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# Analysis of factors influencing milling yield and their association to other traits by QTL analysis in two hexaploid oat populations

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**Abstract** Milling yield, or the grain weight from which 100 kg of rolled groats is obtained upon milling, is an important quality characteristic of cultivated oat (*Avena sativa* L.). Kernel morphology and the groat (caryopsis) percentage of the whole kernel including hull are factors that influence milling yield. We mapped QTLs for kernel area, kernel length, kernel width, and groat percentage in two populations of 137 recombinant inbred lines by RFLP and AFLP analysis to evaluate the prospects of marker-assisted selection (MAS). Phenotypic correlations between kernel morphology traits and groat percentage were not significant. For kernel morphology traits and groat percentage, one to five QTLs were detected, explaining 7.0–60.7% of the total phenotypic variance depending on the trait. One QTL for kernel length in each population and one QTL for kernel width in one population were found at the same location as a QTL for groat percentage, indicating that a change in kernel size or shape could have an influence on groat percentage. The positions and effects of QTLs for kernel morphology and groat percentage were compared to QTLs detected previously for chemical grain composition (oil and β-glucan concentration) and agronomic traits to evaluate the selection response on these traits through MAS. Several regions of the oat genome were identified that contained clusters of QTLs influencing two or more traits. While the allele from one parent at a QTL could simultaneously improve two or more traits in one population, it could have opposite effects on the same traits at another QTL or in the other population. Associations among traits were complex and will require careful consideration when employing QTL-marker associations in MAS to avoid negative selection response. Future research to discover candidate genes for those QTL clusters could provide information about trait associations and help in designing selection programs.

**Keywords** *Avena sativa* · Grain quality · Kernel morphology · Milling yield · QTL

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## Introduction

Milling yield, or the grain weight from which 100 kg of rolled groats is obtained (Forsberg and Reeves 1992), is an important quality characteristic of cultivated oat

(*Avena sativa* L., 2n=6x=42) for the milling industry. One obvious factor affecting milling yield is the groat to whole kernel ratio or groat percentage. The evaluation of groat percentage requires dehulling of the kernel, i.e., removal of the surrounding lemma and palea, or hull, from the caryopsis. This can be accomplished by hand dehulling or using a mechanical dehuller. Hand dehulling is exact but time-consuming, whereas values for groat percentage determined with a dehuller can be inaccurate due to groat breakage and incomplete dehulling. Selection for high groat percentage using recurrent selection in several populations has been achieved by increasing the groat weight, decreasing the hull weight, or both, depending on the population (Stuthman and Granger 1977). Both the method used to dehull the kernels and differences in the populations under investigation may have contributed to different broad sense heritability estimates for groat percentage ranging from values of about 0.3 to as high as 0.9 (Wesenberg and Shands 1973; Stuthman and Granger 1977; Pixley and Frey 1992; Ronald et al. 1999). As a means to better define milling yield in oat breeding programs, kernel weight is routinely measured in addition to groat percentage. Heavier kernels are larger but can have smaller groat to hull ratios than smaller kernels (Peek and Poehlman 1949; Wesenberg and Shands 1971). The ratio of primary and secondary to tertiary kernels is also important because the small tertiary kernel is not easily millable or separated from foreign material. Many millers first size-separate the grain intended for processing; grain size uniformity affects the efficiency of grain dehulling and is therefore an important component of milling yield.

Kernel shape is an additional criteria in oat selection. Oat millers prefer plump kernels over long and thin kernels. Digital Image Analysis (DIA) has allowed researchers to accurately and rapidly measure the average length, width, and area of large samples of kernels to determine grain morphology (Symons and Fulcher 1988). De Koeyer et al. (1993) found that increased kernel weight was an effect of increased kernel width rather than increased kernel length in a recurrent selection program for grain yield. Kernel size or shape is also likely to affect groat percentage and therefore milling yield; however, the effect of single factors defining kernel morphology on groat percentage is not clear.

The chemical composition of the grain, including oil concentration and β-glucan concentration, is an additional important quality factor for food markets. Although there seems to be no biological association between chemical composition and kernel morphology, the selection of a specific kernel type may have an effect on the quality characteristic, or the selection for chemical composition can cause a shift in kernel shape. In a study by Peterson and Wood (1997), high oil concentration was associated with low groat weight and long, slim kernels, suggesting that one mechanism to increase oil concentration is to decrease the volume to surface ratio. Changes in the kernel volume to surface ratio may also influence β-glucan concentration because, in some oat genotypes, β-glucan is distributed more densely in the outer portions of the kernel (Miller and Fulcher 1994). Peterson et al. (1995) reported a positive correlation between groat percentage and β-glucan content in one oat nursery but could not confirm this association in two additional experiments.

The analysis of quantitative trait loci (QTLs) has become an important approach for the identification and characterization of individual loci affecting a trait, with the goal of using QTL-marker associations in marker-assisted selection (MAS). Several studies have been conducted to identify QTLs for important characteristics in oat, including grain yield, plant height, heading date, test weight, disease resistance, and chemical groat composition (Siripoonwiwat et al. 1996; Beer et al. 1997; Holland et al. 1997; Jin et al. 1998; Kianian et al. 1999, 2000).

The identification of QTLs affecting kernel morphology and their comparison to QTLs for groat percentage may facilitate the analysis of trait relationships and their underlying genetic factors. In addition, the comparison of QTLs for kernel characteristics, agronomic traits and chemical composition can be a helpful tool to evaluate the effect of MAS for a specific trait on other related or unrelated traits. The main objective of the study reported here was to identify and characterize QTLs for physical kernel characteristics in two populations of hexaploid oat recombinant inbred lines (RILs) in order to evaluate the prospects of MAS for traits affecting milling yield. A second objective was to compare those QTLs with QTLs affecting agronomic performance and chemical grain composition that might affect the efficiency of germplasm improvement using MAS.

## Material and methods

### Mapping populations

Two previously described populations of RILs were used in this study (O'Donoughue et al. 1995; Kianian et al. 1999). The population Kanota×Ogle (K/O) contained 137 $F_6$-derived lines developed from a cross between the facultative winter-type cultivar Kanota and the spring cultivar Ogle. The population Kanota×Marion (K/M) consisted of 137 $F_6$-derived lines developed from a cross between Kanota and the spring cultivar Marion.

### Restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) assays

RFLP assays were performed on 71 to 137 $F_6$-derived lines in K/O and 137 $F_6$-derived lines in K/M using cDNA and genomic DNA probes as described previously (O'Donoughue et al. 1995; Kianian et al. 1999). Amplified fragment length polymorphism (AFLP) assays were performed on 71 RILs in K/O and 135 RILs in K/M using the method employed by Vos et al. (1995). A detailed description of the AFLP analysis has been presented in Groh et al. (2001). Briefly, template DNA was digested with the restriction enzymes *Mse*I and *Pst*I, and pre-amplification was performed using *Mse*I+1 and *Pst*I+1 primers. Selective amplification primers (*Mse*I+3 and *Pst*I+3) were used for the selective amplification with eight primer combinations. Denatured polymerase chain reaction (PCR) mixtures were loaded on 5% polyacryla-

mide gels and electrophoresed at 85 W for 2.5–3 h. Silver staining using the Silver Sequence staining procedure (Promega, Madison, Wis.) was performed to visualize DNA. The AFLP scores were entered along with the RFLP data in a combined database for linkage mapping.

### Field trials

Phenotypic trait data of the parents and 137 RILs of K/O and K/M were obtained in field trials for kernel morphology (kernel length, kernel width, and kernel area) and groat percentage. Each trait was measured in five environments in K/O in Aberdeen, Idaho, in 1991 and 1992, in St. Paul, Minnesota, in 1994, and in Lincoln, New Zealand, in 1992 and 1993. In K/M, kernel morphology was measured in three environments (Aberdeen 1992 and 1994, St. Paul 1994), and groat percentage was evaluated in two environments (Aberdeen 1992 and 1996). In St. Paul, hill plots with 30 seeds per hill and with a 30-cm space between them were grown in a randomized complete block design with four replicates. In Aberdeen and Lincoln, plants were grown in row plots with one to three replicates. The phenotypic values for the physical kernel characteristics were measured using a balanced sample obtained for each RIL by bulking 5 g of kernels from each replicate in all locations except Aberdeen 1992. In Aberdeen 1992, a sample of each RIL was taken for each of the three replicates and analyzed individually. The average kernel area, kernel length, and kernel width were determined from about 100 kernels of each RIL sample using DIA as described by Symons and Fulcher (1988). The kernel samples were hand-dehulled, and groat percentage was calculated as groat weight/kernel weight×100.

### Data analysis

In the analysis of the phenotypic data, each year-location combination was considered to be one environment. Mean values for each environment in K/O and K/M were calculated from the DIA data for kernel area, kernel length, and kernel width using methods described by De Koeyer et al. (1993). Analyses of variance were performed across environments with genotypes considered to be random effects. The genotypic variance ($\sigma_g^2$) and the broad-sense heritability ($h^2$) were estimated for each trait in both populations. Because analyses were based on means from each environment, the genotype×environment interaction variance could not be estimated and is confounded in the error variance. Parental means in each population were compared using *t*-tests ($P<0.05$). Phenotypic correlation coefficients ($r_p$) were calculated between kernel morphology traits and groat percentage using means across all environments in K/O. In K/M, correlations between traits were calculated using means across those environments in which both traits of the respective trait combination were measured.

In K/O, the linkage map used for QTL mapping contained 307 RFLP markers spanning a total distance of 2,096 cM and was similar to the one used by Kianian et al. (1999). The linkage map of K/M used for QTL mapping combined 103 anchor RFLP and AFLP markers assigned to 27 linkage groups, spanning a total distance of 736 cM (Groh et al. 2001). For the comparative mapping, linkage groups in K/M were assigned to linkage groups defined in K/O (Kianian et al. 2001) using homologous RFLP and AFLP markers as described by Groh et al. (2001). Linkage groups mapped in K/M that could not be assigned to linkage groups in K/O were designated x1 to x7.

The QTL analysis was performed on 71 to 137 RILs in K/O and 137 RILs in K/M using means across environments with the software package PLABQTL (Utz and Melchinger 1996), which employs interval mapping by the regression approach in combination with the use of selected markers as cofactors. Simple interval mapping was performed to identify putative QTL regions. Markers closely linked to those regions were selected as cofactors and used in subsequent composite interval mapping (CIM). To check for multiple QTLs on a chromosome, we extended the cofactor set to all markers of the chromosome under scanning. In K/O, scanning of a chromosome using all markers was not performed because of the unbalanced character of the dataset (71 to 137 RILs were used for mapping); only the most informative markers were chosen as cofactors for QTL analyses. A QTL was declared when the LOD score exceeded the threshold of 2.5 in CIM using the model with pre-selected cofactors. This critical value is equivalent to a significance level of 0.003 for a single comparison in a chi-square distribution with two degrees of freedom. Thirty-six markers in K/O and 32 markers in K/M that remained unlinked in the linkage analysis (Groh et al. 2001; Kianian et al. 2001) were tested for QTL associations using single-factor ANOVA at $\alpha<0.001$.

The additive effect of a QTL was estimated as the overall effect using means across environments. The phenotypic variance ($\sigma_p^2$) explained by a single QTL was obtained as the square of the partial correlation coefficient ($R^2$). The proportion of $\sigma_p^2$ explained by all QTLs for a trait was obtained from a multiple regression of means across environments in a simultaneous fit including the marker that was most closely linked to each QTL.

## Results

### Phenotypic data

No differences were found between cvs. Kanota and Ogle for kernel length, kernel width, kernel area, and groat percentage in trials performed in the K/O population. In K/M, Marion had greater kernel length, kernel width, and kernel area than Kanota but did not differ from Kanota in groat percentage ($P<0.05$). Estimates of $\sigma_g^2$ among RILs were significant ($P<0.01$) for all traits in both populations. The range among the RILs was greater in K/O for kernel length, kernel width, and kernel area than in K/M. The heritability was high for kernel area, kernel length, kernel width, and heading date but only intermediate for groat percentage in both populations (Table 1).

Positive phenotypic correlations were found between kernel area and kernel length and between kernel area and kernel width ($0.66 \le r_p \le 0.75$) in both populations, as expected, since area is a function of length and width. No correlations ($P<0.05$) were detected between kernel length and kernel width or between groat percentage and kernel morphology traits in either population.

### QTL analysis

In K/O, five QTLs for kernel area were found based on composite interval mapping analysis where the LOD score exceeded the threshold of 2.5 (Table 2). These QTLs were located on linkage groups 3–38, 6, 7–10–28, 17, and 27, explaining 60.7% of the total $\sigma_p^2$ in a simultaneous fit. Four QTLs affecting kernel length were detected on linkage groups 3–38, 7–10–28, 17, and 27, explaining 44.1% of $\sigma_p^2$. Four QTLs affecting kernel width were found on linkage groups 5, 9, 16, and 27, explaining 30.5% of $\sigma_p^2$. For groat percentage, two QTLs were detected on linkage groups 3–38 and 9, explaining 22% of $\sigma_p^2$. Alleles from both parents contributed to an increase in the numerical value of each trait. On linkage

**Table 1** Number of environments, means of parents and RILs, range of RILs, and estimates of the genotypic variance and repeatability of RILs from populations Kanota×Ogle and Kanota×Marion for physical kernel characteristics

| Parameter | Kernel area ($mm^2$) | Kernel length (mm) | Kernel width (mm) | Groat percentage (%) |
|---|---|---|---|---|
| Kanota×Ogle | | | | |
| Number of environments | 5 | 5 | 5 | 5 |
| Mean Kanota[a] | 22.7±1.25 | 11.8±0.43 | 2.8±0.08 | 72.6±2.29 |
| Mean Ogle | 24.5±1.25 | 11.9±0.43 | 2.9±0.08 | 69.6±2.29 |
| Mean RILs (*n*=137) | 25.3±0.12 | 12.2±0.05 | 3.0±0.01 | 73.3±0.19 |
| Range | 18.4–37.1 | 9.8–15.7 | 2.4–3.8 | 57.2–82.0 |
| $\sigma_g^2$ | 1.8±0.26** | 0.31±0.04** | 0.010±0.001** | 3.8±0.59** |
| $h^2$ | 0.85 | 0.89 | 0.86 | 0.78 |
| Kanota×Marion | | | | |
| Number of environments | 3 | 3 | 3 | 2 |
| Mean Kanota | 21.5±0.80 | 12.0±0.29 | 2.8±0.05 | 73.4±2.32 |
| Mean Marion | 27.0±0.80 | 13.4±0.29 | 3.0±0.05 | 72.4±2.32 |
| Mean RILs (*n*=137) | 25.1±0.14 | 12.9±0.06 | 2.9±0.01 | 71.7±0.58 |
| Range | 19.8–31.1 | 11.1–15.2 | 2.5–3.3 | 59.0–79.0 |
| $\sigma_g^2$ | 2.8±0.37** | 0.45±0.06** | 0.013±0.002** | 3.6±0.82** |
| $h^2$ | 0.93 | 0.93 | 0.91 | 0.57 |

** Variance component was significant at the 0.01 probability level
[a] Standard errors are attached

groups 3–38, 7–10–28, 9, 17, and 27, QTLs were found for multiple traits at the same position or in adjacent intervals in the K/O population (Fig. 1).

In K/M, only one QTL was found for kernel area, explaining 9.2% of $\sigma_p^2$. Four QTLs were found for kernel length on linkage groups 3, 7–28, 11–41, and x7, explaining 33.3% of $\sigma_p^2$. Only one QTL was found for kernel width, located on linkage group 16. For groat percentage, two QTLs were detected on linkage groups 7–28 and 14, explaining 21.9% of $\sigma_p^2$. QTLs for multiple traits were found on linkage groups 7–28 and 11–41 (Fig. 1).

## Discussion

### Comparison between populations

One objective of this mapping study was the identification of important QTLs for traits affecting milling yield and of associated markers which can be used in MAS. Validation of QTLs across populations is a valuable method to test the consistency of genomic regions significantly affecting a trait. On the other hand, different genotypes may carry different alleles at various QTLs for a given trait. Mapping in different populations can identify donors for favorable QTL alleles that can then be combined into a single improved variety.

Two QTL regions for kernel length and one for kernel width were detected on the same linkage groups in K/O and K/M. Although the number of QTLs detected for these traits was small (1–4), there was a consistency of QTLs across populations. Kernel length and kernel width were both highly heritable with only a small effect of the environment. It seems that the effect of the different genotypic backgrounds was also small, and traits were controlled similarly in K/O and K/M. Among kernel morphology traits, the greatest number of QTLs found in K/O was for kernel area; however, only one QTL for kernel area was found in K/M, and it was not detected in K/O. Kernel area is affected by both kernel length and kernel width; interactions between both factors may have restricted the ability to detect QTLs in K/M and explain the differences between the populations. Mean values for kernel area were significantly different between Kanota and Marion but not between Kanota and Ogle, suggesting that different alleles may exist in Marion and Ogle. No common QTLs were detected in K/O and K/M for groat percentage. As shown by Stuthman and Granger (1977), differences exist between populations for the regulation of groat percentage, which could be the reason for different QTLs in K/O and K/M.

Using two populations with a common parent for QTL detection, we expected to detect common QTLs because the populations share at least one allele. This was the case for kernel length and kernel width but not for kernel area and groat percentage. There might be different alleles segregating in K/O and K/M for these traits. However, there are several factors influencing the accuracy of QTL mapping which would have influenced the results. First, we used a relatively small population size for QTL mapping in both populations (*n*=71 to 137), which resulted in a low power of QTL mapping due to sampling effects. Therefore, chances are good that a QTL will be detected in only one population even though it is actually present in both. With a lower threshold for QTL detection ($\alpha$<0.01), Siripoonwiwat et al. (1996) found a QTL for groat percentage in K/O on linkage group 7–10–28 at the same position as a QTL we detected in K/M. When applying an even less stringent

**Table 2** Parameters associated with QTLs identified by composite interval mapping for physical kernel characteristics estimated from phenotypic data of 137 RILs for the population Kanota×Ogle and Kanota×Marion

| Linkage group[a] | Closest marker locus | LOD score | Kanota allele effect[b] | Phenotypic variance explained (%) |
|---|---|---|---|---|
| Kanota×Ogle | | | | |
| Kernel area ($mm^2$) | | | | |
| 3–38 | *Xcdo549a* | 3.9 | –0.64 | 18.8 |
| 6 | *Xcdo82* | 2.6 | 0.46 | 8.7 |
| 7–10–28 | *Xisu1961* | 2.7 | –0.64 | 16.5 |
| 17 | *Xumn441a* | 3.5 | 0.52 | 11.4 |
| 27 | *Xcdo942* | 2.6 | –0.73 | 19.5 |
| Total[c] | | | | 60.7 |
| Kernel length (mm) | | | | |
| 3–38 | *Xbcd907* | 3.7 | –0.26 | 17.8 |
| 7–10–28 | *Xisu1961* | 3.7 | –0.36 | 21.9 |
| 17 | *Xumn441a* | 2.7 | 0.19 | 9.4 |
| 27 | *Xbcd1872a* | 2.5 | –0.10 | 7.5 |
| Total | | | | 44.1 |
| Kernel width (mm) | | | | |
| 5 | *Xcdo1433* | 2.6 | 0.05 | 15.9 |
| 9 | *Xcdo456* | 5.0 | 0.04 | 13.7 |
| 16 | *Xcdo1360* | 3.7 | –0.04 | 12.2 |
| 27 | *Xcdo942* | 3.0 | 0.04 | 11.3 |
| Total | | | | 30.5 |
| Groat percentage (%) | | | | |
| 3–38 | *Xumn107a* | 2.6 | –0.85 | 13.2 |
| 9 | *Xcdo456* | 3.0 | 0.76 | 12.0 |
| Total | | | | 17.0 |
| Kanota×Marion | | | | |
| Kernel area ($mm^2$) | | | | |
| 11–41 | *Xaaccat146* | 2.7 | 0.55 | 9.2 |
| Kernel length (mm) | | | | |
| 3 | *Xacgcac680* | 3.2 | –0.25 | 12.4 |
| 7–28 | *Xwg420b* | 4.0 | 0.26 | 15.9 |
| 11–41 | *Xaaccat146* | 3.9 | 0.29 | 18.7 |
| x7 | *Xaaccaa490* | 2.6 | –0.31 | 24.2 |
| Total[b] | | | | 33.3 |
| Kernel width (mm) | | | | |
| 16 | *Xcdo1360* | 2.5 | –0.07 | 7.0 |
| Groat percentage (%) | | | | |
| 7–28 | *Xacgcac265* | 3.8 | –0.98 | 12.5 |
| 14 | *Xcdo1358f* | 3.7 | 0.90 | 12.3 |
| Total | | | | 21.9 |

[a] Linkage group designations of the hexaploid oat map have been described by Kianian et al. (2001) for population Kanota×Ogle and Groh et al. (2001) for population Kanota×Marion

[b] A positive QTL effect means that the allele from Kanota increased the numerical value of the trait

[c] Estimates were obtained from a simultaneous fit for all QTLs affecting the trait

threshold in QTL mapping ($\alpha<0.05$), these authors detected a large number (up to 25) of QTLs for several agronomic traits, including groat percentage. With a higher stringency ($\alpha<0.001$), they detected a smaller number of QTLs, comparable to the number detected in our study. Their results show that a large number of QTLs probably exist for each trait but that most of them cannot be detected without risking a high type-I error rate.

Second, the RILs in K/O and K/M were not always grown in the same locations, and data from a greater number of environments were available in K/O than in K/M. Kernel morphology was evaluated in Minnesota, Idaho, and New Zealand in K/O, but only in Minnesota and Idaho in K/M. The additional location of New Zealand contributed to a wider range of values for kernel length, kernel width, and kernel area and may have allowed the detection of additional QTLs in K/O. To test for common QTLs across populations independently from the environment effect, we would only have been able to analyze two environments for kernel morphology (Aberdeen 1992 and St. Paul 1994) and one for groat percentage (Aberdeen 1992). A preliminary analysis using plot means of individual environments revealed a good agreement of QTLs across environments for all traits in each population, although many QTLs were detected at a lower threshold in individual environments. If QTLs are to be employed in MAS, genomic regions that consistently affect a trait across environments are more

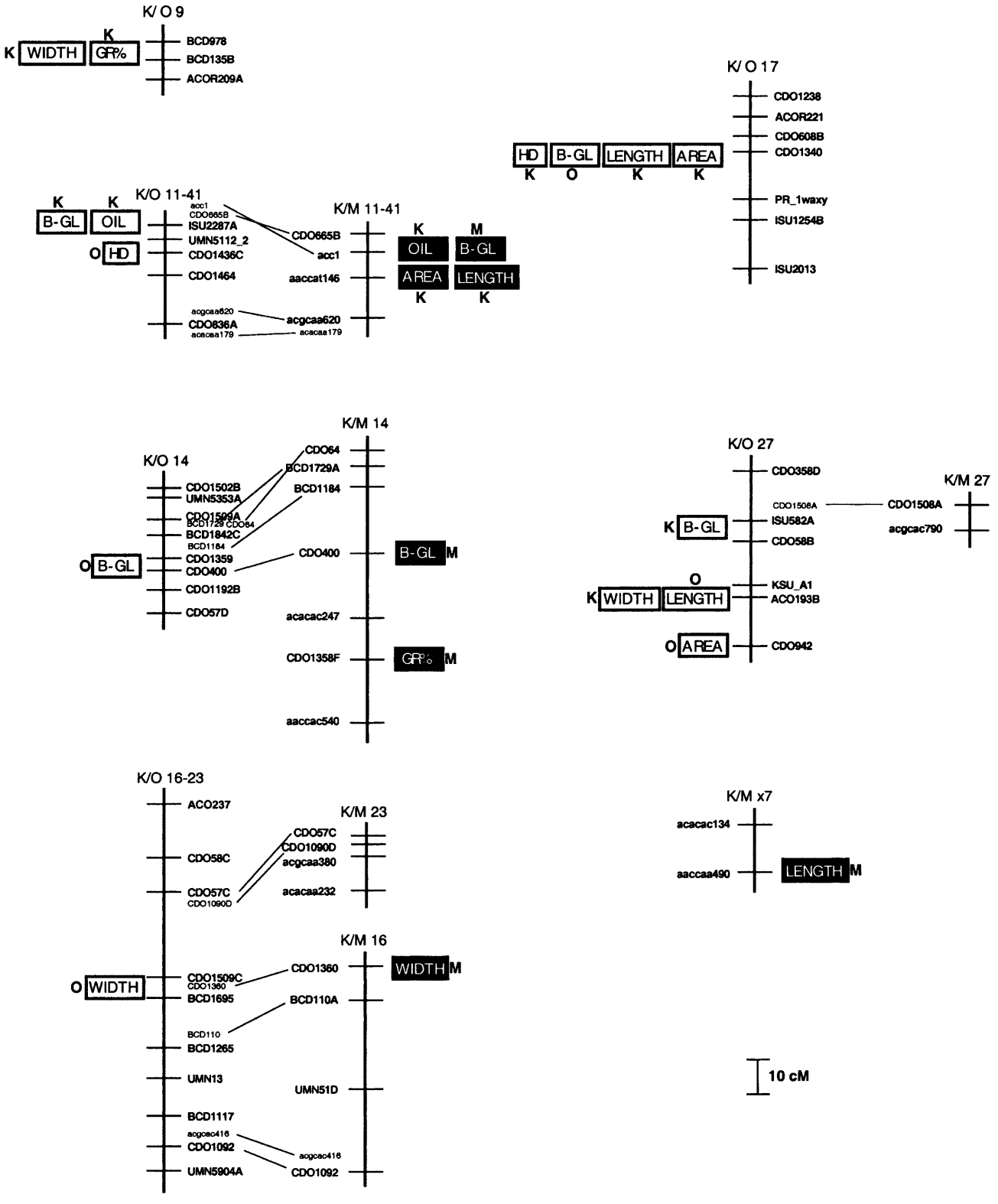


**Fig. 1** Comparative RFLP and AFLP linkage groups in populations Kanota×Ogle (*K/O*) and Kanota×Marion (*K/M*). The positions of anchor markers in the base maps are indicated by *horizontal bars*; additional markers used for the comparison between populations are assigned relative to the anchor markers. For details about the comparative linkage map see Groh et al. (2001). Approximate QTL positions for kernel area (*AREA*), kernel length (*LENGTH*), kernel width (*WIDTH*), groat percentage (*GR%*), oil concentration (*OIL*), β-glucan concentration (*B-GL*), and heading date (*HD*) are indicated for each population. The parental allele that contributed to an increase in the numerical value of a trait is indicated next to the QTL as *K* (Kanota), *O* (Ogle), and *M* (Marion). QTLs for oil and β-glucan concentration ($\alpha<0.01$) have been described by Kianian et al. (1999, 2000). QTLs for heading date ($\alpha<0.01$ in multiple environments) have been identified by Siripoonwiwat et al. (1996)

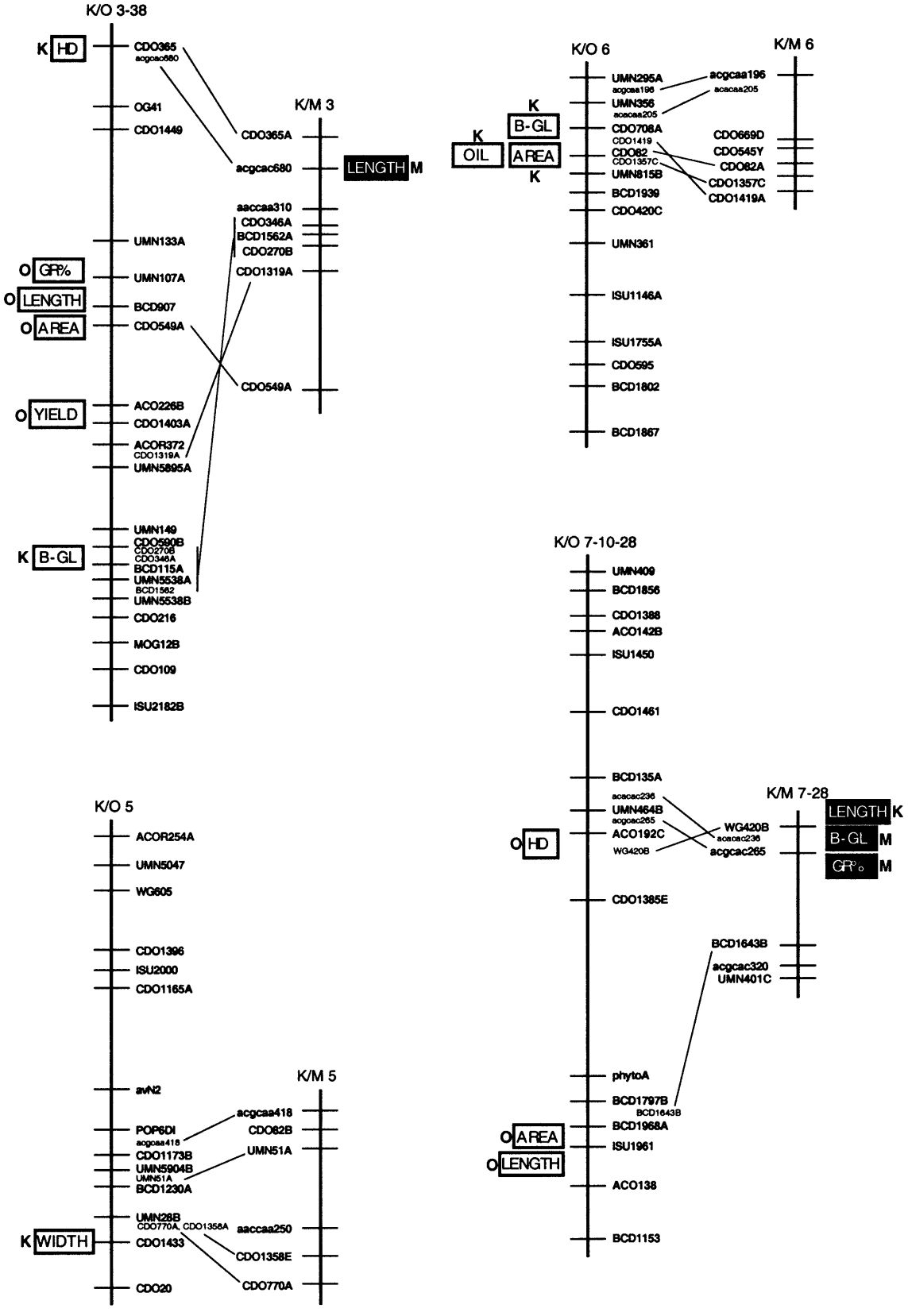


**Fig. 1** (continued)

useful than QTLs detected in individual locations. Also, the main QTL effect across environments should be a better estimator of the actual effect of a QTL. Therefore, means across environments were used for QTL detection in this study, although environmental effects cannot be separated from other effects when comparing the two populations.

Third, map coverage was lower in K/M, with the consequence that the presence of some QTLs in K/O could not be tested in K/M. This applied to the QTL for kernel area on linkage group 27 and the QTL for groat percentage on linkage group 9. In K/M, a QTL was found for kernel length on linkage group x7 which could not yet be assigned to a linkage group in K/O. For all other linkage groups, regions carrying a QTL in one population were also covered by markers in the other population.

## Associations between traits

The analysis of traits potentially affecting the milling yield of oat in two populations revealed several associations among QTLs. Seven linkage groups across populations contained multiple QTLs affecting physical kernel characteristics (Fig. 1). Thus, comparing QTLs of several characteristics across populations may be a good strategy to identify genomic regions of high importance in the expression of complex traits like milling yield.

Significant phenotypic associations were found in both populations between kernel area and kernel length and between kernel area and kernel width, but no correlation was detected between kernel length and kernel width. In addition, all QTLs for kernel length except one were found in different regions than the QTLs for kernel width, showing that different genetic factors seem to control long versus wide kernels. In agreement with these results, De Koeyer et al. (1993) found no association between kernel length and kernel width in a recurrent selection program for yield and concluded that the two traits are independent from each other. In contrast to our results in oat, Backes et al. (1995) found a negative correlation ($r_p$=–0.71) between kernel length and kernel width in barley, indicating that different mechanisms seem to control kernel shape in the two crops. As a consequence, it should be possible to modify the two traits independently in oat. Kernel area is affected by both the length and the width of the kernel; this should also be reflected in the distribution of QTLs. Accordingly, several regions in K/O and K/M contained common QTLs for kernel area and kernel length, and one common QTL was found for kernel area and kernel width.

No phenotypic association was detected between kernel morphology and groat percentage. However, a QTL for kernel length in each population was associated with a QTL for groat percentage, and a QTL in K/O affecting kernel width was found in the same region as a QTL for groat percentage. In K/O, the allele from Ogle gave increased kernel length, kernel area, and groat percentage for the QTLs on linkage group 3–38. On linkage group 9, the allele from Kanota contributed to increased kernel width and groat percentage. Both associations indicate that selection for larger kernels would have positively affected groat percentage. However, in K/M the allele from Kanota on linkage group 7–28 increased kernel length and the allele from Marion increased groat percentage. In this case, selection for shorter kernels would have a positive effect on groat percentage. It seems that a change in kernel morphology can have an effect on groat percentage, or vice versa, but the direction of the change depends on the locus or the genotypic background.

To study the effect of a change in kernel morphology on other important grain quality traits, we compared the physical kernel characteristics with the oil and β-glucan concentrations reported by Kianian et al. (1999, 2000). Seven linkage groups carrying QTLs for kernel morphology also contained QTLs for β-glucan and/or oil concentration (Fig. 1). On linkage group 6 in K/O, the Kanota alleles for the respective QTLs increased kernel area, β-glucan, and oil concentration, while the alleles from Kanota on linkage group 17 increased kernel area and kernel length but decreased β-glucan concentration. On linkage group 7–28 in K/M, the alleles from Kanota increased kernel length and β-glucan concentration, while on linkage group 27, the alleles from Kanota increased β-glucan concentration but decreased kernel area and kernel length. However, the allele from Kanota at the same position increased kernel width, suggesting that kernel shape might be associated more with chemical composition than kernel size. On linkage group 11–41, the alleles from Kanota caused an increase in oil concentration, kernel area, and kernel length but decreased β-glucan concentration in K/M. At the same region in K/O, the allele from Kanota increased oil and β-glucan concentration. Thus, it cannot be concluded that a change in kernel size results in a specific response with respect to chemical grain composition. As has already been found for the physical kernel characteristics, the association between kernel morphology and chemical composition seems to depend on the locus and the genotype.

Mapping studies in hexaploid oat have identified QTLs for plant height, heading date, yield, test weight, groat percentage, and other traits (Siripoonwiwat et al. 1996; Beer et al. 1997; Holland et al. 1997). Most of these studies were conducted using the K/O mapping population. Comparisons between our results and those of these earlier studies reveal that several clusters of QTLs for a variety of traits exist in this population. On linkage group 7–10–28, QTLs for heading date, plant height, and groat percentage were identified around locus *Xwg420b* in K/O, for which the alleles from Kanota accounted for reduced heading date and increased groat percentage (Siripoonwiwat et al. 1996). This could be an indication that maturity can have an influence on grain characteristics, suggesting that plants with reduced days to heading have a higher groat percentage. This heading date QTL was also associated with kernel length and groat percentage in K/M. Here, the allele from Kanota was associated with increased kernel length but de-

creased groat percentage. On linkage group 3–38, a QTL for heading date was located at the same location as a QTL for kernel length in K/M. The allele from Kanota increased days to heading in K/O and decreased kernel length. On the same linkage group, but at a more distant position, we detected QTLs for groat percentage, kernel length, and kernel area in K/O at which the alleles from Kanota decreased the value of each trait. At the same position, Siripoonwiwat et al. (1996) reported a QTL for grain yield at which the allele from Kanota also reduced yield, indicating that earlier flowering RILs had larger kernels thereby contributing to higher yields. Contrary to these findings, at the QTL on linkage group 17 in K/O, the allele from Kanota increasing kernel length and kernel area was associated with a QTL for heading date at which the allele from Kanota increased days to heading. Holland et al. (1997) reported that some of the QTLs for heading date in K/O were indirect effects of response to vernalization, a result of the Kanota parent being vernalization-sensitive. However, none of the heading date QTLs found associated with kernel morphology QTLs in the current study were ones they had reported to be associated with vernalization.

Siripoonwiwat et al. (1996) found clusters of QTLs for heading date, plant height, grain yield, straw yield, and test weight and concluded that loci controlling days to heading had pleiotropic effects on other traits. Holland et al. (1997) also reported common QTLs for heading date and plant height that might be due to pleiotropy. There was also an indication for pleiotropic effects of QTLs affecting heading date and groat percentage. When comparing those results to our study, we observed several associations between QTLs for heading date, grain yield, kernel morphology, and groat percentage across two populations, but the allele from one parent could have opposite effects on two traits depending on the QTL and the population. Some of the associations between QTLs might be due to linked genes, while others seem more likely to be due to single genes with pleiotropic effects.

## Marker-assisted selection in oat

The QTL analyses for groat percentage and kernel morphology in two oat populations revealed only a few loci that were assumed to directly influence milling yield. Several QTLs affecting kernel morphology were detected, but only a few showed an association with groat percentage, and effects were opposite in K/O and K/M. Siripoonwiwat et al. (1996) detected only two QTLs associated with groat percentage in the K/O population at a significance level of $\alpha<0.001$. When the significance level was changed to 0.01 or 0.05, 7 and 22 linkage groups, respectively, were identified carrying QTLs affecting groat percentage. This suggests that many QTLs with presumably small effects on groat percentage exist but could not be detected when applying a more stringent threshold, presumably due to the small population size and intermediate heritability. Stuthman and Granger (1977) showed that groat percentage could be improved by phenotypic selection using recurrent selection. An increased groat percentage was achieved by increasing the groat weight, decreasing the hull weight, or both, depending on the population. Phenotypic selection should be superior to MAS unless experiments can be designed to identify and characterize QTLs accounting for a large amount of the genotypic variance, preferably across environments and populations.

If we want to use MAS to select for QTLs affecting combinations of traits like kernel morphology, groat percentage, and chemical composition, negative associations between characteristics can cause a serious problem. This becomes even more critical when agronomic performance is affected as well. Siripoonwiwat et al. (1996) concluded that knowing the location of QTLs for heading date, plant height, and other traits could help breeders to avoid selecting alleles that can have undesirable pleiotropic effects on yield performance. On the other hand, positive associations between QTLs for different traits could be used for simultaneous improvement. In our study, we found both positive and negative associations between QTLs affecting physical kernel characteristics, grain quality, and agronomic performance. This indicates that the success of MAS will depend on an ability to identify a large number of QTLs and their effects in any given population.

The population sizes employed in this study and the resolution of the QTL mapping are not sufficient to distinguish pleiotropic effects from close linkage. The identification and separation of clusters of QTLs or genes would require large population sizes which cannot easily be obtained and analyzed in mapping studies in oat. To be able to distinguish pleiotropy from linkage with certainty, we need to identify the genes underlying the QTLs and their role in the biochemical pathway of the traits. Even though a large number of QTLs have been identified in many crops for a large number of traits, the genes underlying most QTLs have not been described due to the lack of appropriate strategies to systematically identify them and because of practical limits in genetic studies.

The isolation and genetic mapping of single QTLs in near–isogenic lines (NILs) could be a useful method for characterizing QTLs independently from other loci. NILs are a starting point for the fine mapping of QTLs and map-based cloning (Tanksley and Nelson 1995). The mapping of candidate genes is another approach for identifying genes underlying a QTL. Candidate genes can then be mapped in different sets of NILs to determine their role in trait expression. As information about the location and effect of a large number of genes becomes available in genomics research, it might become feasible to recombine gene clusters and select for the most desired genotypes using marker tools, even if several traits are considered simultaneously.

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