The first doubled haploid linkage map for cultivated oat

Pirjo Tanhuanpää, Ruslan Kalendar, Alan H. Schulman, and Elina Kiviharju

Abstract: To date, all linkage maps of hexaploid oat (*Avena sativa* L.) have been constructed using recombinant inbred lines (RILs). Doubled haploids (DHs), however, have the advantage over RILs of their comprehensive homozygosity. DHs have been used for mapping in several cereal species, but in oats the production of large DH populations has only recently become an option. A linkage map of hexaploid oat was constructed using an anther culture–derived DH population (137 individuals) from the F_1 individuals of a cross between the Finnish cultivar 'Aslak' and the Swedish cultivar 'Matilda'. The map is composed of 28 linkage groups containing 625 DNA markers: 375 AFLPs (amplified fragment length polymorphisms), 3 IRAPs (inter-retrotransposon amplified polymorphisms), 12 ISSRs (inter simple sequence repeats), 12 microsatellites, 57 RAPDs (random amplified polymorphic DNAs), 59 REMAPs (retrotransposon-microsatellite amplified polymorphisms), 105 SRAPs (sequence-related amplified polymorphisms), and 2 SNPs (single-nucleotide polymorphisms). The total map size is 1526 cM. Over half of the markers in the map showed distorted segregation, with alleles from 'Aslak' usually prevailing. This is explained by the better performance of 'Aslak' in anther culture. Quantitative trait loci affecting some important quality and agronomic traits are being localized on the map.

Key words: anther culture, *Avena sativa*, DNA markers, doubled haploid, linkage map, segregation distortion.

Résumé : À ce jour, toutes les cartes génétiques de l'avoine hexaploïde (*Avena sativa* L.) ont été produites avec des populations de lignées recombinantes fixées (« RIL »). Par rapport aux RIL, les haploïdes doublés (HD) ont cependant l'avantage d'une homozygotie exhaustive. Les HD ont été employés en cartographie chez plusieurs espèces de céréales, mais jusqu'à récemment il n'était pas possible de produire de grandes populations de lignées HD chez l'avoine. Une carte génétique de l'avoine hexaploïde a été produite grâce à une population de 137 lignées HD dérivées de la culture d'anthères à partir d'individus F_1 issus du croisement entre le cultivar finlandais 'Aslak' et le cultivar suédois 'Matilda'. La carte compte 28 groupes de liaison et totalise 625 marqueurs : 375 AFLP (polymorphismes de longueur de fragments amplifiés), 3 IRAP (polymorphismes d'amplification inter-rétrotransposons), 12 ISSR (polymorphismes d'amplification inter-microsatellites), 12 microsatellites, 57 RAPD (polymorphismes d'ADN amplifié au hasard), 59 REMAP (polymorphisme d'amplification rétrotransposon-microsatellite), 105 SRAP (polymorphismes d'amplification de séquences apparentées) et 2 SNP (polymorphismes mononucléotidiques). La taille totale de la carte est de 1526 cM. Plus de la moitié des marqueurs de la carte présentaient une ségrégation biaisée où les allèles du parent 'Aslak' étaient généralement favorisés. Ceci s'explique par la meilleure performance de ce cultivar en culture d'anthères. Des travaux de cartographie sont en cours en vue de localiser des QTL contrôlant certains caractères agronomiques et de qualité.

Mots-clés : culture d'anthères, *Avena sativa*, marqueurs moléculaires, haploïdes doublés, carte génétique, ségrégation biaisée.

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Introduction

Cultivated oat (*Avena sativa* L.) is a self-pollinating allohexaploid ($n = 3x = 21$) consisting of 3 basic genomes (A, C, and D; Rajhathy and Thomas 1974) and having a large genome size (1C DNA content 13.7 pg; Bennett and Smith 1976). As a consequence, molecular mapping of the cultivated oat has been rather complicated. To simplify mapping efforts, the earliest oat linkage maps were constructed in diploid oat species (A genome) corresponding to ancestors of the cultivated hexaploid oat. Populations from two different crosses have been used: *A. atlantica*

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Baum et Fedak × *A. hirtula* Lag. (O'Donoughue et al. 1992; Van Deynze et al. 1995) and *A. strigosa* Schreb. × *A. wiestii* Steud. (Rayapati et al. 1994; Yu et al. 1996; Yu and Wise 2000; Kremer et al. 2001).

The first linkage map in hexaploid oat was built using recombinant inbred lines (RILs) from the cross *A. byzantina* C. Koch 'Kanota' × *A. sativa* L. 'Ogle' (O'Donoughue et al. 1995). This KO map has since been extended and used in QTL (quantitative trait locus) studies (Bush and Wise 1996; Holland et al. 1997; Kianian et al. 1999, 2000; Jin et al. 2000; Groh et al. 2001*a*, 2001*b*; Wight et al. 2003; Wooten et al. 2008). Mapping populations of hexaploid oat have also been produced from the crosses between genotypes 'Kanota' and 'Marion' (Kianian et al. 1999, 2000; Groh et al. 2001*a*, 2001*b*), 'Terra' and 'Marion' (De Koeyer et al. 2004), 'Ogle' and 'TAM O-301' (*A. sativa* subsp. *byzantina* C. Koch) (the so-called OT map; Portyanko et al. 2001; Jackson et al. 2008), 'Clintland64' and 'IL86-5698' (Jin et al. 2000), 'Ogle' and 'MAM17-5' (the OM map; Zhu and Kaeppler 2003; Zhu et al. 2003), and 'MN841801' and 'Noble' (Portyanko et al. 2005).

All the maps of hexaploid oat have been constructed using RILs. Compared with F_2 and backcross mapping populations, RILs represent a permanent population. This allows character measurements in several locations and over several years. In addition, because heterozygotes are for the most part lacking, dominant markers are as informative as codominant ones when RILs are used in mapping. In an F_2 population, the information content of dominant markers is low, especially when the markers are in repulsion (Ott 1985). Another permanent population type is a doubled haploid (DH) population. In contrast to RILs, DHs contain no residual heterozygosity. However, because DHs have undergone only one cycle of meiosis compared with the several cycles that are used for development of RILs, RILs have higher recombination. Thus, more accurate maps may be obtained with RILs than with DHs (Ferreira et al. 2006). DHs have been used for mapping in several cereal species (Forster and Thomas 2003). However, in oats the production of DH mapping populations has only recently become an option (Kiviharju et al. 2005).

In this paper, we present the first linkage map of hexaploid oat (*Avena sativa* L.) constructed using a DH population. The map is composed of various types of PCR-based DNA markers; both SRAPs (sequence-related amplified polymorphisms) and the retrotransposon-based marker methods IRAP (inter-retrotransposon amplified polymorphism) and REMAP (retrotransposon-microsatellite amplified polymorphism) were used here for the first time in oat. QTLs for some important quality and agronomical traits are now being placed onto the map, and the results will be presented in a subsequent paper.

Materials and methods

Plant material

The Nordic hexaploid oat cultivars 'Aslak' and 'Matilda' were used as parental lines. 'Aslak' was bred by Boreal Plant Breeding Ltd. (Finland) and 'Matilda' by Svalöf-Weibull Ab (Sweden). Several parents were crossed, and 200 'Aslak' × 'Matilda' F_1 seeds were produced by Boreal. The F_1 plants were sown, and 148 DH plants were derived from anther culture by the method of Kiviharju et al. (2005) in 3 separate experiments. Cold treatment was used for cut tillers and heat treatment for isolated anthers. The induction medium contained W_{14} salts and vitamins (Ouyang et al. 1989) supplemented with 5 mg/L 2,4-dichlorophenoxyacetic acid (Sigma, Oslo, Norway), 0.5 mg/L kinetin (Sigma), 20 mg/L ethylene releasing compound Ethephon (Dr. Ehrenstorfer GmbH, Augsburg, Germany), 50 mg/L L-cysteine (Merck, Espoo, Finland), 500 mg/L myo-inositol (Merck), and 10% maltose, and the pH was adjusted to 6.0. Note that the composition of W_{14} macro salts was incorrectly presented in Kiviharju et al. (2005), the correct one (in mg/L) being KNO_3, 2000; $NH_4H_2PO_4$, 380; $MgSO_4{\cdot}7H_2O$, 200; $CaCl_2{\cdot}2H_2O$, 140; K_2SO_4, 700. A solid medium (solidified with 0.3% Phytagel, Sigma, USA) covered with a liquid layer (with 10% Ficoll 400, Pharmacia Biotech, Sweden) was used for induction culture. Regeneration and rooting media were the same as in Kiviharju et al. (2005). The induction was carried out at 28 °C in the dark or under dim light (approx. 40 μmol photons·m^{-2}·s^{-1}), and regeneration and rooting were carried out at 25 °C under dim light with a 16 h photoperiod. The ploidy level of the regenerants was determined by flow cytometry (Becton Dickinson FACSort, USA), and the haploid genome was doubled by colchicine as described in Kiviharju et al. (2000).

DNA was extracted from oat leaves using a CTAB (cetyltrimethylammonium bromide) method based on Poulsen et al. (1993), except that the DNA was treated additionally with 10 μg/μL RNase (Sigma) for 30 min at 37 °C and was not centrifuged through a CsCl density gradient. DNA concentrations were measured using the GeneQuant II RNA/DNA Calculator (Pharmacia Biotech Ltd., Cambridge, UK).

Markers

Unless mentioned otherwise, PCR amplifications were carried out with Biotools DNA polymerase (Biotools B&M Labs, S.A., Madrid, Spain), with the buffer (containing 2 mmol/L $MgCl_2$) supplied by the enzyme manufacturer, in a PTC-220 DNA Engine Dyad Peltier Thermal Cycler (MJ Research, Waltham, Massachusetts, USA). Oat microsatellites from various sources were used (Li et al. 2000; Holland et al. 2001; Pal et al. 2002; Jannink and Gardner 2005). Two different PCR programs were carried out in a Mastercycler gradient thermal cycler (Eppendorf, Hamburg, Germany) as described by Li et al. (2000), except that the number of cycles was reduced from 48 to 43 (program 1) and from 38 to 33 (program 2) when the whole population was analyzed. The amplification reactions (25 μL) contained 0.75 U of *Taq* polymerase (MBI Fermentas, St. Leon-Rot, Germany) or 0.4 U of Biotools DNA polymerase (Biotools B&M Labs), the buffer supplied by the respective enzyme manufacturer, 1.5–2 mmol/L $MgCl_2$, 100 μmol/L each dNTP, 400 nmol/L each primer, and 20–40 ng of DNA. One primer of each primer pair was labeled with a fluorescent dye, FAM (5-carboxyfluorescein) or TET (6-carboxytetrachlorofluorescein), and the amplification products were resolved and visualized on

a MegaBACE 500 Sequencer (GE Healthcare, Buckinghamshire, UK).

In addition to oat microsatellites, barley (Becker and Heun 1995; Liu et al. 1996; Ramsay et al. 2000) and rye (Hackauf and Wehling 2002; Lochow-Petkus GmbH) microsatellites were tested in the oat parents. Various PCR conditions were used for the amplifications. The only polymorphic barley microsatellite in the DH population (HVM20) was amplified, using the MSA program according to Liu et al. (1996), in a reaction volume of 25 μL containing 0.4 U of Red Hot DNA polymerase (Advanced Biotechnologies, Epsom, Surrey, UK), the buffer supplied by the enzyme manufacturer, 2.5 mmol/L $MgCl_2$, 100 μmol/L each dNTP, 160 nmol/L each primer, and 20 ng of DNA.

RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeat) analyses were carried out basically as described in Tanhuanpää et al. (2006), using Biotools DNA polymerase. The ISSR technique amplifies DNA segments lying between two identical microsatellite repeat regions (Zietkiewicz et al. 1994). The primers contained a microsatellite repeat sequence anchored at the 3′ end.

The IRAP and REMAP marker systems (Kalendar et al. 1999; Kalendar and Schulman 2006) are based on retrotransposons. In the IRAP method, polymorphisms are found among amplification products between the long terminal repeats (LTRs) of retrotransposons, but in the REMAP method they are found between retrotransposons and nearby simple sequence repeats. The polymorphisms thereby reflect retrotransposon insertions that took place following divergence from the last common ancestor. Primers were designed by cloning retrotransposon regions from different plant species (mostly from oat but also from barley, rye, and timothy), identifying the LTRs, and choosing conserved motifs at or near their termini. The microsatellite-based primers contained repeat units (composed of 2 or 3 bases) anchored at their 3′ ends by a single nucleotide. The PCR programs for IRAP and REMAP were as described in Tanhuanpää et al. (2006). IRAP markers were amplified in a reaction volume of 20 μL, using 1 U of polymerase, Failsafe 2× PCR PreMix D (Epicentre, Madison, Wisconsin) containing $MgCl_2$ and dNTPs, 250 nmol/L primers, and 25 ng of DNA. The amplification conditions for REMAP were the same except that 1.5 U of polymerase, 500 nmol/L each primer, and 50 ng of DNA were used.

AFLP (amplified fragment length polymorphism) analysis was based on Vos et al. (1995) and used the restriction enzymes *Eco*RI and *Mse*I. Restriction and ligation were performed simultaneously in a 30 μL volume containing 0.5 μg of DNA, 5 U of each restriction enzyme (New England Biolabs, Ipswich, Massachusetts), 1× NEBuffer2, 100 μg/mL bovine serum albumin, 0.4 mmol/L ATP (MBI Fermentas), 33 U (cohesive-end ligation units) of T4 DNA ligase (New England Biolabs), 2.5 μmol/L *Mse*I adapters, and 0.25 μmol/L *Eco*RI adapters. Adapters were prepared by mixing equimolar amounts of both strands, heating the mixture to 95 °C for 5 min, and then cooling it down to room temperature. The restriction–ligation reaction mixture was incubated for 3 h at 37 °C, 4 h at 21 °C, and 15 min at 70 °C. Pre-selective and selective amplifications were performed in 20 μL reactions containing 0.25 U of polymerase, 200 μmol/L each dNTP, primers, and DNA. The pre-selective amplifications contained 375 nmol/L primers (*Eco*RI + A, C, or G and *Mse*I + A or C) and 50 ng (3 μL from the restriction–ligation reaction) of DNA. The program for pre-selective amplification consisted of an initial incubation at 72 °C for 2 min, followed by 28 cycles of 30 s at 94 °C, 1 min at 56 °C, and 1 min at 72 °C. The selective amplifications contained 250 nmol/L *Eco*RI primers, 500 nmol/L *Mse*I primers, and 5 μL from a 10-fold dilution of the pre-selective amplification reaction as template DNA. The selective amplification reactions were performed for 37 cycles with the following "touchdown" profile: 30 s at 94 °C, 30 s at the annealing temperature, and 1 min at 72 °C. The annealing temperature was 65 °C in the first cycle, was reduced by 1 °C per cycle for the next 8 cycles, and then remained at 56 °C for the following 28 cycles. The selective *Eco*RI primers were labeled with a fluorescent dye — FAM, HEX (hexachloro-6-carboxyfluorescein), or TET — and the amplified fragments were analyzed on a MegaBACE 500 Sequencer (GE Healthcare). AFLP reactions made with different dyes were multiplexed for analysis.

The SRAP marker technique is designed to amplify open reading frames (Li and Quiros 2001). The forward primers ("me") preferentially anneal to exonic regions and the reverse primers ("em") to intronic regions and promoters. SRAP analyses were carried out as described in Tanhuanpää et al. (2007). The primer sequences are presented in Budak et al. (2004). Three SNPs (single-nucleotide polymorphisms) were also analyzed in the progeny. The SNPI11 marker (previously named SNP-RAPD) associated with short straw in oat was described by Tanhuanpää et al. (2006), who also presented its amplification conditions. The development of SNPs for ACCase (acetyl-CoA carboxylase) 1 and 2 will be described in a forthcoming article concerning the localization of an associated QTL on the doubled haploid oat map.

The designation of markers is explained in Table 1. Additional information concerning the markers on the final map, as well as the sequences of primers containing a microsatellite repeat, is presented in the electronic

Table 1. Marker names in the 'Aslak' × 'Matilda' doubled haploid oat map.

Marker type	Designation[a]	No.
AFLP	Selective bases with *Eco* primer – selective bases with *Mse* primer	375
IRAP	Retroprimer (A, D, or F + number)	3
ISSR	Prefix ISSR	12
Microsatellite		
Oat	Prefix AM, and Astavea	11
Barley	HVM20	1
RAPD	Prefix OP	57
REMAP	Retroprimer + ISSR	59
SRAP	me1em1 through me5em16	105
SNP	ACCase1 and 2	2
Total		625

[a]Letters from "a" to "r" at the end of marker names indicate different-sized markers (in order of increasing molecular weight) amplified with the same primer(s).

Table 2. 'Aslak' × 'Matilda' F_1 anther culture.

		No. of ELS		No. of green regenerants			No. of plants in the greenhouse	
Anther culture set	No. of anthers	Total	Per 100 anthers	Total	Per 100 anthers	Per ELS	Total	Per 100 anthers
1	7770	589	7.6	63	0.8	0.11	49	0.6
2	3000	136	4.5	23	0.8	0.17	17	0.6
2[a]	11340	234	2.1	33	0.3	0.14	29	0.3
3	3960	434	11.0	67	1.7	0.15	53	1.3
Total	26070	1393	5.3	186	0.7	0.13	148	0.6

Note: ELS, embryo-like structures.

[a]Induction in this set was made in dim light, in other sets in the dark.

Table 3. Summary data for the doubled haploid oat map.

	No. of loci			Skewed markers ($P < 0.05$) in the map		
Linkage group	Total	In the map	Map length (cM)	Towards 'Aslak'	Towards 'Matilda'	Total
1	50	47	71	9	0	9
2	43	42	46	0	6	6
3	25	25	92	20	0	20
4	19	12	38	11	0	11
5	9	8	34	5	0	5
6	7	7	44	7	0	7
7	73	70	98	61	0	61
8	20	20	42	20	0	20
9	7	7	19	7	0	7
10	54	52	62	19	0	19
11	13	13	23	2	1	3
12	17	17	34	17	0	17
13	58	51	101	17	0	17
14	45	37	107	9	0	9
15	24	24	37	21	0	21
16	18	15	71	0	6	6
17	34	34	69	23	0	23
18	29	28	33	28	0	28
19	20	19	124	0	3	3
20	8	7	42	3	0	3
21	23	23	26	0	20	20
22	10	6	33	1	1	2
23	15	15	50	0	0	0
24	9	9	63	5	0	5
25	9	8	73	4	0	4
26	14	14	36	0	0	0
27	6	6	25	5	0	5
28	9	9	33	7	0	7
Total	668	625	1526	301	37	338

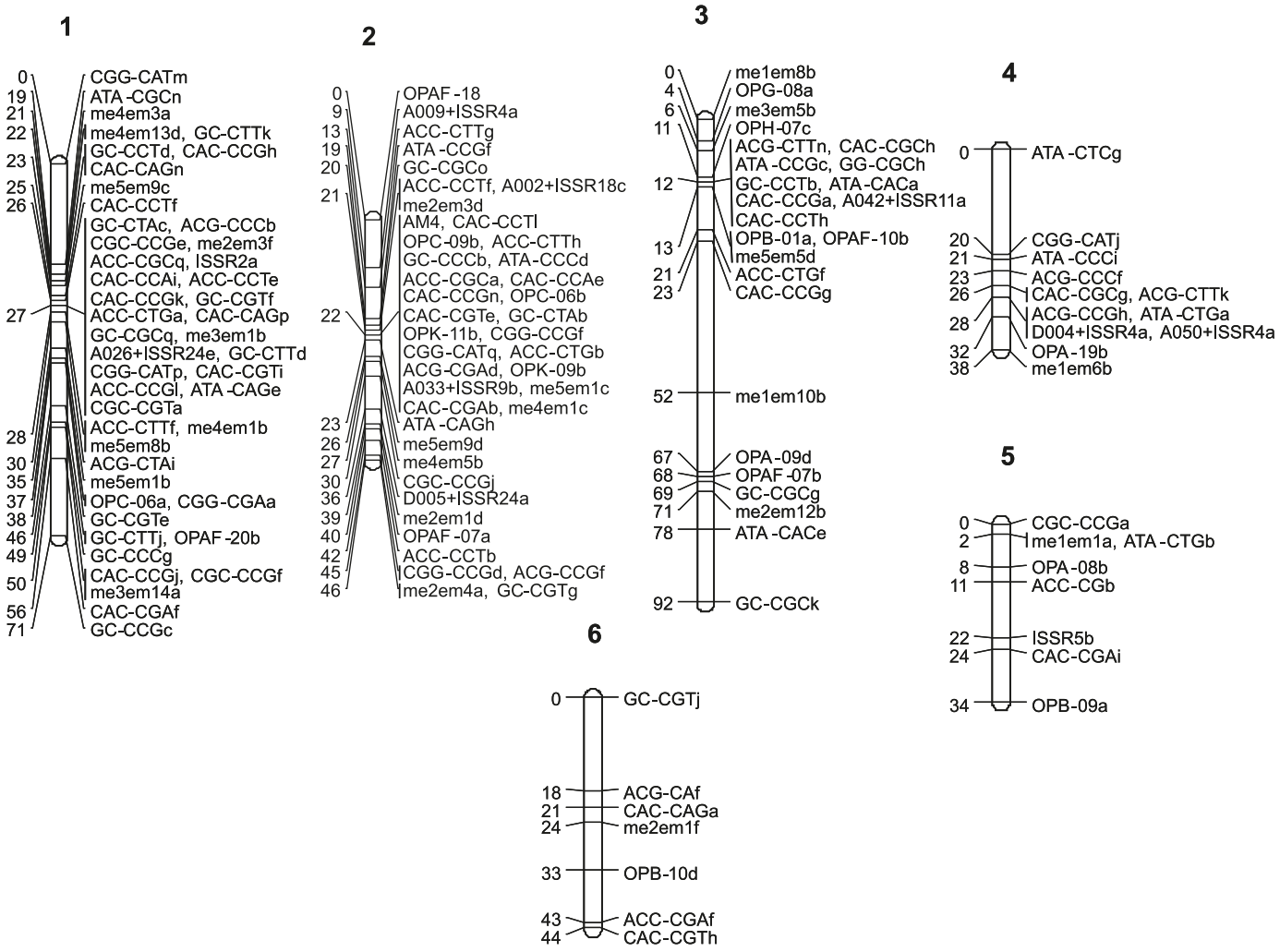
supplementary material (Tables S1 and S2[2]). The primers designed for the oat retrotransposons are owned by Boreal Plant Breeding Ltd. and their sequences can be acquired from them. The primer sequences for a barley retrotransposon (F0004) and a timothy retrotransposon (F0778) that were used in the construction of the map are CGAGTGAGGACAAAGTGCGCA and ACCAGCCCGGGCCGTCGACC, respectively.

Statistical analyses

JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used for map construction, using primarily a LOD (logarithm of odds) score of 9.0 but in some cases 8.0 (groups 8, 23, and 25) or 10.0 (groups 5 and 6). Map distances in centimorgans (cM) were calculated by Kosambi's mapping function (Kosambi 1944). Maps for each linkage group were constructed using first- or second-round maps. The jump threshold was

[2] Supplementary data for this article are available on the journal Web site (http://genome.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3765. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

Fig. 1. A doubled haploid linkage map of oat from the cross 'Aslak' × 'Matilda'. The designations of markers are explained in Table 1. The linkage group identifications are based on microsatellites (marked in bold) that have been located in previous studies: [1]Jannink and Gardner 2005; [2]Pal et al. 2002; [3]Wight et al. 2003; [4]Zhu and Kaeppler 2003. Relationships between KO and OT maps are from Portyanko et al. 2001, and those between KO and OM maps are from Zhu and Kaeppler 2003.



5.0. The segregation of markers was tested against an expected 1:1 ratio using the χ^2 test in the JoinMap program.

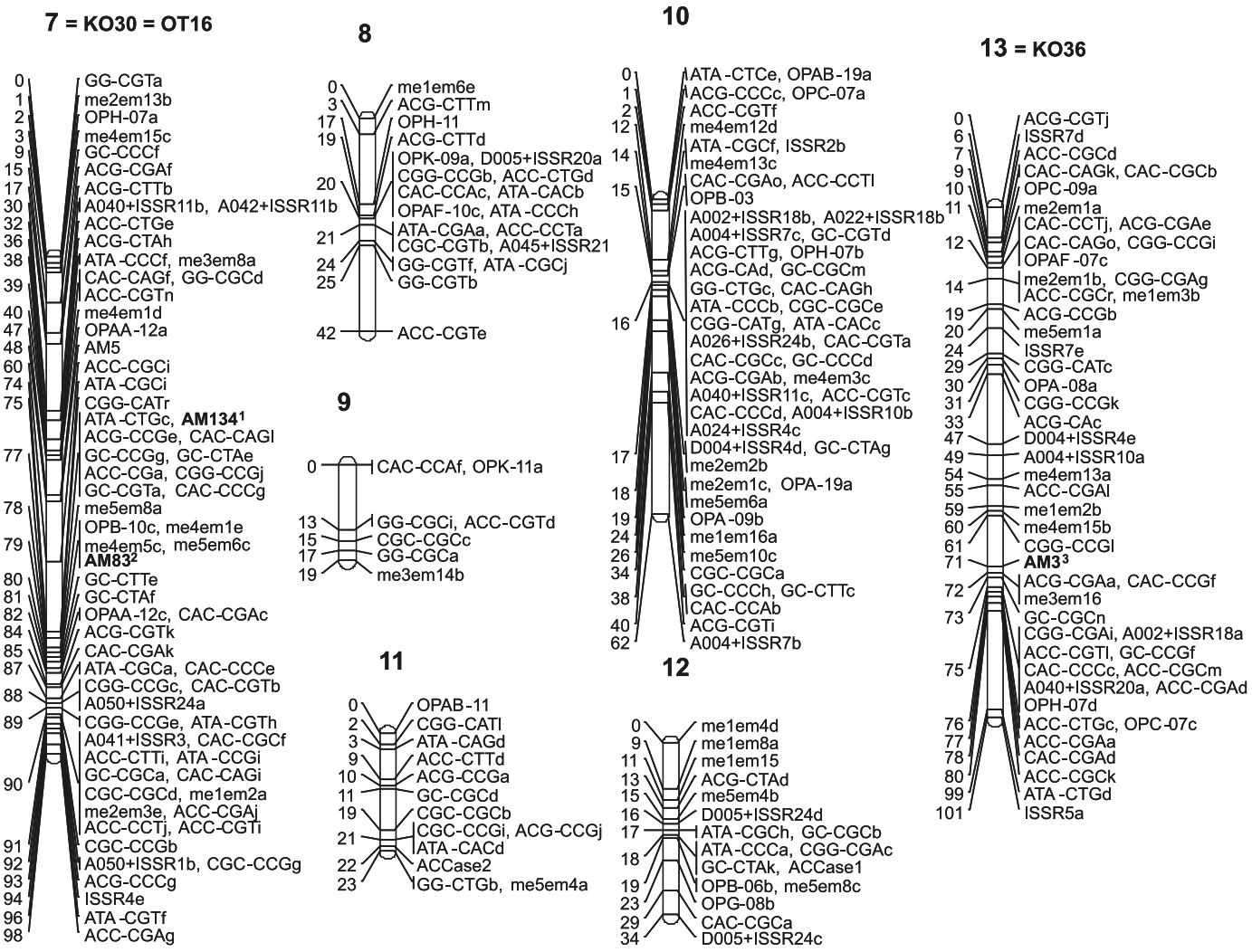
Results and discussion

In total, 148 'Aslak' × 'Matilda' regenerants were produced in 3 separate anther culture experiments (Table 2). Eleven regenerants were rejected because they did not produce seeds (5 individuals) or were potential heterozygotes (5), or for technical reasons (1). As a consequence, the mapping population included 137 DH individuals; the genome of 82 individuals (60%) was doubled by colchicine and 55 individuals were spontaneously doubled haploids. Although the use of dim light in the induction phase of 'Aslak' anther culture previously showed positive effects on the induction of embryo-like structures (Kiviharju et al. 2005), it decreased the response in this experiment. Without that trial, the average green plant regeneration rate would have been 1.1/100 anthers for the 'Aslak' × 'Matilda' progeny. Only a few albino plants were regenerated.

The mapping population was screened with 717 DNA markers polymorphic between the parents; 668 formed 28 linkage groups longer than 10 cM and containing more than 4 markers (Table 3). When only first- or second-round maps were used for determining the order of markers in the groups, the number of markers in the final map was reduced to 625 (Table 3, Fig. 1). The linkage groups contained from 6 to 70 markers, of which 13% were codominant. The SRAPs demonstrated the highest frequency of codominance (18%), whereas among the other marker types the frequency was around 10%. Cultivated hexaploid oat has 21 chromosome pairs, but we found 28 linkage groups. Therefore, some of the linkage groups in our map belong to the same chromosome but probably reside at the extreme ends, thereby becoming impossible to join without additional markers. The total map size was 1526 cM, whereas the estimated complete map size of hexaploid oat is 2932 cM (O'Donoughue et al. 1995).

In the map construction, a LOD score of 9.0 was suitable for creating linkage groups. However, a lower LOD score (8.0) was used for creating linkage groups 8, 23, and 25. In linkage group 8, use of a LOD score of 9 would have

Fig. 1 (*continued*).



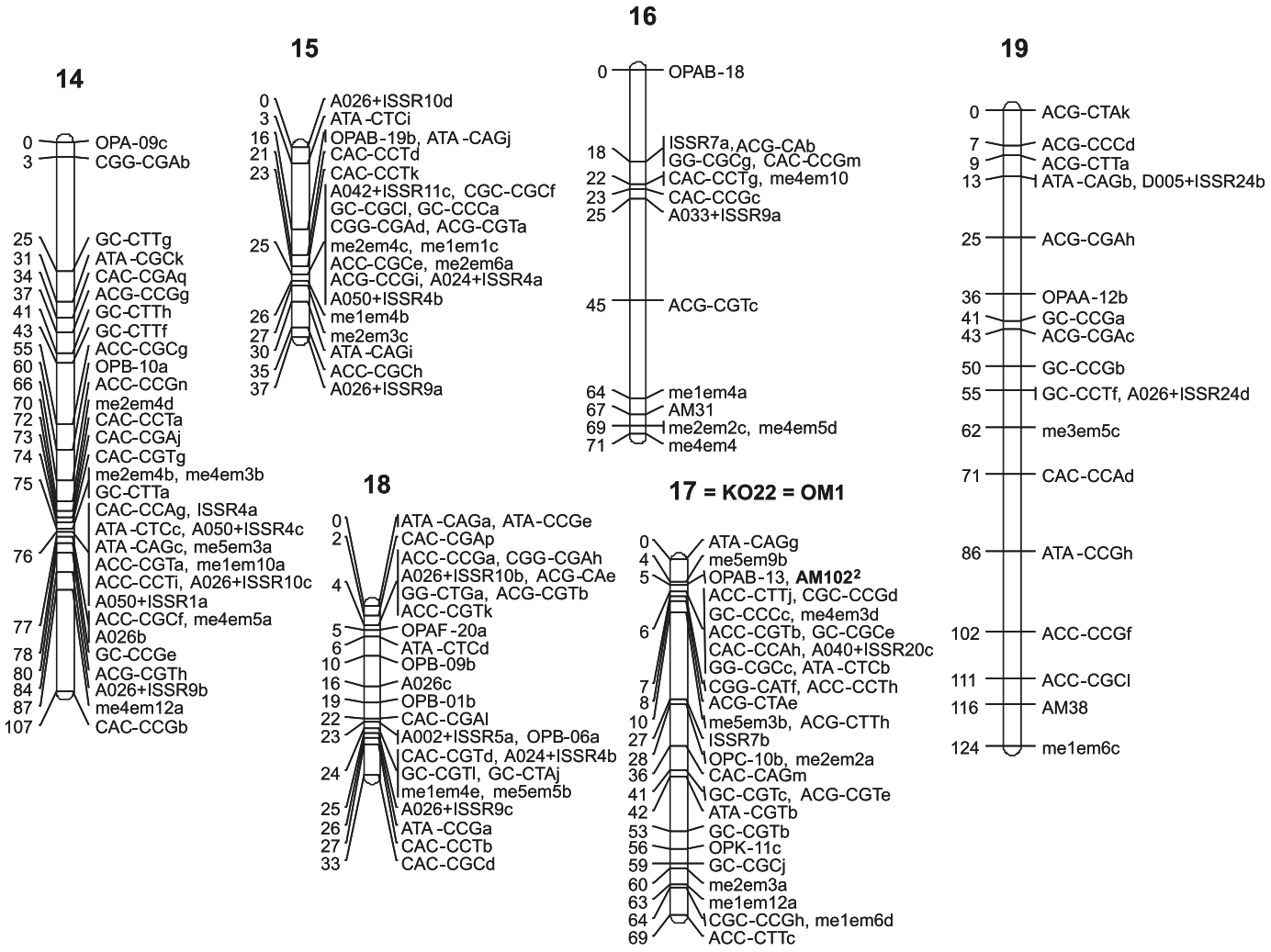
dropped marker ACC-CGTe from the end of the group. However, including this marker lengthens the group from 25 to 42 cM, and the marker seems to be reliable and well linked to other markers. The linkage groups 23 and 25 would split into two parts with a LOD score of 9.0, although all the markers in both groups are well linked to each other, and therefore were kept together with a lower LOD score. On the other hand, linkage groups 5 and 6 were kept apart using a LOD score of 10.0 because with a lower LOD score, half of the markers could not be ordered until the last (third) mapping round. It seems that only one marker connects these groups.

Fifty-four percent of the mapped markers showed distorted segregation ($P < 0.05$, Table 3). About the same level of distortion was observed in every marker type (results not shown). Distorted markers were often confined to certain regions, and sometimes all the markers in a linkage group were skewed. The distortion in these regions was towards only one of the parental alleles, usually 'Aslak' (19 groups), but in 3 linkage groups (7, 16, 21) it was towards 'Matilda'. In linkage group 7, there were distorted regions towards 'Aslak' and 'Matilda'. Only 2 linkage groups (23, 26) contained normally segregating markers exclusively.

Distorted segregation in anther culture–derived progenies is a generally acknowledged feature in many species, as is the clustering of distorted markers (Foisset and Delourme 1996). In the distorted areas, alleles from the parent that responds better in anther culture often prevail among the progeny, implying that there are genes that affect anther culture response (Foisset and Delourme 1996). In our earlier experiments, 'Aslak' produced as many as 8 green plants per 100 anthers, whereas no regenerants have been obtained from 'Matilda'. On the other hand, alleles that favour anther culture traits can also be derived from the less responsive parent, which probably explains the skewing of markers towards 'Matilda' alleles in 3 linkage groups. This has previously been shown in oat (Kiviharju et al. 2004) as well as in other cereals (Martinez et al. 1994; Beckert 1998; Torp et al. 2001). Distorted segregation should not significantly affect linkage analysis in large populations (Devaux et al. 1995; Van Ooijen and Voorrips 2001; Hackett and Broadfoot 2003).

In addition to anther culture, parental heterogeneity may partly explain segregation distortion in the present study. We were forced to use many parents in several crosses to get enough F_1 seed. Although oat is a self-pollinator, culti-

Fig. 1 (*continued*).



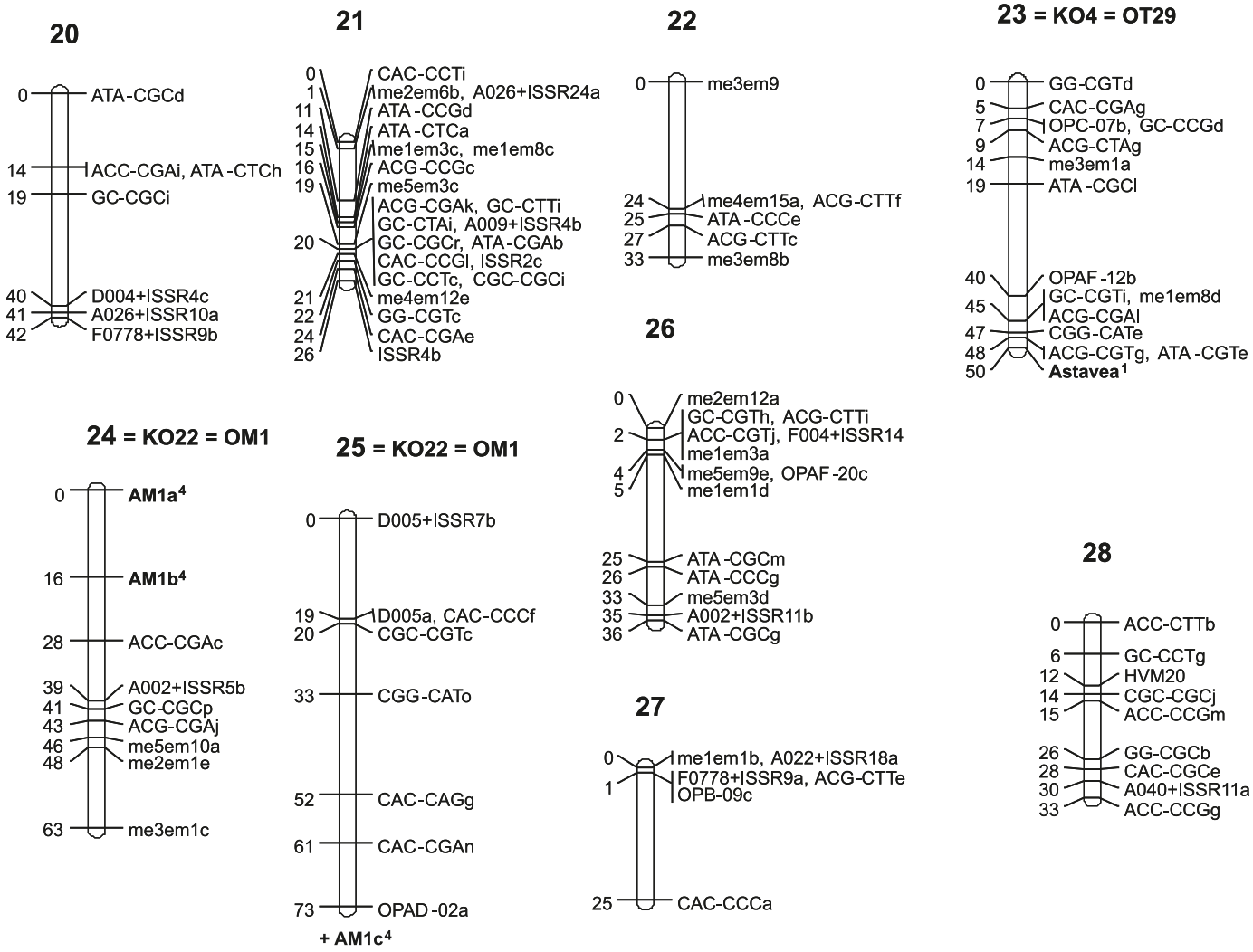
vars are not completely homogeneous. According to our small-scale experiment with DNA markers (results not shown), 'Matilda' is more variable than 'Aslak', which seems to be quite homogeneous.

Dense clusters of normally segregating markers were also observed in several linkage groups and involved all marker types. Clustering is a general phenomenon in linkage maps and is due to non-equal frequency of recombination along chromosomes, recombination being low near centromeres and in heterochromatic and introgressed regions (Young 1994).

Different linkage maps can be compared with each other using anchor markers, usually RFLPs or microsatellites. Because analyzing RFLPs is very laborious, microsatellites were chosen as anchors in the current study even though only a few microsatellites have been located to oat chromosomes (after the current study was completed, some additional microsatellites were reported by Becher 2007). Consequently, our linkage groups could be compared only partly with published maps. Of the 51 tested oat microsatellites, 6 could not be amplified. Twelve were polymorphic in the parents (27% of the functioning microsatellites) and were analyzed in the progeny, producing 14 loci (AM1 amplified 3 dominant loci as in Zhu and Kaeppler 2003). Eleven loci were located on the map and 4 linkage groups could be identified with the aid of microsatellites (Fig. 1). Linkage groups 17, 24, and 25 (AM1c belongs to this group but could not be located until after the third mapping round) all belong to linkage group KO22. Groups 24 and 25 would have been united with a LOD score of 6.

In addition to microsatellites developed from oat, 28 barley and 22 rye microsatellites were tested in the parents of the mapping population. Nineteen (68%) barley and 18 (82%) rye microsatellites were also amplified in oat, although sometimes weakly, probably because of sequence divergence. In previous studies, 42% of wheat (Hu et al. 2007), 38% of barley (Hu et al. 2007), and 12% of ryegrass (Jones et al. 2001) microsatellites were successfully amplified in oat. Li et al. (2000) showed that the percentage of functioning barley microsatellites in oat could be increased from 5% to 26% by lowering annealing temperatures in PCR. In addition to low reproducibility, the polymorphism in microsatellites borrowed from other species is usually low: in our study, only one (5%) of the barley microsatellites and two (11%) of the rye microsatellites were polymorphic in the parents. The barley microsatellite HVM20

Fig. 1 (*concluded*).



mapped to linkage group 28, which thus corresponds to barley chromosome 5 (1H) (Liu et al. 1996). The polymorphic rye microsatellites were not analyzed in the whole progeny because there were problems in their amplification.

Acetyl-CoA carboxylase plays a major regulatory role in fatty acid synthesis (reviewed by Ohlrogge and Jaworski 1997) and is therefore a good candidate gene for oil content. Because our purpose is to locate QTLs for oil content on our map, SNP analyses were done for two different ACCase loci and the SNPs were located on groups 11 and 12. Kianian et al. (1999) identified two ACCase loci, *AccaseA* and *AccaseB*, of which *AccaseA* mapped to linkage group 11 in the KO population. As a consequence, either of our groups, 11 or 12, corresponds to KO11.

Previously, we identified markers associated with the dwarfing gene *Dw6* (Tanhuanpää et al. 2006) and Cd accumulation in oat (Tanhuanpää et al. 2007). One of the markers linked to *Dw6*, SNPI11, belongs to linkage group 14 but could be ordered only after the third mapping round (to the end of the group, between markers CAC-CCGb and me4em12a). The SRAP marker me1em6e, which is linked to Cd accumulation, resides on linkage group 8.

As far as we know, our linkage map of oat is the first one constructed using a DH population and also the first one with Nordic cultivars as parents. In addition, SRAP and retrotransposon-based IRAP and REMAP markers have been located on an oat map for the first time. Although Yu and Wise (2000) used a primer for the barley *BARE1* retrotransposon for SSAP (sequence-specific amplified polymorphism) in oat, which is a hybrid retrotransposon and AFLP technique, here we used a set of native oat retrotransposons for the first time. The 62 retrotransposon-based markers seemed to be uniformly distributed on the map, as could be expected because of the dispersion of retrotransposons and microsatellites in genomes and from previous mapping studies in other species (Schulman et al. 2004). SRAPs were also evenly distributed on the map, as previously reported for *Brassica oleracea* (Li and Quiros 2001). Currently, we are placing QTLs affecting oil content, β-glucan content, leaf blotch disease resistance, and some agronomic characters onto the map.

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