Chapter 10

Genetic diversity for quantitatively inherited agronomic and malting quality traits

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Introduction

Agronomic and quality traits were undoubtedly key issues for the domesticators of barley. According to the archeological record, these early farmers used both wild and cultivated (non-brittle rachis) forms of barley (Harlan, 1995). Crop productivity would clearly have been an attribute of key interest, and the selection of shattering resistant mutants probably led to a quantum leap in yield. Because barley has been used as both a food and as a principal ingredient of fermented beverages from the earliest times, there may well have been conscious selection for end-use properties. The selection of hull-less mutants in areas of the world where barley was a principal foodstuff underscores the importance of end-use properties in domestication. The malting and brewing properties of wild barley accessions and land-races have not been well described and are, in fact, extremely difficult to measure. There are no absolute definitions of malting and brewing quality, due to differences in malting and brewing practices and consumer preferences. Both direct consumers of barley and the first brewers would have selected for uniform, plump kernels. However, only the latter would have selected for the higher levels of enzymatic activity necessary for satisfactory malting and brewing performance.

Plant breeding efforts are directed primarily at traits exhibiting quantitative variation. However, diversity and quantitative traits are not terms that are often encountered together in the plant breeding and genetics literature. This is certainly true for barley, a crop with a rich legacy of genetics research and an impressive record of variety improvement. Genetics was limited to models where phenotypes allowed for Mendelian analysis. Genetic tools, when applied to variety development, were of a statistical nature. In the mid-1980’s, the availability of abundant DNA-level markers, accompanied by the development of computer hardware and software, caused these parallel lines of investigation to change course and head toward intersection. Breeders and geneticists were now able to collaborate in developing and testing hypotheses regarding the number, location, effect, and interactions of genes influencing quantitative traits. These genes were termed "Quantitative Trait Loci" (QTL). In this chapter, we will review (1) diversity in agronomic traits; (2) diversity in malting quality traits; and (3) the current status of QTL analysis in barley and the application of QTL tools to the analysis of genetic diversity in barley and crop improvement.
Agronomic traits

Barley is one of the most widely adapted cereal crops. It is grown in a range of extreme environments that vary from northern Scandinavia to the Himalayan mountains to monsoon paddies. No barley variety is adapted to all environments and, in fact, very different gene pools have evolved in the major barley production areas of the world. The gene pools may be defined by essential physiological parameters that determine adaptation to a production environment - such as vernalization and/or photoperiod response - or they may be defined by evolutionary bottlenecks and the accidents of history, such as regional preferences for two-rowed or six-rowed varieties. Within these gene pools, agronomic performance will be determined by the entire allelic architecture of each genotype. The actual number of genes in barley (with a genome size of 5.3 billion base pairs) is not known at this point. There are at least 26,588 genes in the euchromatic regions of the human genome (2.91 billion base pairs) and there is some evidence for an additional 12,000 genes (Venter et al., 2001). The first higher plant genome – Arabidopsis thaliana, with an estimated genome size of 125 million base pairs – was sequenced and the number of genes was estimated at 25,800 (Pennisi, 2000). Surely, most of the genes in barley are involved in one way or another with yield – the “ultimate” fitness trait. Likewise, a large number of them must be involved in malting quality, which is the end result of the fundamental processes of seed carbohydrate deposition and hydrolysis.

In terms of agronomic traits, growth habit genes may be major “specious” yield genes (see also Chapter 2). For example, highly significant genotype x environment interaction will occur when the progeny derived from a cross of spring x winter habit parents are evaluated under spring and fall-sown conditions. A genetic analysis of such germplasm would likely reveal vernalization requirement, photoperiod reaction, and low temperature tolerance genes as primary determinants of yield. This was indeed the case when Quantitative Trait Locus (QTL) tools were applied to a population derived from the cross of North American winter and spring genotypes (Oziel et al., 1996). Likewise, resistance to biotic stresses can be the primary determinant of yield, if resistance to the disease in question is endemic to a production zone. A “cost”, or lower yield potential of resistant vs. disease susceptible germplasm in disease-free environments, could be due to linkage of resistance and “yield determining” genes or pleiotropic effects of the resistance genes (Hayes et al., 1996).

Effects of inflorescence type on yield

The role of inflorescence type in determining yield is complex. In barley each rachis node has three spikelets, each of them bearing one floret. The two-rowed and six-rowed germplasm groups are defined by the number of fertile florets per rachis node. In two-rowed barley, there is one fertile floret per rachis node, whereas in six-rowed barley all three florets are fertile (Hitchcock, 1971; see also Chapter 2). Most barley varieties of commercial importance are six-rowed or two-rowed inbred lines. This simple genetic system defines the two principal germplasm groups of barley, and these germplasm groups have historically defined end uses. Two-rowed barleys are favored for malting throughout most of the world, except for the USA and Mexico, where six-rowed barleys are used extensively for this purpose (Riggs and Kirby, 1978).

Two-rowed varieties usually have a higher number of tillers per plant and larger, heavier seed than six-rowed varieties. Six-rowed varieties on the other hand, usually have more seeds per inflorescence. Thus the compensatory effects of yield components lead to similar levels of yield potential. However, historical patterns of geographic distribution and end-use of the two-rowed
and six-rowed germplasm groups have led to the idea that the two germplasm groups carry different alleles at other loci in addition to those determining lateral floret fertility (Takahashi et al., 1975). Accordingly, crosses between the two germplasm groups might be expected to produce positive transgressive segregants for economically important phenotypes. The experience of plant breeders, however, has generally been that two-rowed x six-rowed crosses are not suitable for variety development (Harlan, 1957; Kjær and Jensen, 1996). Allard (1988) concluded that “Evidently the two-row/six-row locus affects developmental processes in ways that leave few quantitative characters untouched” and “this locus had large effects on survival and adaptedness”. Large pleiotropic effects on multiple phenotypes have been attributed to alleles at the \textit{vrs1} locus, based on studies of the progeny of two-rowed x six-rowed crosses (Kjær and Jensen, 1996; Jui et al., 1997). However, it is possible that the correlated phenotypes are due to linkage rather than pleiotropy. It is difficult to distinguish between these phenomena in the case of the \textit{vrs1} locus, which is located near the centromere on linkage maps. On the physical map of Künzel et al. (2000), however, the \textit{vrs1} locus is located in one of the higher recombination regions on the long (minus) arm of chromosome 2H. Powell et al. (1990), in a comparative analysis of two types of progeny from a six-rowed x two-rowed cross, concluded that some associations between quantitative phenotypes and the \textit{vrs1} locus were due to linkage rather than pleiotropy. Marquez-Cedillo et al. (2001) used QTL tools to analyze a two-rowed x six-rowed mapping population for agronomic traits. The major QTL related with agronomic traits coincided with the loci that determined inflorescence type.

Two-rowed barley in Japan provides an example of the role of growth habit and maturity genes within a germplasm class. Yasuda et al. (1992) analyzed a collection of two-rowed barleys from Japan, Turkey, Ethiopia and Europe and found that Japanese two-rowed malting cultivars, which were introduced from Europe less than 130 years ago, were quite different from the other germplasm groups. Adaptation to Japanese conditions was achieved within a relatively short period, and a major distinguishing attribute of the Japanese germplasm is early maturity. In Japan, barley should be harvested before the rainy season, when the fields are converted to rice paddies. Yasuda (1964) used hybrid populations derived from winter/spring barley crosses to demonstrate selection for adaptation to Japanese conditions and later showed the effect of four spring growth habit genes on yield performance (Yasuda, 1977; see also Chapter 2).

\textit{Identifying factors that affect yield}

Once the more obvious “yield-limiting genes” are accounted for, what determines yield in barley, and how much genetic diversity is there at these loci? There have been three principal approaches to the study of “yield genes”. Plant physiologists have defined at the cellular, organ, and organism level the fundamental physiological processes that lead to yield in barley (Kirby and Faris, 1972; Gallagher et al., 1975; Lauer and Simmons, 1989; Dreccer et al., 1997). Crop physiologists and plant breeders have defined physiological parameters for production level phenotypes – e.g. yield components, harvest index, and ideotypes – that determine yield (Rasmusson, 1987; Hamblin, 1993). This research has led to a better understanding of fundamental plant processes and better \textit{a posteriori} understanding of crop performance, but in many cases the two approaches have not led to predictive tools that are useful for barley improvement (Beldford and Sedgley, 1991; Rasmusson, 1991). A problem has been that, often of necessity, these studies were conducted in agriculturally irrelevant genetic stocks and environments. Furthermore, it has been exceptionally difficult, as shown in the examples of growth habit and inflorescence type, to separate the effects of individual genes with large effects
from “genetic background”.

Because of these challenges, and the fact that most agronomic traits showed inheritance patterns too complex for Mendelian inheritance, barley breeders and crop geneticists approached the issue of the genetics of yield using biometrical tools. However, as stated by Allard (1988) “Most quantitative genetic models require numerous assumptions, many of which are invalid, thus causing estimates of genetic parameters to be imprecise and even to exceed their theoretical limits. Whatever the cause or causes, the laborious biometrical experiments I conducted provided little information about the numbers of alleles per locus, numbers of loci, types of gene action (additive, dominance, and epistatic), the impact of various single locus or multilocus genotypes on fitness, and other genetic factors about which we must learn more if we are to understand the evolution of adaptedness”.

For over a decade, molecular approaches have been used to map and describe Quantitative Trait Loci that determine yield in barley. As discussed in a subsequent section of this chapter, our understanding of QTL and their effects on agronomic traits is still incomplete. Nonetheless, developments in molecular biology have provided the necessary tools for determining the functional basis of yield and adaptation in barley.

**Malting quality traits**

Historically, the biochemistry and genetics of malting quality have been parallel areas of study. The former has focused on the systematic characterization of the deposition and hydrolysis of starch and proteins. This research has provided a more comprehensive understanding of the underlying processes, but has not provided breeders with a better tool kit for improving malting quality. Genetic studies of malting quality, to date, have provided perspectives on allelic diversity at only a few key loci. Consequently, barley breeders must still conduct expensive tests to determine the malting quality of their experimental germplasm and, because of the expense, can only carry out a limited number of assays. This has precluded the use of extensive population-based analyses of malting quality genetics. Furthermore, when such analyses have been conducted, malting quality phenotypes have shown frequency distributions that defy Mendelian analysis. Breeders have therefore relied upon phenotypic selection of malting quality in agronomically relevant germplasm, with occasional attempts to estimate genetic variances and numbers of “effective factors”. As is evidenced by the quotation from Allard (1988) cited in the previous section on “Agronomic traits”, these procedures have had limited practical applications. Information on allelic diversity in the genes that determine malting quality is limited. Accordingly, we will focus on providing a brief summary of the malting process and an enumeration of the genes known to be involved in these processes. The reader is referred to earlier reviews of the malting process (Burger and LaBerge, 1985; Bamforth and Barclay, 1993) and biochemical aspects of barley seed germination (Briggs, 1992; Fincher and Stone, 1993) for further background information. Expressed Sequence Tag (EST) projects have recently been initiated for malting quality in several laboratories around the world, but results are not yet available. Characterization of the structure and expression of genes expressed at key times and in key tissues relevant to the malting process will provide a foundation for efficient characterization and use of allelic diversity for malting quality traits.

**The malting process**

Malting is an exercise in applied biochemistry, especially enzymology. The starch, protein and nucleic acid molecules that are stored in barley grains are not good nutrients for brewing yeast
nor do they support the fermentation reactions performed by brewing yeasts. These large and structurally complex compounds must be partially or, in some instances, fully degraded into their component sugars, amino acids, and nucleotides before the yeast can use them. When barley seeds germinate, hydrolytic enzymes are synthesized or converted to active forms that can readily degrade these large compounds.

During “malting”, barley seeds are germinated under controlled conditions so that degradative enzymes form and begin to hydrolyze the starch, protein, and nucleic acid molecules into small molecules that are needed at appropriate stages of the brewing process. To arrest the malting process, the green malt is kilned (gently dried, with heat) and the rootlets are removed. By this stage, little of the starch has been converted to sugars, but about 70% of the protein that needs to be solubilized during malting and mashing has already been rendered soluble. There is still some question as to how much free amino nitrogen (FAN) is released during malting.

Modification is a collective term that is used to refer to all of the polymer-degrading processes that occur during malting. If malting is allowed to continue too long, the malt obtained will be overmodified and will not produce beers of optimal quality.

The malt is treated with water under appropriate conditions (a process called “mashing”) to obtain an extract (wort) that must perform several critical functions. The extract must provide adequate nourishment to the yeast so that fermentation can occur. Secondly, the extract must provide sufficient fermentable sugars to enable the yeast to produce the desired levels of alcohol. A high quality malt will contain the right amount of hydrolytic enzymes and metabolites to fulfill these requirements and will have the right degree of friability to allow many of its components to be readily solubilized during mashing. During malting and mashing, the barley starch should be almost completely degraded into sugars that can be utilized by the brewing yeasts, whereas only about 45% of the barley protein should be solubilized. Too much protein solubilization is thought to result in beers with poor foaming characteristics. When insufficient protein hydrolysis occurs, the remaining proteins may interact with polyphenols to form beer haze precipitates.

The malting process, accordingly, involves a host of interacting genes involved in the fundamental processes of seed germination, growth and development. Domestication and selection have accumulated favorable alleles at multiple loci that determine malting quality. The specific alleles that have been accumulated in the major malting barley germplasm groups may differ, based on regional preferences and genetic drift. A summary of current knowledge of the genes and enzymes that control malting quality, and key citations, are presented in Table 10.1.

**Enzymes and genes that control carbohydrate degradation**

Four amylolytic enzymes are generally thought to participate in converting the starch in malted barley into fermentable sugars: these are α-amylase, β-amylase, α-glucosidase and limit dextrinase. Alpha-amylase and α-glucosidase can release α-glucans from native (nongelatinized) starch granules (Sun and Henson, 1990; Sissons and MacGregor, 1994). All four enzymes can hydrolyze gelatinized starch and/or glucan fragments. A fifth carbohydrate, isoamylase, has recently been discovered and it may have a role in degrading starch and/or producing fermentable sugars during mashing (Sun et al., 1999), but the precise role of this enzyme is not yet known.
Table 10.1. Genes and gene products that control malting quality in barley.

<table>
<thead>
<tr>
<th>Enzyme/protein</th>
<th>Function</th>
<th>Gene</th>
<th>Chr.</th>
<th>Citations</th>
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</thead>
<tbody>
<tr>
<td><strong>Carbohydrate-degrading enzymes and inhibitors</strong></td>
<td></td>
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<tr>
<td>α-amylase</td>
<td>Converts native starch, elatinized starch and glucans into sugars</td>
<td>Amy1</td>
<td>7H</td>
<td>Bamforth and Barclay, 1993; Henson and Stone, 1988; Ko and Henry, 1994; Ko et al., 1996</td>
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<tr>
<td></td>
<td></td>
<td>Amy2</td>
<td>6H</td>
<td></td>
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<tr>
<td>β-amylase</td>
<td>Converts gelatinized starch and glucans into sugars; acts as storage protein (globulin)</td>
<td>Bmy1</td>
<td>4H</td>
<td>Bamforth and Berclay, 1993; Eglinton et al., 1998; Erkkila et al., 1998</td>
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<tr>
<td></td>
<td></td>
<td>Bmy2</td>
<td>2H</td>
<td></td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>Converts native starch, gelatinized starch and glucans into sugars</td>
<td>Agl</td>
<td>7H</td>
<td>Henson and Sun, 1995; Im and Henson, 1996; Konishi et al., 1994</td>
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<tr>
<td>limit dextrinase</td>
<td>Breaks down branched starch and amylopectin molecules</td>
<td>LD</td>
<td>7H</td>
<td>Burton et al., 1999; Langridge et al., 1996; Li et al., 1999; MacGregor et al., 1994</td>
</tr>
<tr>
<td>isoamylase</td>
<td>May convert starch to sugars, but precise role not known</td>
<td></td>
<td></td>
<td>Sun and Henson, 1999</td>
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<tr>
<td>β-glucanase</td>
<td>Degrades cell wall β-glucans</td>
<td>Glb1</td>
<td>5H</td>
<td>Loi et al., 1988; Li et al., 1999; MacLeod et al., 1991; Viëtor et al., 1993</td>
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<td></td>
<td></td>
<td>Glb2</td>
<td>7H</td>
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<td></td>
<td></td>
<td>Glb31-37</td>
<td>3H</td>
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<tr>
<td>barley amylase subtilisin inhibitor (BASI)</td>
<td>Inhibits α-amylase encoded by Amy2</td>
<td>Isal</td>
<td>2H</td>
<td>Cannel et al., 1992; Munck et al., 1985</td>
</tr>
<tr>
<td>limit dextrinase inhibitor (LDI)</td>
<td>Inactivates limit dextrinase</td>
<td></td>
<td></td>
<td>MacGregor et al., 1995</td>
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<tr>
<td>Protein Z</td>
<td>β-amylase binding protein; acts as storage protein (globulin)</td>
<td>Paz1</td>
<td>4H</td>
<td>Cannel et al., 1992</td>
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<tr>
<td><strong>Storage proteins, protein-degrading enzymes and inhibitors</strong></td>
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<tr>
<td>B hordein</td>
<td>Storage protein (prolamin)</td>
<td>Hor2</td>
<td>1H</td>
<td>Shewry, 1993</td>
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<tr>
<td>C hordein</td>
<td>Storage protein (prolamin)</td>
<td>Hor1</td>
<td>1H</td>
<td></td>
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<tr>
<td>D hordein</td>
<td>Storage protein (prolamin)</td>
<td>Hor3</td>
<td>1H</td>
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<tr>
<td>hordenin (GluA, GluB, GluC)</td>
<td>Storage and structural protein (glutelin)</td>
<td>GluA</td>
<td>1H</td>
<td>Giese et al., 1994; Kleinhofs et al., 1993</td>
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<tr>
<td></td>
<td></td>
<td>GluB</td>
<td>1H</td>
<td></td>
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<td></td>
<td></td>
<td>Glu.c1</td>
<td>1H</td>
<td></td>
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<tr>
<td>Carboxpeptidase (exopeptidase)</td>
<td>Hydrolyzes storage protein</td>
<td>Cxp1</td>
<td>3H</td>
<td>Kleinhofs et al., 1993</td>
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<td></td>
<td></td>
<td>Cxp3</td>
<td>6H</td>
<td></td>
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<tr>
<td>malt cysteine endoprotease</td>
<td>Hydrolyzes hordeins</td>
<td>Cys1</td>
<td>3H</td>
<td>Cannel et al., 1992; Jones, 1999; Jones and Budde, 1999; Mikkonen et al., 1996</td>
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<tr>
<td></td>
<td></td>
<td>Cys2</td>
<td>3H</td>
<td></td>
</tr>
<tr>
<td>phytasin; aspartic class endoprotease</td>
<td>Hydrolyzes storage protein</td>
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<td></td>
<td>Runeberg-Roos et al., 1991</td>
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<tr>
<td>lipid transfer protein (LTP1); non Probable Amylase Protease Inhibitor (PAPI)</td>
<td>Regulates activities of malt cysteine class endoproteasises</td>
<td>Ltp1</td>
<td>5H</td>
<td>Jones and Marinac, 2000b; Kleinhofs et al., 1993; Lusk et al., 1995; Sorensen et al., 1993</td>
</tr>
<tr>
<td>lipid transfer protein (LTP2)</td>
<td>Inhibits endoprotease</td>
<td>Ltp2</td>
<td></td>
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</table>
The α-amylases are particularly important in the production of fermentable sugars during mashing because they are the only amylolytic enzymes present that are sufficiently thermostable to retain at least some level of activity for the full duration of mashing (Bamforth and Barclay, 1993). Furthermore, most fermentable sugars are produced during the late stages of mashing when temperatures are quite high. The Amy1 and Amy2 loci encode two α-amylase isozymes that differ somewhat in their biochemical and biophysical characteristics. Some allelic variation has been documented in the Amy2 locus of barley (Ko and Henry, 1994; Ko et al., 1996), although the usefulness of this variation is not clear.

Potentially useful allelic variation in the genes that encode the β-amylases have recently been identified. Erkkila et al. (1998) identified three alleles of β-amylase1, including one which might be responsible for very high enzyme activity. Eglinton et al. (1998) identified β-amylase1 alleles that encode proteins with enhanced thermostabilities.

Limit dextrinase activity is thought to be necessary for the complete degradation of starch (MacGregor et al., 1994). Li et al. (1999) identified five limit dextrinase alleles in cultivated barley and an additional 10 different genes or alleles in non-cultivated barley species. It is not yet known if the alleles identified confer useful characteristics for malting and brewing.

**Inhibitors of carbohydrate-degrading enzymes**

There are proteins in barley that interact with and inhibit the activities of some of the enzymes that are involved in degradation of starch and arabinoxylans. The best studied of these proteinaceous inhibitors is the barley amylase subtilisin inhibitor (BASI), which specifically inhibits the α-amylase encoded by Amy2 (Mundy et al., 1986). A portion of the β-amylase in barley and malt is in a bound form that is rendered soluble, and thereby active, via the action of reducing agents or endoproteinases. Genes coding for these β-amylase-solubilizing-proteinases, which have yet to be identified, might affect the amount of β-amylase activity in a malt or mash. One of the proteins that is bound to β-amylase in malt may be “Protein Z”. Additional information on, and citations for, BASI and Protein Z are presented in Table 10.1. Proteins have been isolated from wheat that inhibit the activity of barley malt arabinoxylanases and barley has been shown to contain proteins that cause the same effect (Debyser et al., 1999). The presence of these inhibitors in barley could reduce the rate of arabinoxylan degradation during malting and mashing, leading to problems of high viscosity in the mash. The barley arabinoxylanase inhibitor has not yet been purified, and nothing is known about the gene that codes for its synthesis.

**Enzymes and genes that control protein degradation**

The hordeins (prolamins) and hordenins (glutelins) are the major storage proteins of barley, but Protein Z and β-amylase, which are globulins, also appear to behave as storage proteins (Shewry, 1993). Relatively large amounts of hordenin occur in some cultivars, but since these proteins generally serve structural purposes, they may not play much of a role in determining malting quality. The enzyme systems that reduce the barley storage proteins to ‘soluble protein’ (proteins, peptides and amino acids that are soluble in warm water) are very complicated, and involve both endoproteinases and exopeptidases (mainly carboxypeptidases). The rate-limiting step for protein degradation is the hydrolysis of the original proteins into soluble protein by the endoproteinases, so it is the activities of these enzymes in malt that will usually determine whether a barley genotype is acceptable for malting. There are at least 40 endoproteolytic activities, including representatives of all four of the common endopeptidase classes, in green malt (Zhang and Jones, 1995). It seems likely that the cysteine proteinases play a major role in the degradation of storage
proteins during mashing and malting, and that the aspartic and metalloproteinases also contribute significantly to this process (Jones, 1999). A gene coding for one aspartic class endoproteinase called phytEpsin has been cloned (Runenberg-Roos et al., 1991). Its map location has not been reported. The small peptides and amino acids that are released by the exopeptidases comprise the majority of the free amino nitrogen (FAN) fraction. FAN concentration is measured to indicate how well the original protein material can be utilized by yeasts during brewing. Three carboxypeptidase (Cxp) genes have been cloned and two have been mapped (see also Table 10.1).

**Inhibitors of protein-degrading enzymes**

The activities of the cysteine class malt endoproteinases are strongly inhibited by a series of proteinaceous inhibitors that occur in both barley and malt, with malt containing about 2.5 times as much inhibitory activity as barley. These inhibitors appear to play a role in controlling the rate of protein solubilization during mashing (Jones and Marinac, 2000a). Two of these inhibitors are lipid transfer proteins - LTP1 and LTP2. LTP1 was initially purified and described as a Probable Amylase Protease Inhibitor (PAPI) (Mundy and Rogers, 1986) and its locus, *Ltp1*, has been mapped to chromosome 5H (Kleinhofs et al., 1993; see also Table 10.1).

**Future improvement in malting quality**

By manipulating the carbohydrate and protein-degrading enzymes that occur in malts, as well as their inhibitor proteins, it should be possible to produce even better malting barleys. Because of the large number of enzymes and inhibitors involved, however, it is clear that this will not be an easy process. Similar to the situation for grain yield, malting quality is an economically important phenotype with complex genetic inheritance. Many of the component determinants of malting quality such as malt extract and α-amylase activity are known. However, we are still in the process of developing a comprehensive understanding of how the individual determinants interact to determine the final phenotypes. In terms of genetic diversity, we have yet to develop comprehensive catalogs of the alleles that determine these traits.

**Quantitative Trait Loci (QTL)**

The QTL concept represents an incremental step forward in understanding traits showing quantitative variation. The “QTL” acronym, and the often-used term “quantitative inheritance” may perpetuate the idea that the genes affecting quantitative traits are somehow different from the genes that determine qualitative traits that show Mendelian inheritance. Because alleles at both quantitative and qualitative trait loci can ultimately be reduced to DNA sequences, what are the distinguishing features of QTL? Defining QTL based on assumptions regarding the number of loci determining the target phenotype is not appropriate, since the number of loci that can be characterized in an unbiased fashion is dictated by the terms of a given experiment. These terms include population size, heritability, and the number and types of environments that are sampled. A simpler operative definition may be to consider QTL as genes underlying phenotypes that (i) defy Mendelian classification and (ii) are measured by a quantitative scale. Thus defined, QTL approaches are appropriate for most agronomic and malting quality traits. As elegantly demonstrated in rice and tomato, with sufficient resources and the appropriate genetic stocks, QTL can be "converted" to Mendelian loci and cloned (Yano et al., 1997, 2000; Yamamoto et al., 1998; Frary et al., 2000).

In addition to map-based cloning, a goal of many QTL projects in barley, and other crops, is
to discover, dissect, and manipulate the genes that determine quantitatively inherited phenotypes that pose particular challenges for plant breeders. In essence, this involves a systematic characterization of the existing genetic diversity. The first QTL reports were particularly promising in this regard, as complex phenotypes such as yield, malting quality, and quantitative disease resistance were reduced to relatively few loci that showed little evidence of epistasis or interaction with the environment. Based on such results, it seemed likely that the effectiveness of marker-assisted selection for QTL alleles would be limited only by the availability and cost of markers flanking target QTL. However, as more data were generated on more germplasm combinations, and QTL detection and characterization methodologies were improved, QTL mapping has turned out to be nearly as complex as the phenotypes it was meant to simplify. The essentially descriptive genome mapping and QTL detection projects of the late 20th century have been followed by the functional genomics, proteomics, and large-scale sequencing projects of the early 21st century. With our genetics horizons now limited only by our ability to phrase interesting questions, and to afford the technology, the time is right to summarize results from over ten years of barley QTL studies (Hayes et al., 2000).

The successful cloning of QTL in two model systems (rice and tomato) justifies an attempt to systematically dissect quantitative traits in barley. However, investigators need to recognize that the initial estimates of the number, genome location, and effects of QTL from mapping populations are probably crude representations of very complex biological processes. Finer structure analyses employing alternative genetic stocks and more refined measures of target phenotypes will be required for precise and accurate QTL analysis. When alleles at multiple QTL in multiple germplasm accessions are isolated and characterized at the DNA sequence, expression, and protein levels, techniques for describing, quantifying and classifying genetic diversity can be applied to these data. This will permit hypotheses to be developed and tested regarding the evolution, distribution, and function of QTL alleles in barley germplasm. The results of these experiments will provide a rational basis for germplasm conservation and utilization. The current barley QTL data, while extensive, are too fraught with bias and low precision to undertake such formal analyses of QTL allelic diversity. Additional limitations of the available data are: (1) inadequate sampling of the global diversity in barley germplasm; (2) the use of different QTL analysis procedures; and (3) a lack of common markers. Accordingly, we approached the question of QTL diversity in barley from descriptive and enumerative perspectives.

Summary of QTL diversity

We have summarized the available barley QTL data and addressed the levels of diversity in Figure 10.1 using the following criteria: (1) germplasm sampled; (2) phenotypes sampled; (3) distribution of QTL across and within linkage groups; and (iv) distribution of QTL on the physical map of barley that was reported by Künzel et al. (2000). QTL were assigned to the Bin Map, herein referred to as the “BM”, of Kleinhofs and Graner (2000) as follows: if markers flanking a QTL were also in the BM, we were able to unequivocally assign the QTL to a bin or region. Binned QTL were assigned to the physical map of Künzel et al. (2000) - referred to as the “PM” - as follows. First, we identified markers that occurred in both the BM and the ‘Igri’/‘Franka’ map, referred to as “IF”. The IF map was used in developing both the BM (Kleinhofs and Graner, 2000) and the PM (Künzel et al., 2000). We used markers in common to the BM and IF map as our starting framework. Then, IF markers without BM positions were assigned to the latter using the proportional distances to common flanking markers in the IF and
CM maps. This generated an adjusted "Linkage Bin Map" (LBM). We then aligned the LBM and the PM to generate a "Physical Bin Map" (PBM). The LBM and PBM bins were assigned recombination values, following the nomenclature of Künzel et al. (2000), to generate a "Recombination Bin Map" (RBM). We then aligned the RBM with the PM. As shown in Figure 10.1, this allows for approximations of the physical size of the bins to which QTL are assigned and an estimate of the degree of recombination in each bin. Finally, as shown in Figure 10.1, QTL assigned to each bin in the LBM were drawn proportional to the bin size in the PBM.

For the five trait groups – abiotic stress resistance, agronomic traits, biotic stress resistance, quality, and other – 757 QTL were distributed across the seven chromosomes, with the most QTL on chromosomes 2H and 5H and the fewest on chromosomes 1H and 6H (Table 10.2). The high number of QTL on chromosome 2 is attributable to QTL coincident with, or located near, the *vrs1* locus.

There are QTL for all traits on all chromosomes that are located in bins where there is a low physical:linkage map ratio (Figure 10.1). These QTL are sufficiently resolved to represent defined targets for marker assisted selection, finer structure mapping, and cloning. However, there are also QTL mapping to large physical distances, which often correspond to the centromeric regions on linkage maps. Additional resolution will be required before these QTL can be considered as targets for map-based cloning. For the purposes of marker-assisted selection, linkage drag is clearly an issue. However, procedures for the marker-assisted selection of QTL alleles in such regions are no different from those for QTL in high recombination regions, since the issue is recombination, not physical distance.

The world barley community, particularly in Europe and North America, has been very active in QTL mapping. Of the 757 QTL, the greatest number of reports – 389 – is for agronomic traits, followed by 180 for quality traits (Table 10.3). However, despite the abundance of reports, the wealth of genetic diversity in barley has barely been characterized. Germplasm from important barley production areas of the world, including Africa, Asia, the former USSR, and the Middle East, is underrepresented. Despite the vigorous barley genomics research effort in Australia, there is only one report of these studies (Jeffries et al., 1999). The breadth of the Australian mapping and QTL effort is, however, apparent in the summary posted on GrainGenes (Langridge et al., 1996). Even in the case of the best-characterized germplasm groups, there are only preliminary data that allow for a comprehensive characterization of allelic diversity in any particular set of accessions.

**Table 10.2.** Summary of QTL reports in barley, sorted by chromosome and phenotype. Phenotype classifications correspond to those used by Hayes et al. (2000).

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Number of QTL</th>
<th>Abiotic stress resistance</th>
<th>Agronomic traits</th>
<th>Biotic stress resistance</th>
<th>Quality</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H</td>
<td>77</td>
<td>6</td>
<td>35</td>
<td>9</td>
<td>24</td>
<td>1</td>
<td>113</td>
</tr>
<tr>
<td>2H</td>
<td>145</td>
<td>8</td>
<td>81</td>
<td>16</td>
<td>35</td>
<td>6</td>
<td>113</td>
</tr>
<tr>
<td>3H</td>
<td>113</td>
<td>3</td>
<td>70</td>
<td>17</td>
<td>19</td>
<td>4</td>
<td>75</td>
</tr>
<tr>
<td>4H</td>
<td>97</td>
<td>8</td>
<td>52</td>
<td>15</td>
<td>21</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>5H</td>
<td>135</td>
<td>14</td>
<td>56</td>
<td>14</td>
<td>47</td>
<td>4</td>
<td>85</td>
</tr>
<tr>
<td>6H</td>
<td>75</td>
<td>15</td>
<td>36</td>
<td>13</td>
<td>10</td>
<td>1</td>
<td>59</td>
</tr>
<tr>
<td>7H</td>
<td>115</td>
<td>12</td>
<td>59</td>
<td>19</td>
<td>24</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td>757</td>
<td>67</td>
<td>389</td>
<td>103</td>
<td>180</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>
Applications of QTL analyses

Information about the QTL that influence economically important traits is useful, necessary, and valuable. QTL need to be assigned to regions of the genome – either as a platform for map-based cloning or as a way of assigning functional roles to sequences. Genetic and physical map coordinates for the determinants of phenotypic variation will be essential pieces of information for characterizing and using genetic diversity. The QTL summary we have generated is based on a limited sampling of germplasm, but it is a useful model for addressing two of the key questions in the conservation and use of barley germplasm. “How many genes determine a phenotype?” and “How much allelic diversity is there at these loci?” Answers to these questions will allow conservators to develop representative, non-redundant collections and they will allow plant breeders to make informed decisions regarding the accumulation of favorable alleles in single genotypes. In the following section we have highlighted some of the ways in which QTL research can be of assistance in answering the two fundamental questions we have posed, and we have drawn on the QTL summary to illustrate these applications.

Table 10.3. Summary of barley QTL reports. See also Hayes et al. (2000) for criteria employed in compiling this summary.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Number of phenotypes measured</th>
<th>Number of QTL</th>
<th>Number of populations</th>
<th>Number of citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic stress resistance</td>
<td>26</td>
<td>67</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Agronomic traits</td>
<td>58</td>
<td>389</td>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Biotic stress resistance</td>
<td>15</td>
<td>103</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Quality traits</td>
<td>27</td>
<td>180</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>18</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

1The number of QTL per trait is the number of unique QTL per population. Please refer to Hayes et al. (2000) for QTL for the same trait mapping to the same bin in different populations. 2The number of populations is unique for each trait, but multiple traits are often measured on the same reference population. 3The number of citations per trait is unique, but multiple traits are often described in the same citation.

Catalogs of mapped loci for economically and evolutionarily important phenotypes

A goal of germplasm collection programs is to systematically catalog allelic diversity at key loci. To date, this cataloging has focused, of necessity, on simply scorable morphological descriptors and molecular markers. However, these indicators are often of limited biological utility. What is needed is a characterization of allelic diversity at loci that determine phenotypes showing complex inheritance, and QTL analysis is a first step in this direction. An example of a powerful and immediate application of this is developing catalogs of allelic diversity at genes that confer resistance to biotic stresses. A wealth of theoretical and empirical studies demonstrate that, from the standpoint of probable durability, quantitative resistance may be more desirable than qualitative resistance (Vanderplank, 1978; Johnson, 1981; Parlevliet, 1983). QTL mapping allows for an indirect allelism test via the classification of different germplasm accessions showing the same phenotype. This “QTL allelism” data can be used as a basis for constructing resistance gene pyramids or deploying resistance genes. This strategy has been used to catalog genes that confer quantitative resistance to barley stripe rust (incited by Puccinia striformis fsp. hordei) (Chen et al., 1994; Hayes et al., 1996; Toojinda et al., 2000) and to pyramid quantitative resistance alleles in single genotypes (Castro et al., 2000). QTL conferring resistance to six of the
most important foliar pathogens of barley are reported on all seven chromosomes and in bins corresponding to low, middle, and high recombination regions (Figure 10.1). The proximity of these disease resistance QTL to QTL for agronomic and malting quality traits can be used as a guide for future selection studies, considering the potential for linkage drag. The relationship between physical and linkage map distances can indicate QTL that would be good candidates for finer structure mapping and isolation.

**Understanding correlated responses to selection**

QTL analysis can be used to determine, at a gross level, if trait associations are due to linkage or pleiotropy. In the case of linkage, repulsion phase linkages can be broken via marker-assisted selection, or the QTL information can be used to develop populations that will generate the desired recombinant phenotypes for selection. In the case of coincident QTL, breeding objectives may need to be re-defined, pending a finer dissection of the genetic structure of the region. As discussed in the previous section, the genome-wide distribution of disease resistance QTL, and their associations with agronomic and malting quality QTL can assist in defining breeding objectives. As demonstrated by Zhu et al. (1999), plant architecture, phenology, and morphology have important implications for breeding for Fusarium head blight (FHB) resistance. Finer structure analysis will be required to determine if, in the case of FHB resistance, function follows form (pleiotropy) or if coincident QTL are due to tight linkages. The assignment of QTL to bins that correspond to the degree of recombination on the physical map will assist in prioritizing targets for finer structure mapping and gene isolation. For example, the FHB resistance QTL on chromosome 2H were reported in populations that were derived from crosses between six-rowed genotypes (‘Chevron’ /M64) and between two-rowed genotypes (‘Gobernadora’/CMB). The bin at which QTL are coincident in the two populations contains the vrs1 locus. In progeny of crosses between two-rowed and six-rowed genotypes, the principal FHB rust resistance QTL maps to this region. This is commonly assumed to be a pleiotropic effect of inflorescence morphology (reviewed by Zhu et al., 1999). However, inflorescence type is not segregating in these populations, which suggests that there is potential for recombination between the genes that determine inflorescence type and FHB resistance. The bin to which the FHB resistance QTL and the vrs1 locus map lies at the juncture of middle to high recombination regions, and is thus a reasonable target for genetic dissection.

**Characterization of blocks of the genome that are necessary for essential phenotypes**

Certain complex phenotypes – e.g. quality profiles, resistance to a spectrum of stresses – may be requisites for barley production in some environments. If these regions can be defined via QTL analysis, better efficiencies can be achieved in breeding programs by using a high throughput genotypic screening procedure to identify progeny carrying the target blocks of alleles. Phenotypic and/or genotypic selection can then be carried out for genes that reside outside the target blocks. This strategy has been proposed for malting quality in North American barley germplasm, based on malting quality QTL alleles that are contributed by the reference variety ‘Morex’ in multiple mapping populations (Marquez-Cedillo et al., 2000). The regions which are targeted in the quality footprint are apparent in Figure 10.1. The two regions with the most consistent effects on malting quality are the large blocks on chromosomes 7H and 5H that span the centromeric regions. QTL for multiple malting quality traits map to each of these regions.
Assessment of alternative procedures for measuring the same phenotype

Many complex phenotypes are difficult to improve through breeding because phenotyping is laborious or expensive. When new procedures are developed for measuring a given phenotype, the commonality of QTL detected by the new and the standard procedure can be used as a measure of the correspondence of the two assays. For example, Iyamabo and Hayes (1995) used the consistency of QTL detection as a criterion for comparing grain yield in hill plots and row plots and concluded that the former were useful for detecting large-effect QTL. This has important implications for large-scale germplasm assessment and screening, where the costs of full yield plot assessments are prohibitive. Experiments are underway to compare malting quality QTL that are detected by Near Infrared Spectroscopy (NIR) and conventional wet chemistry analyses, and the summary of observed QTL will be useful in establishing relationships between the two approaches. The efficiencies of NIR would make it an attractive tool for characterizing extensive germplasm arrays for phenotypic diversity in malting quality parameters, and the genetic determinants of this diversity can be assigned to map positions with QTL tools.

Introgression of exotic and/or ancestral germplasm

Exotic germplasm can be a source of alternative alleles at known loci or of entirely new genes, in cases where there is no allelic variation at these loci in cultivated germplasm. Tanksley and Nelson (1996) stimulated widespread interest in discovering and using favorable QTL alleles in phenotypically unattractive tomato germplasm, and this research has borne fruit with the recent cloning of a fruit size QTL (Frary et al., 2000). In barley, there are encouraging preliminary data on the systematic introgression of favorable alleles from ssp. spontaneum into cultivated barley (Matus et al., 2000). The QTL summary does not yet include data from such introgression projects. When QTL are mapped in these studies, a priori information about other loci that are found in the introgression region will be invaluable for minimizing linkage drag and establishing allelism.

Conclusions and Outlook

Despite evidence that there is diversity for traits of economic importance in barley, our understanding of allelic architecture at the many loci determining agronomic performance and malting quality is incomplete. It does not yet provide a basis for systematic classification and utilization of barley germplasm resources in breeding programs.

The focus of future QTL studies could be to thoroughly characterize multiple phenotypes and to develop dynamic profiles of QTL expression in different tissues and at different points in crop development. Most QTL studies have been based on single or end-point measures of a phenotype. To obtain a more complete understanding of the genetic basis of complex traits, it will be necessary to decompose complex traits into their components. For example, QTL analyses of quantitative disease resistance revealed that multiple regions of the genome were associated with symptom expression. Some associations may be the consequence of alleles that condition host pathogen interactions, whereas others may reflect variation in alleles that determine crop architecture, phenology, and morphology. Information that relates QTL to underlying mechanisms of resistance will be invaluable for understanding the genetics of disease resistance and in achieving selection response. The full genetic potential of an organism may not be realized due to temporal and spatial changes in gene expression. Functional genomic tools may be developed to measure these changes in any given tissue or at any point in time. Understanding how developmental expression of alleles determines quantitative traits may reveal
complexities, such as changes in favorable allele phase, that explain some of the unexpected results obtained from selection experiments.

There is a pressing need for more comprehensive sampling and reporting of QTL in barley germplasm. Important barley production areas of the world, with much to offer in terms of genetic diversity, are conspicuously underrepresented in the barley QTL literature. This is, in large part, due to economic constraints that face barley researchers outside of Europe, North America, and Oceania. Every effort should be made to stimulate collaborative and mutually rewarding analysis of the world’s barley genetic resources.

In general, the trend in crop improvement programs has been to rely on ever-narrower germplasm bases to satisfy increasingly stringent yield and quality expectations of producers and consumers. Barley breeders are faced with the challenge of developing cultivars that satisfy multiple end-use requirements for brewing, animal feed, and human consumption. Malting quality standards for barley impose particularly rigid constraints on breeding programs. As a result, the barley industry tends to be conservative about adopting new varieties and exploiting genetic diversity. Nonetheless, impressive progress has been made through the use of narrow germplasm bases, although the genetic mechanisms that account for selection response remain a matter of conjecture (Rasmusson and Phillips, 1997). Cultivars developed from the North American six-row barley germplasm have exceeded the expectations of growers and the malting and brewing industries. However, the extreme susceptibility of these cultivars to fusarium head blight clearly demonstrates the risk of reliance on such a narrow germplasm base. In response to recent devastating epidemics of this disease in the Midwest, breeders have screened exotic germplasm and are incorporating diverse sources of resistance into new cultivars adapted to the region. This is eloquent testimony to the need for systematically characterizing diversity in barley and maintaining barley germplasm resources.

Barley possesses enormous genetic diversity that enables it to grow under a wide range of environmental conditions and to tolerate stresses such as drought and salinity. In addition to its utility in malting and brewing, barley can provide food security in regions where other cereal crops cannot be produced. It can be readily crossed with its wild ancestor (ssp. spontaneum), which may also represent a tremendous reservoir of variation in disease resistance and stress tolerance that remains to be exploited. Although it is difficult to quantify the variation in agronomic and malting quality traits that is present in barley, due to the complex inheritance of these phenotypes, progress is being made in this regard. Efficient strategies for discovering, characterizing, and using alleles in exotic and ancestral germplasm will help to ensure the sustainability, productivity, and profitability of barley production.

References


Genetic diversity for quantitatively inherited agronomic and malting quality traits


Figure 10.1. Quantitative Trait Loci (QTL) determining economically important traits in barley, assigned to bins on the consensus linkage map (Kleinhofs and Graner, 2000) and the physical map of Künzel et al. (2000). The criteria used for QTL assignment to map locations were described by Hayes et al. (2000).
<table>
<thead>
<tr>
<th>QTL Map</th>
<th>Linkage distance</th>
<th>Bin Map</th>
<th>Physical distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agronomic QTL</td>
<td>Phytoalexin</td>
<td>Proteinase inhibitors</td>
<td>Anthocyanin formation</td>
</tr>
<tr>
<td>Malting quality QTL</td>
<td>Beta-amylase</td>
<td>Alpha-amylase</td>
<td>Grain protein</td>
</tr>
<tr>
<td>Disease Resistance QTL</td>
<td>Fusarium HB</td>
<td>Powd. mildew</td>
<td>Stripe rust</td>
</tr>
<tr>
<td></td>
<td>Leaf rust</td>
<td>Net blotch</td>
<td></td>
</tr>
</tbody>
</table>