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ORIGINAL PAPER

New Diversity Arrays Technology (DArT) markers for tetraploid oat (*Avena magna* Murphy et Terrell) provide the first complete oat linkage map and markers linked to domestication genes from hexaploid *A. sativa* L.

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Abstract Nutritional benefits of cultivated oat (*Avena sativa* L., $2n = 6x = 42$, AACCDD) are well recognized; however, seed protein levels are modest and resources for genetic improvement are scarce. The wild tetraploid, *A. magna* Murphy et Terrell (syn *A. maroccana* Gdgr., $2n = 4x = 28$, CCDD), which contains approximately 31% seed protein, was hybridized with cultivated oat to produce a domesticated *A. magna*. Wild and cultivated accessions were crossed to generate a recombinant inbred line (RIL) population. Although these materials could be used to develop domesticated, high-protein oat, mapping and quantitative trait loci introgression is hindered by a near absence of genetic markers. Objectives of this study were to develop high-throughput, *A. magna*-specific markers; generate a genetic linkage map based on the *A. magna* RIL population; and map genes controlling oat domestication. A Diversity Arrays Technology (DArT) array derived from 10 *A. magna* genotypes was used to generate 2,688 genome-specific probes. These, with 12,672 additional oat clones, produced 2,349 polymorphic markers, including 498 (21.2%) from *A. magna* arrays and 1,851 (78.8%) from other *Avena* libraries. Linkage analysis included 974 DArT markers, 26 microsatellites, 13 SNPs, and 4 phenotypic markers, and resulted in a 14-linkage-group map. Marker-to-marker correlation coefficient analysis allowed classification of shared markers as unique or redundant, and putative linkage-group-to-genome anchoring. Results of this study provide for the first time a collection of high-throughput tetraploid oat markers and a comprehensive map of the genome, providing insights to the genome ancestry of oat and affording a resource for study of oat domestication, gene transfer, and comparative genomics.

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Introduction

Cultivated oat (*Avena sativa* L. and *A. byzantina* C. Koch, $2n = 6x = 42$, AACCDD genomes) is the world's fifth or sixth most important cereal crop. Its importance in the human diet is increasing due to its nutritional benefits, which include a high content of serum LDL cholesterol-lowering soluble fiber, good-quality protein, and high oil

content (Braaten et al. 1994; Eggum et al. 1989). Of these, increasing protein content has been an important breeding objective in oat for at least three decades (McFerson and Frey 1991; Zhu et al. 2004). This objective is becoming increasingly important as continued research highlights health benefits of dietary protein, particularly protein from plant sources (Nutall et al. 1984; Wang et al. 2008). Compared to the protein composition of other major cereals, oat is higher in essential amino acids, making oat a nutritionally superior crop and an excellent target for increased seed protein content (Jones et al. 1948; Young and Pellett 1994).

The wild Moroccan oat species *A. magna* Murphy et Terrell (syn. *A. maroccana* Gdgr., $2n = 4x = 28$, CCDD genomes) is a potentially valuable resource for protein content, with a measured value of 31% protein in the grain (Ladizinsky and Fainstein 1977). This value could be commercialized via direct domestication, following the model of tetraploid wheat (Distelfeld et al. 2006), or through creation of synthetic hexaploid oat containing identified quantitative trait loci (Ladizinsky 2000). Although *A. magna* carries resistance to crown rust (*Puccinia coronata* f. sp. *avenae*) and powdery mildew (*Erysiphe graminis*) (Ladizinsky 1995; Ohm and Shaner 1992), it has several undesirable characteristics that need to be addressed before cultivation, including lack of domestication and susceptibility to barley yellow dwarf virus.

Avena magna is part of the secondary gene pool for *A. sativa* because hybrids are completely self-sterile (Harlan et al. 1973; Ladizinsky 1995; Ladizinsky and Fainstein 1977; Thomas 1992), but $5x$ hybrids can be backcrossed as females via pollination with tetraploid or hexaploid oat. Fluorescent in situ hybridization (FISH) experiments with pAs120a, an A genome-abundant sequence from *A. strigosa*, indicate that *A. magna* may carry the C and D genomes that are also found in hexaploid oat (Linares et al. 1998).

Ladizinsky (1995) described the sexual transfer of the domestication syndrome, including genes for reduced awn size (*A*), yellow lemma color (*Lc*), glabrous lemma (*Lp*), and non-shattering spikelet (*Ba*), from *A. sativa* to *A. magna* through two successive backcrosses. The first pentaploid hybrids were pollinated by *A. magna* in the field, yielding a small number of seeds, including a seed germinated to produce a partially fertile $2n = 32$ plant designated Aa2. The F_2 seeds derived from Aa2 having 28 chromosomes, along with the domestication syndrome, were then raised and either (1) crossed with wild *A. magna* strains to evaluate segregation of the four domestication traits; or (2) crossed passively with *A. sativa* pollen to initiate a second backcross cycle. Cross-group #1 was used to verify that the four genes segregated essentially in a 3:1 manner and that they formed a single linkage group spanning approximately 19 map units. Cross-group #2 formed $5x$ hybrids that were backcrossed a second time to *A. magna*, this time to domesticated Aa2-3 (a fertile, selfed $2n = 28$ offspring of Aa2). One of these plants, designated Ba 13-13, was selected due to its stable chromosome number (28) and elevated protein content (24.07 vs. 18.05–18.75% *A. sativa* controls). Apart from the report of Gardner and Latta (2006), there are no mapping populations with corresponding linkage maps for any of the allotetraploid *Avena* species. These authors assembled a rudimentary linkage map for the aggressive weed *A. barbata* (AABB genomes) using mostly dominant AFLP markers. Their map contained 129 marker loci distributed among 19 linkage groups, for a total genome size of 644 cM, with a maximum linkage group size of 61.5 cM. Although their dominant AFLP markers provided little or no information regarding sub-genome relationships, Gardner and Latta demonstrated the usefulness of markers for identifying at least 11 putative QTL: in their case for genomic regions affecting environmental fitness.

Valid molecular markers are needed for construction of a complete tetraploid linkage map ($n = 14$) and for genome comparisons between tetraploid and hexaploid oat. Historically, genetic marker development has been focused on hexaploid oat. Available markers currently encompass SCAR (Chong et al. 2004; Orr and Molnar 2008), SSR (Dumlupinar, unpublished; Li et al. 2000; Pal et al. 2002), AFLP (Groh et al. 2001; Kremer et al. 2001), RFLP (O'Donoughue et al. 1992), and recently Diversity Arrays Technology (DArT) (Tinker et al. 2009). The DArT platform is based on microarray hybridizations of complexity-reduced genomic representations, and provides comprehensive genome coverage with no prior sequence information (Jaccoud et al. 2001; Wenzl et al. 2004). These characteristics make DArT markers well suited to genotyping of little-characterized genomes.

Objectives of the present study were to: (1) expand the existing DArT marker resource to included representation from *A. magna* (CCDD) genomes; (2) elucidate the polymorphism content of newly-developed DArT and hexaploid-derived SSR and SNP markers in *A. magna*; (3) develop the first complete linkage map for a tetraploid oat; and (4) map the hexaploid domestication syndrome genes. This work should provide the foundation for further domestication of tetraploid oat, leading perhaps to cultivation of a high-protein oat resource. It should also provide a unique resource for evolutionary studies aimed at determining ancestral genome donors, and for measuring the degree of rearrangement during and since the polyploidization of tetraploid and hexaploid *Avena*.

Materials and methods

Plant material and DNA extraction

A population of 117 recombinant inbred lines (RILs) was developed through hybridization of the wild tetraploid *A. magna* accession #169 with domesticated *A. magna* (Ba 13-13), a BC_1 domesticated, inbred selection from a cross of *A. sativa* (undefined cv., either 'Ogle', '86-4189', '86-4467' or '86-5698') × *A. magna* #169 (Ladizinsky 1995). Domesticated *A. magna* Ba 13-13 was tetraploid, but morphologically akin to common hexaploid oat. The Ba 13-13/*A. magna* #169 (BAM) RIL population was increased by single seed descent to the F_8 stage and cytologically examined for ploidy level at the F_2 and F_8. Nine additional *A. magna* lines (Cc 7069, Cc7070, Cc7071, Cc7073, Cc7237, Cc7238, Cc7240, Cc7244, CIav 8330) were selected for construction of a dedicated *A. magna* DArT array. All genotypes originated from Morocco. Plants were grown in 4-inch square pots, in a greenhouse with an approximately 16-h photoperiod and a daytime temperature ranging from 22 to 30°C.

DNA was extracted from fresh, flash-frozen leaf tissue using a cetyl trimethyl ammonium bromide (CTAB) protocol. Tissue was ground in liquid N_2 and 600 µl extraction buffer (0.35 M sorbitol, 0.3 M Tris–HCl pH 8.0, 5 mM EDTA pH 8.0, 2 M NaCl, 2% CTAB, 5% (w/v) *N*-lauroylsarcosine, 2% (w/v) polyvinylpyrrolidone (PVP40, K29-32), and 0.5% (w/v) sodium metabisulfite) was added to the powder in a 2-ml centrifuge tube. After mixing, the solution was incubated at 65°C for 60 min, 600 µl chloroform was added, and the solution was mixed by inversion and centrifuged at 10,000*g* for 20 min. The aqueous phase was transferred to a fresh tube, 600 µl chilled isopropanol was added, and the solution was mixed by inversion, held at 4°C for approximately 2 h, and centrifuged at 10,000*g* for 30 min. Supernatant was then discarded, and the pellet was washed twice with 70% EtOH, dried, and resuspended in 1× TE buffer.

Morphological markers

Domestication traits were scored phenotypically at the F_2 (117 RILs) and F_8 (112 RILs) generations. Wild-type plants were characterized by dominant lemma pubescence; presence of two large, geniculate awns also dominant; and recessive basal disarticulation, defined by a prominent abscission scar and seed shattering at maturity. Domesticated types were glabrous and non-shattering, with a single, small awn. The BAM RILs represented the two parental and two intermediate phenotypes (Fig. 1): one non-shattering, single awned, and pubescent (Fig. 1c); one shattering, with two geniculate awns, and only basal pubescence (scored as glabrous, Fig. 1d). Segregation ratios were analyzed for each trait at the F_2 generation using expected Mendelian ratios of 3:1 with standard Chi-square tests.

Fig. 1 BAM population phenotypes. Four phenotypes were present in the Ba 13-13 × #169 RIL mapping population, including domesticated (**a**), wild (**b**), and two intermediate phenotypes (**c**, **d**)

A distinct heterochromatic region in the long-arm telomere of chromosome 3 (morphologically homologous to *A. sativa* chromosome 5C) was observed to be associated with the wild *A. magna* phenotypes (Jellen and Ladizinsky 2000; Jellen et al. 1993). This trait was cytologically scored in the BAM population, with nitrous oxide treatment of root tissue according to Kato (1999) and chromosome preparation and banding generally according to Jellen et al. (1993), but using Giemsa instead of Wright's stain. To evaluate linkage relationships, pairwise comparison was performed at the F_2 generation with the knob and domestication traits conforming to a single-gene model. Analyses were based on the expected 1:2:1 ratio.

DArT assay

Development of DArT markers followed published methods (Jaccoud et al. 2001; Tinker et al. 2009; Wenzl et al. 2004). To ensure adequate coverage of the *A. magna*

genome, two dedicated libraries were constructed, using standard *Pst*I/*Taq*I complexity reduction. The first library, representing genetic diversity, contained 1,152 clones from 9 *A. magna* genotypes (Cc 7069, Cc7070, Cc7071, Cc7073, Cc7237, Cc7238, Cc7240, Cc7244, CIav 8330). The second library included 1,536 clones from *A. magna* #169.

These clones, along with 12,672 supplementary oat clones (1,536 from wild species; 11,136 from cultivated materials) were used to print a dedicated array for mapping and diversity analysis. Genomic representations of BA 13-13, #169, and 112 F_8 RILs of the BAM mapping population were prepared using *Pst*I/*Taq*I complexity reduction and hybridized to the DArT arrays. Polymorphism was detected using dedicated software, DArTsoft (Diversity Arrays Technology Pty Ltd, Australia), which applies fuzzy *K*-means clustering to classify samples in a binary manner (0/1) for a particular array feature.

SNP assay

SNP markers used in this study were identified by comparison of expressed tag (EST) sequences from the hexaploid oat cultivars 'Ogle1040' (Brown and Jedlinski 1983), 'TAM O-301' (McDaniel 1974), 'Gem' (Duerst et al. 1999), and 'HiFi' (McMullen et al. 2005) using a new *in silico* SNP selection pipeline (Oliver et al. 2011). Fifty-three assays were validated using the BAM parents (Ba 13-13 and #169) and polymorphic markers were mapped across 112 F_8 RILs of the BAM population. Genotyping was performed by high-resolution melt analysis, using a BioRad C1000 thermal cycler with a CFX96 optics module. Reactions comprised 1× SsoFast EvaGreen Supermix (BioRad) with 55 ng genomic DNA and 0.5 μM forward and reverse primers in a 12.5 μl reaction volume. Thermocycling conditions comprised an initial denaturation at 98°C for 2 min; 46 cycles of 98°C for 2 s and 55°C for 5 s, with fluorescence measured at the end of each cycle; and a melt curve analysis, with a melt gradient from 65 to 95°C, increasing in 0.2°C increments every 10 s, with fluorescence measured at the end of each increment. Melt curves were analyzed using BioRad Precision Melt Analysis Software Version 1.0.534.0511 and genotypes were assigned based on differences in relative fluorescence units as a function of melting temperature.

SSR assay

SSR markers used in this study were identified from enriched genomic libraries using the hexaploid cultivar Ogle1040. Assays for selected microsatellites were validated across a panel of nine hexaploid mapping parents [TAM O-405 (Texas A&M breeding line), Otana (Stewart et al. 1978), Ogle1040, TAM O-301, Sun-II (National Genetic Resources Program, USA), Kanota (Stanton 1955), MN841801-1 (Univ. MN breeding notes), Noble-2 (Univ. MN breeding notes) and Makuru (Baum 1972)] and Ba 13-13 and #169. Microsatellites polymorphic between the BAM parental lines were screened across 112 F_8 RILs and mapped.

Polymerase chain reaction (PCR) was performed in 25-μl PCR reaction volumes containing approximately 120 ng DNA, 5 μl each primer (10 μmol L^{-1}), 5 μl 10× buffer (NEB, #B9014), an additional 1.2 μg $MgCl_2$ (NEB, #B0267S), 1 μl dNTPs (2.5 mol L^{-1}; NEB, #N0447L), and 2.5 U *Taq* polymerase (NEB, #B9014). The conditions were 94°C for 4 min followed by 40 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min, with a final elongation of 4 min at 72°C. Samples were held at 4°C until prepared for electrophoresis. Reaction products were separated on 3% SFR agarose (Amresco, AMR-J234) and band sizes were estimated by comparison with a 100 base pair (bp) ladder (NEB, #N0467S).

Map construction

Alleles for the 112 BAM RILs at each locus were determined by comparison with parental alleles (Online Resource 1). Using this information, preliminary mapping of the domestication syndrome genes was carried out with Chi-square and pairwise analyses of morphological markers. Further linkage analysis and map construction were performed using the MultiPoint package (Mester et al. 2003, 2004; Korol et al. 2009). Multilocus ordering was determined using an algorithm based on the evolutionary optimization strategy (Mester et al. 2003, 2004), using maximum likelihood estimation to calculate pairwise recombination fractions (rf) for all marker pairs. Preliminary clustering and assignment of markers to a linkage group (LG) was evaluated at an $rf = 0.05$ threshold. Selection of framework markers and stability of marker neighborhoods were evaluated concurrently by jackknife resampling, with repeated verification of marker order and removal of unreliable markers. Markers mapping to the same location were grouped and represented by a single delegate. Stable LGs were joined terminally by incrementally increasing the recombination threshold, with a final rf of 0.30. Remaining markers were then attached to a framework marker or an optimal interval. To avoid erroneous linkage groups based on incorrect marker phase, genotypes of unlinked loci or loci in fragment groups were converted to the alternate phase, reclustered, and assigned to linkage groups.

Diversity analysis

Genotypes of DArT markers across 11 *A. magna* accessions were used to assess linkage disequilibrium (LD) and

to determine marker utility based on hierarchical clustering. All analyses were performed using JMP Genomics 4.1 (SAS Institute, Cary, NC) and included 2,014 polymorphic DArT markers, of which 974 were mapped on the BAM population. Markers with one or more null genotypes within the *A. magna* panel were not included in the analysis. Linkage disequilibrium was calculated using marker variables as numeric genotypes and BAM linkage map groups and positions as the group and distance units. Maximum likelihood was used for haplotype estimation. Hierarchical clustering was performed using the Ward method (1963) and three principal components, with dendrogram construction based on the distance scale.

Results

Morphological markers

Chi-square analysis suggested that awnedness and basal articulation followed a 3:1 segregation ratio in the F_2 and segregated 1:1 in subsequent generations, indicating monogenic control (Table 1; Online resource 1). Segregation of lemma pubescence did not follow a recognizable ratio, although Ladizinsky (1995) considered this trait to be controlled by a single gene. Segregation of lemma color was ambiguous, with phenotype distinctions blurred by a range of intermediates, and was not scored in this study.

Cytogenetic analysis of the heterochromatic knob in the F_2 generation followed seed phenotypes (Fig. 1): wild types had the knob on both 5C homologs, domesticated types lacked the knob, and intermediate types generally had a knob on only one chromosome (Fig. 2). Pairwise analyses indicated that awnedness and basal articulation are linked to the heterochromatic knob at the long-arm terminus of chromosome 5C (Table 2).

Molecular markers

A total of 15,360 DArT probes were analyzed for hybridization, resulting in 2349 (15.29%) that produced a detectable signal. Ninety-five of these DArT loci mapped, but were ambiguous on the *A. magna* panel and were consequently not included in the diversity analyses. Of the remaining loci, 447 (21.03%) were new and specific to the CCDD genome of *A. magna*, while 1,679 (78.97%) were from existing *Avena* arrays (Tinker et al. 2009). Seventy-six loci were not polymorphic across the diversity panel and removed from subsequent analyses. Of the 2,050 loci remaining, 36 had a minor allele frequency (MAF) <0.10 and were null in at least one of the eleven diversity lines. These were removed, leaving a total of 2,014 loci.

Assays for 67 *in silico* SNP loci derived from hexaploid oat sequence were evaluated across the BAM parental lines. Fourteen (20.90%) were polymorphic and robust across the BAM population. One of these had segregation distortion that did not follow the trend within the linkage group and was discarded; remaining markers were incorporated in the map. Of these, all but one (RA lrc40347_1) have been mapped in a hexaploid RIL population (Oliver et al. 2010). Similarly, assays for 244 robust hexaploid oat derived microsatellites (Dumlupinar, unpublished) were evaluated across the parental lines. Twenty-six were polymorphic and clearly mapped in the BAM population. Unfortunately, only four loci had shared polymorphism between the tetraploid and hexaploid maps.

Diversity analysis

To determine the utility of these markers, MAF and hierarchical clustering based on Ward's method were applied to a panel of 2,014 DArT loci across 11 *A. magna* genotypes. The analysis indicated significant relationships, partitioning accessions into three major clusters, with both BAM parents as outliers (Fig. 4). Ba 13-13, the domesticated *A. magna* accession, clustered independently from the first node, suggesting an influence of hexaploid DNA. Other cluster relationships could be based on geographic origin.

Genetic map

Based on consistent segregation ratios and minimal distortion in the BAM population, 974 DArT loci, 26 microsatellites, 13 SNP markers, and 4 morphological markers were selected to construct the genetic linkage map. The

Table 1 Chi-square analysis of domestication traits segregating in a wild × cultivated *A. magna* F_2 recombinant inbred line population

Trait	Phenotype	No. observed	χ^2	*p* value
Basal disarticulation	Non-shattering	88	0.00285	0.9574
	Shattering	29		
Awnedness	Two awns	87	0.02564	0.8728
	One awn	30		
Lemma pubescence	Pubescent	100	6.8404	~0.009
	Glabrous	17		

Analyses were based on an expected 3:1 segregation ratio

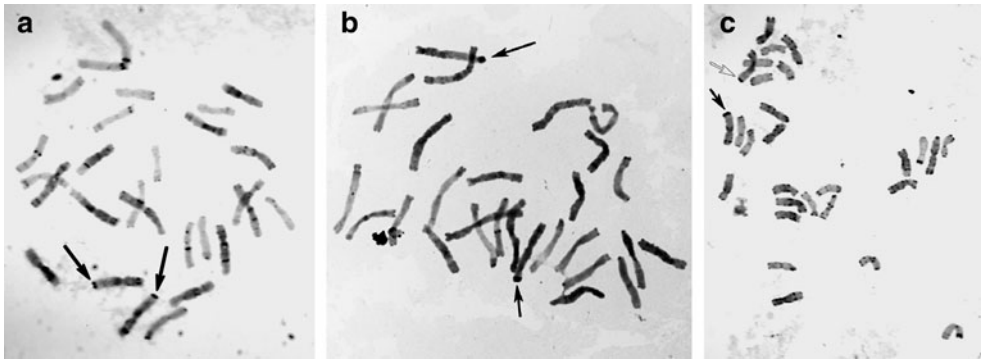

Fig. 2 Photomicrographs of heterochromatic cytological knob. C-banded root-tip metaphase cells of the segregating BAM [Ba 13-13 (non-5C knob) × #169 (5C knob)] population. **a** BAM-21 F_2 plant, homozygous non-5C knob (*arrows*), **b** BAM-108 RIL plant, homozygous 5C knob (*arrows*), **c** BAM-25 F_2 plant, heterozygous non-knob (*white arrow*)/knob (*black arrow*). Magnification is ×1,000

Table 2 Pairwise comparison of domestication traits with the cytogenetic knob on chromosome 5C

Trait	Phenotype	No. observed			χ^2	*p* value
		Knob phenotype				
		+	+/−	−		
Basal articulation	Non-shattering	9	51	28	93.120	1.48E−18
	Shattering	26	2	1		
Awnedness	Two awns	35	52	0	115.547	2.75E−23
	One awn	0	1	29		

Analyses were performed on traits conforming to a single-gene model and based on an expected 1:2:1 ratio in the F2 generation

+ homozygous knob, +/− heterozygous knob,− homozygous non-knob

MultiPoint mapping software was used to establish linkage groups and to simultaneously determine marker order and select informative framework or skeleton markers while removing problematic markers. Map construction involved 1,017 markers and 112 RILs, giving a maximum recombination resolution of nearly 1 cM. The framework map comprised 14 linkage groups and 362 markers at 214 unique loci, with a total map distance of 1,411.1 cM. An additional 655 markers were attached to the framework at the optimal interval. Framework markers comprised 332 DArTs (66 from the *A. magna* array, 228 from hexaploid arrays, and 38 from a wild species array); 16 microsatellites; 10 SNPs; and 4 morphological markers (Table 3). Markers were generally well distributed, with dense coverage on two linkage groups (BAM-1, BAM-2) and occasional marker intervals of 25–35 cM (BAM 9, 10, 11, 14) (Fig. 3; Table 3).

Loci for basal abscission, awnedness, and the cytological knob mapped to the terminus of BAM 13, providing an anchor to associate this linkage group with chromosome 5C. Lemma pubescence mapped to the end of linkage group BAM 11, suggesting the phenotype is likely to be controlled, at least predominantly, by a major QTL in this region.

Linkage disequilibrium

Linkage disequilibrium analysis was not meaningful based on the small sample of genotypes ($n = 11$) included in this study. However, linkage group-specific heat plots based on mapped marker-to-marker correlation coefficients were used to discriminate among redundant markers at shared loci and to reveal conserved genetic blocks within the linkage group.

Mapped marker-to-marker correlation coefficients were readily visualized by two-way color profiles within a heat plot, disrupting the random pattern and resulting in identical color blocks based on hybridized (red) and non-hybridized (blue) alleles (Online Resource 2). Markers generating identical profiles along both axes, mapping to the same locus, and not in an area of obvious linkage disequilibrium were considered redundant. Within the map framework, 68 loci were represented by more than one marker. At these shared loci, 82 of 201 markers (40.8%) were redundant, with coverage representing approximately half (52.94%) of the 68 loci. Thus, 47.06% of shared loci were comprised of only unique markers.

Clusters of loci containing minor alleles suggested conservation of genetic linkage blocks. Within these regions, the origin of the hybridized allele was non-random, deriving either from Ba 13-13 or from #169. This clustering by allelic origin indicated two types of linkage block conservation, and divided linkage groups into two sets of seven (Fig. 3; Online Resource 2). In linkage groups BAM 1, 2, 4, 7, 9, 10, and 12, regions contained minor alleles matching the wild or domesticated parent. Conversely, linkage groups BAM 3, 5, 6, 8, 11, 13, and 14 conserved the minor allele matching #169.

Table 3 Marker distribution and map length of linkage groups in a genetic map constructed from a wild × cultivated *A. magna* recombinant inbred line population

Linkage group	DArT		SNP		SSR		Total no. markers	Length (cM)	Largest gap (cM)	Avg. marker dist. (cM)[c]	Avg. marker dist. (cM)[d]
	Total[a]	Framework[b]	Total	Framework	Total	Framework					
BAM 1	106	23 (52)	2	1 (2)	2	1 (1)	110	109.1	10.8	4.36	0.99
BAM 2	118	20 (40)	2	1 (1)	3	3 (3)	123	123.5	13.7	5.15	1.00
BAM 3	26	12 (13)	0	0	2	1 (1)	28	95.6	12.2	7.35	3.41
BAM 4	64	14 (22)	0	0	2	2 (2)	66	143.4	20.5	8.96	2.17
BAM 5	112	11 (27)	4	1 (3)	2	1 (1)	118	61.7	13.4	4.75	0.52
BAM 6	78	14 (20)	0	0	0	0	78	104.9	22.3	7.49	1.34
BAM 7	32	15 (19)	1	0	0	0	33	87.6	19.8	5.84	2.65
BAM 8	112	9 (17)	1	1 (1)	5	4 (4)	118	85.2	17.4	6.09	0.72
BAM 9	60	15 (20)	0	0	2	2 (2)	62	134.0	24.8	7.88	2.16
BAM 10	72	12 (18)	1	1 (1)	1	0	74	90.4	36.5	6.95	1.22
BAM 11	50	9 (18)	2	2 (2)	4	1 (1)	57[e]	104.8	36.5	8.06	1.84
BAM 12	37	13 (22)	0	0	0	0	37	73.2	22.9	5.63	1.98
BAM 13	80	9 (24)	0	0	3	1 (1)	86[f]	93.5	22.1	7.79	1.09
BAM 14	27	12 (20)	0	0	0	0	27	104.2	26.0	8.68	3.86
Total	974	188 (332)	13	7 (10)	26	16 (16)	1,017	1411.1			

[a] Includes framework markers, shared markers, and attached markers

[b] Number of framework markers at unique loci. Values in parenthesis include shared markers

[c] Average marker distance between framework markers. Calculations were based on a single marker representative at each locus

[d] Average marker distance including shared and attached markers

[e] Includes one morphological marker

[f] Includes three morphological markers

Discussion

Here, for the first time, an allotetraploid oat genome has been resolved into 14 linkage groups defining two genome classes. Delineation of linkage groups, selection of framework markers, and resolution of marker order were performed using the MultiPoint mapping package, with algorithms based on marker order and incrementally increasing recombination thresholds (Korol et al. 2009; Mester et al. 2004). Closely linked markers were grouped and represented by a delegate at a single locus, and iterative resampling was performed to simultaneously determine marker order and to detect and remove markers causing local instability in a linkage group. These features allowed selection of the most informative and reliable markers within the resolution of recombination, minimizing map distortion and achieving an unambiguous ordering of framework markers.

The current map, consisting of 1,017 markers over 1,411.1 cM, has an average marker interval of 1.39 cM, which is more dense than in previous oat maps, including 'Kanota' × 'Ogle' (1.6 cM) (Wight et al. 2003) and 'Ogle1040' × 'TAM O-301' (3.8 cM) (Oliver et al. 2010). Marker density is attributed largely to DArT markers, with coverage enhanced by a dedicated array accounting for multiple *A. magna* genotypes. As observed with previous oat DArT studies (Tinker et al. 2009), markers tended to cluster in various regions, with little or no coverage in other regions. Other restriction-based marker technologies have shown the same predisposition, especially those based on methylation-sensitive enzymes (Peng et al. 2000; Young et al. 1999) as is the case with DArT. Overall marker dispersal may reflect general gene distribution throughout the linkage group, with regions of sparse marker distribution corresponding to regions of reduced gene frequency and increased recombination around methylation-sensitive markers targeting gene-rich areas. For example, research in wheat (Lukaszewski and Curtis 1993; Werner et al. 1992), barley (Künzel et al. 2000), and, to a lesser extent, rice (Cheng et al. 2001; Wu and Tanksley 1993) has indicated differing levels of recombination and marker frequency within the chromosomes of these species, with crossovers generally suppressed near the centromere and increased in telomeric regions. Several mapping studies with DArT markers showed clear marker clustering through the enrichment for subtelomeric, gene-rich, euchromatic regions, compared to other marker types both in barley (Wenzl et al. 2006) and wheat (Akbari et al. 2006). This trend, coupled with the apparent marker distribution bias, may suggest

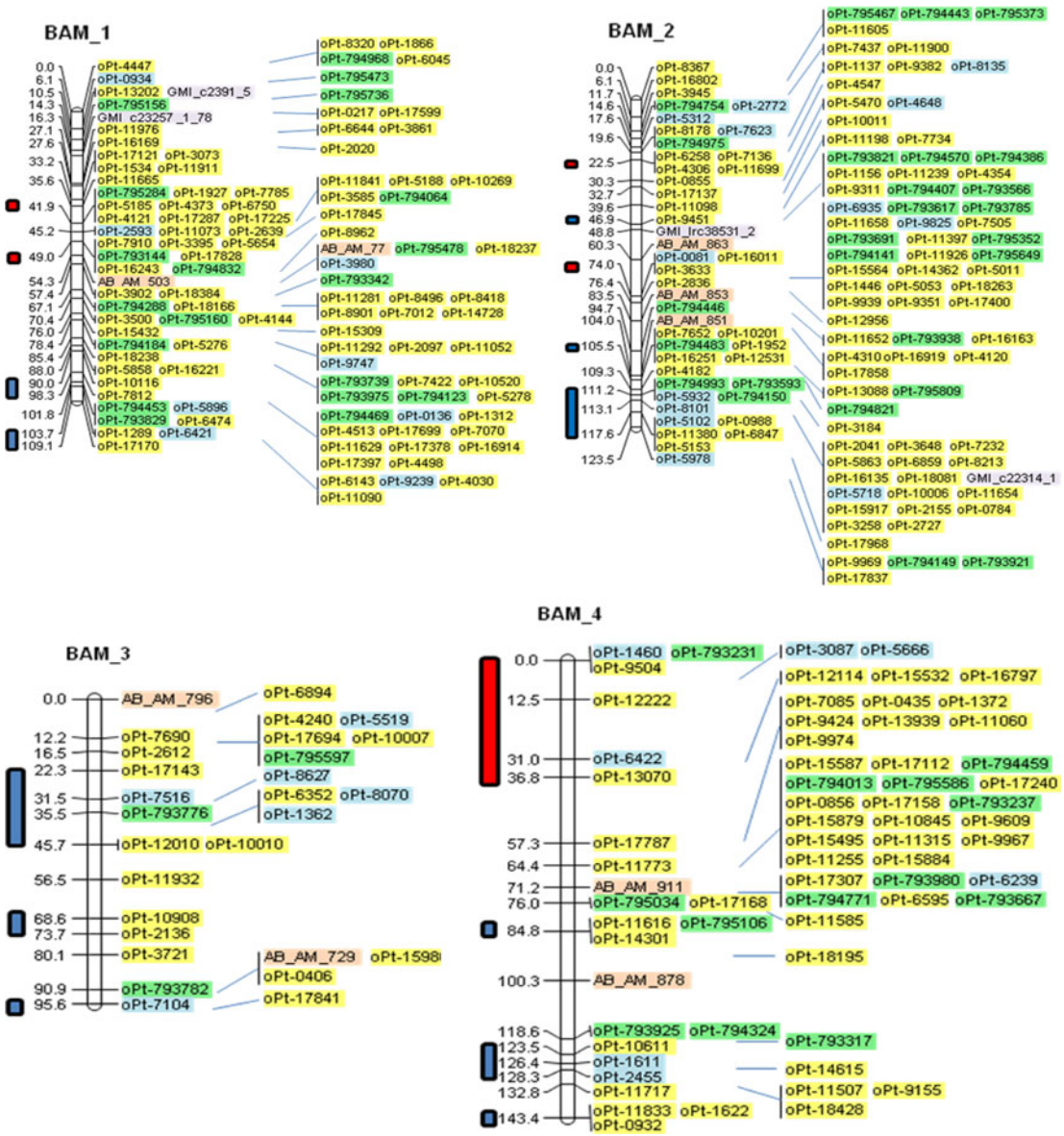

Fig. 3 BAM linkage map. A fourteen-linkage-group genetic map of tetraploid oat constructed from an *A. magna* recombinant inbred line population. Attached markers are mapped to an optimal interval within the framework. Markers with the "oPt-" prefix are DArTs from the cultivated, *A. magna*, and wild species arrays. Microsatellite markers have the "AB_AM_" prefix. SNP markers have the "GMI_" prefix and domestication-associated traits Lp, Awn, Ba, and Knb (cytological knob) are indicated. Bars to the left of each linkage group indicate haplotypic blocks of minor alleles matching domesticated Ba 13-13 or wild #169. Major blocks matching Ba 13-13 are at 0.0–36.8 on BAM_4, 16.6–34.8 on BAM_9, 70.5–87.7 on BAM_10, and in the top portion of BAM_12

that oat chromosomes are similarly arranged, with areas of lower gene frequency and recombination interspersed with gene clusters and recombination hotspots.

In previous studies, DArT markers sharing the same map positions were considered redundant loci (Tinker et al. 2009). Using the mapped marker-to-marker correlation coefficients based on a panel of diverse lines, we were able to differentiate between truly redundant DArT markers and tightly linked markers that could not be resolved with available numbers of RILs. This technique provides a method to separate informative from redundant markers in other studies with small population sizes.

The domestication syndrome genes in this study fit the model for a single Mendelian locus and mapped accordingly on BAM 11 and BAM 13. This result concurs with the domestication profile in *Triticum dicoccoides*, the wild tetraploid progenitor of cultivated wheat, where domestication syndrome factors were controlled predominantly by QTL in a single genome (Peng et al. 2003). Like wheat and other grass species (Middleton 1938; Morishima 1984;

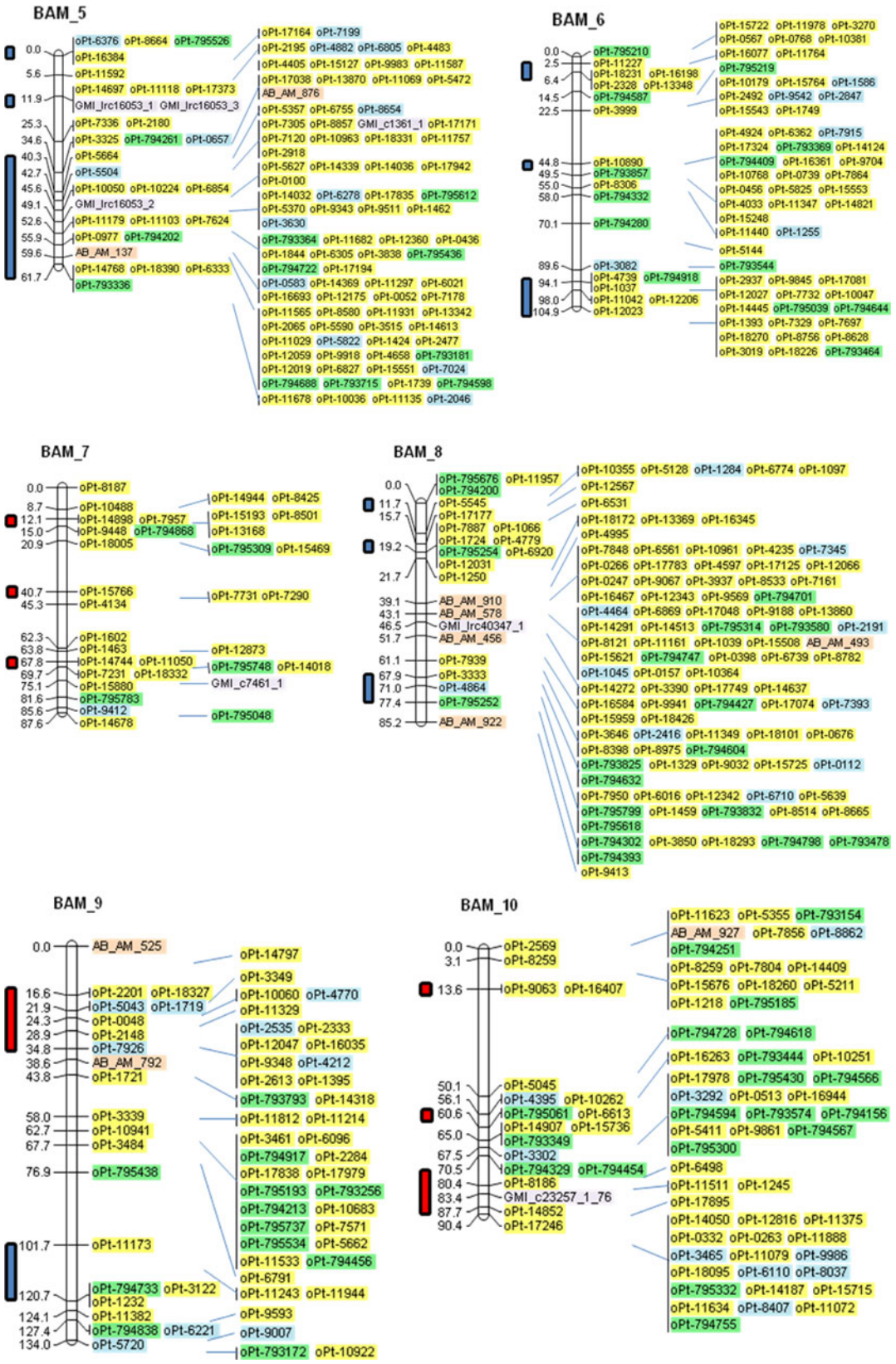

Fig. 3 continued

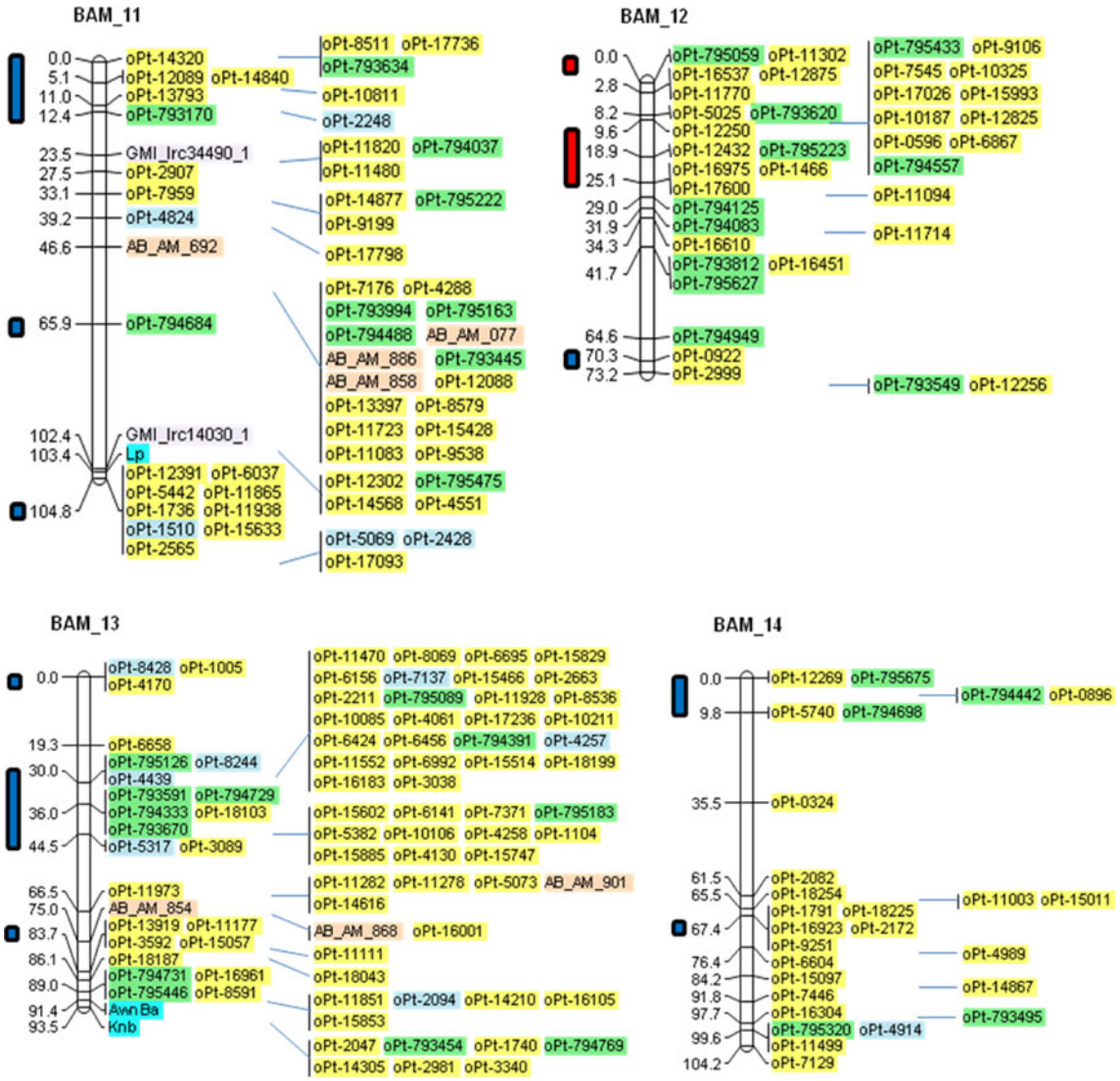

Fig. 3 continued

Paterson et al. 1995; Peng et al. 2003; Poncet et al. 2000; Xiong et al. 1999), the oat domestication traits are controlled by a small number of genes with relatively large phenotypic effects. On the BAM linkage map, these genes are positioned near the telomeres, regions typically recognized for high gene density and frequent recombination (Sandhu and Gill 2002). Conveniently, the subtelomeric localization of these genes was made possible by cytologically scoring a knob that cosegregated with the wild alleles. The small number of domestication genes, which correspond loosely in genomes of various grass species, has been suggested as an indication of rapid domestication (Paterson et al. 1995), a possibility made more feasible by the genetic location within the chromosome. The current map opens the way for further study of the domestication syndrome elsewhere in the oat genus, such as the A_sA_s diploid *A. strigosa* Schreb., as well as the AABB tetraploid *A. abyssinica* Hochst.

Within the panel of 11 *A. magna* genotypes, cluster resolution appeared to be influenced by geographic origin (Fig. 4). Although all genotypes (with the exception of Ba 13-13) were collected from the northern coastal region and adjacent lowlands of Morocco, clustering was to some extent associated with localities within this region. For example, Cc 7069 and Cc 7237 are both from the same village, while Cc 7244 is from a neighboring town. However, proximity of the collection sites and unknown travel and dispersal patterns make it impossible to clearly correlate cluster divisions with geographic origin.

Patterns of minor allele clustering provide significant clues for genome discrimination, and for dissection of genome origin and conservation within *Avena* species.

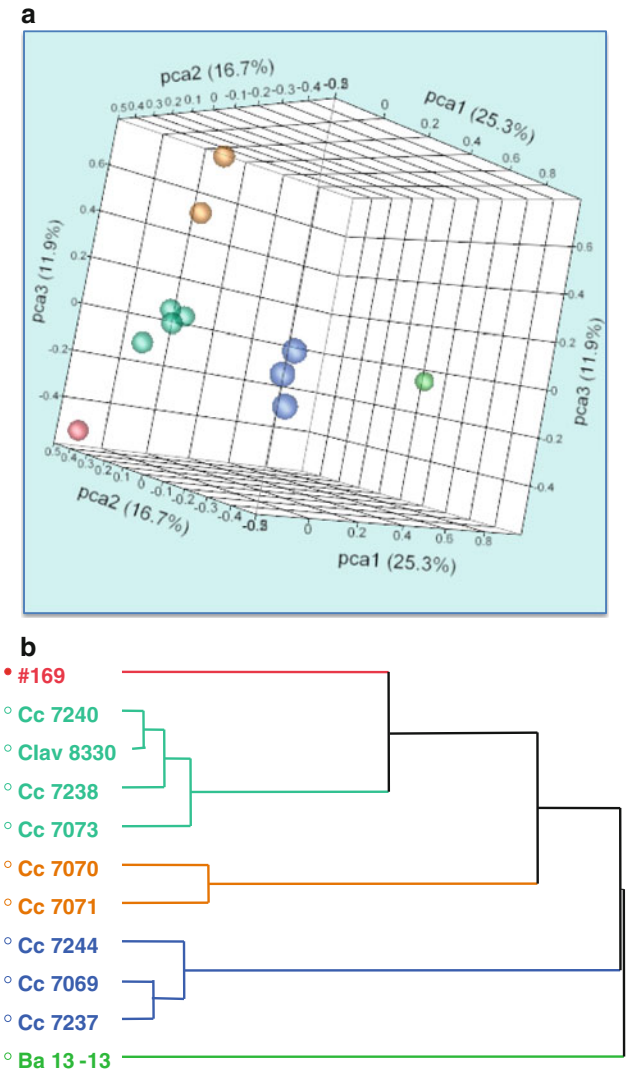

Fig. 4 Genetic diversity analysis. Principal component analysis (**a**) and hierarchical clustering (**b**) of allelic diversity at 2,014 DArT loci was performed using 11 *Avena magna* lines, including the parents of the BAM mapping population. Analysis was performed using JMP Genomics (SAS Institute, Cary, North Carolina)

Seven linkage groups contained regions of loci with minor alleles matching #169, while seven groups contained regions with minor alleles matching both wild and domesticated parents. This observation hints at possible conservation of #169-like alleles representing haplotypic blocks of *A. magna* DNA that are conserved within the species and distinct from similar subgenomic regions of hexaploid oat. Cytogenetic evidence for intra-genome variation among C-banded chromosomes of the C genomes of Sections Pachycarpa (Baum) and Avena has been reported (Jellen and Ladizinsky 2000). Thus, it seems likely not only that the linkage groups with conservation of #169 alleles represent C-genome chromosomes, but also that the C genome represented in *A. magna* is distinct from that of hexaploid oat. This supports the contention, based on chromosome morphological similarities (Jellen and Ladizinsky 2000) and hybrid pairing data (Ladizinsky 2000), that *A. insularis* rather than *A. magna* or *A. murphyi* is the immediate tetraploid ancestor of hexaploid oat. Additional confirmation for the identity of C-genome chromosomes is provided by the cytological knob associated with non-domestication alleles. Cytogenetic investigations of numerous wild and domesticated *Avena* accessions of Sections Pachycarpa (4*x*) and Avena (6*x*) have suggested an association between this chromosome 5CL telomeric knob and spikelet-dispersed phenotypes (Jellen and Ladizinsky 2000; Jellen unpublished). In this study, the knob was mapped to linkage group BAM 13, which had conserved minor-allele clusters from *A. magna*. Interestingly, the domestication gene controlling lemma pubescence (*Lp*) mapped to BAM 11, which similarly contained *A. magna* minor-allele clusters. As an extension of this logic, linkage groups represented by *A. magna* minor-allele clusters should define the C-genome chromosomes.

In contrast, the alternate genome of *A. magna* (DD) appears to be similar to that of hexaploid oat. Broad interspecific similarities within the D genome suggest that the diploid donor was cross-compatible and hybridized widely with various species. In this study, the distribution of loci containing minor alleles matching both wild and domesticated accessions indicates that conservation across *Avena* species is caused by selection of key phenotypes such as disease resistance, and not ancestral bottlenecks. Recently, the Ogle1040/TAM O-301 (OT) linkage group OT32 containing several crown rust resistance QTL (Hoffman et al. 2006; Jackson et al. 2010; Jackson et al. 2007) was anchored to chromosome 9D using DArT deletion analysis on monosomic stocks (Jackson et al. 2009). This same chromosome was also found to contain a QTL for partial crown rust resistance in MN841801 (Acevedo et al. 2010). Although shared DArT markers between the BAM and OT linkage maps were insufficient to facilitate a study of synteny, five DArT loci linked to the aforementioned QTL were found in a region containing minor alleles on BAM 1. This suggests that this region is homeologous to chromosome 9D in cultivated oat and was conserved among *Avena* species due to selection for crown rust resistance. In the present study, the average linkage group length of the D-genome chromosomes was greater than those of the C genome (Table 3; Fig. 3). Since the C genome in oat is highly heterochromatic (Jellen et al. 1993; Linares et al. 1992) and heterochromatic DNA is methylated in grasses like rice (Yan et al. 2010), this result supports C- and D-genome assignments.

The large number of molecular markers developed in this study allowed for construction of the first complete oat

linkage map and provided important insight on C- and D-genome ancestry and oat domestication. Mapped marker-to-marker correlations allowed discrimination of redundant loci and assignment of linkage groups to C and D genomes. Oat domestication phenotypes were shown to be controlled primarily by single genes clustered in telomeric regions, suggesting potential for rapid adaptation. Taken as a whole, these results provide clues on the evolutionary development of *A. magna*, and, more broadly, on possible genome interrelationships in the context of polyploidization. Unfortunately, syntenic relationships between tetraploid and hexaploid linkage maps could not be resolved based on the lack of shared markers on published maps. Future work should be aimed at specific development of shared markers to facilitate comparative mapping between genomes of these species. Overall, these results facilitate deployment of key *A. magna* characteristics and provide a unique resource for future studies of oat evolution and comparative genomics.

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References

Acevedo M, Jackson EW, Chong J, Rines HW, Harrison S, Bonman JM (2010) Identification and validation of quantitative trait loci for partial resistance to crown rust in oat. Phytopathology 100:511–521

Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A (2006) Diversity arrays technology (DArT) for high-throughput profiling of the hexaploid wheat genome. Theor Appl Genet 113:1409–1420

Baum BR (1972) Material for international oat register. Cat No A52-4772

Braaten JT, Wood PJ, Scott FW, Wolynetz MS, Lowe MK, Bradley-White P, Collins MW (1994) Oat beta-glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. Eur J Clin Nutr 48:465–474

Brown CM, Jedlinski H (1983) Ogle spring oat. Crop Sci 23:1012

Cheng Z, Presting GG, Buell CR, Wing RA, Jiang J (2001) High-resolution pachytene chromosome mapping of bacterial artificial chromosomes anchored by genetic markers reveals the centromere location and the distribution of genetic recombination along chromosome 10 of rice. Genetics 157:1749–1757

Chong J, Reimer E, Somers D, Aung T, Penner GA (2004) Development of sequence-characterized amplified region (SCAR) markers for resistance gene *Pc94* to crown rust in oat. Can J Plant Path 26:89–96

Distelfeld A, Uauy C, Fahima T, Dubcovsky J (2006) Physical map of wheat high-grain protein content gene Gpc-B1 and development of a high-throughput molecular marker. New Phytolog 169:753–763

Duerst RD, Kaeppler HF, Forsberg RA (1999) Registration of 'Gem' oat. Crop Sci 39:879–880

Dumlupinar Z, Jellen EN, Anderson J, Bonman JM, Carson M, Chao S, Obert DE, Hu G, Jackson EW (2011) The art of attrition: Development of robust oat microsatellites (unpublished)

Eggum BO, Hansen I, Larsen T (1989) Protein quality and digestible energy of selected food determined in balanced trials with rats. Plant Foods Hum Nutr 39:13–21

Gardner KM, Latta RG (2006) Identifying loci under selection across contrasting environments in *Avena barbata* using quantitative trait locus mapping. Mol Ecol 15:1321–1333

Groh S, Zacharias A, Kianian SF, Penner GA, Chong J, Rines HW, Phillips RL (2001) Comparative AFLP mapping in two hexaploid oat populations. Theor Appl Genet 102:876–884

Harlan JR, DeWet JMJ, Price EG (1973) Comparative evolution of cereals. Evolution 27:311–325

Hoffman DL, Chong J, Jackson EW, Obert DE (2006) Characterization and mapping of a crown rust resistance gene complex (*Pc58*) in TAM O-301. Crop Sci 46:2630–2635

Jaccoud D, Peng K, Feinstein D, Kilian A (2001) Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Res 29:E25

Jackson EW, Obert DE, Menz M, Hu G, Avant JB, Chong J, Bonman JM (2007) Characterization and mapping of oat crown rust resistance genes using three assessment methods. Phytopathology 97:1063–1070

Jackson E, Jellen E, Oliver R, Lazo G, Tinker N, Rossnagel B, Anderson J, Bonman JM (2009) Resolving the oat mapping story by weaving together a consensus map. In: Tinker NA (ed) Proc Plant Animal Gen Conf XVII, San Diego, W337

Jackson EW, Obert DE, Avant JB, Harrison SA, Chong J, Carson ML, Bonman JM (2010) Quantitative trait loci in the Ogle/TAM O-301 oat mapping population controlling resistance to *Puccinia coronata* in the field. Phytopathology 100:484–492

Jellen EN, Ladizinsky G (2000) Giemsa C-banding in *Avena insularis* Ladizinsky. Genet Res Crop Evol 47:227–230

Jellen EN, Phillips RL, Rines HW (1993) C-banded karyotypes and polymorphisms in hexaploid oat accessions (*Avena* spp.) using Wright's stain. Genome 36:1129–1137

Jones DB, Caldwell A, Widness KD (1948) Comparative growth-promoting values of the proteins of cereal grains. Cereal Chem 35:639–649

Kato A (1999) Air drying method using nitrous oxide for chromosome counting in maize. Biotech Histochem 74:160–166

Korol A, Mester D, Frenkel Z, Ronin Y (2009) Methods for genetic analysis in the Triticeae. In: Feuillet C, Muehlbauer GJ (eds) Genetics and genomics of the *Triticeae*, 1st edn. Springer, New York, pp 163–199

Kremer CA, Lee M, Holland JB (2001) A restriction fragment length polymorphism based linkage map of a diploid *Avena* recombinant inbred line population. Genome 4:192–204

Künzel G, Korzun L, Meister A (2000) Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics 154:397–412

Ladizinsky G (1995) Domestication via hybridization of the wild tetraploid oats *Avena magna* and *A. murphyi*. Theor Appl Genet 91:639–646

Ladizinsky G (2000) A synthetic hexaploid ($2n = 42$) oat from the cross of *Avena strigosa* ($2n = 14$) and domesticated *A. magna* ($2n = 28$). Euphytica 116:231–235

Ladizinsky G, Fainstein R (1977) Domestication of the protein-rich tetraploid wild oats *Avena magna* and *A. murphyi*. Euphytica 26:221–223

Li CD, Rossnagel BG, Scoles GJ (2000) The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor Appl Genet 101:1259–1268

Linares C, Vega C, Ferrer E, Fominaya A (1992) Identification of C-banded chromosomes in meiosis and the analysis of nucleolar activity in *Avena byzantine* C. Koch cv 'Kanota'. Theor Appl Genet 83:650–654

Linares C, Ferrer E, Fominaya A (1998) Discrimination of the closely related A and D genomes of the hexaploid oat *Avena sativa* L. Proc Natl Acad Sci USA 95:12450–12455

Lukaszewski AJ, Curtis CA (1993) Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor Appl Genet 86:121–127

McDaniel ME (1974) Registration of TAM O-301 oat. Crop Sci 14:127–128

McFerson JK, Frey KJ (1991) Recurrent selection for protein yield of oat. Crop Sci 31:1–8

McMullen MS, Doehlert DC, Miller JD (2005) Registration of 'HiFi' oat. Crop Sci 45:1664

Mester D, Ronin Y, Minkov D, Nevo E, Korol A (2003) Constructing large-scale genetic maps using an evolutionary strategy algorithm. Genetics 165:2269–2282

Mester DI, Ronin YI, Nevo E, Korol AB (2004) Fast and high precision algorithms for optimization in large-scale genomic problems. Comput Biol Chem 28:281–290

Middleton GK (1938) Inheritance in a cross between *Avena sativa* and *Avena sterilis* ludoviciana. J Am Soc Agron 30:193–208

Morishima H (1984) Wild plants and domestication. In: Tsunoda S, Takahashi N (eds) Biology of rice. Jap Sci Press/Elsevier, Tokyo/Amsterdam, pp 3–30

Nutall FQ, Mooradian AD, Gannon MC, Billington C, Krezowsi P (1984) Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. Diabetes Care 7:465–470

O'Donoughue LS, Wang Z, Röder M, Kneen B, Leggett M, Sorrells ME, Tanksley SD (1992) An RFLP-based linkage map of oats based on a cross between two diploid taxa (*Avena atlantica* × *A. hirtula*). Genome 35:765–771

Ohm HW, Shaner G (1992) Breeding oat for resistance to diseases. In: Marshall HG, Sorrells ME (eds) Oat science and technology. Amer Soc Agron, Madison, WI, pp 657–698

Oliver RE, Obert DE, Hu G, Bonman JM, O'Leary-Jepsen E, Jackson EW (2010) Development of oat-based markers from barley and wheat microsatellites. Genome 53:458–471

Oliver RE, Lazo GR, Lutz JD, Rubenfield MJ, Tinker NA, Anderson JM, Wisniewski-Morehead MH, Adhikary D, Jellen EN, Maughan PJ, Brown-Guedira GL, Chao S, Beattie AD, Carson ML, Rines HW, Obert DE, Bonman JM, Jackson EW (2011) Model SNP development based on the complex oat genome using high-throughput 454 sequencing technology. BMC Genomics 12:77

Orr W, Molnar SJ (2008) Development of PCR-based SCAR and CAPS markers linked to β-glucan and protein content QTL regions in oat. Genome 51:421–425

Pal N, Sandhu JS, Domier LL, Kolb FL (2002) Development and characterization of microsatellite and RFLP-derived PCR markers in oat. Crop Sci 42:912–918

Paterson AH, Lin YR, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu SC, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718

Peng J, Korol AM, Fahima T, Röder MS, Ronin YI, Li YC, Nevo E (2000) Molecular genetic maps in wild emmer wheat, *Triticum dicoccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Peng J, Ronin Y, Fahima T, Röder MS, Li Y, Nevo E, Korol A (2003) Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc Natl Acad Sci USA 100:2489–2494

Poncet V, Lamy F, Devos KM, Gale MD, Sarr A, Robert T (2000) Genetic control of domestication traits in pearl millet (*Pennisetum glaucum* L., *Poaceae*). Theor Appl Genet 100:147–159

Sandhu D, Gill KS (2002) Gene-containing regions of wheat and the other grass genomes. Plant Physiol 128:803–811

Stanton TR (1955) Oat identification and classification. USDA-ARS Tech Bull No 1100. US Govt Print Office, Washington

Stewart VR, Wesenberg DM, Hayes RM, Petr FC (1978) Registration of Otana oats. Crop Sci 18:693

Thomas H (1992) Breeding oat for resistance to diseases. In: Marshall HG, Sorrells ME (eds) Oat science and technology. Amer Soc Agron, Madison, WI, pp 473–507

Tinker NA, Kilian A, Wight CP, Heller-Uszynska K, Wenzyl P, Rines HW, Bjørnstad Å, Howarth CJ, Jannink J-L, Anderson JM, Rossnagel BG, Stuthman DD, Sorrells ME, Jackson EW, Tuvesson S, Kolb FL, Olsson O, Federizzi LC, Carson ML, Ohm HW, Molnar SJ, Scoles GJ, Eckstein PE, Bonman MJ, Ceplitis A, Langdon T (2009) New DArT markers for oat provide enhanced map coverage and global germplasm characterization. BMC Genomics 10:39

Wang YF, Yancy WS, Yu D, Champagne C, Appel LJ, Lin P-H (2008) The relationship between dietary protein intake and blood pressure: results from the PREMIER study. J Human Hypertens 22:745–754

Ward JH (1963) Hierarchical grouping to optimize an objective function. J Am Stat Assoc 58:236–244

Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A (2004) Diversity Arrays Technology (DArT) for whole-genome profiling of barley. Proc Natl Acad Sci USA 101:9915–9920

Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7:206

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311

Wight CP, Tinker NA, Kianian SF, Sorrells ME, O'Donoughue LS, Hoffman DL, Groh S, Scoles GJ, Li CD, Webster FH, Phillips RL, Rines HW, Livingston SM, Armstrong KC, Fedak G, Molnar SJ (2003) A molecular marker map in 'Kanota' × 'Ogle' hexaploid oat (*Avena* spp.) enhanced by additional markers and a robust framework. Genome 46:28–47

Wu K-S, Tanksley TD (1993) Genetic and physical mapping of telomeres and macrosatellites of rice. Plant Mol Biol 22:861–872

Xiong LZ, Liu KD, Dai XK, Xu CG, Zhang Q (1999) Identification of genetic factors controlling domestication-related traits of rice using and F_2 population of a cross between *Oryza sativa* and *O. rufipogon*. Theor Appl Genet 98:243–251

Yan H, Kikuchi S, Neumann P, Zhang W, Wu Y, Chen F, Jiang J (2010) Genome-wide mapping of cytosine methylation revealed dynamic DNA methylation patterns associated with genes and centromeres in rice. Plant J 63:353–365

Young VR, Pellett PL (1994) Plant proteins in relation to human protein and amino acid nutrition. Am J Clin Nutr 59:1203S–1212S

Young WP, Schupp JM, Keim P (1999) DNA methylation and AFLP marker distribution in the soybean genome. Theor Appl Genet 99:785–790

Zhu S, Rossnagel BG, Kaeppler HF (2004) Genetic analysis of quantitative trait loci for groat protein and oil content in oat. Crop Sci 44:254–260