Quantitative Trait Loci and Epistasis for Oat Winter-Hardiness Component Traits

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ABSTRACT
Winter hardiness is a complex trait and poor winter hardiness limits commercial production of winter oat (Avena spp.). The objective of this study was to identify quantitative trait loci (QTL) for five winter-hardiness component traits in a recombinant inbred line population derived from a cross between the winter-tender cultivar Fulghum and the winter-hardy cultivar Norline. Crown freezing tolerance, vernalization response, and photoperiod response were evaluated in controlled environment studies. Heading date and plant height were evaluated over two seasons in Kinston, NC, and winter field survival was evaluated in five environments over two seasons in the mountains of North Carolina and Virginia. A partial genetic linkage map of regions believed to affect winter hardiness was developed using restriction fragment length polymorphism and simple sequence repeat markers. Most QTL were located on linkage groups FN3, FN22, and FN24. Quantitative trait loci were identified for all traits except photoperiod response, and epistatic interactions were identified for winter field survival, crown freezing tolerance, vernalization response, and plant height. Major QTL for winter field survival ($R^2 = 35\%$) and crown freezing tolerance ($R^2 = 53\%$) were identified on linkage group FN3, which was associated with an intergenomic reciprocal translocation between chromosomes 7C and 17.

Poor winter hardiness limits fall-sown oat (Avena spp.) production in much of North America. Although fall-sown oat production is possible in portions of the extreme southern United States using cultivars bred for spring production in the Midwest, reliable production throughout most of the region requires cultivars that overwinter in the juvenile stage. Cereal winter hardiness is influenced by several quantitative component traits, including crown freezing tolerance, vernalization and photoperiod responses, heading date, and plant height (Fowler et al., 1999). Crown freezing tolerance is the most important winter-hardiness component trait (Olien, 1967). Photoperiod and vernalization responses combined with per se lateness act as cold-temperature avoidance mechanisms that delay growth of freezing-sensitive reproductive tissues until warmer temperatures arrive. Plant height tends to be
correlated with these traits because plants that flower later tend to grow taller.

Genomic regions controlling winter-hardiness component traits have been identified in diploid wheat (*Triticum monococcum* L.) (Vagujfalvi et al., 2003; Yan et al., 2003), common wheat (*T. aestivum* L.) (Fowler and Limin, 2004; Kobayashi et al., 2005; Limin and Fowler, 2002; Toth et al., 2003), and barley (*Hordeum vulgare* L.) (Francia et al., 2004; Hayes et al., 1993; Pan et al., 1994). Quantitative trait loci (QTL) for different winter-hardiness component traits, for example, vernalization and freezing tolerance, are frequently linked.

There are relatively few reports of QTL for oat winter-hardiness component traits. Quantitative trait loci for vernalization and photoperiod responses have been identified in spring parent by winter parent oat mapping populations (Holland et al., 1997, 2002; Locatelli et al., 2006). Wooten et al. (2008) identified QTL for crown freezing tolerance in a spring parent by winter parent population, but to our knowledge this is the first published report on QTL for winter-hardiness traits in a population developed from a cross between two winter parents. A reciprocal translocation involving chromosomes 7C and 17 (T7C-17) has been reported to be associated with crown freezing tolerance and winter field survival in two populations developed from crosses between two winter parents. A reciprocal translocation involving chromosomes 7C and 17 (T7C-17) has been reported to be associated with crown freezing tolerance and winter field survival in two populations developed from crosses between two winter parents: ‘Fulghum’ × ‘Wintok’ (Santos et al., 2006) and Fulghum × ‘Norline’ (Wooten et al., 2007).

Fulghum is a winter oat cultivar selected in the late 19th century from the heterogeneous land race ‘Red Rustproof’ (Coffman, 1977). It has limited winter hardiness (Livingston and Elwinger, 1995) and does not contain T7C-17 (Jellen and Beard, 2000). Norline is a winter oat cultivar developed in Indiana in 1960 (Patterson and Schaffer, 1978). It is winter hardy (Livingston and Elwinger, 1995) and does contain T7C-17 (Wooten et al., 2007).

Although previous studies identified an important cytological marker, the identification of QTL for winter-hardiness component traits would provide a tool for improving winter hardiness through marker-assisted selection. This approach is particularly suitable because winter hardiness is sporadically expressed in field nurseries. An additional benefit would be an enhanced understanding of the relationships among different winter-hardiness component traits. The objective of this study is to build on the results from Wooten et al. (2007) and identify QTL for the winter-hardiness component traits winter field survival, crown freezing tolerance, heading date, plant height, and photoperiod and vernalization responses in a recombinant inbred line (RIL) population derived from the cross of Fulghum (winter tender, non–7C-17 translocation) × Norline (winter hardy, 7C-17 translocation).

**Controlled Crown Freezing Tolerance Test**

The experiment was an incomplete blocks within complete replications design. There were 15 incomplete blocks of 10 entries within each of five complete replications over time. Each complete replication consisted of 129 RILs plus seven entries of each parent and the winter-hardy check cultivar Wintok (Livingston and Elwinger, 1995). Ten F$_{6,7}$ seeds of each entry were germinated for 4 d on moist paper in petri dishes with one dish per entry. Five seedlings of each entry were then planted 1.5 cm deep in five adjacent 20-cm-long nursery tubes held in racks of 100 tubes. Plants were grown in Metromix 200 (Scotts-Sierra Horticultural Products Co., Marysville, OH) and lightly watered daily with a complete nutrient solution. The plants were grown for 5 wk in a 9-m$^2$ growth chamber in the Southeastern Plant Environment Laboratory at North Carolina State University. The chamber was illuminated for a 10-h photoperiod with a photosynthetic photon flux density of 300 μmol m$^{-2}$ s$^{-1}$ with a day temperature of 13°C and night temperature of 10°C. At approximately the five-leaf stage, plants were transferred to a hardening growth chamber for a 3-wk acclimation treatment. The hardening chamber was held at a constant 3°C with a 10-h photoperiod of photosynthetic photon flux density of 300 μmol m$^{-2}$ s$^{-1}$. While cold acclimating, plants were watered with a complete nutrient solution (Livingston, 1991) three times per week and watered with deionized water on alternate days.

Crows were prepared and frozen at −10°C as described in Santos et al. (2006), with a change in experimental design. Within each replication the entries were assigned to an incomplete block of 10 entries using an α (0,1) lattice structure. The same 10 entries were frozen in each of five sponges within each replication. One of the five congruent sponges from each incomplete block was placed on each shelf in a freezer.

After the crows and sponges thawed, the roots were trimmed from the crows to prevent the growth of microbes as roots deteriorate, and the crows were planted in 50- by 30-cm plastic flats filled 5 cm deep with moist Metromix 200. The flats were returned to the growth chamber in the Southeastern Plant Environment Laboratory. After 3 wk of regrowth, recovery for each crown was visually measured on a scale of 0 to 10 (0 = complete plant death, 10 = no freezing damage).

**Field Evaluations**

Heading date and plant height were evaluated using a randomized complete block design with two replications. F$_{6,7}$ seed of each RIL and the two parents were planted on 23 Oct. 2002 at the Cunningham Research and Education Center, Kinston, NC. Plots were 1.3 m long and row spacing was 0.3 m. Heading date was recorded as the day of the year when 50% of panicles had emerged. Severe lodging prevented measurement of plant height in 2002–2003. Each plot was harvested, and seed was bulked across replications for subsequent winter survival evaluation. The heading date and plant height evaluations were repeated in 2003–2004 using remnant F$_{6,7}$ seed and a similar protocol, except plot size was increased to two adjacent 1.3-m-long rows with row spacing of 0.6 m. Plant height was

**MATERIALS AND METHODS**

**Plant Material**

The experiment was conducted using a population (FN hereafter) of 129 F$_6$-derived RILs developed via single-seed descent from the cross of Fulghum (winter tender, non–7C-17 translocation) × Norline (winter hardy, 7C-17 translocation).
Winter field survival was evaluated using a randomized complete block design with five replications in each of five environments. F6:7 seed of the RILs plus the parents was used. The experiment was planted on 16 Sept. 2003 at the Upper Mountain Research Station (elevation 895 m) near Laurel Springs, NC, and 9 Oct. 2003 at the Mountain Research Station (elevation 727 m) near Waynesville, NC. Plots were hand-planted with 6 g of seed plot−1 in single rows 2.3 m long with a mean row spacing of 0.3 m. Fall plant emergence and growth were recorded for each plot in early November. Laurel Springs reached a minimum temperature of −14.5°C on 31 Jan. 2004 and Waynesville reached a minimum temperature of −14.2°C on 7 Jan. 2004. Field survival was estimated for each plot in March 2004 as the percent survival for the plots corrected for plot variation in germination or fall growth. The experiment was repeated in the 2004–2005 season at both North Carolina locations with the addition of the Virginia Tech College of Agriculture and Life Sciences Kentland Research Farm (approximate elevation 530 m), near Blacksburg, VA. Plot size in 2004–2005 were two collinear row segments each 1.3 m long with a row spacing of 0.3 m. Laurel Springs was planted on 24 September, Waynesville on 8 October, and Blacksburg in the second week of October. In winter 2004–2005, Laurel Springs reached a minimum temperature of −19.2°C, Waynesville reached a minimum temperature of −15.7°C, and the city of Blacksburg reached a minimum temperature of −17.9°C. All three locations reached their maximum temperature of −17.9°C. In winter 2004–2005, Laurel Springs reached a minimum temperature of −14.2°C on 7 Jan. 2004. No winter damage was observed in Waynesville in either season, so the data were not included in the analysis of winter field survival.

Photoperiod and Vernalization Responses

Photoperiod and vernalization responses were evaluated in a growth chamber experiment at the Southeastern Plant Environment Laboratory at North Carolina State University. A split-plot factorial design with three replications over time was used. Photoperiod was the whole plot factor, and vernalization and genotype were factorial subplot factors. Seedlings for the nonvernalized treatment were germinated in moist paper towels for 4 d at 20°C. Seedlings with the vernalized treatment were germinated in moist paper towels for 4 wk in the dark at 2°C. Two plants of each treatment were planted in separate 10-cm2 square pots with all plants in a replication planted on the same day. Long- and short-day effects were simulated in two separate growth chambers. Both chambers were illuminated for a 10-h photoperiod with photosynthetic photon flux density of 550 μmol m−2 s−1, and the long-day treatment was simulated using a 2-h mid-night interruption with low-intensity incandescent lights. This provided long-day stimulus to the plants, while minimizing the difference in photosynthetically active radiation. After 42 d with differing photoperiod treatments, both chambers were increased to 16-h photoperiod to facilitate flowering. Temperature was held at 25/20°C photoperiod/darkness. Each plot consisted of two plants grown in the same pot, and the days to flowering was recorded as the number of days from seedling emergence to panicle exertion for each plant; each plant was a plot subsample. The mean flowering date of the two plants for each pot was used as the response variable in the analysis of variance.

Molecular Markers

Plant tissue was harvested from young tillers of each F6 plant in addition to Fulghum and Norline. DNA was extracted according to the large-scale DNA extraction protocol (CIMMYT, 2005). Briefly, DNA was extracted from ground frozen tissue using a CTAB extraction buffer, precipitated with isopropanol, and washed with ethanol solutions. Suitable quantities of DNA were extracted for simple sequence repeat (SSR) markers from the initial plant samples, but the extraction was repeated using tissue harvested from 7 to 10 seedlings grown from F6 seed of each RIL to extract enough DNA to complete restriction fragment length polymorphism (RFLP) analysis.

Sixty-two oat and barley SSR primers polymorphic in cultivat ed oat (Holland et al., 2001; Jannink and Gardner, 2005; Li et al., 2000; Pal et al., 2002) were evaluated for polymorphism in Fulghum and Norline. Twenty-two showed possible polymorphism between the parents. These primers were evaluated in the population and parents. All SSR screening methods were described in Srnić et al. (2005), except that polymerase chain reaction (PCR) amplifications were performed in a 10-μl total volume containing 1X PCR buffer with 2.0 mM Mg2+, 0.25 mM dNTPs, 0.4 pmol forward primer, 1.5 pmol reverse primer, 1.5 pmol M13-labeled primer, 0.2 μg μL−1 BSA, 0.75U Taq polymerase, and 50 ng genomic DNA.

The RFLP probes chosen had previously been associated with the 7C–17 translocation (Fox et al., 2001), vernalization or photoperiod response in oat (Holland et al., 1997, 2002). These probes were provided by the GrainGenes probe repository (http://wheat.pw.usda.gov/GG2/index.shtml [verified 9 Aug. 2009]) or by Dr. Howard Rines, USDA-ARS, St. Paul, MN. Probes were amplified from plasmids using PCR (CIMMYT, 2005) but without the use of mineral oil. Probe DNA was then purified using a QIAEX II agarose gel extraction protocol (Qiagen Inc., Valencia, CA). Probes were P32 labeled using a random prime labeling kit (Invitrogen, Carlsbad, CA), and unincorporated P32 was removed with a sephadex G50 (Sigma-Aldrich Corp., St. Louis, MO) gravity column.

Twenty micrograms of DNA per sample were digested with 60 units of restriction enzyme in the appropriate buffer with the addition of 0.1 mg mL−1 of BSA (Promega Corp. Madison, WI). After digestion, the DNA was loaded into a 1% agarose gel in 1× TBE and electrophoresed for 24 h at 35 V. DNA was transferred to Hybond-XL membrane (GE Healthcare, Little Chalfont, UK), using the neutral Southern blotting technique described in the Hybond manual. DNA was fixed on the membranes by baking at 70°C for 2 h. Prehybridization, hybridization, and membrane washing were conducted as described in Qi et al. (2003), and membranes autoexposed X-ray film for 2 to 21 d at −70°C. Parental polymorphism screens were conducted using Fulghum and Norline DNA digested with BamHI, DnaI, EcoRI, EcoRV, and HinIII. Putatively codominant polymorphisms were preferentially selected for population genotyping.

Data Analysis

The phenotypic data were analyzed using the MIXED procedure of SAS (Littell et al., 1996) with the Satterthwaite option for calculating degrees of freedom. For the vernalization and photoperiod study, days to flowering were transformed with the natural logarithm function to normalize the error variance.
Narrow-sense entry mean heritabilities with standard errors were estimated for the population excluding checks. All effects were treated as random in the model which followed the method described by Holland et al. (2003, Table 2.1: sections 11 and 12). Subsequently, RILs plus parents were considered a fixed effect, and the entry means were estimated with the LSMEANS statement. The DIFF option ($\alpha = 0.05$) was used to test for transgressive segregation. Differences between the treatment levels of vernalization and photoperiod least-squares means (LSmeans) were estimated for each genotype. These differences served as response variables in the analyses for vernalization and photoperiod. Pearson product–moment correlations were estimated among phenotypic traits using the CORR procedure. The LSmeans for each RIL were combined with genotypic data, and single-marker analyses for each marker were conducted for each trait using PROC GLM. The SAS program EPISTACY (Holland, 1998) was used to check for epistasis.

A genotypic linkage map was constructed with Mapmaker/EXP version 3.0 (Lander et al., 1987). A minimum log of the odds score of 4 for grouping markers and the Kosambi mapping function were specified. Linkage groups were assigned names according to their likely homology with linkage groups in the ‘Kanota’ × ‘Ogle’ map (KO) (Wight et al., 2003) or Ogle × ‘TAM O-301’ map (OT) (Portyanko et al., 2001) based on SSR loci or multiple RFLP probes. A region of one linkage group was associated with chromosome 7C. The T7C-17 region was assigned to a linkage group using the marker data from a line in this population identified as nullisomic for chromosome 7C. The RIL that was nullisomic for chromosome 7C was not included in the population for QTL detection.

Multiple interval mapping (MIM) in Windows QTL Cartographer (Wang et al., 2006) was used to map QTL and to test for epistatic interactions (Kao et al., 1999). Briefly, the MIM QTL search was initiated with no initial model. The likelihood ratio for the presence of a QTL was calculated every 1 cM. Multiple interval mapping models were created and tested in an iterative, stepwise fashion in a search for new QTL to add to the model. New models were accepted if they decreased the Bayesian Information Criterion (Piepho and Gauch, 2001). The Schwarz (1978) Bayesian Criterion was used as the penalty function to prevent over-fitting the model (Basten et al., 2004; Zeng et al., 1999). The Schwartz Bayesian Criterion was chosen because it was the most conservative in the software package. Epistatic interactions and corresponding QTL detected with EPISTACY were manually added to the MIM model. Genomic positions involved in epistatic interactions remained in the MIM model if the corresponding interaction term was significant according to the Bayesian Information Criterion. Thus, genomic positions with nonsignificant main effects could remain in the MIM model if the term was involved in a significant interaction. To further model epistasis, a two-locus marker with interaction analysis of variance was fitted in PROC GLM for each two-way epistatic interaction. Markers linked nearest to genomic positions identified from MIM that interacted epistatically were markers in the model. The LSmeans statement was specified to estimate the four two-locus marker classes.

RESULTS

Genetic Linkage Map

Thirty-four polymorphic loci were successfully screened in the population and 20 loci were linked in seven linkage groups. The length of the linkage map was 129 cM (Fig. 1). Three linkage groups, FN3, FN22, and FN24, were similar to linkage groups 3+38, 22_44_18, and 24_26_34 in the KO map, respectively (Wight et al., 2003). One linkage group, FN11, was similar to group 11 in the OT map (Portyanko et al., 2001). The linkage groups not aligned with previous oat maps were numbered FN41, FN42, and FN43 to avoid confusion with previously numbered oat linkage groups. The T7C-17 translocation mapped on the largest linkage group, FN3. The linkage group corresponded with

Figure 1. Linkage map from a ‘Fulghum’ × ‘Norline’ (FN) recombinant inbred line population with quantitative trait loci (QTL) for winter-hardiness component traits. Locus marker names are shown on right side of linkage groups, with absolute position in centimorgans on the left. Approximate locations of main-effect QTL are indicated with shaded bars representing the equivalent of a one–log-of-the-odds (LOD) support interval and extending lines representing the equivalent of a two-LOD support interval. Quantitative trait loci are labeled with trait abbreviations winter field survival (WFS), crown freezing tolerance (CFT), vernalization response (VR), heading date (HD), and plant height (PHT), and the QTL number for each trait (Table 3). The portions of linkage group FN3 that represent chromosome 7C and the translocated region are identified with labeled QTL bars.
chromosomes 7C and 17 in the KO map (Fox et al., 2001; Wight et al., 2003). The RIL that was nullisomic for chromosome 7C confirmed that linkage group FN3 included the translocation region. The breakpoint between chromosome 7C and the translocation was between loci HVM20 on chromosome 7C and AM270s_1 on the translocation.

Segregation distortion was detected for several markers. All markers on linkage group FN3 had significantly more Norline alleles than predicted by a 1:1 segregation ratio, but none differed significantly from a 2:1 segregation ratio. The unlinked marker AM31, the marker CDO1523 on group FN24, and the markers Astaveb and Avenin on group FN42 all showed segregation distortion with more Fulghum alleles than expected.

Phenotypic Data
Five of the six traits measured had entry mean heritabilities >70% in this population (Table 1). The entry mean heritability of photoperiod response was low (h² = 25%), so the data are not presented. Fulghum and Norline were significantly different from each other for winter field survival, crown freezing tolerance, growth chamber vernalization response, and heading date, but not for plant height (Table 1). Winter field survival and crown freezing tolerance showed a bimodal distribution, while heading date and vernalization response followed a normal distribution (data not shown). Plant height had a skewed distribution due to several lines with very short height. Transgressive segregants were identified for increased crown freezing tolerance, vernalization response, heading date, and plant height, and for decreased vernalization response and plant height. Winter field survival was significantly correlated with crown freezing tolerance and heading date but not vernalization response or plant height (Table 2). Heading date was significantly correlated with all of the other traits. Vernalization response was not correlated with crown freezing tolerance.

Quantitative Trait Loci
Quantitative trait loci were identified for all traits, and epistatic interactions were identified for all traits except heading date (Table 3 and Fig. 1). Four main-effect QTL plus two epistatic interactions were identified for winter field survival. The Fulghum alleles decreased winter field survival at three of the four main-effect QTL, and two of those were on linkage group FN3. Examination of the interaction between marker loci AM2 and CDO1523 showed that RILs with nonparental allelic contributions at these loci had better performance than either parental genotype as indicated by the negative sign of the interaction.

The interaction between marker loci UMN433 and CDO1523 followed a dominant or recessive pattern for epistasis with the gene near CDO1523 as the hypostatic gene. Fulghum alleles at UMN433 masked the effect of alleles at CDO1523. Specifically, winter field survival was near 53.4% for lines with Fulghum alleles at UMN433 regardless of the alleles at CDO1523. Mean winter field survival of RILs with Norline alleles at UMN433 varied depending on the alleles at CDO1523. The RILs with Norline alleles at CDO1523 were slightly more freezing tolerant than those with Fulghum alleles.

One major QTL on linkage group FN3 was identified for crown freezing tolerance, accounting for 46% of the phenotypic variation (Table 3 and Fig. 1). Three minor additive QTL were also identified. Fulghum alleles at the QTL near the marker loci HVM20 and BCD1261 on FN3 and near the marker loci CDO504 on FN22 decreased freezing tolerance, while Fulghum alleles near marker locus Astaveb on FN42 increased crown freezing tolerance. The epistatic interaction between the QTL near HVM20 and Rast1_4 behaved as described by the sign of the interaction in the MIM model, with the nonparental marker genotype classes having greater crown freezing tolerance than the parental marker genotype classes. However the interaction between HVM20 and CDO504 showed a pattern of

<table>
<thead>
<tr>
<th>Trait</th>
<th>Crown freezing tolerance</th>
<th>Vernalization response</th>
<th>Heading date</th>
<th>Plant height</th>
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<tbody>
<tr>
<td>Winter field survival</td>
<td>0.73***</td>
<td>0.06</td>
<td>0.41***</td>
<td>-0.07</td>
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<td>0.20*</td>
<td>-0.12</td>
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<td>-0.15</td>
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<tr>
<td>Heading date</td>
<td></td>
<td></td>
<td>-0.22*</td>
<td></td>
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*Significantly different from zero at P < 0.05.
**Significantly different from zero at P < 0.001.
Table 3. Quantitative trait loci (QTL) for the oat winter-hardiness component traits winter field survival (WFS), crown freezing tolerance (CFT), vernalization response (VR), heading date (HD), and plant height (PHT) evaluated in a recombinant inbred line (RIL) population derived from a cross of ‘Fulghum’ and ‘Norline’ (FN).

<table>
<thead>
<tr>
<th>Trait</th>
<th>QTL</th>
<th>Linkage group</th>
<th>Position</th>
<th>Nearest marker(s)</th>
<th>Additive effect†</th>
<th>$R^2$</th>
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<td>AM2</td>
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<td>CDO1319</td>
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<td>2.1</td>
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<tr>
<td>PHT 1</td>
<td>FN3</td>
<td>24</td>
<td>AM270S_1</td>
<td>2.1</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>PHT 2</td>
<td>FN24</td>
<td>0</td>
<td>CDO1523</td>
<td>4.5</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>PHT 1 x 2 Epistatic interaction</td>
<td>AM270S_1 x CDO1523</td>
<td>-3.0</td>
<td>5.4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Additive effect of a single Fulghum allele, equivalent to the mean of RILs homozygous for Fulghum alleles minus the mean of RILs homozygous for Norline alleles divided by 2.

DISCUSSION

Linkage Map

The RFLP mapped in this population targeted specific regions of the oat genome believed to carry winter-hardiness component genes based on previous research. Most of these loci mapped to three linkage groups that were homologous to the linkage groups 3+38, 22_44_18, and 24_26_34 in the KO map (Wight et al., 2003). In contrast, we used all available polymorphic SSR markers regardless of genome location, so these markers were not as likely to be in specific genomic regions of interest. They tended to map into the small linkage groups.

Segregation distortion was found for several markers, especially those in linkage group FN3. Segregation distortion appears to be a common phenomenon in oat (Portyanko et al., 2001; Wight et al., 2003). The prevalence of Norline alleles in linkage group FN3 was consistent with previous studies that found a preponderance of translocation types in segregating progeny (Santos et al., 2006; Wooten et al., 2007).

The observed segregation distortion with a preponderance of Fulghum alleles was more difficult to explain. It is possible that one or both of the regions near Astaveb and CDO1523 had genes that were homologous to a critical gene hypothesized to be on or near the T7C-17 (Jellen and Beard, 2000; Wooten et al., 2007). No RILs were found to have T7C-17 absent (Fulghum translocation type) and Norline alleles at both the Astaveb and CDO1523 loci. This observation perhaps supported the theory that critical genes reside on the translocation in Norline, but the critical genes are in different locations in Fulghum, possibly near Astaveb or CDO1532. The position of a QTL near Astaveb (Table 3 and Fig. 1) where Fulghum alleles increased winter field survival and crown freezing tolerance suggested that this

dominant or recessive epistasis. Fulghum alleles at HVM20 masked expression of alleles at CDO504. Among RILs that had Norline alleles at HVM20, those with Norline alleles at CDO504 had significantly greater crown freezing tolerance than those with Fulghum alleles at CDO504.

Two main-effect QTL and two epistatic interactions were identified for vernalization response (Table 3 and Fig. 1). The Fulghum allele at the larger main-effect QTL on linkage group FN22 exhibited lower vernalization response than the Norline allele, reflecting the parental responses to vernalization. At the smaller main-effect QTL on linkage group FN3, the Fulghum allele exhibited an increase in vernalization response. The two epistatic interactions each involved a locus on either end of FN3. They accounted for a greater percentage of variation than the two main-effect QTL (30 vs. 17%). Both epistatic interactions had a negative sign because RILs with nonparental allelic combinations at these loci had greater vernalization response than either parental genotypes. For both interactions, the two-locus marker class mean with the greatest vernalization response had Fulghum alleles at the locus on linkage group FN3 (either UMN433 or UMN220B) and Norline alleles at the other loci (either CDO1461A or CDO549). This indicated that linkage group FN3 had QTL with large but complex effects on vernalization response.

Three QTL were identified for heading date (Table 3 and Fig. 1). The QTL near marker locus CDO1319 on linkage group FN3 and near marker locus CDO484B on linkage group FN43 both had additive effects with Fulghum alleles decreasing heading date. Fulghum alleles at the unlinked locus CDO187SSR increased heading date. Two QTL were identified for plant height, and there was an epistatic interaction between them.

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hypothesized critical gene(s) was, or was linked to, a gene or genes for winter-hardiness component traits.

Linkage group FN3 included parts of both chromosomes 7C and 17 and T7C-17, but the length of the linkage group was much shorter than in the KO map (Wight et al., 2003). The T7C-17 was expected to reduce recombination and that may explain the small size of the linkage group FN3; however, the KO population also segregated for T7C-17, so it was not expected that linkage group FN3 in this map would be much shorter than in the KO map. In contrast, linkage groups FN22 and FN24 expanded portions of reduced recombination in the KO map. All of the markers on linkage group FN22 mapped near position 100 on group 22_44_18 in the KO map, and all of the markers on linkage group FN24 mapped near position 41 in the linkage group 24_26_34 in the KO map (Wight et al., 2003). Wight et al. (2003) noted that Kanota and Ogle differ for several translocations in addition to T7C-17 and the difference in recombination between the KO and FN maps have resulted from additional translocations segregating in the KO population.

The nullisomic 7C/17 line allowed us to place the breakpoint of T7C-17 between marker loci HVM20 and AM270s_1. The identified translocation breakpoint also delineated the confidence intervals for most QTL on linkage group FN3 (Fig. 1).

Phenotypic Data
All traits showed continuous variation with most having near normal distributions. The bimodal distributions of the winter field survival and crown freezing tolerance traits were explained by major genes for each trait located on linkage group FN3. These accounted for 31% of the variation in winter field survival and 46% of the variation in crown freezing tolerance. This was similar to the results of Santos et al. (2006), who reported that T7C-17 status accounted for 22% of the variation in crown freezing tolerance and 27% of the variation in winter field survival in a RIL population derived from Fulghum × Wintok (FW population). Clearly there were major genes on or near T7C-17 which conferred increased winter field survival and crown freezing tolerance and caused a bimodal distribution for these two traits. The lack of significant correlations between vernalization response and crown freezing tolerance or winter field survival contrasts with previous results in small grains (Doll et al., 1989; Mahfoozi et al., 2001). This relationship in oat may not be as clear (Wooten et al., 2008). Field heading date was correlated with all traits. The much lower correlation between heading date and crown freezing tolerance compared with the correlation with winter field survival suggested that genes increasing heading date and field survival do not increase crown freezing tolerance to the same degree. Late heading date was likely a freeze stress avoidance mechanism (Prasil et al., 2004), not a stress tolerance mechanism.

Quantitative Trait Loci
Many of the QTL identified in this study were associated with multiple winter-hardiness component traits (Fig. 1 and Table 3). Many authors have reported tight linkage between winter-hardiness traits in winter cereals (Kobayashi et al., 2005; Pan et al., 1994; Storlie et al., 1998; Sutka et al., 1999; Toth et al., 2003). While it is not possible to discern linkage from pleiotropy in this population, the similarity between these results and those from other winter cereals supports the linkage hypothesis for most of the QTL. Because most of the markers mapped in this population previously were associated with winter-hardiness component traits, there were some similarities between the QTL identified in this study and those identified previously.

Linkage group FN3 had QTL for all of the traits measured; however, the configuration of vernalization QTL was contrary to the expected configuration. Typically QTL for increased freezing tolerance are linked to QTL for increased vernalization response; however, on linkage group FN3, Fulghum alleles increased vernalization response but reduced crown freezing tolerance and winter field survival. Santos et al. (2006) found an association between T7C-17 and the traits crown freezing tolerance and winter field survival. Our results support the importance of this genomic region, with two additive QTL accounting for 52% of the variation for crown freezing tolerance and two additive QTL accounting for 35% of the variation in winter field survival in the region of the translocation. The primary QTL mapped for crown freezing tolerance (near HVM20) did not concur with the results of a previous spring × winter oat QTL study in the KO population (Wooten et al., 2008). Quantitative trait loci for crown freezing tolerance were identified in the KO population for the markers on the ends of linkage group FN3, UMN220 (on KO LG 24_26_34) and UMN433 (on KO 3+38) (Wooten et al., 2008). These QTL were also identified as vernalization response and heading date QTL (Holland et al., 1997; Siripoonwiwat et al., 1996). Further, the QTL near UMN220 in the KO population was involved in several epistatic interactions (Holland et al., 1997), possibly similar to its epistatic interaction in the FN population evaluated in this study (Table 3). These results demonstrated the importance of examining winter-hardiness component traits in a winter oat population, as vernalization response QTL identified in this study corresponded to freezing tolerance QTL mapped in a spring × winter population, whereas the primary crown freezing tolerance QTL in this winter oat population were not mapped in previous spring × winter studies.

Linkage groups FN22 and FN24 were both aligned with portions of the linkage groups in the KO populations and carried similar QTL to those found in the KO and OT mapping populations. Linkage group FN22 carried QTL for winter field survival, crown freezing tolerance,
and vernalization response. The QTL for vernalization response, crown freezing tolerance, and winter field survival located on FN22 must be linked in coupling, as the Fulghum alleles at these loci decreased all these traits. The linkage of freezing tolerance, vernalization response, and winter field survival QTL is common across winter cereal species (Galiba et al., 1995; Kobayashi et al., 2005; Pan et al., 1994). The markers on linkage group FN22 were associated with vernalization response in both the KO and OT populations (Holland et al., 1997, 2002). Linkage group FN24 had QTL for plant height as well as epistatic interactions for crown freezing tolerance and winter field survival. In the KO and OT mapping populations, this region carried a major QTL for vernalization response and heading date, as well as epistatic interactions for vernalization response (Holland et al., 1997, 2002; Siripoonwiwat et al., 1996).

A comparison of the QTL configuration on FN3 with FN22 and FN24 indicated the arrangement on FN3 was unusual. The major crown freezing tolerance and winter field survival QTL on FN3 were not previously identified in other oat populations and were linked in repulsion to vernalization response QTL. It appeared that the T7C-17 created an unusual arrangement in this region that greatly increased the most important winter-hardiness traits even when linked in repulsion to genes for vernalization response. Wooten et al. (2007) noted that T7C-17 had nearly identical effects for crown freezing tolerance in the FN population and the FW population examined by Santos et al. (2006), but the translocation had a substantially larger effect on winter field survival in the FW population. The linkage of vernalization response QTL in repulsion in the FN provided an explanation for these observations. We suspect that the vernalization response QTL near T7C-17 were linked in coupling with crown freezing tolerance QTL in the FW population. The Wintok vernalization response alleles likely increased winter field survival without having much of an effect on crown freezing tolerance. This agreed with the observed differences in the translocation effect on crown freezing tolerance and winter field survival in the FN and FW populations (Santos et al., 2006; Wooten et al., 2007). The translocation likely disrupted the recombination in the region, caused segregation distortion in both populations, and complicated linkage mapping and identification of QTL in this region. Further QTL studies in populations derived from parents that do not segregate for the translocation should be informative in precisely mapping the winter-hardiness QTL in this region.

Several QTL and epistatic interactions were identified on the small linkage groups of unknown location. The loci on FN42 had a QTL where Fulghum alleles increased winter field survival and crown freezing tolerance. The Astaveb loci is in a region where both the OT map and the corresponding region of the KO map had QTL for heading date (Holland et al., 1997, 2002; Jannink and Gardner, 2005; Siripoonwiwat et al., 1996). The Avenin locus maps to OT8 (Jannink and Gardner, 2005), but it is approximately 60 cm away from the location of the nearest winter-hardiness component trait QTL identified by Holland et al. (2002). We suspect this region did not correspond to the QTL we mapped in the FN population. The epistatic interaction for winter field survival involving AM2 on FN41 could not be associated with any previous QTL studies because neither SSR marker on FN41 has been mapped to linkage groups in the KO or OT mapping populations. The QTL for heading date near CDO484B on linkage group FN43 was difficult to explain. There were several QTL for heading date traits near CDO484 on KO 24_26_34 (Holland et al., 1997), but this region was mapped as CDO484A which was in the linkage group FN24. These regions may be homeologous to FN24, but there were too few markers to make that determination.

Three unlinked markers (CDO187SSR, CDO549, and CDO1461) were identified as main-effect QTL or involved in epistatic interactions. The Fulghum alleles at marker locus CDO187SSR caused late heading date in the FN population. In the KO population the marker CDO187 was associated with a QTL for heading date (Siripoonwiwat et al., 1996), and these data seemed to fit the same pattern. A QTL for vernalization response was identified near the marker locus CDO549 on linkage group 13 in the KO population, but unlike the FN population the marker did not interact epistatically (Holland et al., 1997). No QTL for winter-hardiness component traits have been associated with the RFLP probe CDO1461 in either the KO or OT populations.

The portion of the phenotypic variation explained by the QTL (the sum of the $R^2$ values) did not approach the heritability for any trait (Tables 1 and 3). Further, the sample size of the population limited the QTL detection power, and because only a portion of the true QTL were included in the model, the estimated effect of those included QTL was biased upward (Beavis, 1994). Although we were able to identify several major QTL for the winter-hardiness component traits, there likely were other relevant QTL in regions of the genome not covered with molecular markers, or QTL that were not identified as significant in this sample of the population.

**SUMMARY**

Significant variation was observed for the winter-hardiness component traits winter field survival, crown freezing tolerance, heading date, plant height, and vernalization and photoperiod responses. Quantitative trait loci were identified for all traits except photoperiod response. Multiple QTL for related winter-hardiness component traits were identified on three linkage groups. The linkage group FN3 had major QTL for winter field survival ($R^2 = 35\%$) and crown freezing tolerance ($R^2 = 53\%$). These QTL were in
the region of the T7C-17, which was previously associated with winter-hardiness traits. Vernalization response QTL alleles were linked in repulsion phase to the QTL alleles for increased winter hardiness on linkage group FN3. Linkage group FN22 had QTL alleles for decreased crown freezing tolerance, winter field survival, and vernalization response linked in coupling phase. These results identified a major QTL associated with winter-hardiness component traits near T7C-17, which illustrated the importance of this genomic region to winter hardiness.

References
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