, probably due to the quality of our genotyping data, although MST_{MAP} assembled maps significantly faster than JoinMap. Since not all the individual maps had the same SNP coverage, we preferred not to force the number of LGs to match the number of chromosomes and to use a set of stringent parameters with MST_{MAP}, taking advantage of the wealth of genetic map information to link the disjointed LGs. Specifically, we used the Close et al. (2009) consensus map to join and orientate LGs.

All constructed individual maps were then validated by visualizing with CheckMatrix from MadMapper (West et al., 2006; Kozik, 2006). First, we confirmed the high quality of the genetic maps by generating two-dimensional heat plots, which show all pairwise recombination values for nonredundant markers. An example of a heat map from one of the individual maps is shown in Supplemental Fig. S2. Second, we generated a graphical genotyping plot from each map to easily identify all double crossovers. Double recombination events can be real or indicative of genotyping errors. We manually inspected all double recombinants that were not supported by large centimorgan distances between markers. In total, 98 singletons were replaced with missed calls. Most of these were identified in FC (49) and VB (34) mapping populations, probably because of their lower marker density compared to other maps, although in the case of FC some of these 49 double crossovers might be real, given the higher opportunity for recombination of RILs than DHs. We preferred to be conservative and err on the side of caution. The remaining rare singletons occurred in SM1, SM2, OWB_{A.C.}, HA, MH, and ID mapping populations.

We generated each of the 11 component maps from the filtered genotype datasets, and both the individual maps and the genotyping data used for their construction are presented in Supplemental Table S4. A total of four markers could not be placed into individual genetic

maps: marker 3_1434 from OWB_{H.b.}, marker 2_0029 from MB, and markers 1_0739 and 1_0780 from FC. The rest of the SNPs were distributed among the seven barley chromosomes in each of the component maps (Table 2), with average densities ranging from one SNP per 2.52 cM in the MB genetic map to one marker per 5.02 cM in the ID genetic map. Genetic map sizes varied among the different populations, from 954.1 cM for FC to 1257.8 cM for OWB_{A.C.} (Table 2).

A higher number of loci exhibiting segregation distortion were detected in OWB_{A.C.}, HA, and VB genetic maps, but segregation distortion loci were present in almost every population and regions affected by distortion were not always coincident among individual maps (Supplemental Fig. S3). It is unclear whether or not the method for population development (RIL or doubled haploidization via *H. bulbosum* or anther or microspore culture) is associated with a greater degree of segregation distortion.

Development of an Integrated Consensus Map

Individual genetic maps were merged into a consensus map using MergeMap (Wu et al., 2011; Wu, 2008b), a freely available software tool that implements an algorithm based on graph theory (Jackson et al., 2005, 2008; Yap et al., 2003) to integrate linkage maps. Although JoinMap (Van Ooijen, 2006) has been one of the most commonly used softwares to build consensus maps, MergeMap outperforms JoinMap in marker order accuracy and speed of operation (Wang et al., 2011; Wu et al., 2011) and has been successfully used to generate previous SNP consensus maps of barley (Close et al., 2009) and cowpea [*Vigna unguiculata* (L.) Walp.] (Muchero et al., 2009). Given the high number of individual maps and differences in population size, which affect the accuracy of the marker positioning, a few ordering conflicts were found in all chromosomes except 6H and 7H. MergeMap resolved most of these conflicts by deleting the smallest set of marker occurrences necessary to remove the conflicts. However, in chromosome 5H there was a case of equal probability of marker removal between the two maps in conflict (SM1 and SM2). We then used the option of assigning “weights” to individual maps that the software offers (Wu et al., 2011) to give priority to the marker order of SM2, due to the greater number of lines in this population (Table 1). Since genetic distances in the consensus map were expanded relative to the individual maps, which is an algorithmic anomaly of the coordinate system used in MergeMap, chromosomal lengths were normalized after consensus map construction (see Materials and Methods).

Although a comparison of the consensus genetic map to the individual component maps showed a good consistency in the locus order between the populations, a total of four markers were found to map twice in the consensus map due to their different chromosomal position in the component maps. In particular, markers 1_0349 and 1_0716 mapped on both 1H and 3H, marker 2_1055 mapped on 1H and 6H, and 2_0029 was found to map on both 5H and 6H (Supplemental Table S5). Map data from flow-sorted chromosomes (see below) were

Table 2. Distribution of single nucleotide polymorphism loci in the individual component maps and the consensus map.

Map [†]	Characteristic	Chromosome							
		1H	2H	3H	4H	5H	6H	7H	All
OWB _{H.b.}	Markers	153	225	240	206	262	191	191	1468
	Bins	64	71	89	57	86	59	64	490
	cM [‡]	154.0	179.3	199.3	123.4	229.0	150.8	195.3	1231.1
SM1	Markers	139	211	233	125	217	119	171	1215
	Bins	46	57	62	48	78	41	56	388
	cM	138.9	146.6	154.7	141.5	187.3	151.5	140.8	1061.3
MB	Markers	208	259	236	142	285	205	238	1573
	Bins	60	72	77	41	74	51	64	439
	cM	133.7	151.9	178.0	138.2	194.7	133.8	158.9	1089.2
HO	Markers	97	135	128	97	116	98	88	759
	Bins	47	63	59	48	63	44	47	371
	cM	141.4	166.5	158.0	126.9	182.6	122.0	185.4	1082.8
SM2	Markers	99	154	162	84	149	75	112	835
	Bins	50	61	69	49	80	36	59	404
	cM	133.8	136.9	151.2	127.3	179.9	158.3	134.8	1022.2
OWB _{A.C.}	Markers	134	189	207	179	224	174	164	1271
	Bins	70	78	76	65	72	51	73	485
	cM	178.1	185.1	198.9	151.1	189.9	139.5	215.2	1257.8
HA	Markers	86	125	120	100	127	88	88	734
	Bins	34	46	35	26	52	31	35	259
	cM	155.6	180.6	149.4	100.4	190.1	132.4	157.0	1065.5
MH	Markers	49	82	99	55	65	76	65	491
	Bins	26	39	34	26	35	28	33	221
	cM	122.1	152.9	154.0	117.9	161.8	103.6	150.1	962.4
VB	Markers	32	94	41	76	89	42	66	440
	Bins	26	72	34	55	58	29	56	330
	cM	145.0	197.8	180.7	145.3	207.5	137.9	198.7	1212.9
FC	Markers	51	86	65	48	52	50	57	409
	Bins	33	45	38	33	30	29	33	241
	cM	126.2	167.3	146.2	113.6	139.8	102.4	161.0	956.6
ID	Markers	50	71	95	49	73	68	40	446
	Bins	27	39	38	30	39	35	22	230
	cM	147.5	191.3	148.4	136.2	165.1	132.4	199.6	1120.5
Mean	cM	143.3	167.7	165.4	129.2	184.3	133.1	172.4	1095.5
Consensus	Markers	345	491	487	359	540	357	415	2994
	Bins	145	191	180	155	198	126	168	1163
	cM	143.2	172.9	180.1	146.5	189.9	142.2	162.5	1137.3

[†]FC, Foster × Clho 4196; HA, Haruna Nijo × Akashinriki; HO, Haruna Nijo × OHU602; ID, Igri × Dobla; MB, Morex × Barke; MH, Mikamo Golden × Harrington; OWB_{A.C.}, anther culture-derived Oregon Wolfe Barley; OWB_{H.b.}, Oregon Wolfe Barley created with the *Hordeum bulbosum*-based approach; SM1, Steptoe × Morex 1; SM2, Steptoe × Morex 2; VB, Vlamingh × Buloke.

[‡]Centimorgans estimated using the Kosambi function.

used to manually curate two of these markers (1_0349 and 2_0029), for which 3H and 6H map positions were retained, respectively, while the second position was removed. Removal of one of the map positions for SNPs 1_0716 and 1_1055 was done based on population consistency and rice synteny, with the 1H and 6H map positions retained for these two markers, respectively.

The resulting consensus genetic map contained 2994 SNP loci in 1163 marker bins (unique loci) in an aggregate map size of 1137 cM (Table 2; Supplemental Table

S6), providing an average marker bin density of 0.99 cM. The map has only one large gap of 11 cM in the long arm of chromosome 4H (Fig. 1; Supplemental Table S6) with the remaining gaps smaller than 5 cM. Although the genotyping of most of the additional individual populations with a subset of the total number of available OPA-SNP markers (Table 1) limited the mapping of new markers on the consensus map, 167 new SNPs were placed into the new consensus map compared to the Close et al. (2009) map, most of them mapping to

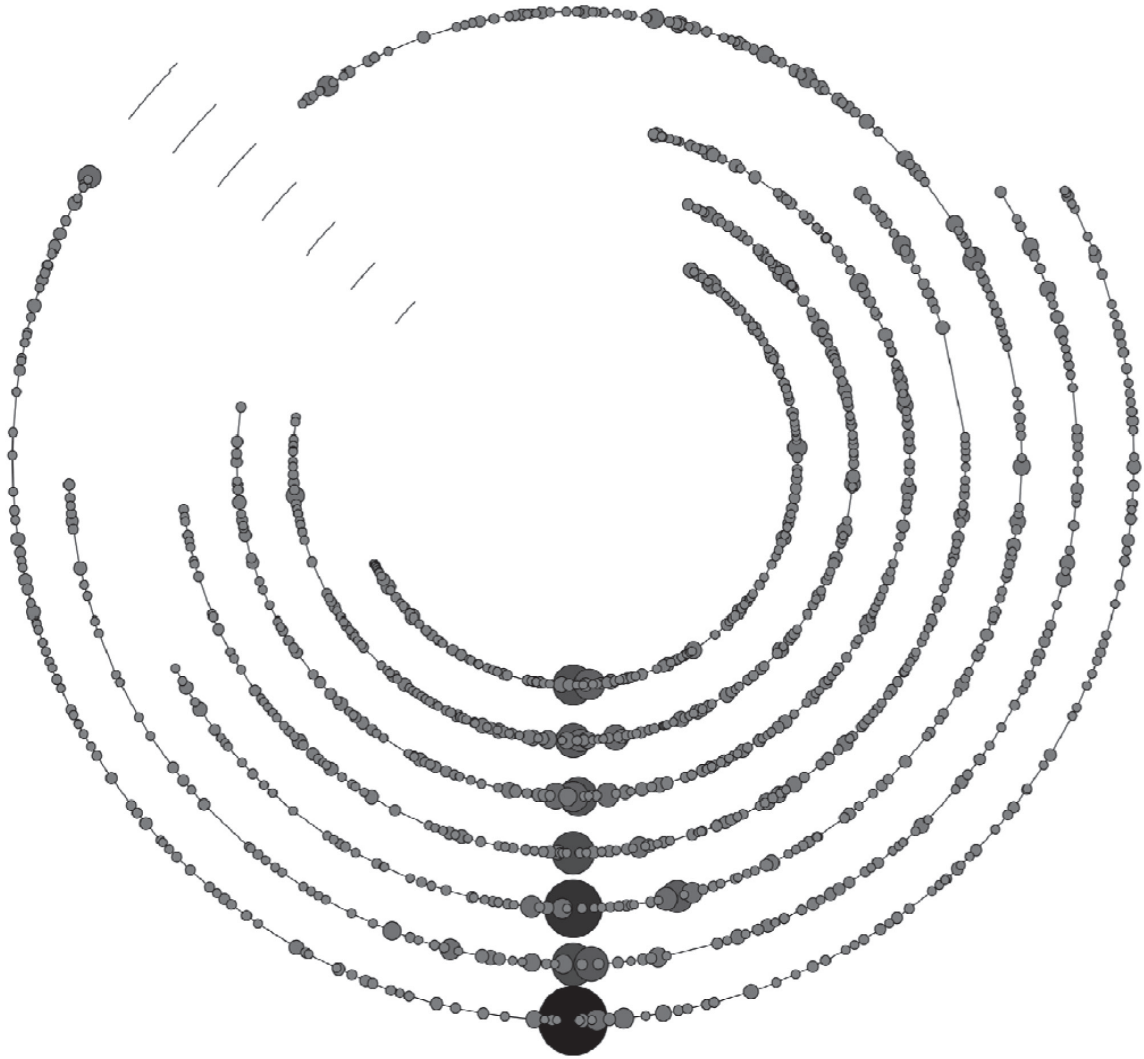


Figure 1. The improved single nucleotide polymorphism-based consensus map of barley. The consensus genetic map is represented as concentric circles, from 1H (most central) to 7H (most outer). Chromosomes are anchored based on their respective pericentromeric regions determined with flow-sorted chromosome arms (except for 1H, where homology to rice was used). Circles represent the number of markers at each position, with both size and color representing total number of markers (blackest black = 53; grayest gray = 1). A scale bar representing 5 cM is shown as concentric arcs beside each chromosome.

chromosomes 2H, 3H, and 4H (Supplemental Table S7). This is an increase of 51 SNPs after subtracting 116 SNPs that were used previously but not included in the new map (Table 3). However, the resolution of the consensus linkage map was clearly higher with the inclusion of the seven additional individual maps, as shown by the increased number of bins in all chromosomes, with a total increase in map resolution of almost 20% (Table 3). Chromosomes 1H, 4H, and 6H had the smallest number of markers and bins and were also the smallest in size (Table 2). Close et al. (2009) also showed similar results, although we were able to increase both the number of markers and marker resolution for the three LGs, especially in chromosome 4H (Table 3). In general, small rearrangements were observed when comparing the two consensus maps, with the largest near pericentromeric regions. Since both maps were generated using the same software, these differences mainly reflect the greater

resolution of the current SNP consensus map as a result of the addition of populations that have informative recombination events between closely linked markers or regions with little recombination. This improved consensus genetic map is publicly available at HarvEST:Barley (version 1.82 and higher; HarvEST, 2011) and Grain-Genes 2.0 (USDA-ARS, 2011a).

To assess the impact of individual populations on the integrated map, we performed a leave-one-out analysis in the construction of the consensus genetic map. As shown in Table 4, the MB population had the greatest impact on the number of markers, with 9.0% reduction in the number of mapped SNPs if it is removed from the integrated map. This can be associated with the application of the three POPAs for genotyping this population and the use of both cv. Morex and Barke in the design of the GoldenGate assays (Close et al., 2009). As expected, leaving out SM2 had no impact on marker number, since SM2

markers are included in SM1. Of the new populations used in this study, FC added the most markers to the updated consensus map (1.4%; Table 4). Regarding their contribution to the consensus map resolution, OWB_{H,b} was the population that had the highest impact in the number of unique bins followed by OWB_{A,C} (6.5 and 5.2% bin reduction, respectively; Table 4) which reveals the importance of including this anther culture-derived population in the development of the new SNP-based consensus map. The inclusion of VB, FC, and the additional individuals of the SM population (SM2) also had a relevant influence on increasing map resolution, with numbers of marker bins reduced by 3.9, 2.4, and 2.1% due to their absence from the consensus map, respectively (Table 4). Surprisingly, the exclusion of HA increased the number of bins by 0.9% (9 bins) and is likely a direct result of the small population size (54 lines). Regardless of the negative impact on map resolution, the HA population contributed to an increase in the number of SNP markers on the consensus map by 0.6% (Table 4), which led us to keep it as a component population of the integrated consensus map.

For reference to previous data sets, historical markers were integrated into the SM and OWB_{H,b} individual populations (Costa et al., 2001; Kleinhofs et al., 1993) and the consensus map was reconstructed (Supplemental Table S8).

Gene Mapping using Flow-Sorted Chromosome and Arms

The BOPA1 and BOPA2 platforms were applied to amplified, flow-sorted material to rough map genes to chromosome 1H and the chromosome arms of 2H to 7H. For the purpose of anchoring markers to individual chromosomes or arms, SI was more important than the clustering results as the chromosome location is independent of the allele. This approach was extremely robust, as 2930 genes were mapped with BOPA1 and BOPA2, which represents 96.1% of the genes surveyed (Table 5). An overlap of 2560 genes was mapped with both flow-sorted chromosome or arms and genetic maps, with an agreement of 99.4% (2545 genes) between mapping approaches. A clear correspondence observed between the number of genes mapped using both approaches indicates no significant bias based on chromosome or arm in mapping. An additional 370 genes were mapped using flow-sorted materials, which were not genetically mapped in any of the 10 populations (Supplemental Table S9).

An advantage with gene mapping using flow-sorted material is that it is an accurate method for determining the physical position of genes relative to chromosome arm. Applying this mapping information from the arms of chromosomes 2H to 7H permits the definition of the pericentromeric region when coupled with the consensus genetic map. Bins in the genetic map were evaluated for an admixture of genes mapped to both the short and long chromosome arm. The pericentromeric region was defined as the set of bins still containing this mixed

Table 3. Comparison of the new single nucleotide polymorphism (SNP)-based consensus genetic map with the Close et al. (2009) consensus map.

		Close et al. (2009)	New consensus map
Number of individuals		373	1133
Number of SNPs	1H	341	345
	2H	485	491
	3H	475	487
	4H	338	359
	5H	535	540
	6H	352	357
	7H	417	415
	Total	2943	2994
Number of bins	1H	125	145
	2H	161	191
	3H	152	180
	4H	113	155
	5H	180	198
	6H	111	126
	7H	133	168
	Total	975	1163

Table 4. Leave-one-out population analysis for the consensus genetic map.

Population left out [†]	Consensus genetic map		Percent reduction in the number of bins	Percent reduction in marker inclusion
	Bins	Markers		
FC	1135	2953	2.4	1.4
HA	1174	2977	-0.9	0.6
HO	1140	2983	2.0	0.4
ID	1151	2987	1.0	0.2
MB	1121	2726	3.6	9.0
MH	1153	2989	0.9	0.2
OWB _{A,C}	1103	2990	5.2	0.1
OWB _{H,b}	1087	2880	6.5	3.8
SM1	1150	2891	1.1	3.4
SM2	1139	2994	2.1	0.0
VB	1118	2989	3.9	0.2
None	1163	2994	NA	NA

[†]Population left out of the construction of the consensus genetic map. FC, Foster × Clho 4196; HA, Haruna Nijo × Akashinriki; HO, Haruna Nijo × OHU602; ID, Igrī × Dobra; MB, Morex × Barke; MH, Mikamo Golden × Harrington; OWB_{A,C}, anther culture-derived Oregon Wolfe Barley; OWB_{H,b}, Oregon Wolfe Barley created with the *Hordeum bulbosum*-based approach; SM1, Steptoe × Morex 1; SM2, Steptoe × Morex 2; VB, Vlamingsh × Buloke.

state of physically mapped genes from both arms. These regions are shown in Table 6 for chromosomes 2H to 7H. A unique characteristic of these regions is the significant increase in gene density, likely caused by a complete lack of recombination. This is clearly observed in Fig. 1, where pericentromeric regions were used to anchor chromosomes on the horizontal axis.

Conflicts between consensus genetic map position and flow-sorted chromosome or arm was observed for 15 genes (Supplemental Table S5), and these may be explained by low-level error in the mapping of genes with the

Table 5. Genes mapped using flow-sorted chromosome or arms and genetic maps.

Chromosome	Arm	Method of mapping		Unique to flow-sorted chromosome or arms	Correspondence between flow sorting and genetic mapping
		Flow-sorted chromosomes or arms	Genetic map		
1H	NA	432	345	116	316
2H	2HS	153	157	21	129
	2HC ^c	32	38	NA	32
	2HL	275	296	27	248
	2HB ^b	3	NA	1	2
3H	3HS	118	128	12	102
	3HC ^c	62	69	0	62
	3HL	270	290	28	243
4H	4HS	116	104	18	97
	4HC ^c	25	30	0	25
	4HL	202	225	15	186
5H	5HS	95	85	12	81
	5HC ^c	34	43	NA	34
	5HL	370	412	45	325
	5HB ^b	14	NA	1	11
6H	6HS	113	117	11	101
	6HC ^c	48	56	0	48
	6HL	170	184	17	152
	6HB ^b	1	NA	0	1
7H	7HS	174	182	24	149
	7HC ^c	51	57	0	51
	7HL	168	176	22	146
	7HB ^b	4	NA	0	4
Total	NA	2930	2994	370	2545

^cC indicates a gene is located in the pericentromeric region based on information from flow-sorted chromosomes and the consensus genetic map.

^bB indicates a gene was detected on both the short and long chromosome arm.

flow-sorting approach or the mapping of paralogs. Reevaluation of the 22 SNP loci markers detected on both short and long arms of a chromosome (Supplemental Tables S5 and S6) found four in the pericentromeric region (Supplemental Table S5). Flow-sorted chromosome or arm mapping supported the improved quality of SNPs included in the consensus genetic map. Of the 167 new SNPs included in this consensus genetic map, 86 were mapped using flow-sorted material. In contrast, the 116 SNPs removed with respect to Close et al. (2009) only had 32 genes mapped using flow-sorted material. An attempt was made to allocate SNP loci to chromosomes using DNA isolated from disomic wheat–barley addition lines. However, a much higher incidence of marker position conflicts than with flow-sorted materials indicates that flow sorting with subsequent amplification is the more robust approach for OPA-based gene mapping (data not included).

Supplemental Information Available

Supplemental figures and tables associated with this manuscript are located at <http://www.crops.org/publications/tpg>.

Table 6. Pericentromeric regions of chromosomes 2H to 7H.

Chromosome	Bins	cM region
1H	NA	NA
2H	74–76	67.1–68.1
3H	56–59	65.3–67.9
4H	57	56.2
5H	39	42.4
6H	48–50	59.3–60.6
7H	86–88	80.3–81.8

Acknowledgments

This work was supported by the USA National Science Foundation (DBI-0321756 to TJC and SL), the Human Frontier Science Program (LT000218/2011-L to MJM) and Gatsby Charitable Foundation (MJM and BBHW), the Czech Ministry of Education Youth and Sports and the European Regional Development Fund (LC06004 and CZ.1.05/2.1.00/01.0007 to JD, JB, HŠ and PS), the Ministry of Science and Innovation of Spain (AGL2007-62930/AGR and GEN2006-28560), and the Agriculture and Food Research Initiative Plant Genome, Genetics and Breeding Program of USDA's Cooperative State Research and Extension Service (2009-65300-05645 to TJC, SL and GJM). Authors thank Dr. Adam J. Lukaszewski for seeds of wheat–barley addition lines.

References

- Baik, B.-K., and S.E. Ullrich. 2008. Barley for food: Characteristics, improvement, and renewed interest. *J. Cereal Sci.* 48:233–242. doi:10.1016/j.jcs.2008.02.002
- BarleyWorld. 2006. Oregon Wolfe barleys. Available at <http://barleyworld.org/oregonwolfe.php>. (verified 10 Nov. 2011). The Barley Project, Crop and Soil Science Department, Oregon State University, Corvallis, OR.
- Bilgic, H., S. Cho, D.F. Garvin, and G.J. Muehlbauer. 2007. Mapping barley genes to chromosome arms by transcript profiling of wheat–barley ditelosomic chromosome addition lines. *Genome* 50:898–906. doi:10.1139/G07-059
- Cheema, J., and J. Dicks. 2009. Computational approaches and software tools for genetic linkage map estimation in plants. *Brief. Bioinform.* 10:595–608. doi:10.1093/bib/bbp045
- Cho, S., D.F. Garvin, and G.J. Muehlbauer. 2006. Transcriptome analysis and physical mapping of barley genes in wheat–barley chromosome addition lines. *Genetics* 172:1277–1285. doi:10.1534/genetics.105.049908
- Chutimanitsakun, Y., R.W. Nipper, A. Cuesta-Marcos, L. Cistue, A. Corey, T. Filichkina, E.A. Johnson, and P.M. Hayes. 2011. Construction and application for QTL analysis of a restriction site associated DNA (RAD) linkage map in barley. *BMC Genomics* 12:4. doi:10.1186/1471-2164-12-4
- Cistué, L., A. Cuesta-Marcos, S. Chao, B. Echávarri, Y. Chutimanitsakun, A. Corey, T. Filichkina, N. Garcia-Marino, I. Romagosa, and P.M. Hayes. 2011. Comparative mapping of the Oregon Wolfe Barley using doubled haploid lines derived from female and male gametes. *Theor. Appl. Genet.* 122:1399–1410. doi:10.1007/s00122-011-1540-9
- Close, T.J., P.R. Bhat, S. Lonardi, Y. Wu, N. Rostoks, L. Ramsay, A. Druka, N. Stein, J.T. Svensson, S. Wanamaker, S. Bozdag, M.L. Roose, M.J. Moscou, S. Chao, R.K. Varshney, P. Szűcs, K. Sato, P.M. Hayes, D.E. Matthews, A. Kleinohs, G.J. Muehlbauer, J. DeYoung, D.F. Marshall, K. Madishetty, R.D. Fenton, P. Condamine, A. Graner, and R. Waugh. 2009. Development and implementation of high-throughput SNP genotyping in barley. *BMC Genomics* 10:582. doi:10.1186/1471-2164-10-582
- Cockram, J., J. White, D.L. Zuluaga, D. Smith, J. Comadran, M. Macaulay, Z. Luo, M.J. Kearsey, P. Werner, D. Harrap, C. Tapsell, H. Liu, P.E. Hedley, N. Stein, D. Schulte, B. Steuernagel, D.F. Marshall, W.T.B. Thomas, L. Ramsay, I. Mackay, D.J. Balding, A.C. The, R. Waugh, and D.M. O'Sullivan. 2010. Genome-wide association mapping

- to candidate polymorphism resolution in the unsequenced barley genome. *Proc. Natl. Acad. Sci. USA* 107:21611–21616. doi:10.1073/pnas.1010179107
- Comadran, J., L. Ramsay, K. MacKenzie, P. Hayes, T.J. Close, G. Muehlbauer, N. Stein, and R. Waugh. 2011. Patterns of polymorphism and linkage disequilibrium in cultivated barley. *Theor. Appl. Genet.* 122:523–531. doi:10.1007/s00122-010-1466-7
- Costa, J.M., A. Corey, P.M. Hayes, C. Jobet, A. Kleinhofs, A. Kopisch-Obusch, S.F. Kramer, D. Kudrna, M. Li, O. Riera-Lizarazu, K. Sato, P. Szűcs, T. Toojinda, M.I. Vales, and R.I. Wolfe. 2001. Molecular mapping of the Oregon Wolfe Barleys: A phenotypically polymorphic doubled-haploid population. *Theor. Appl. Genet.* 103:415–424. doi:10.1007/s001220100622
- Cuesta-Marcos, A., P. Szűcs, T.J. Close, T. Filichkin, G.J. Muehlbauer, K.P. Smith, and P.M. Hayes. 2010. Genome-wide SNPs and re-sequencing of growth habit and inflorescence genes in barley: Implications for association mapping in germplasm arrays varying in size and structure. *BMC Genomics* 11:707. doi:10.1186/1471-2164-11-707
- FAOSTAT. 2009. Production of crops. Available at <http://faostat.fao.org/site/567/default.aspx> (verified 10 Nov. 2011). FAO, Rome, Italy.
- Hamblin, M.T., T.J. Close, P.R. Bhat, S. Chao, J.G. Kling, K.J. Abraham, T. Blake, W.S. Brooks, B. Cooper, C.A. Griffey, P.M. Hayes, D.J. Hole, R.D. Horsley, D.E. Obert, K.P. Smith, S.E. Ullrich, G.J. Muehlbauer, and J.-L. Jannink. 2010. Population structure and linkage disequilibrium in U.S. barley germplasm: Implications for association mapping. *Crop Sci.* 50:556–566. doi:10.2135/cropsci2009.04.0198
- HarvEST. 2011. HarvEST:Barley. Available at <http://harvest.ucr.edu> (verified 10 Nov. 2011). University of California, Riverside, CA.
- Horsley, R.D., D. Schmierer, C. Maier, D. Kudrna, C.A. Urrea, B.J. Stefenson, P.B. Schwarz, J.D. Franckowiak, M.J. Green, B. Zhang, and A. Kleinhofs. 2006. Identification of QTLs associated with Fusarium head blight resistance in barley accession CIho 4196. *Crop Sci.* 46:145–156. doi:10.2135/cropsci2005.0247
- Illumina. 2008. Illumina BeadStudio 3.2. Illumina, Inc., San Diego, CA.
- Illumina. 2010. GenomeStudio genotyping module v.2010.3. Available at http://www.illumina.com/software/genomestudio_software.ilmn (verified 10 Nov. 2011). Illumina, Inc., San Diego, CA.
- International Barley Sequencing Consortium. 2011. International barley sequencing consortium. Available at <http://barley-genome.org> (verified 10 Nov. 2011). Iowa State Univ., Ames, IA.
- Islam, A.K.M.R. 1983. Ditelosomic additions of barley chromosomes to wheat. p. 233–238. *In* S. Sakamoto (ed.) *Proc. Int. Wheat Genet. Symp.*, 6th, Kyoto, Japan. 28 Nov.–3 Dec. 1983. Kyoto University, Kyoto, Japan.
- Jackson, B.N., S. Aluru, and P.S. Schnable. 2005. Consensus genetic maps: A graph theoretic approach. *Proc. IEEE Comput. Syst. Bioinform. Conf.* 2005:35–43.
- Jackson, B.N., P.S. Schnable, and S. Aluru. 2008. Consensus genetic maps as median orders from inconsistent sources. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 5:161–171. doi:10.1109/TCBB.2007.70221
- Kleinhofs, A., A. Kilian, M.A. Saghai Maroof, R.M. Biyashev, P. Hayes, F.Q. Chen, N. Lapitan, A. Fenwick, T.K. Blake, V. Kanazin, E. Ananiev, L. Dahleen, D. Kudrna, J. Bollinger, S.J. Knapp, B. Liu, M. Sorrells, M. Heun, J.D. Franckowiak, D. Hoffman, R. Skadsen, and B.J. Steffenson. 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. *Theor. Appl. Genet.* 86:705–712. doi:10.1007/BF00222660
- Kosambi, D.D. 1943. The estimation of map distances from recombination values. *Ann. Eugen.* 12:172–175. doi:10.1111/j.1469-1809.1943.tb02321.x
- Kozik, A. 2006. MadMapper. Available at <http://cgpdp.ucdavis.edu/XLinkage/MadMapper/> (verified 10 Nov. 2011). Univ. of California Davis Genome Center, R. Michelsmore Lab, Davis, CA.
- Marcel, T.C., R.K. Varshney, M. Barbieri, H. Jafary, M.J. de Kock, A. Graner, and R.E. Niks. 2007. A high-density consensus map of barley to compare the distribution of QTLs for partial resistance to *Puccinia hordei* and of defence gene homologues. *Theor. Appl. Genet.* 114:487–500. doi:10.1007/s00122-006-0448-2
- Massman, J., B. Cooper, R. Horsley, S. Neate, R. Dill-Macky, S. Chao, Y. Dong, P. Schwarz, G. Muehlbauer, and K. Smith. 2011. Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. *Mol. Breed.* 27:439–454. doi:10.1007/s11032-010-9442-0
- Mayer, K.F., M. Martis, P.E. Hedley, H. Šimková, H. Liu, J.A. Morris, B. Steuernagel, S. Taudien, S. Roessner, H. Gundlach, M. Kubaláková, P. Suchánková, F. Murat, M. Felder, T. Nussbaumer, A. Graner, J. Salse, T. Endo, H. Sakai, T. Tanaka, T. Itoh, K. Sato, M. Platzer, T. Matsumoto, U. Scholz, J. Doležel, R. Waugh, and N. Stein. 2011. Unlocking the barley genome by chromosomal and comparative genomics. *Plant Cell* 23:1249–1263. doi:10.1105/tpc.110.082537
- Moragues, M., J. Comadran, R. Waugh, I. Milne, A.J. Flavell, and J.R. Russell. 2010. Effects of ascertainment bias and marker number on estimations of barley diversity from high-throughput SNP genotype data. *Theor. Appl. Genet.* 120:1525–1534. doi:10.1007/s00122-010-1273-1
- Muchero, W., N.N. Diop, P.R. Bhat, R.D. Fenton, S. Wanamaker, M. Pottorf, S. Hearne, N. Cisse, C. Fatokun, J.D. Ehlers, P.A. Roberts, and T.J. Close. 2009. A consensus genetic map of cowpea [*Vigna unguiculata* (L) Walp.] and synteny based on EST-derived SNPs. *Proc. Natl. Acad. Sci. USA* 106:18159–18164. doi:10.1073/pnas.0905886106
- Ramsay, L., J. Comadran, A. Druka, D.F. Marshall, W.T. Thomas, M. Macaulay, K. MacKenzie, C. Simpson, J. Fuller, N. Bonar, P.M. Hayes, U. Lundqvist, J.D. Franckowiak, T.J. Close, G.J. Muehlbauer, and R. Waugh. 2011. *INTERMEDIUM-C*, a modifier of lateral spikelet fertility in barley, is an ortholog of the maize domestication gene *TEOSINTE BRANCHED 1*. *Nat. Genet.* 43:169–172. doi:10.1038/ng.745
- Rostoks, N., S. Mudie, L. Cardle, J. Russell, L. Ramsay, A. Booth, J.T. Svensson, S.I. Wanamaker, H. Walia, E.M. Rodriguez, P.E. Hedley, H. Liu, J. Morris, T.J. Close, D.F. Marshall, and R. Waugh. 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Mol. Genet. Genomics* 274:515–527. doi:10.1007/s00438-005-0046-z
- Roy, J.K., K.P. Smith, G.J. Muehlbauer, S. Chao, T.J. Close, and B.J. Stefenson. 2010. Association mapping of spot blotch resistance in wild barley. *Mol. Breed.* 26:243–256. doi:10.1007/s11032-010-9402-8
- Sato, K., T.J. Close, P. Bhat, M. Muñoz-Amatrián, and G.J. Muehlbauer. 2011a. Single nucleotide polymorphism mapping and alignment of recombinant chromosome substitution lines in barley. *Plant Cell Physiol.* 52:728–737. doi:10.1093/pcp/pcr024
- Sato, K., Y. Motoi, N. Yamaji, and H. Yoshida. 2011b. 454 sequencing of pooled BAC clones on chromosome 3H of barley. *BMC Genomics* 12:246. doi:10.1186/1471-2164-12-246
- Sato, K., and K. Takeda. 2009. An application of high-throughput SNP genotyping for barley genome mapping and characterization of recombinant chromosome substitution lines. *Theor. Appl. Genet.* 119:613–619. doi:10.1007/s00122-009-1071-9
- Schulte, D., R. Ariyadasa, B. Shi, D. Fleury, C. Sasaki, M. Atkins, P. Dejong, C.C. Wu, A. Graner, P. Langridge, and N. Stein. 2011. BAC library resources for map-based cloning and physical map construction in barley (*Hordeum vulgare* L.). *BMC Genomics* 12:247. doi:10.1186/1471-2164-12-247
- Schulte, D., T.J. Close, A. Graner, P. Langridge, T. Matsumoto, G. Muehlbauer, K. Sato, A.H. Schulman, R. Waugh, R.P. Wise, and N. Stein. 2009. The international barley sequencing consortium – At the threshold of efficient access to the barley genome. *Plant Physiol.* 149:142–147. doi:10.1104/pp.108.128967
- Šimková, H., J.T. Svensson, P. Condamine, E. Hřibová, P. Suchánková, P.R. Bhat, J. Bartoš, J. Šafář, T.J. Close, and J. Doležel. 2008. Coupling amplified DNA from flow-sorted chromosomes to high-density SNP mapping in barley. *BMC Genomics* 9:294. doi:10.1186/1471-2164-9-294
- Stein, N., M. Prasad, U. Scholz, T. Thiel, H. Zhang, M. Wolf, R. Kota, R.K. Varshney, D. Perovic, I. Grosse, and A. Graner. 2007. A 1000-loci transcript map of the barley genome: New anchoring points for integrative grass genomics. *Theor. Appl. Genet.* 114:823–839. doi:10.1007/s00122-006-0480-2
- Suchánková, P., M. Kubaláková, P. Kovářová, J. Bartoš, J. Čihliříková, M. Molnair-Laing, T.R. Endo, and J. Doležel. 2006. Dissection of the

- nuclear genome of barley by chromosome flow sorting. *Theor. Appl. Genet.* 113:651–659. doi:10.1007/s00122-006-0329-8
- USDA-Agricultural Research Service (USDA-ARS). 2011a. GrainGenes: A database for Triticeae and Avena. 2011. Available at <http://wheat.pw.usda.gov/GG2/maps.shtml> (verified 11 Nov. 2011). USDA-ARS, Washington, DC.
- USDA-Agricultural Research Service (USDA-ARS). 2011b. The Steptoe × Morex barley mapping population. Available at <http://wheat.pw.usda.gov/ggpages/SxM/> (verified 10 Nov. 2011). USDA-ARS, Washington, DC.
- Van Ooijen, J.W. 2006. JoinMap version 4.0, software for the calculation of genetic linkage maps. Kyazma BV, Wageningen, the Netherlands.
- Varshney, R.K., T.C. Marcel, L. Ramsay, J. Russell, M.S. Roder, N. Stein, R. Waugh, P. Langridge, R.E. Nix, and A. Graner. 2007. A high density barley microsatellite consensus map with 775 SSR loci. *Theor. Appl. Genet.* 114:1091–1103. doi:10.1007/s00122-007-0503-7
- Vrána, J., M. Kubaláková, H. Šimková, J. Číhalíková, M.A. Lysák, and J. Doležel. 2000. Flow sorting of mitotic chromosomes in common wheat (*Triticum aestivum* L.). *Genetics* 156:2033–2041.
- Wang, J., D.J. Lydiate, I.A.P. Parkin, C. Falentin, R. Delourme, P.W.C. Carion, and G.J. King. 2011. Integration of linkage maps for the amphidiploid *Brassica napus* and comparative mapping with *Arabidopsis* and *Brassica rapa*. *BMC Genomics* 12:101. doi:10.1186/1471-2164-12-101
- Wenzl, P., H. Li, J. Carling, M. Zhou, H. Raman, E. Paul, P. Hearnden, C. Maier, L. Xia, V. Caig, J. Ovesná, M. Cakir, D. Poulsen, J. Wang, R. Raman, K.P. Smith, G.J. Muehlbauer, K.J. Chalmers, A. Kleinhofs, E. Huttner, and A. Kilian. 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* 7:206. doi:10.1186/1471-2164-7-206
- West, M.A., H. van Leeuwen, A. Kozik, D.J. Kliebenstein, R.W. Doerge, D.A. St Clair, and R.W. Michelmore. 2006. High-density haplotyping with microarray-based expression and single feature polymorphism markers in *Arabidopsis*. *Genome Res.* 16:787–795. doi:10.1101/gr.5011206
- Wu, Y. 2008a. MSTmap online. Available at <http://138.23.178.42/mstmap> (verified 10 Nov. 2011). Timothy Close, Department of Botany & Plant Sciences, University of California, Riverside, CA.
- Wu, Y. 2008b. MergeMap online. Available at <http://www.mergemap.org> (verified 10 Nov. 2011). Timothy Close, Department of Botany & Plant Sciences, University of California, Riverside, CA.
- Wu, Y., P.R. Bhat, T.J. Close, and S. Lonardi. 2008. Efficient and accurate construction of genetic linkage maps from the minimum spanning tree of a graph. *PLoS Genet.* 4:e1000212. doi:10.1371/journal.pgen.1000212
- Wu, Y., T.J. Close, and S. Lonardi. 2011. Accurate construction of consensus genetic maps via integer linear programming. *IEEE/ACM Trans. Comput. Biol. Bioinform.* 8:381–394. doi:10.1109/TCBB.2010.35
- Yap, I.V., D. Schneider, J. Kleinberg, D. Matthews, S. Cartinhour, and S.R. McCouch. 2003. A graph-theoretic approach to comparing and integrating genetic, physical and sequence-based maps. *Genetics* 165:2235–2247.
- Zhou, T., I. Takashi, H. Naohiko, K. Makoto, H. Takehiro, K. Ryouichi, and S. Kazuhiro. 2011. Malting quality quantitative trait loci on a high density map of Mikamo Golden x Harrington cross in barley (*Hordeum vulgare* L.). *Mol. Breed.* 10.1007/s11032-011-9602-x.