AN ABSTRACT OF THE THESIS OF

Sarah Windes for the degree of Master of Science in Crop Science presented on June 12, 2020.

Title: Integrating Solutions at the Intersection of Climate Change and Flavor: Breeding for Sustainable Malting Barley and Beer Sensory Characteristics.

Abstract approved:

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Patrick M. Hayes

The following thesis consists of four sections: a general introduction, two manuscripts, a general conclusion, and an overall bibliography. The two manuscripts report on: (1) discussing the prospects of developing a perennial malting barley and potential alternative sustainable crop management practices and (2) further exploring the contributions of barley variety to beer and hot steep flavor characteristics. The first manuscript discusses four possible paths to achieving the conversion from annual to perennial growth habit while maintaining expected levels of malting quality and agronomic performance: direct domestication, wide hybridization, manipulation of the vernalization and photoperiod sensitivity genes, and mapping annual and perennial forms of ryegrass (*Lolium multiflorum* L., and *L. perenne*. L, respectively) as a basis to identify genes conferring perenniality. The second manuscript used two independent sets of barley germplasm to address the contributions of other, different barley genotypes to beer flavor. Pedigree, malt quality, beer quality, sensory attributes, and metabolomic profiles were compared within and between the two sets. Differences in malt hot steep and lager beer sensory that are attributable to barley genotype, as assessed by laboratory research and/or consumer panels, were investigated, along with differences in abundance of metabolomic compounds. The observations within this study lead to the conclusion that the variable metabolites

observed among the two sets of barley germplasms are a direct result of genetic differences that lead to differential responses within the malting and brewing processes

Integrating Solutions at the Intersection of Climate Change and Flavor: Breeding for Sustainable Malting Barley and Beer Sensory Characteristics

by
Sarah Windes

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APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Sarah Windes, Author

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CONTRIBUTION OF AUTHORS

Dr. Patrick M. Hayes initiated, advised and supervised all aspects of the project. He supported and contributed substantially during hypothesis, discussion and revision process of this manuscript.

Chapter 2: Dr. Daniela Carrijo advised and contributed substantial material for the writing and editing of this chapter. In particular, she was essential in addressing agronomic points and revisions. Dr. Colin Curwen-McAdams contributed critical knowledge in theoretical perennial crops and cropping practices.

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DEDICATION

To my family, both biological and chosen.

Chapter 1: General Introduction

Barley (*Hordeum vulgare L.* of the grass family *Poaceae*) is an ancient cereal crop that has advanced alongside human species through the evolution of agronomy, the rise and fall of civilizations and cultures, and advancements in brewing and genetics. It was one of the first domesticated crops at the dawn of agriculture and has played an important role in the transition of humans from wandering hunter-gatherers into stable agrarians (Ullrich, 2011).

Though its origin lies within the Fertile Crescent, today barley is grown worldwide with a total report of over 156 hundred thousand metric tons produced as of January 2020. The European Union produced around 62 thousand metric tons, followed by Russia (20,000) and Canada (10,400). Total production has been steadily declining in the United States over the past few decades until very recently, with 2019 production at around 3.6 thousand metric tons (USDA).

Barley grown in the United States focuses primarily on malting purposes, with a smaller percentage secured for livestock feed, and a very small portion for human consumption (USDA). Currently, an average of 15% of all barley grown worldwide is used for the purposes of making malt, a key ingredient in beer production (FAOSTAT). Though in theory any grain can be malted, barley accounts for the vast majority of the cereals used for fermentation end-use (Ullrich, 2011). Malt is an essential component of beer: it is the perfect substrate for yeast nutrition and contributes essential aromas and flavors (Herb et al., 2017). Therefore, barley is an indirect but essential contributor to the US economy.

The craft brewing sector has played an essential role in stabilizing barley production within the United States. Craft beer accounts for 14% of the beer market and >40% of malt (Brewers Association, 2019). Over 96% of the malting barley, from 2014 through 2018, was contracted and grown in the states of Colorado, Idaho, Minnesota,

Montana, North Dakota, and Wyoming. The majority of this barley is malted in Colorado, Idaho, Minnesota, Montana, Washington, and Wisconsin (AMBA). Strict quality parameters for barley to be used in the malting industry are rigorously observed to ensure high performance within the malthouse and brewhouse (Ullrich, 2011).

As a consequence, growers and potential growers need to consider many different aspects before deciding to plant malting barley. Environmental factors such as regional climate, soil fertility, and hardiness zone as well as suitable infrastructure (grain storage, elevators, and rail accessibility) are key considerations. Additionally, the barley variety itself is vital, with deliberations from growth habit to the list of suggested malting varieties released by the American Malting Barley Association (AMBA). If the strict malting specifications enforced by maltsters and brewers are not met, barley growers may have to sell their crop as feed barley at a deficit (AMBA).

Modern barley breeding programs, such as those found at Oregon State University, rigorously screen for competitive, high yielding, high quality barley varieties in order to meet the demands of growers, maltsters, and brewers. Recently, with the surge of craft brewing pushing the boundaries of the status quo, there has been increasing interest in unique flavors attributable to the genomic contributions of barley. Heritage varieties such as Marris Otter and Golden Promise lend the suggestion that barley variety and distinctive flavors could be interconnected.

Barley Habitat and Production

Barley is a widely adapted species with ample drought, cold, and salt tolerance typically produced in temperate and semiarid subtropical climates and capable of yielding satisfactory harvests in areas unsuitable for many other cereals. However, it is most suited to well-drained loam soils in a moderate rainfall (400-800 mm/year) and moderate temperature climates (15-30°C). Additionally, barley has a low

tolerance of warm, humid conditions that lead to disease-rich environments (Ullrich, 2011).

Currently, around three-quarters of barley produced in the United States is used for malting purposes. As of 2017, two-rowed barley, the primary barley grown for malting purposes, was produced largely in Idaho, Montana, and North Dakota. Central Oregon, east of the Cascade Mountains and an ideal location for growing malting barley, produced an estimated 2.3 thousand bushels (USDA-NASS). Production in this region of the United States is typically grown under high elevation, dryland conditions or irrigation, which is favorable for reaching malt quality (Ullrich, 2011).

In order to achieve malting quality standards, barley grain must have a total protein between 11.0 and 13.5%, have greater than 70% plump kernels, and achieve more than 98% germination (AMBA, https://ambainc.org/amba-publications/guidelines-for-malting-barley-breeders/). These, among other quality parameters, are critical targets growers must attain in order to sell their crop at the premium prices of malting barley.

Barley and Growth Habit

Growth habit generally falls into three categories: winter, facultative or spring. This classification is based on vernalization response, short-day photoperiod response, low temperature tolerance, and seeding-time (von Zitzewitz et al., 2011). Winter types require vernalization, have low temperature tolerance, are often sensitive to short-day photoperiod (<12 h), and are fall planted. Spring type barleys do not require vernalization, are more sensitive to lower temperatures, are not short-day photoperiod sensitive, and are planted in the spring. In between these two growth habit types lies facultative types. Facultative barleys are similar to spring barleys whereas they do not require vernalization. However, like winter barleys, they must be sensitive to short-day photoperiod to prevent an untimely transition from a vegetative to reproductive

phase while the threat of low temperatures is relevant. They can either be planted in winter or spring and have an improved low temperature tolerance over spring habit types.

Although most US barley varieties are of spring growth habit, and their production meets current US market needs, there is increasing interest in fall-planted barley (winter and facultative). This demand is driven by the potential yield advantages of fall-seeded types over spring types (University of Minnesota, 2019), earlier maturation, better use of water resources, and flexibility in planting time afforded by facultative types. Winter and facultative varieties are a priority for the US malting industry, based on the aforementioned potential yield advantage, planting date flexibility, and the prospect of winter and facultative growth habits to assist in meeting the challenges of variabilities in climate.

Malting

Starches and other simple sugars within the barley endosperm are the primary sources of energy utilized by yeasts during fermentation. These carbohydrates account for 75-80% of the grain's dry weight (Briggs, 1998). In order to access this rich source of carbohydrates, raw barley undergoes an advanced process called malting, a kind of forced germination of the barley grains. This includes soaking the grain in water for a period of time (steeping), instigating embryo growth under warm, moist conditions for several days (germination), then gradually drying and curing with forced warm air through the grain bed (kilning) (Jones, 2005).

The chemical changes that occur within the barley kernel during this process of steeping, germination, and drying are complex. Hydrolyzing enzymes, mainly α- and β-amylase, cause cell walls within the endosperm to break down, degrading complex starches and carbohydrates embedded within a matrix of proteins (Shewry and Ullrich, 2014). The proteins contained in this medium become the primary source of reserve amino acids, β-amylase, and debranching enzymes that are vital for the

brewing process. In addition, nitrogenous material such as nucleic acids, phosphates, vitamins, and lipids are liberated and degraded during germination. These are highly important resources for yeast growth during fermentation and can contribute to the flavor profiles of finished beer (Briggs, 1998).

Flavor

Malting, in particular kilning, is the major source of barley's contribution towards beer flavor. Certain malt quality traits are known to impact flavor, most notably through Maillard reactions produced through kilning. Darker malts have significantly more large-molecular-weight substances from Maillard reactions, mainly melanoidins. Small-molecular-weight substances formed through Maillard reactions are highly important to flavor and aroma characteristics and are found in both dark and lighter malts. The small-molecular-weight substances primarily include acids, alcohols, aldehydes, ketones, esters, heterocyclic substances, as well as polyphenols. These substances may be further utilized by yeasts, contributing to the formation of additional flavor and aroma compounds (Briggs, 1998). For example, post-fermentation surplus saccharides lend sweet characteristics, while high amounts of 2,3-butanedione and diacetyl come through as butterscotch or buttered popcorn flavors. Maillard reaction specific flavors tend to be influenced by compounds such as maltoxazine, maltol, isomaltol, and ethyl maltol. These are described as bready, caramel, and cotton candy. These compounds, produced by Maillard reactions, are influenced by interactions between amino acids and saccharides derived from germinated barley. Thus, due to the fact that the type of Maillard reaction product is affected by precursor amino acids and saccharides, it is hypothesized that genetics and subsequent varying metabolite composition in barley influences flavor (Bettenhausen, 2018, 2020).

Metabolomics

Varying metabolite (i.e., small molecule) content within barley malt is believed to contribute unique flavor profiles within beer that could theoretically be traced back to

the germplasm of the barley itself. There are several studies suggesting correlations between barley genetics and malt amines, amino acids, alkaloids, phenolics, and lipids. All of these contribute to malt and/or beer sensory characteristics. Though many of the malt metabolite compounds are lost through boiling wort during the brewing process, there are several that are found within the finished beer such as maltols and hordatines. Additionally, flavor stability components can be traced back to malt metabolites. Malt free amino nitrogen (FAN), key for yeast nutrition, can affect flavor stability through variability in how yeasts utilize amino acids in different metabolic pathways. Malt lipids, lypoxigenase enzymes, and lysine, all undesirable off-flavors, are additional components that can be attributed to malt metabolite chemistry. In contrast, desirable flavors such as roasted, nutty, and caramel, can be credited to pyrazines, pyrroles, and furans, small molecules derived from Maillard reactions and affected by the composition of precursor amino acids and saccharides. These Maillard reaction products also influence stability through oxidation potential of finished beer and could be especially influential during storage (Bettenhausen, 2018, 2020).

With all of these in consideration, it is no wonder that barley plays an integral part in the making of beer from where it is grown to flavor stability in the finished product. As barley breeders continually strive to supply superior varieties to growers that meet malting and brewing standards, an important consideration must be acknowledged: what changes in the genetic makeup of a barley plant will affect the flavor of a beer produced with it? Will breeding to combat increasing climate variability that brings instability to traditional growing regions as well as opens up non-traditional areas for barley cultivation come with consequences that is tasted in our beer? These are questions to be considered as breeders are tasked with an ever-expanding variation of agronomic qualities to be introduced into a variety. If, for example, barley was to be bred for a perennial growth habit, instead of annual, would that be feasible and produce desirable results? If so, how would that change be reflected in the flavor and aromatic characteristics of the beer?

This thesis explores two ideas 1) whether breeding for perennial growth habit in barley is viable and feasible and 2) building on the foundation of the contributions of barley genotype towards beer flavor as established by Herb et al. (2017) and Bettenhausen et al. (2018, 2020). The first chapter investigates viable possibilities towards developing a perennial malting barley through literature review and practical considerations. Additional deliberation is put towards the discussion of whether or not a perennial malting barley would be practical with the current infrastructure centered around an annual plant. The second chapter contributes to the growing literature surrounding how barley genotype affects sensory attributes and metabolite profiles of a finished lager beer. Two different groups of barley varieties were used i) five established commercial winter varieties grown in Condon, Oregon and ii) four spring barley types, Full Pint and three progeny selections, grown in Madras, Oregon. Agronomic data, barley quality, malting quality, beer analytical data, sensory characteristics, and beer volatile metabolite profiles were all generated for both groups and compared within each group. This was accomplished in order to test the hypothesis that variations within the barley genome can be detected with sensory testing, quantified through volatile profiles, and traced back to their phenotypic origins.

Chapter 2: Improving the Sustainability of Malting Barley Production: Prospects for Perennial and Annual Growth Habit Varieties

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Abstract

Malted grains—principally barley (*Hordeum vulgare* L.) —are essential raw materials for brewing. There is an increasing demand for more sustainable crop production practices. At the same time, climate change makes it imperative to identify new production zones, systems, and crops. These demands and imperatives have stimulated interest in converting staple cereal crops, including barley, from annual to perennial growth habit. Most effort has been devoted to wheat (*Triticum aestivum* L.), and the most progress made in domesticating a perennial relative of wheat. These results prompt the questions: what are the prospects for developing perennial malting barley and is developing perennial malting barley the most direct path to sustainability? Malting barley is a challenge for growth habit conversion due to stringent quality parameters and the extensive infrastructure required for production, processing, and distribution. We discuss four possible paths to achieving the conversion from annual to perennial growth habit while maintaining expected levels of malting quality and agronomic performance: direct domestication, wide hybridization, manipulation of the vernalization and photoperiod sensitivity genes, and mapping annual and perennial forms of ryegrass (*Lolium multiflorum* L., and *L. perenne* L., respectively) as a basis to identify genes conferring perenniality. We conclude that any one of these approaches would require significant, long-term investment. Until such investment is forthcoming, we conclude that there are more cost-effective, short-term solutions—notably no-till, multiple cropping, and increased emphasis on fall-seeded barley—that could enhance the sustainability and viability of annual malting barley production.

Key Words

Barley, growth habit, malting, production

Introduction

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is a crop of worldwide importance, ranking fourth amongst the cereals (FAOSTAT, 2019). In the United States, >800,000 ha of barley was grown in 2017 (USDA-NASS 2017), with an estimated total economic impact of over US$1 billion yr^{-1}. This impact is due to the added value generated by malting and brewing. Although exact figures are not available due to the confidential and proprietary nature of the malting business, in the United States, most of the barley grown is seed of malting varieties under contracts with end users (S. Heisel, personal communication, 2019). Malt is an essential component of beer; it is the perfect substrate for yeast nutrition and contributes essential aromas and flavors (Herb et al., 2017). Therefore, barley is an indirect but essential contributor to the US economy.

The craft brewing sector in the United States has played an essential role in stabilizing barley production. Craft beer accounts for 14% of the beer market and >40% of malt (Brewers Association, 2019). From 2014 through 2018, over 96% of the malting barley was contracted in the states of Colorado, Idaho, Minnesota, Montana, North Dakota, and Wyoming. The vast majority of this barley is malted in Colorado, Idaho, Minnesota, Montana, Washington, and Wisconsin (American Malting Barley Association, 2019). Therefore, there is currently a geographically narrow and defined supply chain between farm and brewery. There is increasing interest in modifying this supply chain in many states, with locally grown barley and malting. There are currently over 100 craft malting facilities in production in 28 US states and Canada (C. Swersey, personal communication, 2019). The barley varieties

that meet the requirements of brewers of all scales follow a path of rigorous testing prior to final recommendation by the American Malting Barley Association (AMBA). Currently, 20 of the 25 two-row varieties recommended by AMBA are of spring growth habit (American Malting Barley Association, 2019).

Growth habit in barley can be defined as winter, facultative, or spring. The classification is based on vernalization response, short-day photoperiod response, low temperature tolerance, and seeding-time (von Zitzewitz et al., 2011). Winter types require vernalization, are often sensitive to short-day (<12 h) photoperiod, are planted in the fall, and are tolerant of low temperatures. At the other extreme, spring types do not require vernalization, short-day sensitivity is not relevant, they are planted in the spring, and they are sensitive to low temperatures. Facultative types do not require vernalization, need to be sensitive to short-day photoperiod to delay the vegetative to reproductive transition until risk of low temperature injury has passed, can be planted in the fall or spring, and are low-temperature tolerant. Facultative growth habit is advantageous from a breeding standpoint since more cycles of breeding can be performed per year than with winter types. Breeding for facultative growth habit is being facilitated by the availability of markers in key vernalization and photoperiod genes (Cuesta-Marcos et al., 2015) and information on low-temperature tolerance quantitative trait loci (QTLs) (von Zitzewitz et al., 2011).

Five of the barley varieties on the AMBA recommended list for two-row barley are of winter growth habit. The first facultative type (DH130910) was submitted to the AMBA testing program in 2018 and passed the first year of testing. Although the potential advantages of facultative barley in the United States are theoretical, there has been a long-term, consistent effort in Australia to develop facultative varieties of wheat (*Triticum aestivum* L.) and barley for multiple environments (Penrose et al., 1996; Eagles et al., 2009; Sprague et al., 2018). All Australian malting barleys are spring types. In Europe, winter malting barley is of commercial importance, accounting for 15% of malting barley purchases in the United Kingdom and 50% in

France (H. Maubach, personal communication, 2019). No facultative malting barley varieties have been released in Europe.

Although most US barley varieties are of spring growth habit, and their production meets current US market needs, there is increasing interest in fall-planted barley (winter and facultative types). This demand is driven by the potential yield advantages of fall-seeded types over spring types (University of Minnesota, 2019), earlier maturity, better use of water resources, and flexibility in planting time afforded by facultative types. Winter and facultative varieties are a priority for the US malting industry, based on the aforementioned potential yield advantage, the actual planting date flexibility, and the prospect of winter and facultative growth habits to assist in meeting the challenges of climate change. Climate change poses challenges for plant breeders, farmers, processors and consumers and—with foresight—was identified as a research priority by the Tri-Societies (ASA, CSSA, and SSSA, 2011). Building on the recognition that alternative growth habits may be useful for malting barley production, we asked "what are the prospects for extending growth habit changes in barley to include perennial growth habit?"

The conversion of cereal crops from annual to perennial growth habit has been proposed as a strategy to mitigate negative impacts of agricultural production on the environment. Compared with annual crops, perennial crops store more water and C in the soil and are able to utilize nutrients more efficiently (Cox et al., 2006). Reasons for this include, but are not limited to, : (i) the elimination or reduction of soil tillage, which translates into less soil organic matter decomposition, less fossil fuel consumption, and lower greenhouse gas emissions associated with the operation of tillage; (ii) the maintenance of a near-permanent soil cover reduces the potential for soil erosion; and (iii) perennials' deeper root systems provide greater ability to scavenge water and nutrients from the soil, thus minimizing percolation and leaching losses (Pimentel et al., 2012). Given the environmental benefits of perennial systems, many breeding programs have made efforts to develop perennial grains, focusing

especially on the staple cereals wheat and rice (*Oryza sativa* L.) (Crews and Cattani, 2018). The development of perennial malting barley has not been studied, or implemented, as extensively. In this Scientific Perspective, we present possible routes that can be explored in the development of perennial malting barley and the challenges of this quest, and, finally, we discuss improving the sustainability of malting barley and beer production using existing annual barley cultivars.

Possible Paths Forward to Developing Perennial Malting Barley

The genetic basis of perennial growth habit in annual growth habit crop plants is still far from unraveled (Heidel et al., 2016; Kiefer et al., 2017). To date, efforts to develop perennial crops have relied heavily on the use of perennial relative species either for direct domestication or in wide hybridization (Crews and Cattani, 2018). The first approach involves subjecting the perennial relative to several cycles of selection for improved agronomic traits: an example is the ongoing domestication of *Silphium integrifolium* Michx., a perennial relative of sunflower (Vilela et al., 2018). The second approach involves crossing the cultivated annual species with its perennial relative in an attempt to introgress genes for perennial habit into a cultivar with good agronomic performance. This approach was employed in rice and wheat, using their perennial relatives *Oryza longistaminata* A. Chev. & Roehr. (Zhang et al., 2017) and *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey (Gazza et al., 2016), respectively. There are parallels with, and possible parallels to, both direct domestication and wide hybridization in *Hordeum.* Alternatively, two other approaches would be to manipulate vernalization and photoperiod sensitivity genes in annual barley, and to map perennial and annual forms of ryegrass (*Lolium multiflorum* L., and *L. perenne*. L, respectively) as a basis to identify genes conferring perenniality.

Direct Domestication

Within the genus *Hordeum*, there are an estimated 24 perennial species (Blattner, 2009). *Hordeum bulbosum* L. is the closest perennial relative of annual barley and has

been identified as a promising genetic resource for the development of perennial barley (Westerbergh et al., 2018). Further, this species is relatively well characterized due to its usefulness for making doubled haploids of cultivated barley (Kasha and Sadasivaiah, 1971).

When employing the strategy of domestication of barley perennial relatives, it is important to consider the adaptability of the wild species to the regions where barley is currently grown. This is especially important for malting barley, because the many unique processes involved in the supply chain (grain production, grain transport, malting, malt transport, and brewing) suggest that the production areas will remain the same. Using the United States as an example, the principal barley production states are Idaho, Montana, and North Dakota. Most varieties grown in these states are spring growth habit types, due to the risk of low-temperature injury. However, as noted in the introduction, there is increasing interest in fall-planted varieties due to potentially high yields and input use efficiencies (see below).

In a study evaluating a set of 17 wild perennial *Hordeum* species in a cold climate, six species, [*H. brevisubulatum* (Trin.) Link, *H. bulbosum* L., *H. fuegianum* Bothmer, N. Jacobsen & R.B. JØrg, *H. jubatum* L., *H. lechleri* (Steud.) Schenck, and *H. secalinum* Schreb.) were identified as having good winter survival and productivity (Westerbergh et al., 2018). *Hordeum bulbosum* L. has been genetically mapped and has the advantage of having high levels of conserved synteny with annual barley (Wendler et al., 2017). Thus, selection within perennial *H. bulbosum* for traits important in contemporary malting barley would benefit from available information on key genes related to domestication (e.g., shattering resistance, seed size, and synchronous flowering; Doebley et al., 2006). This is analogous to what is being done with wild relatives of wheat before hybridization (DeHaan et al., 2014).

The domestication of *Thinopyrum intermedium*, a perennial wheat-like grass, which is currently being developed by the Land Institute under the marketing name Kernza, is a useful example for the domestication path to perennial barley. *Thinopyrum*

intermedium is the focus of breeding and genomics based on its potential to provide ecosystem services and meet the end uses of annual wheat (Jungers et al., 2019; Ryan et al., 2018). There is preliminary acceptance for the crop in malting and brewing, despite processing challenges. Unmalted Kernza is currently being used as an adjunct (at a 15% inclusion rate) in Long Root Ale, brewed by Hopworks Urban Brewery in collaboration with Patagonia Provisions (Hopworks Urban Brewery, 2016). The precedent established by the *Th. intermedium* domestication effort, which involves a network of institutions involved in breeding, management, processing, and utilization, is impressive. Responses to selection of up to 13% per cycle for important traits (DeHaan et al., 2014) are reported. If such a network could be established for domestication of *H. bulbosum*, and such responses to selection achieved, the path to simultaneous perennial growth habit and acceptable malting quality could be successful.

Wide Hybridization

Wide hybridization requires identifying perennial species or genera that can produce viable offspring with barley by cross-pollination (or laboratory techniques) (Feuillet et al., 2008). Once they have been identified, there are two main routes a breeding program can attempt: introgression of small amounts of genetic information related to perennial habit or stabilizing the interspecific or intergenic hybrid at a new ploidy that incorporates genomes from each parent.

The first strategy requires homology between the chromosomes of the perennial donor species with barley to allow recombination (Seberg and Petersen, 1998). Then, a program of backcrossing, or recurrent selection for population development, can be developed based on the overall objectives of the program. This situation also allows for the development of mapping populations to identify markers or QTLs related to perennial habit. These tools are especially important in breeding for perennial habit conversion because the unknown number of genes and long testing cycle are significant costs to the program.

If, as in the case of wheat, there are no perennial relatives that readily recombine, then the option is to stabilize hybrids that contain an additional genome from the relative. This approach has been shown to be possible in practice with ×*Triticosecale* (Stace, 1987) and ×*Tritipyrum* (Curwen-McAdams et al., 2017a). Because a genome contains a large amount of genetic information, and not just what is needed for perennation, the end result will likely be quite different from either parent (Renny-Byfield and Wendel, 2014), and the only way to see if a combination might work is through experimentation. The result of this path is more likely an entirely new crop type rather than a slight modification of the desirable qualities of existing annual growth habit cultivars of barley.

Compared with the other major cereals, few traits have been brought into cultivated barley from wild relatives (Hajjar and Hodgkin, 2007). Hybridization between *H. bulbosum* and annual barley is, as noted, possible, and the development of introgression lines derived from a *H. bulbosum* × *H. vulgare* hybrid, followed by sequential backcrossing with *H. vulgare*, are reported (Johnston et al., 2009). These lines have been explored mainly for the introgression of traits determined by a single or few genes, such as disease resistance (Pickering et al., 1995; Yu et al., 2018). This is, at least in part, due to the low recombination frequencies obtained in the interspecific cross (Wendler et al., 2015). Perennial growth habit is the result of numerous responses timed to react to environmental cues, controlled by multiple structural and regulatory genes (Thomas et al., 2000); thus, successful transfer of all of them in a single cross is unlikely. In addition, verifying perenniality is a major task.

Despite it being possible, with the use of molecular techniques, to locate and confirm the presence of an introgression in the annual barley genome (Pickering et al., 2000), it is not possible to know that the gene(s) introgressed are the ones conferring perenniality, given that these are yet unknown. From research on wheat, it was found

that the addition of one of the chromosomes from *Thinopyrum elongatum* was enough to encode a polycarpic habit, but not enough for long-lived perennation (Lammer et al., 2004). Building the baseline knowledge of genome homology through experimental crossing seems a good place to begin.

Manipulating Vernalization and Photoperiod Sensitivity Genes in Annual Barley

Ofir and Koller (1974) hypothesized that the vernalization and photoperiod responses of perennial *H. bulbosum* are involved in triggering the transitions from vegetative to reproductive and back to vegetative growth stages. In other words, vernalization and/or photoperiod sensitivity could have a role in the perennial habit of *H. bulbosum*. Subsequently, the vernalization response pathway has been extensively studied in the model plant, *Arabidopsis thaliana* (L.) Heynh. (Kim and Sung, 2014, and citations therein). The message of this body of work is that vernalization in *Arabidopis* is an epigenetic phenomenon, with a dependable environmental trigger (temperature). Oliver et al. (2009) extended this work to barley and demonstrated that vernalization responses in cereals are associated with changes in histone methylation. In annual growth habit barley, this means that the transition from vegetative to reproductive growth is repressed until sufficient exogenous low temperature signals are received. Once these are received, the *VRN-H1* gene is in an active chromatin state and the plant transitions to the reproductive growth stage. Reproductive growth culminates in seed formation and a return to methylation of *VRN-H1* in the seed, and later, in the new plant that arises from this seed. By extension, uncoupling the methylation of *VRN-H1* from the signals for plant senescence (reviewed by Thomas, 2013) and coupling them with signals for a return to vegetative growth could be a first step to manipulating epigenetic responses and ensuring an extended vegetative (perennial) state. Epigenetic control of photoperiod sensitivity in the Triticeae has not been demonstrated. In rice, however, Ding et al. (2012) demonstrated the role in methylation in photoperiod-mediated male sterility in rice (Ding et al., 2012; Song and Cao, 2017).

This potential key role for vernalization and photoperiod in long-term cyclical growth of a perennial grass that is crossable with barley makes these traits a potential starting point for development of perennial malting barley. This strategy has the advantage of working within the known framework of annual malting barley by manipulating key genes involved in physiological responses to exogenous environmental signals (temperature and/or photoperiod). These cloned and characterized genes include *VRN-H1*, *VRN-H2*, *VRN-H3*, *PPD-H1*, and *PPD-H2* (Cuesta-Marcos et al., 2015). Recent work elucidating regulatory circuits involved in flowering in perennial and annual forms of *Arabidopsis* (Hyun et al., 2019) provides insights that may be useful to inform further work on perennial *Hordeum*.

In-Depth Comparative Analysis of the Cultivated Annual and Perennial Forms of Ryegrass

There is potentially much knowledge to be gained from the distant relationship of barley with ryegrass (*Lolium* spp.) and the close relationship between the cultivated forms of annual and perennial ryegrass (*L. perenne* L. and *L. multiflorum* Lam., respectively). Both forms are used for forage, and the perennial form is also used for turf (Hannaway et al., 1999). The Noble Foundation provides an overview of the species, with particular reference to the "double-edged sword" of annual ryegrass as a crop and a weed (Glidewell, 2010). Although described as separate species, the two growth habits are interfertile and in a population derived from crossing perennial and annual forms, such as that reported by Warnke et al. (2004), the genes responsible for growth habit could potentially be mapped and targeted for map-based cloning. Mapping would involve implementing phenotypic assays for degree of perenniality. The phenotyping protocols developed in the course of perennial wheat would be an excellent starting point (Armstrong and Stevenson, 1947). With phenotypic data, the statistical significance of marker traits relationships identifying QTLs can be established (van Ooijen, 1999). A starting point for identifying candidate genes for these QTLs would be the draft genome sequence of *Lolium* (Byrne et al., 2015). Capitalizing on the syntenic relationships of *Lolium* with the Triticeae, the search for candidate genes could be further narrowed down using crop genome sequences (e.g.,

wheat [Alaux et al., 2018] and barley [Mascher et al., 2017]), and model genome sequences (e.g., *Brachipodium* [IBI, 2010]). The identification of candidate genes could then be followed by a thorough process of validation using gene editing (Gasparis et al., 2018) or transgenic (Hisano and Sato, 2016) strategies appropriate for barley (Gasparis et al., 2018, and Hisano and Sato, 2016, respectively). Assuming that this process was successful in terms of achieving perenniality in barley suitable to the industry for malting and brewing purposes, challenges to commercializing the resulting variety (or varieties) would remain in terms of the financial costs of associated intellectual property (Schinkel and Schillberg, 2016) and regulatory approval of transgenics (FAS, 2011). As of March 2019, the US Food and Drug Administration had not yet announced the status of CRISPR (clustered regularly interspaced short palindromic repeats)-derived plant varieties (FDA, 2019).

Challenges of Creating a Perennial Analog of an Annual Crop

Despite perennial malting barley being an exciting proposition, no annual grain crop to date has been converted to a successful perennial analog (Curwen-McAdams and Jones, 2017b). Converting an annual plant to perennial growth habit faces a multitude of theoretical and practical obstacles. Belowground allocation of resources, mainly in the form of starches, is vital for perennial plants to survive adverse conditions in a vegetative state and to resume reproductive growth when conditions are optimal. A consequence of this is, within a genus, perennial species allocate proportionally more resources belowground than to seed production, compared to annual species (Vico et al., 2016). Diverting C from a one-time allocation to seed production to subterranean resources leads to lower grain yields in perennials than in annuals (Jaikumar et al., 2012), with a few exceptions reported (e.g., Steinwand et al., 2013). Using perennial grain development in wheat as an example, in trials of advanced intergeneric lines, none have been long lived or high yielding enough to compete with annual wheat (Hayes et al., 2012).

In addition to resource allocation, another consideration in converting an annual plant to perennial growth habit relates to diseases. With annual crops, there is typically a seasonal break between successive plantings of the same crop, and this break often includes a rotation crop. With perennial crops, there is no break—it is tantamount to continuous production of the same crop (Curwen-McAdams and Jones, 2017). This continuity can lead to a buildup of multiple diseases (bacterial, fungal, and viral) and a consequent decline in crop productivity (Bailey et al., 2001). This can be particularly threatening if the perennial crop consists of a single genotype. There are ample historical examples of genetic vulnerability resulting from extensive monocultures over time (e.g., the Irish potato [*Solanum tuberosum* L.] famine, southern corn leaf blight [*Bipolaris maydis* (Y. Nisik. & C. Miyake) Shoemaker], sigatoka [*Mycosphaerella musicola* J.L. Mulder in J.L. Mulder & R.H. Stover] in banana (*Musa acuminata* Colla)). A solution to homogeneity is to build genetic heterogeneity (e.g., by developing phenotypically uniform populations composed of multiple genotypes), which requires considerable extra breeding effort to meet processing quality standards. In malting barley in particular, uniformity of processing during malting and brewing is essential (Ullrich, 2011).

Another key constraint relates to genetics. As already noted, the genetic basis of perennial vs. annual growth habit is not known. Although there are many wild relatives of annual grain crops, the homology of the related genomes, along with specific genetic incompatibility mechanisms, will determine the ability to make crosses and incorporate genetic information through recombination or genomic addition (Curwen-McAdams and Jones, 2017). Despite years of crossing and genetic analysis, there are no mapping populations of wheat or barley clearly segregating for perennial vs. annual growth habit. The international barley community has made great strides in stocking the-omics tool kit (Beier et al., 2017). However, technologies such as transgenics and gene editing have no applicability when the target genes are not known.

Alternatives to Developing Perennial Malting Barley

Considering the challenges regarding the development and deployment of perennial malting barley, another option is to improve the sustainability of existing annual barley systems by focusing on alternative annual growth habits (e.g., winter and facultative) and changing management practices. Two major environmental benefits of converting a crop from annual to perennial growth habit are the elimination of soil tillage and the maintenance of a near-permanent soil cover (Pimentel et al., 2012), although it is important to note that even perennial systems are part of a crop rotation and thus will be subject to soil tillage and soil exposure within some timeframe. These benefits can be similarly achieved in annual barley with the adoption of no-till practices and double cropping, respectively.

Results of a global meta-analysis indicated that barley, among other cereals, yields the same under no-till and conventional tillage systems (except when irrigated in a humid environment) (Pittelkow et al., 2015). This study also showed that there was a general trend among many agricultural crops in which yields decreased in the first years after no-till adoption but eventually increased to match yields obtained in conventional tillage systems. The authors attributed that to, among other factors, a lag period in which adjustments in management, labor, and equipment were made before a full transition from conventional tillage to no-till system was established. This demonstrates the importance of acknowledging that, when transitioning from a conventional to a no-till system, other changes in management will likely be required. For example, shifts in weed populations (Chauhan et al., 2006; O'Donovan and McAndrew, 2000) and N cycling (Nyborg et al., 1995) are common, thus requiring different weed and N management practices.

Double cropping can serve to increase the span of soil cover throughout the year (Watson et al., 2014). This strategy has been explored in spring barley followed by a summer crop in tropical environments (Alvarez-Prado et al., 2013; Camper et al., 1972). However, in temperate environments, which represent most of the barley

growing regions, the growing season is often too short for such a system. In temperate regions, double cropping can be achieved with fall-seeded (winter or facultative) barley, followed by a summer annual of short duration (i.e., soybean). Fall-planted barley is normally harvested 1 to 2 weeks before fall-planted (usually winter growth habit) wheat, which provides the additional time many regions may need for successful stand establishment of soybean as the second crop. Although double cropping with a fall-seeded barley is ideal from the standpoints of (i) providing soil coverage for a maximum amount of time, (ii) introducing genetic diversity into the farming system, and (iii) adding organic matter and fertility into the system, fall-seeded barley alone has the environmental benefit of a longer ground cover span and of utilizing water more efficiently than spring-seeded barley (Kaspar et al., 2001).

Conclusion

Increasing the sustainability of producing grains for malting—in the face of climate change—is a necessary and worthy goal. By providing ecosystem services and acting as a buffer against climate change, perennial malting barley is an attractive proposition. However, despite the wealth of genetics tools available, it would take considerable effort and time to develop a malting barley cultivar with perennial growth habit. Should the option be considered with the investment in time and resources, starting points could include domestication of, or hybridization with, existing perennial *Hordeum* species, manipulation of growth habit genes in annual barley, and/or comparative genomic analyses of *Lolium* and *Hordeum*. Perhaps the simplest path is providing enhanced ecosystem services with annual malting barley. Possibilities include the adoption of no-till and/or double cropping (e.g., fall-planted barley–soybean [*Glycine max* (L.) Merr.]) to eliminate soil tillage and maintain near-continuous ground cover, respectively. Facultative varieties could be of particular use, given the options they provide for fall and spring planting. These management practices could be adopted using current and upcoming annual barley varieties with known malting and brewing profiles.

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Chapter 3: Comprehensive analysis of different contemporary barley genotypes enhances and expands the scope of barley contributions to beer flavor

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Abstract

Recent research has demonstrated contributions of barley genotype to beer flavor based on the progeny of a cross between an heirloom and a more contemporary barley variety. To advance this line of research, the current study used two independent sets of barley germplasm to address the contributions of different barley genotypes to beer flavor. Pedigree, quality of malt and beer, and beer metabolomic profiles were compared within and between the two sets. Utilizing both laboratory and consumer panels, differences in sensory attributes of malt hot steeps and lager beers that are attributable to barley genotype were investigated. Results concur with previous studies: the two sets of barley germplasm were found to have distinct but subtle differences in flavor profiles of malt hot steeps and finished lager beers. Distinct metabolomic profiles, attributable to barley genotype, were detected. Further, covariation of metabolomic profiles and sensory attributes were identified in both sensory panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasm are a direct result of genetic differences that lead to differential chemical responses within the malting and brewing processes.

Key Words

Barley, malt, beer, malt hot steep, metabolomics, flavor

Introduction

Malted barley is the primary source of fermentable sugars used to ferment most beers. Until recently, barley contributions to beer flavor were mostly attributed to Maillard Reaction Products (MRPs) developed during malt kilning and the interactions of malts with hops. However, recent research exploring the relationship between genetic variation of barley and beer flavor has shown that genotype does impact beer flavor (Herb et al., 2017a, 2017b; Bettenhausen et al., 2020). The degree of malt modification and growing environment were also determined to impact the sensory characteristics of beer, based on a large number of nano-brews, malt analytics, and a research sensory panel (Herb et al., 2017a, 2017b). Bettenhausen et al. (2020) carried this research a step further with *i*) larger, pilot scale malts and beers, *ii)* brewery, consumer, and laboratory sensory panels, and *iii*) measurement of volatile and non-volatile metabolites.

The interactions between malt chemistry traits and genotypes have been demonstrated to contribute unique beer flavor characteristics. Genetic differences and resulting metabolite composition differences lead to variation in the amount and composition of precursor amino acids and saccharides within the barley kernel. Through the process of malting, these precursors have the potential for biochemical reactions during germination to produce metabolites and MRPs vital for flavor characteristics. Our previous research on the contributions of barley to beer flavor was based on the progeny of a cross between an heirloom (Golden Promise) and a more contemporary barley variety (Full Pint) with a unique malting quality profile (Herb et al., 2017a, 2017b; Bettenhausen et al., 2020, 2018). The current study addresses the next question by expanding the scope of the evaluated germplasm: what are the contributions of other, different barley genotypes to beer flavor?

To address this question, we chose two different sets of barleys: 1) commercially available winter two-row malting varieties and 2) potential spring two-row varieties with Full Pint as one parent and varieties other than Golden Promise as the other parent. Pedigree, malt quality, beer quality, sensory attributes, and metabolomic profiles were compared within and between the two sets. The commercially available varieties were grown near Condon, Oregon in collaboration with the Western Rivers Conservancy (WRC; http://www.westernrivers.org/) within the framework of a project designed to enhance riparian habitat around the John Day River and its tributaries. The acquisition of the Rattray Ranch, historically used to produce dryland winter wheat, allowed for assessing the potential for winter malting barley as an alternative crop. Strips of four commercially available barley varieties were embedded within a commercial field of Wintmalt. The second set was derived from the Next Pint (NP) project, a collaboration between Mecca Grade Estate Malt (MGEM; https://www.meccagrade.com/) and Oregon State University to develop a variety to replace Full Pint, the current MGEM estate variety. Three advanced lines and Full Pint were grown, with irrigation, near Madras, Oregon at MGEM facilities.

The two sets of barley lines followed an experimental pipeline similar to that described in Bettenhausen et al. (2020). Briefly, each line underwent *i*) pilot scale malting and brewing, *ii*) quality analysis of malts and beers, *iii*) sensory analysis of beer by a trained laboratory panel and a consumer panel, and *iv*) metabolomic profiling of finished beer. In addition, sensory analysis of malt hot steeps was conducted. Since its development, the hot steep malt sensory evaluation method has piqued the interest of brewing and malting industries as an improved approach to evaluate malt sensory, as well as predict beer sensory characteristics derived from malts (Liscomb, 2016; ASBC Methods of Analysis). Though widely used and discussed, there are few formal comparisons of hot steep malt and beer sensory. Therefore, the potential of hot steep malt sensory evaluation as an economical, effective tool for assessing barley/malt impacts on beer flavor was investigated. The

current study advances research examining contributions of barley genotype to sensory characteristics of malt and finished beer.

Materials and Methods

Plant materials

Two independent sets of barley germplasm were used in this experiment, designated WRC set (Western Rivers Conservancy) and NP set (Next Pint) (Table 1). The WRC set included five released cultivars all of which are two-row winter growth habit types, four of European origin and one developed at Oregon State University (https://barleyworld.org/). Three of the five cultivars are approved by the American Malting Barley Association (Wintmalt, Thunder, Violetta; https://ambainc.org/2020-amba-recommended-malting-barley-varieties/). The NP set included three advanced lines and a Full Pint "check", all of which are two-row spring growth habit types developed by the Oregon State University barley breeding program. None of the barleys in the NP set are on the AMBA approved list. The three advanced lines were bred and selected over three years of testing from a larger set of 126 doubled haploid progeny derived from crosses with Full Pint.

The WRC set was grown at the Rattray Ranch, near Condon, Oregon (45°14′8″N 120°11′6″W). Briefly, the varieties were planted in the fall of 2017 and harvested in the summer of 2018. No irrigation was applied, as is standard practice in this summer-fallow dryland production area. Each variety, except Wintmalt, was grown in in 1.6 ha strip. The strips were embedded in a 197ha field of Wintmalt. The strips were planted, maintained, and harvested using commercial equipment. The NP set was grown at the Klann Farm, near Madras, Oregon (44°46'29.3"N 121°10'17.0"W). Briefly, the three advanced lines were planted in the spring of 2018 in 0.05 ha strips. Irrigation was applied following regular practices. The strips were embedded in a commercial field of wheat. The strips were planted and harvested using OSU Barley Project research equipment. Full Pint grain was sourced from an adjoining field managed by Oregon State University.

Malting and malt quality

Approximately 230 kg subsamples of grain were obtained for each of the barley lines in the WRC and NP sets. Each barley line was malted, in 90 kg batches, using the OSU mini-malter (https://barleyworld.org/), as previously utilized by Bettenhausen et al. (2020). Steeping conditions were the same for both sets and supplemental moisture was provided during the first day of germination by spraying if required. In order to optimize modification of the grain, the WRC set had a target moisture of 46% and the target for the NP set ranged from 45-51% based on results from micro-malting. Both sets were germinated for four days (WRC at 16°C and NP at 18°C) and had identical kilning conditions. Malt quality analyses were conducted by the Hartwick Center for Craft Food & Beverage (https://www.hartwick.edu/about-us/centers-institutes/center-for-craft-food-and-beverage/) following standard ASBC testing methods. The malting quality traits (and results) are shown in Table 2.

Brewing

Using an Esau and Hueber 2.5hl brewery at Oregon State University (OSU), lager beers were prepared in collaboration with the OSU Brewing Science Lab. Each malt variety/selection was mashed and brewed separately in two different batches 1) WRC malts in May 2019, 2) NP malts in July 2019, yielding 1.2hL each of Pilsner-style, malt-forward lager. The brewing recipe and protocol were adapted from a single-malt, lager protocol supplied by Rahr Malting intended to emphasize malt forward characteristics and achieve a drinkable, "commercial style" lager. Key ingredients were the neutral yeast (Bohemian Lager Strain 2124, Wyeast Labs), hop extracts (Isohop, John I. Haas, Inc.) and hop pellets (Kazbek hops, Brewers Supply Group). The brewing protocol was similar to Bettenhausen et al. (2020) but with modifications. Analysis of the beer was performed by the OSU Brewing Science Lab as described in Table 3.

Beer sensory

A beer sensory pipeline was performed as described in Bettenhausen et al. (2020), and two types of sensory studies were conducted 1) a consumer panel and 2) a laboratory panel.

The consumer panel testing was performed in collaboration with the Oregon State University Center for Sensory & Consumer Behavior Research (http://agsci-labs.oregonstate.edu/sensoryresearch/). WRC beers were tested in August 2019 while NP beers were tested in January 2020. The procedures were performed as described by Bettenhausen et al. (2020). Briefly, participants (WRC n = 152; NP n = 155) were asked to answer a series of questions per beer, including 1) overall liking (scale from 1-9), 2) Check All That Apply (CATA) for sensory characteristics, 3) ideal lager attributes, and 4) demographics.

The laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in October 2019. Thirteen (13) panelists (6 M, 7 F; 22 - 55 years old), who had prior experience on beer and wine descriptive analysis sensory panels, were trained over three separate sessions with the beers in question using the Projective Mapping (or Napping®) with Ultra Flash Profiling sensory method (Risvik et al., 1994; Perrin and Pages, 2009). WRC beers and NP beers were assessed in duplicate for sensory attributes on two separate days (WRC n = 10; NP n = 8). During each testing session, panelists assessed the orthonasal aroma and flavor by mouth of the beer in two separate tests, with new blind codes for the samples.

Hot steep malt sensory

Sensory analysis was performed on liquid extract produced from hot steeps of the malts in question, which were prepared in accordance with ASBC Methods of Analysis – Sensory Analysis 14 (ASBC Methods of Analysis, 2017). Descriptive data were collected using Projective Mapping (also known as Napping®) combined with Ultra Flash Profiling (Risvik et al., 1994; Perrin and Pages, 2009). Due to changes in panelist availability between the beer and hot steep malt sensory analyses, a new laboratory panel was recruited and trained over four, hour-long sessions. This 15-

member panel (8 M, 7 F; 23-68 years old) consisted of some of the same members as the beer sensory panel, but also included some new members, most of which had prior experience performing sensory analyses on other foods such as wine. Laboratory panel testing was performed in collaboration with the OSU Brewing Science Lab in March 2020. Malt hot steeps from five WRC malts and four NP malts were assessed for sensory attributes on separate days. During each testing session, panelists assessed both the orthonasal aroma and the flavor by mouth of the malt hot steeps in two separate tests. Half the panel carried out the orthonasal testing session followed by a five-minute break and then the flavor session, while the other half of the panel proceeded in the opposite order. Unique blind codes were used for each test, and the serving order was randomized for each panelist. The WRC malt hot steep sessions were carried out with 15 panelists held over two days, while the NP malt hot steep session was carried out with ten panelists on a single day.

Sensory data analysis

All sensory data were were collected via Compusense Cloud Software (Version 20.0.7404.31336, Guelph, Ontario, Canada). Projective Mapping combined with Ultra Flash Profiling provides both attribute counts and coordinate data for each sample evaluated. Coordinate data was analyzed using XLSTAT Multifactor Analysis (MFA) (Addinsoft, New York, NY). Individual MFA plots for aroma and flavor were created for both WRC and NP sample sets in both beer and malt hot steeps. Attribute data was processed in order to combine specific descriptors under the more broad descriptors, in accordance with the Base Malt Flavor Map. Post processing, descriptor data were then analyzed by Correspondence Analysis (CA) in XLSTAT. Attributes were ranked according to frequency of use summed across all of the samples in the set. Those attributes that were used at a rate of at least 45% of the most frequently used attribute were included in the CA plot for the laboratory panel beer sensory data. For the malt hot steeps, aroma and flavor CA plots were created individually before being combined and plotted together with the attributes used being those that were used by the overall panel with a frequency of > 25% of that of the most frequently used top attribute.

Detection of the metabolome in beer

Volatile metabolites in beer were detected using a non-targeted metabolomics approach. The methods included analysis of volatiles using headspace solid-phase microextraction gas chromatography-mass spectrometry (HS/SPME-GC-MS) with methods as previously described (Bettenhausen et al., 2020). Briefly, Mass spectra from the MS platform was converted to the .cdf file format and processed and annotated using the workflow described in (Bettenhausen et al., 2020, 2018). Metabolite quantities were established as previously described (Bettenhausen et al., 2018). Briefly, each sample resulted in a matrix of molecular features (defined by retention time and mass (m/z) generated using XCMS software in R v. 3.2.4 (Smith, 2006). Mass spectra were deconvoluted using the RamClust algorithm (Broeckling et al., 2016) and normalized to total ion current (TIC); the relative abundance and variance of each molecular feature was determined by the mean area of the pooled quality control (QC) injection. Volatile metabolites were annotated by spectral matching in RamSearch software (Broeckling et al., 2016) to an in-house database of ~1,500 compounds and to external and theoretical databases including NIST v14 (http://www.nist.gov), Metlin (Tautenhaun et al., 2016), Golm Metabolome Database (Hummel et al., 2013), MSFinder software (v. 3.26, RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa, Japan) (Tsugawa et al., 2016; Lai et al., 2017), Human Metabolome Database (Wishart et al., 2013), and FooDB (FooDB, 2017); Spectra were also evaluated using the findMAIN function of the interpretMSSpectrum R package (Lisec, 2017) and chemical ontologies were established using HMDB and the ClassyFire package in R (Djoumbou et al., 2016).

Statistics (metabolomics)

Volatile metabolite abundances for each dataset (WRC and NP) were compared independently, by Analysis of Variance (ANOVA) with the aov function in the R statistical environment v. 3.5.1(Team, 2014), and false discovery rate (FDR) adjustment was performed on the ANOVA p-values using the Benjamini-Hochberg

algorithm (Benjamini and Hochberg, 1995). Principal Components Analysis (PCA) was conducted on unit-variance scaled metabolites and sensory traits from each panel with SIMCA software v. 15 (Sartorius Stedim Biotech, Umea, Sweden) (Biotech SS, 2017). Respective sensory attributes of each independent sensory panel were integrated with the volatile metabolites for further multivariate analysis. Biplots of scores and loadings values from O2PLS models were conducted in SIMCA software on unit variance scaled data for sensory attributes (y) and volatile metabolites (x). Predictive power (Q^2) was determined via cross-validation, by which the data was divided into seven parts and 1/7th of the data was removed, and the model was built on the remaining 6/7th of data remaining, and the removed 1/7th of data are predicted from the model. Heat maps were created by after z-transformation of the metabolite data. The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r_s) between metabolite and sensory trait values (Zar, 1972).

Results

Barley, malting quality, and beer quality associated with barley genetics

As shown in Table 1, there were genetic relationships among the barley varieties/selections used in this study. In the WRC, comprised of winter growth habit two-row varieties, Opal is a parent shared by Wintmalt and Violetta. Wintmalt, in turn is a parent shared by Thunder and Flavia. Calypso does not have Wintmalt or Opal in its pedigree. Both of its parents have Puffin in their pedigrees, and Puffin has Maris Otter in its pedigree. Thunder has Charles, the first North American two-row malting barley approved by the American Malting Barley Association (AMBA), as its other parent. Thunder is unique in this set in having European and North American parentage. The NP set, comprised of spring growth habit two-row experimental varieties and the variety Full Pint, has an unusual genetic structure in that the three selections are derived from "wide" crosses between European winter two-rows (Violetta and Maris Otter) and a North American two-row (Full Pint). Two of the selections, DH131144 and DH131756, are sisters derived from the cross of Full Pint

x Violetta; Violetta was the male parent of the former and female parent of the latter. In this set, DH120270 is unique in having Maris Otter as a parent. Violetta and Maris Otter are, therefore, genetic commonalities between the WRC and NP sets.

There were notable similarities and some key differences in malting quality within and between the WRC and NP sets (Table 2), using the AMBA specifications for adjunct and all-malt quality. Within the WRC, all varieties were highly friable. Calypso, Flavia, and Violetta were well-modified and the most similar to each other. They met most criteria for the all-malt specifications but were too low in free amino nitrogen (FAN), diastatic power (DP), and alpha-amylase (AA) for the adjunct specifications. Wintmalt was the least modified of the set, with the highest β-glucan and lowest S/T (soluble/total protein), not meeting all-malt or adjunct criteria. Thunder was the most modified and notable for its high extract, FAN, AA, and S/T. Entries within the NP set came closest to meeting adjunct criteria, rather than all-malt criteria. DH131756 and DH131144 were well-modified and met most if not all AMBA adjunct specifications. DH120270 was under-modified, with low friability, high ß-glucan, lower extract, S/T, FAN, DP, and AA. It did not meet all-malt or adjunct criteria. Full Pint was less modified than DH131756 and DH131144, with lower friability and higher ß-glucan. It met AMBA adjunct specifications for most criteria but was slightly over specifications for ß-glucan and total protein (TP). Comparisons between the two sets show that the WRC malts were more friable and - except for Thunder - had lower extracts, TP, FAN, DP, and AA than the NP set. Overall, Calypso came closest to meeting the all-malt criteria and DH131144 met all the criteria for adjunct malting.

Despite the variation in malting quality (Table 2), all beers fell within range for German lager-style, Pilsener beer guidelines – except for color and ABV, as described by the Brewers Association Beer Style Guidelines (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles). All the NP beers were high in color. The IBU values

were similar for all beers, but below the BA guidelines for this beer style (Table 3). As might be expected based on their malt profiles, Wintmalt had the lowest alcohol by volume (ABV) and real degrees of fermentation (RDF) out of the WRC set and DH120270 had the lowest ABV within the NP set. Comparing the WRC vs. the NP sets, DH120270 and Full Pint had the lowest ABV and RDF values while Thunder had the highest RDF. As a point of comparison collectively across both data sets, ABV ranged from 4.99 – 5.42% while RDF ranged from 65.44 – 69.64%.

Sensory characteristics for malt hot steeps

Projective Mapping was used to evaluate both aroma and flavor attributes of malt hot steeps made from the WRC (15 panelists) and NP (10 panelists) samples. In each sample set, one malt was randomly selected to be presented as a duplicate. For the WRC malts, Flavia was replicated giving six total malt hot steep samples. Based on aroma evaluation only, panelists grouped duplicates closely together, meaning they perceived similarities between them, and dissimilarities between other samples. During the flavor evaluation, the Flavia duplicates were not placed as close to one another. *Thin body* was the only mouthfeel attribute used frequently enough to be plotted. Coordinate data from aroma evaluation showed that Thunder and Violetta were different from the other samples. During the aroma evaluation, *grainy* was used consistently among the samples, but showed more variable usage during flavor evaluation (Figure 1). In both aroma and flavor evaluations, *grassy* had a large variation in usage among the samples, with Calypso being described as *grassy* most frequently. Additionally, Calypso's aroma was described by *vegetal*, while its flavor was described by *cracker*. Both Flavia samples were high in *grassy,* and on average were high in *earthy*. Thunder and Violetta were each much lower in *grassy* than the rest of the samples. Thunder was consistently described by sweet aromatic, *breakfast cereal*, and *sweet bread*. Violetta was also more closely associated with *bread*. Descriptors used for Wintmalt varied between aroma and flavor, but *grassy* was used in both.

For the NP malts, Full Pint was replicated, giving five malt hot steep samples. The coordinate data showed similar configurations between aroma and flavor evaluations, with the exception of a Full Pint duplication moving positions. In both MFA plots, DH120270 appeared distinct from the other samples. *Grainy* was the most used descriptor for the NP aroma and flavor evaluations (Figure 1). There were large differences in usage for *grassy* in both flavor and aroma, and *sweet aromatic* via aroma only. Additionally, *sweet bread*, *earthy*, and *breakfast cereal* highlighted the differences between the samples during the flavor evaluation. In both aroma and flavor evaluations, Full Pint was described by *breakfast cereal*. The duplicates were more tightly grouped with the aroma than the flavor evaluation. DH120270 was the most unique sample of the group and was highly *grassy* and *earthy* across both evaluations. DH131144 and DH131756 were described by sweet bread, *sweet aromatic*, and other descriptors within the *bread* category.

Beer sensory – consumer panel

Results from the consumer panel indicate that barley variety had a borderline significant impact on beer flavor for the WRC beers. Overall Liking data showed Violetta was liked significantly more than Calypso (Tukey's Post Hoc HSD test $p=0.06$). Consumers were able to distinguish significant differences in attributes *citrus*, *floral*, *hoppy*, and *sweet* between the five WRC beers (Cochran's Q test, $p<0.05$). Thunder was significantly less *citrus* than the other four varieties, more *hoppy* than Violetta, and more *toasted* than Wintmalt; Violetta was found to be significantly more *sweet* and *floral* than Calypso, Flavia, and Wintmalt, and more *refreshing* than Calypso; And Wintmalt and Violetta were significantly more *crisp* than Thunder (McNamara's multiple pairwise comparison, $p<0.05$).

There were no significant differences in Overall Liking for the NP beers (ANOVA, $p= 0.72$). However, consumers were able to distinguish significant differences in the *bitter* attribute between the four beers (Cochran's Q test, $p<0.05$). Full Pint was found to be significantly less *bitter* than DH120270; DH120270 was found to be

significantly more light in *mouthfeel* than DH131756; and DH131144 and more *thin/watery* than either DH131756 or Full Pint (McNamara's multiple pairwise comparison, $p<0.05$).

Consumer panelists identified important attributes for an Ideal Lager from the list of common descriptors given in the CATA. *Crisp* and *refreshing* were selected as key attributes for an "ideal lager" in both the WRC and NP sets. *Citrus* and *light* were also selected as key attributes for the WRC and NP sets, respectively.

Beer sensory – laboratory panel

Projective Mapping was used to assess both aroma and flavor attributes of the WRC (13 panelists) and NP (10 panelists) beers in duplicate (10 and 8 beers per set, respectively). Multifactor Analysis (MFA) plots of the WRC aroma coordinate data showed that both Calypso and Violetta duplicates were grouped more closely together than the duplicates of the Wintmalt, Flavia, and Thunder beer samples. This pattern was also present in the coordinate data from the WRC flavor test. The separation of the duplicates indicates that the differences between the beers were subtle. Correspondence Analysis (CA) with attribute data showed Calypso duplicates were close together and were described by *fruity* and *floral* in aroma (Figure 2), and *fruity* in flavor (data not shown for concision). Aroma attribute data showed differences between duplicates for the other 4 beer samples. *Fruity* was the most commonly used descriptor for this sample set, while *earthy*, *grainy*, and *floral* helped discriminate the samples from one another. Additionally, the flavor data showed Flavia duplicates were similar and described by *grainy* and *grassy*. Wintmalt duplicates were close together and described by *sweet aromatic*, *fl*oral and *vegetal*. On average, Violetta duplicates were higher in *dough* and *sweet bread* than the other samples, which did not match its description by orthonasal evaluation. Thunder duplicates showed differences in use of *sweet bread* and *sweet aromatic* between them. In summary, panelists had some difficulty in describing and grouping the WRC samples, as indicated by inconsistencies in descriptions of duplicate beer samples.

The MFA plots for the NP aroma sample set (8 beers) showed that, with the exception of DH131756, the duplicates are placed closely together, indicating that they were perceived as similar by the panel. In the plot for the NP flavor sample set, DH131756 and DH131144 duplicates were mixed together, indicating that panelists were confusing these four beer samples. For both aroma and flavor evaluation, *grainy* was the most frequently used attribute for the sample set and thus unhelpful for discriminating samples (Figure 2). In both aroma and flavor, the frequency of *dough* had high variation in usage among the samples. DH120270 was described by *grassy* and *sweet aromatic* via orthonasal evaluation but was described by *vegetal* via taste evaluation. In both the aroma and flavor evaluations, the duplicates for DH131144 varied somewhat. In general, they were described with both sweet aromatic or *sweet bread*, as well as *dough*, *pasta,* or *cracker*. Although there were differences between the DH131756 duplicates they were both high in *fruity* in the aroma evaluation, and high in *sweet aromatic* in the flavor evaluation. Full Pint duplicates varied in their attribute counts for various descriptors but was consistently associated with *dough* in both aroma and flavor. Overall, duplicates were more similarly described for the NP sample set than the WRC sample set.

Metabolomics

Metabolite variation among beers within the WRC and NP sets

From HS/SPME-GC-MS, 1,342 metabolites were detected and 130 were annotated within the WRC set and within the NP set, 676 metabolites were detected and 160 were annotated (Figure 3). Volatile beer metabolites were annotated and assigned to a super and sub-class based on chemical ontology (Tables 4, 5). Classes of metabolites varied between WRC and NP datasets (Figure 3 A, B). Of those 130 annotated volatile metabolites from the WRC set, 15 metabolites varied among the 5 varieties (ANOVA for genotype $q < 0.05$). Of those 160 annotated volatile metabolites within the NP set, 24 metabolites varied among the 4 varieties (ANOVA for genotype $q < 0.05$).

PCA was conducted on the 130 volatile compounds with the five WRC beers and this demonstrates that variation was attributed to the barley variety (Figure 4A). PCA generated three principal components and was able to explain 86.6% of the variation in the data for the WRC varieties. In this scores plot, PC1 (33.1%) explained the separation between Wintmalt, Flavia, and Violetta vs. Thunder and Calypso. The loadings plot (Figure 4B) of volatile metabolites attributed to these WRC varieties did not explain any trends among the varieties.

PCA was conducted on the 160 volatile compounds detected in the NP set resulting in three principal components (Figure 4C) which explained 87.1% of the variation in the data for the three selections and Full Pint. In this scores plot, PC1 (61.4%) explained the separation between DH120270 and DH131756 vs. DH131144 and Full Pint. The loadings plot (Figure 4B) of volatile metabolites attributed to these varieties demonstrates a high content of lipids (fatty acid esters), terpenoids, and organoheterocyclic compounds (potential MRPs), specifically for DH120270.

O2PLS modeling

To investigate relationships between the beer volatiles and each of the beer descriptors from the Consumer Panel, an O2PLS model was developed with the sensory and volatile metabolite data. The O2PLS algorithm for the WRC set resulted in two predictive and two orthogonal components that explained 76.1% of the variation, with a predictive power of Q^2 = 59.2% to support that the model was not over-fit. The O2PLS algorithm for the NP set resulted in two predictive and two orthogonal components that explained 86.9% of the variation, with a predictive power of Q2 = 0%. This score indicates that the model is not at all predictive. This is probably due to the low number of samples (n =4) and/or low variation among the samples. Although there is separation among varieties, it may not be sufficient for effective cross-validation and prediction (Umetrics, 2015).

Analysis of the scores and loadings indicate most of the 19 sensory attributes from the Consumer Panel can be linked to beer volatile metabolites within the first two O2PLS components, as indicated by correlation coefficient values of greater than 0.5 ($p < 0.50$) (Figure 5). A SIMCA 'distance to model' function was applied to characterize the metabolites with the largest contribution to explaining the variation in significantly different sensory traits. The data indicate associations with organic acid esters, fatty acid esters, and benzoic acids, which are known classes of aroma compounds.

The sensory/chemistry cluster within the WRC beers along O2PLS Component 1 demonstrates co-variation of Violetta and traits such as crisp, overall liking, refreshing, citrus, and floral, but displays a negative association with traits such as astringent, bitter (such as are associated with Calypso) and with hoppy, honey, and toasted (such as are associated with Thunder) (Figure 5A). Metabolites that were positively correlated with attributes covarying with Violetta included benzenoids (4), fatty acid esters (5), organic acids (7), coumarins (2), ketones (2), and varying other classes. Two of the most correlated metabolites were an hydroxycinnamic acid (putatively identified as chicoric acid, WRC0679) which may impart a woody and nutty flavor (however, there are three other phenylpropanoids that are highly correlated, as well) and isomaltose (WRC0156, fatty acyl glycoside/oligosaccharide) which may contribute to sweetness, isopentyl acetate (WRC0390,banana, fruity). Other fatty acid esters and organic acid esters also had higher rates of correlation and have been associated to not only light, fruity flavors, but also to floral, refreshing flavors. Negative correlations included compounds of many of the same classes, but included many metabolites putatively identified as Maillard Reaction Products (such as WRC08606, Ethanoic acid ester; furans, pyrazines, pyrans). The relationships of metabolites to sensory were validated using Spearman's Rank Correlation performed on the sensory traits.

The sensory/chemistry cluster along O2PLS Component 1 demonstrates co-variation of Full Pint and traits such as *crisp*, *fruity (tropical)*, and *sour/tart* to a lesser extent, *honey*, *caramel*, *toasted*, *astringent*, and *molasses*, and co-variation of DH131144 with both *fruity (tropical)* and *fruity (non-tropical)*. By contrast, they are negatively correlated with *sweet*, *refreshing*, and *bitter*. DH120270 demonstrates co-variation with *bitter* and *thin/watery*. Metabolites that were positively correlated with attributes co-varying with the "most liked" DH131144, albeit by a slight margin over the other three beers, are fatty acid esters (6) which are known volatiles related to fruity (tropical and non-tropical) attributes, specifically, diethyl maleate (NP477), ethyl hexanoate (NP025), a pentanoic acid ester (NP145), methyl caprylate (NP197), 10-undecenoic acid ester (NP013), and ethyl decanoate (NP021). Other classes which co-vary with DH131144 include benzenoids (benzoic acid esters, 4), organoheterocyclic compounds (potential MRPs, 9), and others. The heterocycles of note include 5-methylquinoxaline (NP150), known to contribute to coffee and roasted attributes, and a thiophene (NP564), which can be attributed to garlic or onion flavors or aromas, potentially leading to the *cracker* and *dough* attributes assessed by the laboratory panel. Full Pint had a similar profile, with many similar co-varying metabolites. Three metabolites of note include: one fatty acid ester, ethyl hexanoate isomer (NP027), known to contribute many tropical and non-tropical attributes, some of which were found in DH131144, octyl benzoate, a benzoic acid ester (NP035), which can contribute lemon balm, and 2,6-dimethylbenzenethiol (NP565), a thiophene, which can contribute Maillard-type attributes, such as meaty, roasted, and sulfur. DH131756, which contained the most abundant metabolite profile, co-varied with the consumer panel sensory attributes *sweet*, *refreshing*, and *molasses*. Metabolites which contributed to this are heterocyclic compounds (9), fatty acid esters (9), organic acid esters (4), benzenoids (2), and others. Fatty acid esters of note were ethyl-9-decenoate (NP006), decyl propionate (NP047), and methyl caprylate isomers (NP026, NP019) which all are known to contribute to sweeter, more complex, fruity attributes. Vanillylmandelic acid, a benzenoid (phenol, NP011) can contribute to sweet and vanilla attributes; ethyl lactate, an organic acid ester, can contribute to butterscotch, fruity, and tart flavors. DH120270 had a unique profile, co-varying with light,

thin/watery, floral, citrus, and bitter sensory attributes. Metabolite classes included heterocyclic compounds (15), fatty acid esters and terpenoids (11), organic acid esters (6), and others. Two heterocyclic compounds of note are 4-methylpyridine (a pyridine, NP629), known for tea and fig properties, and 5-methylquinoxaline, known for roasted properties. There are many metabolites which are known to have phenolic and bitter sensory properties that may contribute to the co-variation with bitter, assessed by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes. The relationships of metabolites to sensory were validated using Spearman's Rank Correlation performed on the sensory traits.

Other trends among chemical classes

The data were evaluated to determine if broad trends of metabolite classes could distinguish each of the beers within the sets: specifically, for lipids (to include fatty acid ester formation), nitrogenous compounds, organic acids, and phenolics. Metabolite abundances were z-transformed to express the data as a profile within a variety, therefore a range in color denotes range in variation of a compound class within a variety, with very blue (high) or very yellow (low) indicate proportions of a metabolite's contribution to the profile (Figure 6 A,B).

The heatmap for the WRC beers showed Calypso had a unique profile, abundant in alkanes, alkenes, and benzoic acid esters that were not abundant in the other four varieties, also being more abundant in prenol lipids (terpenoids) including linalool (WRC0071), p-methan-1-ol (WRC1030), alpha-cadinol (WRC0284), alpha-cuebene (WRC0196), and geraniol (WRC0182). These metabolites have been associated, in literature, not with *bitter* and *astringent* sensory attributes, as denoted from the sensory panel, but with the *grassy* and *vegetal* (among other attributes noted in the literature, such as floral, citrus, and menthol) noted in the aroma factor analysis from

the laboratory panel (Roth et al., 2014; Chambers et al., 2013; Beal, 1994). Calypso was also abundant in a class of organoheterocycles known as "quinolines," which have been shown to be attributed to a tea-like flavor (bitter, astringent) in the literature (FooDB, 2017). Among the five beers, there were no trends among lipids/fatty acid esters, as they were equally distributed. The nitrogenous compounds shared by Wintmalt and Flavia included 42-diethoaminoethanol (WRC0626), pyridine-like compounds (WRC0374, WRC0493, WRC0489) which may contribute to or overpower the other sensory attribute of citrus and instead contribute to the malty seen in the consumer panel and *breakfast cereal*, *bready*, and *earthy* attributes from the laboratory panel. Organic acids predominate Violetta, and to a lesser degree, Thunder (Figure 6). One organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, is seen to covary with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188). Violetta, Wintmalt, and Flavia displayed negative correlations with the prevalent benzenoid class which was shown to covary with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes, 4-hydroxybenzyl alcohol (WRC 0481), and benzaldehyde (WRC1013), associated with more almond, bitter attributes.

The heatmap for the NP beer set displays trends between Full Pint/DH131144, and within certain classes between DH131756/DH120270, although DH120270 again was recognized as having the most unique profile (Figure 6). The trends between Full Pint and DH131144 include higher abundances of aldehydes and ketones such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132). Full Pint and DH131144 also shared many abundant fatty acid esters, noted in the previous section. Trends within the organic acid ester class occurred between DH131756 and

DH120270, including many isomers of acetic acid, keto acids, and an acetamide of note (NP097) which, in literature has been known to contribute a mousy attribute.

Metabolomics: considering both sets of beers

To assess the Next Pint and WRC beers together, PCA and a two-way orthogonal projection to latent squares (O2PLS) was performed on all nine beers. Only metabolites which were annotated and shared among all varieties were included in the analysis, abundances were unit variance normalized. 94.8% of the data was able to be explained by four principal components. PC1 (68%) and PC2 (16.6%) were able to explain significant variation among these data. The differences may be attributable to "environment" (i.e. two completely different locations, one dryland the other irrigated); genetic relationships (i.e. Full Pint a parent of all NP lines and no WRC lines); growth habit (one set winter and the other spring); degree of selection (one set commercially available, the other set comprised of three experimental varieties and the control); and/or to the higher abundance of metabolites in the WRC set (Figure 7).

Discussion

Barley, malting quality, and beyond

The barleys used for this research form two distinct groups that are confounded by three factors: growth habit, commercial status, and production environment. The WRC set is comprised of winter growth habit, commercially available varieties grown under dryland conditions while the NP set is comprised of spring growth habit experimental selections and a control, grown under irrigated conditions. Although the two sets were treated identically through brewing, beer sensory, and beer metabolomics, these treatments occurred at different time points. Therefore, it is necessary to discuss the results of each set separately. However, there are commonalities between sets that merit some further discussion and integration, both *inter se* and with prior research.

The first commonality is genetic relatedness. Violetta, a member of the WRC set, is also a parent of two members of the NP set (DH131756 and DH131144). Because it is the female parent in one cross and the male parent in the other cross that could have some bearing on the flavor differences between the two sister lines: in Angiosperms, organelles show maternal inheritance: therefore, the chloroplast and/or mitochondrial genomes these two selections could be genetically different and those polymorphisms could lead to flavor differences. However, most phenotypes of commercial importance in barley studied to date (e.g. agronomic and malting quality traits) show nuclear, rather than cytoplasmic, inheritance. In this regard, it is not surprising that these two doubled haploid siblings could have contrasting malting quality and other downstream phenotypes based on contributions from the nuclear genome only. Further exploring pedigree records and possible genetic contributions to beer flavor, Maris Otter (an iconic heirloom variety from the United Kingdom) is a direct parent of one NP member (DH120270) and figures in the pedigree of only one WRC member (Calypso). Full Pint, the control in the NP set and a parent of all three experimental varieties in the NP set, was also a parent of the Oregon Promise lines analyzed by Bettenhausen et al. (2020) and Herb et al. (2017a, 2017b). Going further back in the pedigrees, the old European landraces Criewener 403, Pflugs Intensiv, Bavaria, and Danubia feature in all nine lines. The Czech landrace Hanna and English landrace Spratt are in eight of the nine pedigrees, missing from Thunder and Full Pint respectively. More contemporary lines that feature most frequently in the pedigrees of the 9 lines are Isaria, Kenia, and Gull (all 9), Puffin and Malta (missing from Full Pint and DH120270), and Klages (in the pedigrees of Full Pint, Thunder, and the NP set). While pedigree doesn't provide the full picture of the genetic relationships between these nine barleys, it is valuable in showing common and different ancestries that may explain some of the phenotypic flavor contrasts. DNA fingerprinting of these nine genotypes is underway, and that will provide objective measures of genetic relatedness and perhaps identify specific alleles that could be contributors to differences in beer flavor.

Capitalizing on this genetic relatedness to identify the genetic drivers of differences in quality parameters, flavor, and metabolic profiles will be the topic of a future paper - where sample size is larger and complete genotype data are available. At this point, however, specific differences and commonalities between the two sets can be pointed out that relate to variety and therefore impact on one of the questions driving this research: "do barley genotypes contribute to beer flavor?" These differences and commonalities will be highlighted during this Discussion, which will proceed sequentially by feature (e.g. malt analysis, sensory analyses, metabolomics) but progressively integrating results for each trait and its impacts on other traits.

Malting quality specifications are key metrics for barley variety release. Within the WRC set, the lower degree of modification of Wintmalt and higher degree of modification and enzyme-related trait values for Thunder were notable. Both varieties are on the AMBA recommended variety list, which requires thorough vetting for quality and brewery performance. Although every effort was made to produce optimum malts for all varieties, for reasons unknown Wintmalt did not achieve target specifications in this project. The overall higher grain proteins of the NP set may have affected downstream flavor, sensory, and metabolite composition.

Sensory attributes of malt hot steeps and beer, and their relationships

Hot steep malt sensory

Prior to the establishment of the hot steep malt sensory method, Congress worts were used for sensory evaluation of malt samples (Coghe et al., 2004). Since its development, the hot steep malt sensory evaluation method has piqued the interest of the brewing and malting industries to improve analysis of malt sensory and predict beer sensory for malts of interest (Liscomb, 2016; ASBC Methods of Analysis, 2017). It is helpful when only a small quantity of malt is available and is more convenient than making beer. The predictive ability of this method, though much more rapid than brewing, has yet to be fully understood. With the analysis pipeline implemented in this research, we can identify relationships of hot steep malt sensory

with other traits. However, determining if relationships are causal and predictive will require further experiments.

Within the WRC set, Thunder and Calypso were standout samples for hot steep malt sensory. The former was higher in *sweet bread* and *sweet aromatic* for both aroma and flavor while the latter was *grassy* and *vegetal* in aroma and *cracker* in flavor. Considering the other varieties in this set, Thunder and Violetta were lower in *grassy* thus separating them from the other samples. DH120270 was a standout sample within the NP set. In both the aroma and flavor evaluations, it was consistently described by panelists as more *grassy* and *earthy* than the other samples. Malt analytics provide clues that Thunder was more modified than Calypso, thus leading to differences in hot steep malt sensory. While it seems likely that the *sweet bread* and *sweet aromatic* descriptors for malt hot steeps are attributable to the higher enzyme profiles of Thunder, DH131144, and DH131756, further research is necessary. The basis of the *grassy* profile for Calypso is not obvious, however in the case of DH120270, it could be ascribed to under-modification. Attribution of this characteristic to Maris Otter heritage requires further research. From a plant breeding perspective, the poor modification of DH120270 and its *grassy* and *earthy* profile, which were not selected as ideal lager traits, in the hot steep malt sensory would be grounds for not advancing it on to brewing and beer sensory. In this sense, sensory evaluations using hot steep malt sensory could be a tool in variety selection. In order to assess its value for the malting and brewing industries, the key question remains "is hot steep malt flavor predictive of beer flavor"? The current research provides some insights into this relationship, but other experiments will be required. Within the current experiment, the connection between malt and beer sensory is best explored using the laboratory panel data, given the commonality of protocol and lexicon.

Laboratory beer sensory

The laboratory beer sensory panel had some difficulty matching duplicates within the WRC set to one another, with the exception of Calypso. However, differences in

sensory attributes were still perceived among the beer samples. This pattern suggests that stringent selection for commercial potential led to barleys that, despite differences in malt and beer analytics produced beers that are only subtly different in sensory profiles. The nuanced differences may result from inconsistencies in malt-modification (Herb et al., 2017a). There is evidence to show that undermodified malts may result in higher *grassy* qualities (Bettenhausen et al., 2020). In the NP set, duplicates were more similarly described for both aroma and flavor, indicating that panelists not only found differences among the beers but that these differences could be identified with consistency. DH120270 duplicates were closely grouped, with consistent *grassy* aroma and *vegetal* flavors. The other NP samples, DH131756, DH131144, and Full Pint, are less stable throughout testing indicating that they have less distinct profiles from one another.

Comparing beer and hot steep malt sensory

While beer samples were all duplicated, only one malt hot steep sample per set was duplicated. Therefore, there was only one measurement of panelist consistency for the malt hot steep evaluations. While mashing and steeping processes mirror one another, it is important to note that mashing takes place at a higher temperature for a longer time than steeping. It is clear that the differences among beers were more subtle and nuanced than those of the malt hot steeps. For example, once the malt was brewed into beer, the *grassy* characteristic of DH120270 decreased, making it more similar to the profiles of the other NP samples. The standout samples for the malt hot steeps, DH120270 (*grassy*) and Thunder (*sweet bread* and *aromatic*), were less noticeably different in the beer sensory evaluation. Observing patterns of descriptor usage across the two sensory methods can give us insights into the connection between the two. Both *grassy* and *grainy* were used more in malt hot steep characterization than beer characterization. *Floral* was used only once in the description of malt hot steep aroma but became an important attribute for beer sensory. Similarly, *fruity* was used infrequently to describe malt hot steep samples but very frequently to describe the resulting beers. *Floral* and *fruity* aromas were likely present in beer due to the addition of hops and the production of esters by yeast during fermentation (Kishimoto

et al., 2006; Verstrepen et al., 2003). Nonetheless, some attributes were stable across both malt hot steep and beer sensory. For example, Thunder retained its *sweet bread* quality from malt hot steep to beer. Results from this study indicate that hot steep malt sensory profiles are more distinct than those of their resulting beers. It is important to note that beer sensory profiles will also be influenced by fermentation byproducts and interactions with hops. More evidence is needed to make further conclusions about the predictive ability of the hot steep malt method.

Comparing consumer and laboratory beer sensory

Differences in lexicon, panel size, methodology (including panel training), and goals preclude directly comparing the sensory results from laboratory panel and consumer panels. Nevetherless, both panels identified differences in beer flavor within the WRC set; in particular, the consumer panel identified *citrus, floral, hoppy*, and *sweet as the differentiating attributes within the set.* For the laboratory panel, *dough, sweet bread fruity,* and *floral* were key attributes that differentiated the finished beer samples. It is important to note that a set of lexicons were preselected and provided to consumers to describe each beer sample due to panelists lacking specific sensory training. The lexicon provided to consumers had fewer attributes related to the *bread* category, while adding more options that fell under *sweet aromatic* (*caramel, honey*). Beers brewed from Violetta and Calypso - at opposite ends of the overall liking spectrum - had very similar malt and beer analytics, suggesting that these objective measures are not necessarily predictive of hedonic assessment. This finding also indicates that there can be differences in beer flavor, attributable to barley variety, in the relatively small number of commercially available winter two-row malting barley varieties.

In contrast to the WRC set, no significant differences were found in overall liking of NP beers evaluated by the consumer panel. However, both laboratory and consumer panels coincided in differentiating DH120270 from other samples: *lighter* and *thin/watery* by the consumer panel and *grassy* by the laboratory panel. DH120270,

therefore, is consistently different from the other selections and the Full Pint check, indicating that this experimental variety could have been eliminated at the malt analysis stage, with no need to go on to the expense of malt and beer sensory. In the bigger picture, the lack of significant differences in liking between DH131756 and DH131144 indicates that either of them could potentially be selected to replace Full Pint without an adverse consumer perception of beer flavor. The decision could be based primarily on agronomics and malt analytics. The latter, while not necessarily predictive of beer flavor in this research, can be key in variety approval and malt sales.

Beer metabolomics: connecting chemistry with sensory analysis and analytics

Metabolomics and sensory

Of the WRC beers, Violetta produced the beer with the highest score for *overall liking*, encompassing previously described desirable traits for a lager – namely *refreshing, crisp, citrus, sweet,* and *light* (Bettenhausen et al., 2020). This variety had reduced MRPs and a unique profile of fatty acid esters (Figures 3, 6). Calypso, unique in pedigree, similar to the other varieties in malt and beer analysis, and a standout in hot steep malt sensory and beer sensory, had a unique chemical profile. It was also the *least liked* of the WRC beers. Because the PCA revealed separation of the WRC varieties that did not match any of the similarity groupings according to malting quality, beer analytics, or laboratory/consumer sensory, we looked to specific variety:metabolite associations.

The stringent selection applied to varieties during breeding and commercialization – which may not have included consumer sensory assessment - may have led to minor differences in volatile compounds, including an increase in compounds that convey *bitter* or *astringent.* As noted in the results, Calypso was more abundant in prenol lipids (terpenoids) and in a class of organoheterocycles known as “quinolines,” which are associated with a tea-like flavor (bitter, astringent) (FooDB, 2017).

There were no trends among lipids/fatty acid esters among the five varieties, as the lipid/fatty acid ester class (acetate esters) was generally equally distributed. The medium-chain fatty acid ethyl esters (ethyl hexanoate and ethyl octanoate), however, co-varied with Calypso (Figures 5, 6) (Saerens et al., 2010; Thurston et al., 1982).The nitrogenous compounds shared by Wintmalt (less modified malt) and Flavia (well-modified malt) included 42-diethoaminoethanol (WRC0626) and pyridine-like compounds (WRC0374, WRC0493, WRC0489) which may contribute to, or overpower, the sensory attribute of *citrus* and instead contribute to *malty* noted by the consumer panel and the *breakfast cereal, bready*, and *earthy* attributes identified by the laboratory panel. Organic acids predominate in Violetta, and to a lesser degree, Thunder (Figure 6). An organic acid ester, triethyl citrate (WRC0375), which is known to contribute to vinous and non-tropical fruity attributes, co-varied with Thunder and the *fruity (non-tropical)* sensory attribute from the consumer panel, as well as the *sweet aromatic* attribute from the laboratory panel. The organic acids most unique to Violetta included acetic acid ester (WRC0035), triethyl citrate (WRC0047), ethyl propanoate (WRC0194), isopentyl acetate (WRC0390), 4-isopropylphenylacetic acid (WRC0638), dimethyl malonate (WRC0384), and heptyl-2-methylpropanoate (WRC0188) (Table 5). Violetta, Wintmalt, and Flavia had negative correlations with the prevalent benzenoid class, which covaried with Calypso. This class included 1,2-benzenedicarboxylic acid ester (WRC0153), known to be associated with almond, floral, herbal, green, and more phenolic attributes; 4-hydroxybenzyl alcohol (WRC 0481); and benzaldehyde (WRC1013), which is associated with more almond, bitter attributes.

In the NP set, Full Pint and DH131144 had higher abundances of aldehydes and ketones - such as 2-nonen-4-one (NP428), 1-hexene (NP255), and 1-pentanol (an alcohol, NP132) - and they shared many abundant fatty acid esters. Although Full Pint, DH131144, and DH131756 were similar in sensory attributes, DH131756 and DH120270 shared many isomers of acetic acid, keto acids, and an acetamide of note (NP097) which is noted in literature to contribute a mousy attribute. There are many metabolites that are known to have phenolic and bitter sensory properties that may

contribute to the co-variation with *bitter* in DH120270, identified by the consumer panel and with the *cracker* and *sweet aromatic* assessed by the laboratory panel. Examples of these metabolites include 2-phenyl-2 butenal (NP146), a phenylacetaldehyde known to contribute a bitter, black tea note and 2-methoxy-4-vinylphenol (NP381), recognized for the contribution of clove, smoky, and spicy attributes.

Given the distinctiveness of the WRC and NP germplasm sets in terms of growth habit, production environment, and commercialization status, the causes of similarities and differences are confounded, but notable. Some of these differences could be attributed to genetic relatedness: e.g. Full Pint is unique to the NP set as a member and as a parent. When DNA fingerprint data are available for the WRC and NP sets, causal effects based on genetic differences may be identifiable. The WRC varieties, as a group, contained fewer organoheterocycles (potential MRPs) than the NP varieties (Figure 3). As discussed in Bettenhausen et. al. (2020), MRPs play a major role in beer flavor. Two metabolites, furfural and 2-pentylfuran belong to the class of organoheterocycles known as furans, furfural serving as a precursor to 2-pentylfuran, which contributes fruity, grassy flavors (NP148 and WRC0228, Figure 6, denoted in red text). All varieties contained this furan, but normalized abundances differed among all varieties. Lower abundances of MRP in the WRC may be related to the lower grain protein, overall. Since degree of modification involves protein breakdown (through protease activity), incomplete modification would leave these varieties lacking in components for the Maillard Reaction (proteins, saccharides) (Nursten, 2007). In the NP set there were fewer instances of phenylpropanoids (a class including cinnamic acid esters and coumarins) and more benzenoids (phenols, benzoic acid esters) than in the WRC set. Phenolic compounds are formed via the shikimate pathway and are known to contribute to more *bitter* and *astringent* attributes, such as those found in DH120270. Fatty acid esters, especially ethyl dodecanoate, (WRC0012 and NP031, denoted in HMap in green text) were present in DH120270 and Wintmalt. Abundances of ethyl dodecanoate in other varieties were well below the amounts in DH120270 and Wintmalt. Wintmalt and DH120270 were

also the least modified (Table 2) and differed the most for beer analytics. The development of these fatty acid esters, through esterification of ethanol with fatty acids, is crucial for development of flavors, but the lipids that are present in each variety (type and amount) may play a role in how much of that flavor is developed and at what rate. The presence of these compounds (ethyl octanoate, ethyl-9-decenoate, n-decanoic acid) in conjunction with the low MRP/organoheterocycle profile of WRC suggests not only that these compounds contribute to desirable attributes associated with Violetta, but that they could also contribute to off-flavors during aging (Heuberger et al., 2016; Vanderhaegen et al., 2007; Vandergaegen et al., 2006).

Metabolomics, malting quality, and beer analytics

Calypso met all parameters, regarding malting quality specifications, to produce an "ideal lager" (Table 2), but, in fact, it did not produce the most "liked" beer within the consumer panel. Wintmalt met the least parameters in its malting quality profile and yet it produced an acceptable beer by consumer panel standards and no negative attributes were noted by the laboratory panel. Violetta and Flavia were noted as having more complex flavor profiles; this is potentially due to variable (on the edge of acceptability) S/T, total protein, and FAN levels (Table 2), leaving less room available for Maillard Reactions to create *roasted* and *caramel* attributes out of proteins and saccharides (Nursten, 2007; Mottram, 1993). The lack of MRP attribute creation leaves more room for lipid conversion into fatty acid esters (and therefore room for lighter *fruity*, *floral* attributes to potentially shine). Thunder, as an example, whose higher enzyme levels indicated the potential for a more *crisp* and *dry* beer, with no residual sweetness, showed co-variation with the *caramel*, *honey*, *toasted*, and *non-tropical fruity* from the consumer panel; *sweet bread* and *sweet aromatic* from the laboratory panel. Thunder, despite the high enzymatic potential, was the most attenuated, expressing residual saccharides and higher FAN levels that likely contributed with *sweet*, *grainy*, *caramel*, and *malty* flavors due to residual saccharides. The higher FAN in Thunder, as opposed to the level found in Violetta (which expressed lighter flavors driven by fatty acid esters (Olaniran et al., 2017))

may indicate that the MRPs, driven by amino acid presence, can be an indicator of potential flavors in beer, as the ability to detect MRPs (during sensory) is greater because MRPs are generally more overpowering (i.e. the smell of a cooking steak vs. the smell of soap). The esterase activity in beer also makes the detection of lighter, fruitier, floral flavors created by fatty acid esters less detectable in comparison with MRPs. The lower degree of modification of Wintmalt and DH120270 could produce beers with *grassy* attributes due to the presence of acetaldehyde, hexanal, hexanol and general "greenness" of the malt (Olaniran et al., 2017). Under-modified malt leads to low sugar extraction during mashing, and therefore lower than target ethanol concentrations after fermentation, as seen in Table 3 of beer analytics. Wintmalt and DH120270 also had the haziest wort (potentially due to either low modification or high molecular weight beta-glucans, leading to possible unintentional flavor outcomes). Full Pint and DH131144 are chemically the most similar of the NP varieties; they also are similar from a malting quality standpoint, with the exception of friability and beta-glucan, they also are similar from a malting quality standpoint, with the exception of friability and beta-glucan, where the former – or simply less modification - may account for the differences in laboratory panel sensory perceptions (*sweet bread* of DH131144 vs. *fruity/vegetal* of Full Pint).The NP set, overall, was "less friable" than the WRC set, which could lead to lower brewing efficiencies and undesirable flavor outcomes. There was, however, improvement in friability in DH131756 and DH131144 compared to Full Pint. Despite these improvements, the resulting beers were still similar.

Conclusions

This study contributed to the body of knowledge by examining the effects of more and different barley genotypes on beer flavor. The current results support our previous findings that barley genotype does lead to differences in flavor profiles of lager beer. Two sets of barley germplasm 1) commercially available winter barleys and 2) Full Pint and three advanced progeny breeding lines were found to have

distinct, subtle differences that contributed to nuanced flavor profiles of both malt hot steeps and finished lager beer. Variations between and among barley germplasm sets were greatest for malt analytics, and this variation declined for beer analytics and then again for sensory profiling. Consumer and laboratory panels detected differences in sensory attributes of beer and malt hot steeps, but the basis of these differences was not always obvious. It is important to emphasize, in this context, that the descriptors and preferences reported are applicable only to these research beers and should not be taken as representative of the specific barley varieties and/or selections and their production environments.

Nonetheless, the research findings support the value of sensory assessments of pilot and commercial-scale beers of potential and new varieties. While common practice in the final stages of the variety recommendation and/or adoption processes, brewing and sensory assessment may also have value earlier in the variety development pipeline. Sensory assessments can continue to play an important role for defect elimination and can be expanded to include discovery of new flavor opportunities. In the case of the WRC set, a variety with acceptable malt and beer analytics was not favored by the sensory panels while a variety with less favorable malt and beer analytics was acceptable. In the case of the NP set, one potential variety could be eliminated based on flavor as well as on poor malting and brewing quality attributes. The remaining two selections were not appreciably different in sensory profile from the reference variety, which simplifies the variety selection process to decisions based on agronomics, malting quality, and/or beer quality.

All measures and procedures used in this research have value in guiding decisions regarding variety selection, but none were directly predictive of another. For example, malt analytics can guide maltster decisions on what barley varieties are likely to produce consistent malt using existing malting protocols in order to meet brewers' expectations. Additionally, while exploring the ability of hot steep malts as an economical and efficient predictive tool for beer flavor profiles, there were some

attributes that were stable across both beer and hot steep malt sensory analysis. Hot steep malt sensory profiles were found to be more distinct than those of their resulting beers. The current research provides some insights into this relationship, but other experiments are justified in order to define the basis of this relationship: the hot steep malt sensory may provide a useful common language for maltsters and brewers. Moreover, metabolomics can provide insights into the chemical basis of specific sensory descriptors and consumer preference. Distinct metabolomic profiles were detected within and between germplasm sets which were attributable to variety. Covariation of metabolomic profiles and sensory attributes was identified in both panels. These observations lead to the conclusion that the variable metabolites observed among the two sets of barley germplasms are a direct result of genetic differences that lead to differential responses within the malting and brewing processes. When metabolites are connected to genes, barley breeders will have additional targets for selection in order to meet target, or novel, beer flavor profiles. Until then, the new knowledge generated by this research can be capitalized upon by extending it to additional barley genotypes, different malts of the same varieties, and different beer styles.

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Tables

Table 1: Pedigree and developer or provider of barley lines per project/set.

Project/set	Variety/selection	Pedigree	Developer/Provider
WRC	Wintmalt	(Opal*3087/96, F1)*(8751/Magie)	Ackermann Saatzucht GmbH & Co. KG
	Thunder	Wintmalt/Charles	Oregon State University
	Violetta	Opal x Br 2324b616	Saatzucht Josef Breun GmbH & Co.
	Flavia	(((Carrrero * NIKS.2230) * Aquarelle) * Metaxa) * Wintmalt	Ackermann Saatzucht GmbH & Co. KG
	Calypso	Sunbeam/Suzuka	Limagrain Cereal Seeds
NP	DH131756	Violetta/Full Pint	Oregon State University
	DH131144	Full Pint/Violetta	Oregon State University
	DH120270	Maris Otter/Full Pint	Oregon State University
	Full Pint	Orca/Harrington	Oregon State University

Pedigree based on breeding annotated method mother/father. DH, doubled haploid, experimental barley selection that has not been released.

Table 2: Malt quality of barley lines per project/set.

Project/set	Variety	Moisture %	Friability %	Extract %	Color °SRM	β-glucan mg/L	SP %	TP %	S/T %	FAN mg/L	DP °L	AA DU	Filtration Time	Clarity	pH
WRC	Wintmalt	4.6	91.2	80.3	1.56	128	3.78	10	37.8	123	102	43.4	normal	hazy	6.07
	Thunder	4.8	97.0	83.9	1.97	58	4.89	9.1	53.7	202	124	78.7	normal	clear	5.91
	Violetta	4.6	95.2	80.3	1.69	29	3.89	9.5	40.9	141	113	40.2	normal	clear	6.06
	Flavia	4.6	96.8	80.0	1.57	33	3.64	9.2	39.6	127	111	44.1	normal	clear	6.06
	Calypso	4.3	99.2	81.3	1.73	31	3.83	8.8	43.5	150	114	46.6	normal	clear	6.04
NP	DH131756	4.6	82.5	82.5	1.94	77	5.8	13.8	42	237	163	70.2	normal	clear	5.83
	DH131144	4.7	84.7	81.4	2.22	38	5.62	12.2	46.1	236	174	83.9	normal	clear	5.98
	DH120270	4.5	72.1	78.5	1.41	272	4.35	13.1	33.2	150	161	58.5	normal	clear	5.98
	Full Pint	4.7	69.4	82.9	1.84	110	5.32	12.9	41.2	220	208	91.9	normal	clear	5.99
Adjunct Malt Criteria		NA	NA	> 81%	1.6-2.5	< 100	4.8-5.6%	≦ 13%	40-47%	> 210	> 140	> 50	NA	NA	NA
All-malt Criteria		NA	NA	> 81%	1.6-2.8	< 100	< 5.3%	≦ 12%	38-45%	140-190	110-150	40-70	NA	NA	NA

All-malt and Adjunct malt criteria are based on parameters suggested by American Malting Barley Association (https://ambainc.org/wp-content/uploads/2019/10/Malting_Barley_Breeding_Guidelines_June_2019.pdf)
Color, based on Standard Reference Method (SRM); SP, soluble protein, based on dry basis; TP, total protein; S/T, soluble/total ratio; FAN, free amino nitrogen; DP, diastatic power, based on Lintner units; AA, alpha amylase, based on diastatic units (DU); Clarity and Filtration Time are dependent upon multiple factors.

Table 3: Beer quality of barley lines per project/set.

Project/set	Sample Name	ABV	°P	RE	AE	Color	RDF	IBU
WRC	Wintmalt	5.12	12.14	4.38	2.52	3.79	65.44	22.94
	Thunder	5.41	12.05	3.82	1.87	4.01	69.64	23.6
	Violetta	5.42	12.27	4.04	2.09	3.17	68.47	20.74
	Flavia	5.40	12.31	4.11	2.16	3.16	68.03	21.35
	Calypso	5.31	12.06	3.99	2.07	4.09	68.29	23.88
NP	DH131756	5.21	12.08	4.18	2.30	6.57	66.85	21.11
	DH131144	5.34	12.12	4.01	2.08	7.89	68.33	23.94
	DH120270	4.99	11.70	4.11	2.30	4.72	66.29	22.33
	Full Pint	5.10	11.64	3.86	2.01	6.21	68.17	22.1
BA Guidelines	German-style Pilsener	4.6-5.3	11-12.9	NA	1.5-3.1	3-4	NA	25-50

From beer produced from each malt; ABV, alcohol by volume; ºP, Degrees Plato, concentration of dissolved solids in wort to quantify the concentration of extract; RE, real extract, based on attenuation of wort; AE, apparent extract, RDF, real degree of fermentation; Color, based on SRM method; IBU, international bittering units based on dissolved solids. German Pilsner guidelines provided by the Brewers Association (https://www.brewersassociation.org/edu/brewers-association-beer-style-guidelines/#Lager%20Styles).

Table 4: WRC metabolite data.

Code	Class	Subclass	Metabolite	Sensory (Lit)*	Pvals (FDR adjusted)
WRC0490	alkaloids	alkaloids	piperine	animal, pepper	0.706908739
WRC1013	benzenoids	benzaldehydes	benzaldehyde	almond, bitter, burnt sugar, cherry, sweet	0.615626002
WRC0487		benzamines	benzoic acid, 2-amino-4-methyl-	NA	0.574021995
WRC0437		benzenoids	1-phenyl-2-pentanol	earthy, green, mild, sweet	0.871431342
WRC0118			4-hydroxybenzyl alcohol	astringent	0.003209971
WRC0058			benzamide, 4-ethyl-n-butyl-n-tetradecyl-	NA	1.01E-05
WRC0303			n,n-dimethyl-3-methylaniline	NA	0.930437122
WRC0031			phenylethyl alcohol	alcoholic	0.694068216
WRC0153		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	0.020408376
WRC0240			benzoic acid, 3-amino-	bitter	0.77550619
WRC0081			m-anisic acid, cyclobutyl ester	NA	0.426151565
WRC0485			phenoxyacetic acid	sour, sweet	0.751673025
WRC0481			4-hydroxybenzyl alcohol	almond, bitter, coconut, fruity, sweet	0.000454381
WRC0195		phenols	phenol	phenolic	0.777237092
WRC0162		xylenes	2-thiophenecarboxaldehyde	NA	0.988374331
WRC0632	hydrocarbons	alkanes	pentane	alkanes	0.599809451
WRC0298		hydrocarbons	(+/-)-n,n-dimethyl menthyl succinamide	cool, minty	0.354995976
WRC0087			2-butene, 2-methyl-	NA	0.027641517
WRC0183			3-octen-1-yne	NA	0.478134928
WRC0549	lipids	fatty acid esters	2-hexenyl valerate	apple, banana, cognac, fruity, green, pineapple	0.424406565
WRC0017			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	0.730798863
WRC0038			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	0.825596072
WRC1280			butanedioic acid ester	apple, apricot, chocolate, cooked, cranberry, fruty, grape, musty	0.503947227
WRC0395			cis-3-hexenyl 3-methylbutanoate	apple, fresh, fruity, green, pineapple, tropical	0.60551798
WRC0042			decanoic acid, 2-methylbutyl ester	apple, brandy, fruity, grape, pear, sweet, waxy	0.561543604
WRC0054			dodecanedioic acid ester	clean, floral, soapy, sweet	0.002795841
WRC0016			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	0.356235187
WRC0012			ethyl dodecanoate	clean, floral, soapy, sweet	0.994188846
WRC0010			ethyl nonanoate isomer 1	fruity, rose, rum, tropical, wine	0.907131038
WRC0021			ethyl nonanoate isomer 1	fruity, rose, rum, tropical, wine	0.175222868
WRC0034			ethyl nonanoate isomer 2	fruity, rose, rum, tropical, wine	0.719632188
WRC0037			ethyl nonanoate isomer 3	fruity, rose, rum, tropical, wine	0.101493802
WRC0838			hexanedioic acid ester	NA	0.312289708
WRC1258			hexanoic acid, 2-ethyl-, 1,1-dimethylethyl ester	apple peel, banana, fruity, pineapple, sweet	0.550649618
WRC0044			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	0.839726605
WRC0055			hexanoic acid, ethyl ester	apple peel, banana, fruity, green, pineapple, sweet	0.051542526

Code	Class	Subclass	Metabolite	Sensory (Lit)*	Pvals (FDR adjusted)
WRC0850			hexyl butyrate	apple, apple peel, fruity, green, soapy, sweet, waxy	0.879457245
WRC0492			maltitol	NA	0.910463183
WRC0502			methyl stearate	oily, waxy	0.646711115
WRC0428			methylglutaric acid	NA	0.112482816
WRC0025			n-capric acid isobutyl ester	green, herbal, aldehydic, orange, sweet, vegetable	0.548793871
WRC0018			n-decanoic acid	apple, brandy, fruity, grape, pear, sweet, waxy	0.141449297
WRC0056			octadecanoic acid, 2-(2-hydroxyethoxy)ethyl ester	fatty, waxy	0.581557905
WRC0053			octanoic acid, 3-methylbutyl ester	coconut, fruity, green, pineapple, soapy, sweet	0.87901237
WRC0039			pentadecanoic acid ester	NA	0.853079933
WRC0033			pentadecanoic acid, ethyl ester	NA	0.187855393
WRC0048			pentanoic acid, 3-methyl-, ethyl ester	apple, fruity, green, nutty, pineapple, sweet	0.339326951
WRC0152			picolinyl 2,5-octadecadienoate	NA	0.337535919
WRC1067			propionic acid, ethyl ester	fruity, grape, juicy, pineapple, rum, sweet	0.99407921
WRC0011			stearic acid	NA	6.80E-08
WRC0098			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	0.738933198
WRC0036		fatty alcohols	1,2-hexanediol	NA	0.00910715
WRC0288			5-hexenol	green	0.435604014
WRC0028			octadecane-1,2-diol	NA	3.33E-06
WRC0045			octadecane-1,2-diol	NA	9.27E-09
WRC1044		fatty amides	butyramide	nutty	0.997343871
WRC0638	organic acids	carboxylic acid esters	4-isopropylphenylacetic acid	cumin	0.437490166
WRC0035			acetic acid, 2-phenylethyl ester	acidic, vinegar	0.940262587
WRC0149			acetic acid, hydroxy-, ethyl ester	vinegar, acetic	0.210652518
WRC0679			chicoric acid	NA	0.394625739
WRC0063			cyclohexanecarboxylic acid, hexyl ester	NA	0.166237978
WRC0384			dimethyl malonate	fruity	0.234273712
WRC0188			heptyl 2-methylpropanoate	apple, apricot, cherry, floral, fruity, grape, green, orange, pear, raspberry	0.371211838
WRC0390			isopentyl acetate	banana, bitter, fruity, solvent, sweet	0.485697221
WRC0813			methoxyphenylacetic acid	NA	0.635309546
WRC0194			propanoic acid, ethyl ester	fruity, grape, juicy, pinapple, tropical, rum, sweet	0.642669574
WRC0047			triethyl citrate	acidic	0.798920969
WRC0375			triethyl citrate	fruity, wine	0.987804974
WRC0806		thioesters	ethanethioic acid, s-(1-methylethyl) ester	coffee, fruity, garlic, meaty, onion, sulfur	0.748236927
WRC0061	organoheterocycles	benzodiazines	quinoxaline	NA	0.512520368
WRC0304		benzopyrans	9h-xanthene-9-carboxylic acid 4-iodo-phenyl ester	NA	0.001524444

Code	Class	Subclass	Metabolite	Sensory (Lit)*	Pvals (FDR adjusted)
WRC0299		furanones	2,5-dimethyl-4-(1-pyrrolidinyl)-3(2h)-furanone	cereal	0.001400672
WRC0228		furans	2-pentylfuran	butter, green bean	0.75194507
WRC0020		indoles	1h-indole	NA	0.08572782
WRC0187		lactones	4-hydroxybutanoic acid lactone	NA	0.641354112
WRC0483		lactones	5-methyl-delta-valerolactone	herbal, sweet	0.607247746
WRC0621		pyridines	2-methyl-5-(methylthio)pyrazine	NA	0.232120622
WRC0113			2-pyridinecarboxaldehyde	orange, beer	0.991241272
WRC0374			3-acetoxypyridine	NA	0.280219263
WRC0198			3-butenoic acid, 2-oxo-4-phenyl-	caramel, green, radish, sweet, walnut	0.689164082
WRC0514			3-pyridinecarboxamide	NA	0.946175962
WRC0493			4-pyridinecarboxylic acid	NA	0.280005203
WRC0489			pyridine	amine, fishy, putrid, rancid, sour	0.211677035
WRC0144		pyrimidines	6-amino-4-phenyl-1h-quinazolin-2-one	NA	0.246713835
WRC0015		quinolines	4,8-dimethylquinoline	tea	1.19E-05
WRC0027			quinoline	tea	4.87E-06
WRC0626	organonitrogen compounds	amines	2-diethylaminoethanol	NA	0.530161311
WRC0231		aminoalcohols	ethanol, 2-mercapto-	meaty, sulfur	0.009879577
WRC0146		aminoxides	trimethylamine n-oxide	NA	0.003719152
WRC0049		monoalkylamines	1,2-diamino-2-methylpropane		0.688684214
WRC0377	organooxygen compounds	alcohols	1,3-propanediol	bitter	0.419987827
WRC0095			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.325990024
WRC0079			2,3-butanediol	buttery, creamy, fruit, fruity, onion	0.629553019
WRC0672		aldehydes	5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy	0.551687176
WRC0154			pyrrole-2-carboxaldehyde	ethereal	0.588979868
WRC0041		alkenes	1,2-dimethoxy-ethene	NA	0.975031657
WRC0050		cyclic ketones	5h-inden-5-one, 1,2,3,3a,4,7a-hexahydro-7a-methyl-, trans-	NA	0.403817198
WRC0686		enals	2-butenal, 3-methyl-	almond, cherry, fruity, nutty, sweet	0.285570647
WRC0522			2-propenal	almond, cherry	0.537975444
WRC0642			2-propenal isomer	almond, cherry	0.557811565
WRC0110		ketones	2,4,6-tri-isopropylacetophenone	NA	0.434039049
WRC0376			5-methyl-3-hexen-2-one	berry, cheese, sweet	0.792292059
WRC0184			benzyl ethyl ketone	tea	0.76195861
WRC0631			p-pentylacetophenone	NA	0.405683094
WRC0378		monosaccharide phosphates	.alpha.-d-mannose 1-phosphate	NA	0.345338389
WRC0156		o-glycosyl compounds	isomaltose	sweet	0.330814644
WRC0088			hydroxylamine, o-methyl-	NA	0.994184058

Code	Class	Subclass	Metabolite	Sensory (Lit)*	Pvals (FDR adjusted)
WRC0089	organosulfur compounds	sulfonyls	methyl methanethiosulfonate	sulfur	0.370350209
WRC0013		thiols	1-propene-1-thiol	sulfur	0.000390225
WRC0285			3-mercapto-3-methyl-1-butanol	meat, meat broth, roasted, spicy, sweet, vegetable	0.247417155
WRC0503			ethanethiol	sulfur	0.800788743
WRC0604	phenylpropanoids	chalcones	2,2',4'-trihydroxychalcone	bitter	0.626114597
WRC1022		cinnamaldehydes	3-(4-methylphenyl)-2-propenal	cinnamon, spicy	0.723883409
WRC0230		cinnamic acid esters	isoamyl cinnamate	cocoa, floral, musty, orchid	0.002186943
WRC1015		coumarin glycosides	7-diethylaminocoumarin		0.78508669
WRC0383		coumarins	3,4-dihydro-2h-1-benzopyran-2-one	almond, cinnamon, coconut, coumarin, creamy, herbal, sweet, tobacco	0.518789385
WRC0496			3-hydroxycoumarin	NA	0.515208909
WRC0817			7-methoxycoumarin-4-acetic acid	NA	0.357304062
WRC0125		curcuminoids	curcumin	NA	0.733301025
WRC0173		flavonoids	quercetin 3'-methyl ether	NA	0.960851397
WRC0830			kaempferol 3-o-rutinoside	NA	0.708276963
WRC0207			quercetin 3,5,7,3',4'-pentamethyl ether	orange, oregano	0.28958032
WRC0266		hydroxycinnamic acid esters	trans-ferulic acid	NA	0.527637399
WRC0322		phenols	phenol	NA	0.319535801
WRC0071	prenol lipids	monoterpenoids	linalool	citrus, floral, green, lavender, lemon, orange, sweet	0.372124568
WRC1030			p-menthan-1-ol	NA	0.603670996
WRC0182			trans-geranic acid methyl ester	tea	0.819417786
WRC0284		sesquiterpenoids	alpha-cadinol	herb, woody	0.221398507
WRC0196			alpha-cubebene	herbal	0.512497438
WRC0155			epicubenol	NA	0.571142891

*FoodDB (FooDB, 2017)

Table 5: NP metabolite data.

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP163	alkane	alkane	18-methyl-nonadecane-1,2-diol	alkane, bland	0.820977507
NP110	benzenoids	benzaldehydes	benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	0.317850335
NP225			benzaldehyde-like	almond, bitter, burnt sugar, cherry, sweet	0.118233935
NP496		benzenoids	1-(3,4-dimethylphenoxy)-4-(3,4-dimethylphenylsulfonyl)benzene	benzene	0.803746293
NP034			2-phenylethanol	bitter, floral, honey, lilac, rose, spice	0.311930896

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP105		benzoic acid esters	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester	almond, floral, herb, lettuce, phenolic, prune, sweet, wintergreen	0.180790962
NP397			4-methoxybenzyl phenylacetate	anise, balsam, honey, woody	0.820819112
NP083			allyl benzoate	berry, cherry, floral, sweet	0.763528462
NP045			amyl salicylate	azalea, chocolate, clover, floral, green, herbal, sweet	0.535297473
NP046			benzamide-like	bitter	0.228087936
NP041			butyl salicylate	clover, bitter, harsh	1.70E-05
NP390			ethyl benzoate	anise, balsam, banaba, berry, bitter, cherry, cranberry, fruit, grape, minty, musty, sweet	0.293353318
NP035			octyl benzoate	lemon balm, balsam, fruity	0.662480588
NP226			phenylacetate	flower, honey	0.235788474
NP113			salicylic acid ester	azalea, chocolate, clover, floral, green, herbal, sweet	0.195765125
NP354			4-hydroxybenzoic acid	nutty, phenolic	0.733978339
NP298			benzoic acid-like	bitter	0.770579691
NP146		phenlyacetaldehydes	(e)-2-phenyl-2-butenal	phenolic, black tea	0.036702854
NP407		phenols	1,2-benzenediol	NA	0.354950226
NP122			2-ethylphenol	coffee	0.439152248
NP381			2-methoxy-4-vinylphenol	clove, curry, peanut, smoky, spicy	0.092670956
NP091			phenol-like	phenolic, bitter	0.003886208
NP221			phenol-like	phenolic, bitter	0.002213033
NP379			phenol-like	phenolic, bitter	0.005171709
NP348			vanillylmandelic acid	sweet, vanilla	0.879033502
NP565		thiophenols	2,6-dimethylbenzenethiol	meaty, metallic, phenolic, roasted, sulfurous	0.631834542
NP062	dithioles	1,2-dithioles	dithiole-like	sulfur	0.903187155
NP068	hydrocabons	alkanes	2-methylheptane	NA	0.450605604
NP013	lipids	fatty acid esters	10-undecenoic acid, ethyl ester	clean, cognac, creamy, fruity, musty, soapy, waxy	0.433218647
NP642			2-butenoic acid, phenyl ester	caramel, green, radish, sweet, walnut	0.074371422
NP014			3-methylbutyl octanoate	coconut, fruity, green, pineapple, soapy, sweet	0.450882706
NP398			3-nonenoate	fruity, green, melon, pear, watermelon	0.762275434

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP416			butanoic acid, butyl ester	apple, banana, berry, fruity, peach, pear, pineapple, sweet	0.97634633
NP024			decanoic acid ester	citrus, fatty, rancid, sour	0.323517362
NP047			decyl propionate	cognac, ether, fatty, fruity, rum	0.798749338
NP375			diethyl decanedioate	fruity, melon, quince, wine	0.275910396
NP477			diethyl maleate	banana	0.898365603
NP011			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	0.80589457
NP033			ethyl 9-decenoate	fatty, fruity, green, soapy, waxy	0.718639799
NP012			ethyl decanoate isomer 1	apple, brandy, fruity, grape, pear, sweet, waxy	0.455700326
NP021			ethyl decanoate isomer 2	apple, brandy, fruity, grape, pear, sweet, waxy	0.429589309
NP031			ethyl dodecanoate	clean, floral, soapy, sweet	0.032454099
NP061			ethyl nonanoate isomer 1	fruity, rose, rum, tropical, wine	0.588719884
NP020			ethyl nonanoate isomer 2	fruity, rose, rum, tropical, wine	0.405839502
NP016			ethyl nonanoate isomer 3	fruity, rose, rum, tropical, wine	0.495002938
NP044			ethyl nonanoate isomer 4	fruity, rose, rum, tropical, wine	0.386257527
NP096			ethyl propionate isomer 1	fruity, grape, juicy, pineapple, rum, sweet	0.647053189
NP295			ethyl propionate isomer 2	fruity, grape, juicy, pineapple, rum, sweet	0.105924421
NP325			glutaric acid ester	NA	0.1926714
NP165			glutaric acid, 2-ethylphenyl decyl ester	NA	0.072587176
NP065			heptanoic acid, ethyl ester isomer 1	berry, floral, fruit, green, sweet, waxy	0.636182694
NP066			heptanoic acid, ethyl ester isomer 2	berry, floral, fruit, green, sweet, waxy	0.635579699
NP051			hexadecanoic acid, ethyl ester	balsam, creamy, fruity, milky	0.002178063
NP048			hexanoic acid, ethyl ester isomer 1	apple peel, banana, fruity, green, pineapple, sweet	0.162907235
NP023			hexanoic acid, ethyl ester isomer 2	apple peel, banana, fruity, green, pineapple, sweet	0.301691695
NP025			hexanoic acid, ethyl ester isomer 3	apple peel, banana, fruity, green, pineapple, sweet	0.468028327
NP027			hexanoic acid, ethyl ester isomer 4	apple peel, banana, fruity, green, pineapple, sweet	0.484436555
NP302			isopropyl 2-methylbutanoate	ethereal, fruity, green, pineapple, sweet, tropical	0.651481889
NP197			methyl caprylate isomer 1	green, herbal, aldehydic, orange, sweet, vegetable	0.433209373

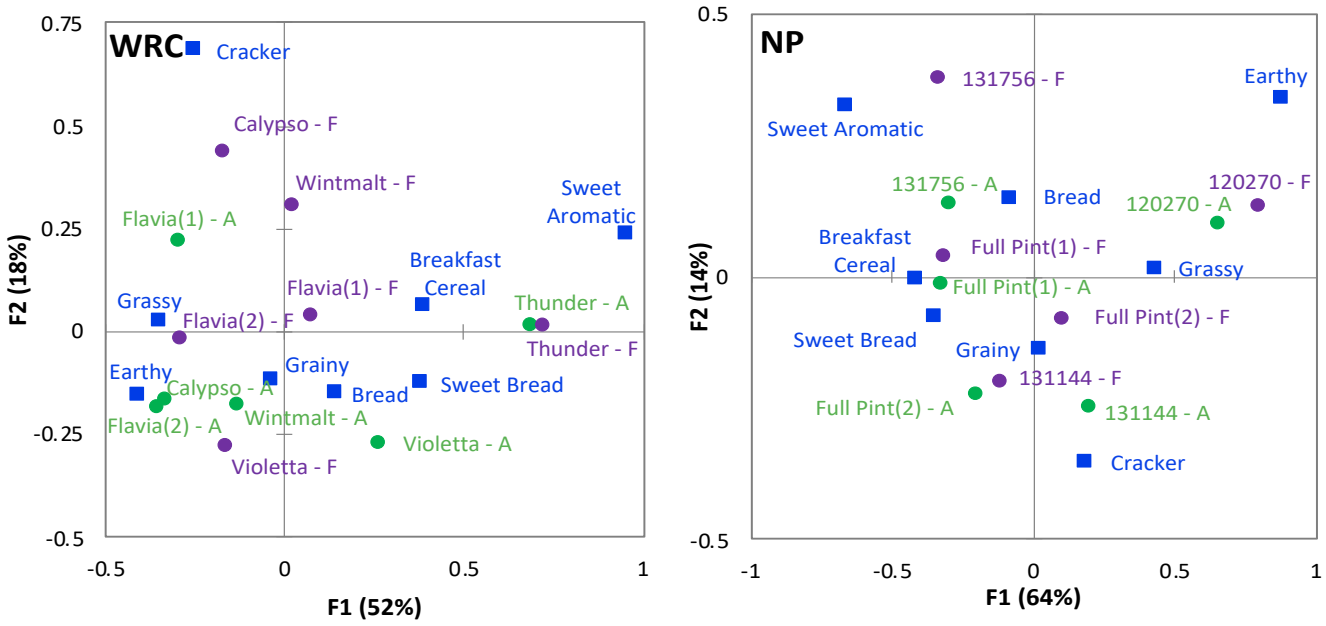
Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP019			methyl caprylate isomer 2	green, herbal, aldehydic, orange, sweet, vegetable	0.558356061
NP026			methyl caprylate isomer 3	green, herbal, aldehydic, orange, sweet, vegetable	0.598024264
NP154			octadecanoic acid, 17-methyl-, methyl ester	fatty, waxy	0.002225769
NP028			pentadecanoic acid, ethyl ester	NA	0.479388132
NP018			pentanoic acid ester	fruity	0.058209287
NP145			pentanoic acid, 2,4-dimethyl-, methyl ester	apple, fruity, green, nutty, pineapple, sweet	0.560264404
NP222			pentanoic acid, 2-methyl	apple, berry, fruity, hazelnut, tropical	0.449636432
NP218			tetradecanoic acid, ethyl ester	ether, soapy, sweet, violet, waxy	0.007519268
NP194		fatty alcohols	1,2-hexanediol	NA	0.107444266
NP064			2-nonen-1-ol	cardboard	0.024587848
NP288			cis-4-decenol	fatty, fruity, waxy	0.009848283
NP097	organic acids	carboximidic acid esters	acetamide	mousy	0.230221297
NP007		carboxylic acid esters	3-mercaptohexyl acetate	floral, fruity, passion fruit, pear, tropical	0.167846964
NP558			3-mercaptopropionic acid	roasted, sulfurous	0.273612561
NP253			acetic acid, 2-methylphenyl ester	vinegar, acetic	0.269204167
NP037			acetic acid, 2-phenylethyl ester	vinegar, acetic	0.274619641
NP038			acetic acid, methyl ester	vinegar, acetic	0.384540956
NP077			acetic acid-like	vinegar, acetic	0.435787556
NP206			acetic acid-like	vinegar, acetic	0.592722648
NP200			ethyl acetate	anise, balsam, ethereal, fruity, green, pineapple, sweet	0.914454224
NP003			ethyl lactate	butter, butterscotch, fruity, tart	0.937872796
NP216			fumarate	NA	0.014266695
NP101			oxalic acid ester	NA	0.754187949
NP040			1-butanol, 2-methyl	banana, fruity, juicy, overripe fruit, peanut, sweet	0.974910926
NP030			isopentyl acetate	banana, bitter, fruity, solvent, sweet	0.829578943
NP022		hydroxy acids	beta-hydroxypyruvic acid	cabbage, sour, radish	0.097822798
NP141			ethyl 2-(methylthio)acetate	apricot, citrus, earthy, floral, fruity, green, herbaceous, meaty, nutty	0.811869944

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP001			ethyl (±)-3-hydroxybutyrate	NA	0.557623706
NP008			hydroxybutyric acid	NA	0.455775994
NP002			malic acid	NA	0.502705377
NP454		keto acids	ketobutyric acid	NA	0.001083814
NP056		benzodiazines	5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	0.008126284
NP150			5-methylquinoxaline-;like	burnt, coffee, corn, nutty, roasted, toasted	0.636594277
NP213		benzopyrans	3,4-dihydro-6-methoxy-2,2-dimethyl-2h-1-benzopyran-4-ol	mushroom	0.135434255
NP036			4-methylene-3,4-dihydroisocoumarin	NA	0.436122366
NP220		benzothiazoles	benzothiazole	coffee, gasoline, meat, nutty, rubber, sulfur, vegetable	0.0089315
NP195		furanones	2(5h)-furanone, 5-methyl-5-phenyl-	NA	0.82022869
NP387			5-methyl-3(2h)-furanone	NA	0.596415839
NP198		furans	2-furoic acid ester	fruity, fungal, mushroom, sweet, tobacco	0.127349619
NP148			2-pentylfuran	NA	0.965172114
NP259			3,4-furandicarboxylic acid	maillard	0.645056711
NP464		heteroaromatic compounds	2-(methoxymethyl)furan	coffee, roasted	0.774502934
NP393			2-(methylthiomethyl)furan	garlic, horseradish, onion, sulfur, vegetable	9.78E-05
NP076			2,5-dimethyl-3-(methylthio)furan	coffee, roasted	0.955702239
NP563			2-propylthiophene	NA	0.715513578
NP049			5-ethyl-(3h)-furan-2-one	spice	0.085242377
NP289			dimethyl furan	onion	0.982367845
NP052			furfuryl ethyl ether-like	coffee, roasted	0.23576453
NP069			furfuryl ethyl ether-like	coffee, roasted	0.0128696
NP497			furfuryl ethyl ether-like	coffee, roasted	0.289188146
NP564			thiophene	garlic, onion	0.551916285
NP545		isocoumarans	isobenzofuranone-like	celery, herbal	0.005936539
NP515		lactones	6-butyloxan-2-one	coconut, coumarin, milky, sweet	0.272856274
NP006		purines	hypoxanthine	NA	0.569643337
NP306			purine-like	maillard	0.078521055
NP102		pyrazines	isopropyl pyrazine	green, honey, minty, nutty	0.135067166
NP541			pyridine-4-carboxylic acid, 2,2,6,6-tetramethyl-4-oxo-1-piperidinyl ester	NA	0.98320676

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP088		pyrazoles	3-nonyl-1h-pyrazole	NA	0.42506264
NP336		pyridines	3-butenoic acid	NA	0.397407913
NP629			4-methylpyridine	tea, fig	0.005421012
NP189			4-vinylpyridine	tea	0.546820787
NP050			4-vinylpyridine-like	tea	0.425012019
NP300			5-methoxypyrimidine	NA	0.457036089
NP278		pyrimidines	2,4-diamino-5,6-dihydroxypyrimidine	NA	0.383821407
NP512		pyrrolidines	2-pyrrolidinone	NA	0.814726786
NP461		pyrrolines	3-acetyl-1h-pyrroline	NA	0.307331654
NP391			1-(4-methyl-1h-pyrazol-1-yl)ethanone	bread, nut, walnut	0.457498715
NP396		quinolines	4,8-dimethylquinoline	tea	0.713273029
NP094		thiazolidines	4,4-dimethyl-thiazolidine	NA	0.589456441
NP333	organooxygen compounds	alcohols	1-(2-furyl)-3-buten-1-ol	fruity, sweet	0.009457476
NP132			1-pentanol	balsam, balsamic, fusel, oil, sweet, vanilla	0.511788116
NP147			2,3-butanediol	buttery, creamy, fruit, fruity, onion	0.64057463
NP262			2-buten-1-ol	NA	0.667521173
NP455			shikimate	NA	0.000122921
NP427		aldehydes	2-methyl-2-heptenal isomer 1	almond, fatty, fresh, green, pungent, soap, vegetable	0.220361903
NP560			2-methyl-2-heptenal isomer 2	almond, fatty, fresh, green, pungent, soap, vegetable	0.685765642
NP426			5-hydroxymethyl-2-furancarboxaldehyde	caramel, cardboard, musty, waxy, fatty	0.47023777
NP386			5-methyl-2-furancarboxaldehyde	almond, burnt sugar, caramel, maple, spice	0.938099444
NP493			nonanal	citrus, fatty, fishy, fresh, grapefruit, lime, orange peel	0.301605406
NP126		aryl alkyl ketones	2-acetylfuran	almond, balsam, beef, caramel, cocoa, coffee, peanut, potato, sweet	0.788318139
NP478		carbonyl compounds	1-phenyl-1-pentanone	balsam, valerian	0.339068118
NP042			2,5-dihydroxybenzaldehyde	NA	0.488085791
NP106			2-acetyl-3-(1-methyl-2-pyrrolyl)-1,4-benzenediol	bread, nut, walnut	0.302971184
NP255			1-hexene	caraway, celery, green, pepper, rooty, spicy	0.526089072
NP176		ethers	1-hexene, 4-methyl-	earthy, green, leafy, mushroom, violet	0.535987054
NP638		ketones	2-nonen-4-one	fruity	0.423825614

Code	Class	Subclass	Metabolite	Sensory (Lit	Pvals (FDR adjusted)
NP428			3-penten-2-one	acetone, fishy, fruity, phenolic	0.439440631
NP060			9-heptadecanone	NA	8.08E-05
NP269		sugar alcohols	galactitol	NA	0.253079402
NP231	organosulfur compounds	thioethers	3-(methylthio)thiophene	NA	0.575457929
NP373		thiols	3-mercapto-3-methyl-1-butanol	meat broth, roasted, spicy, sweet, vegetable	0.541277302
NP299	phenylpropanoids	chalcones	2,4-dihydroxychalcone	NA	0.523516602
NP109		cinnamic acid esters	1-(m-methoxycinnamoyl)pyrrolidine	NA	0.385367507
NP134			propyl cinnamate	amber, musty, vine	0.332854611
NP205			ferulic acid	NA	0.188169394
NP004		flavonoids	epicatechin	NA	0.582793055
NP072	prenol lipids	monoterpenoids	4-isopropylbenzoic acid	NA	0.412935839
NP131			alpha-terpineol	anise, citrus, floral, lilac, mint, oil, pine, terpene, woody	0.104706838
NP634			citral	citrus, lemon, mint	0.044578987
NP039			linalool	citrus, floral, green, lavender, lemon, orange, sweet	0.012505061
NP559			p-menthan-2-one	herbal, minty, spearmint	0.090617749

*FoodDB (FooDB, 2017)



Figures

Figure 1: Hot Steep Malt Sensory Evaluation

Figure 1: Correspondence Analysis from hot steep Projective Mapping (left pane: Western Rivers Conservancy samples, right pane: Next Pint samples) Blue squares indicate sensory attributes, green and purple circles indicate malt steep samples. 1 and 2 designates duplicate observations of the same

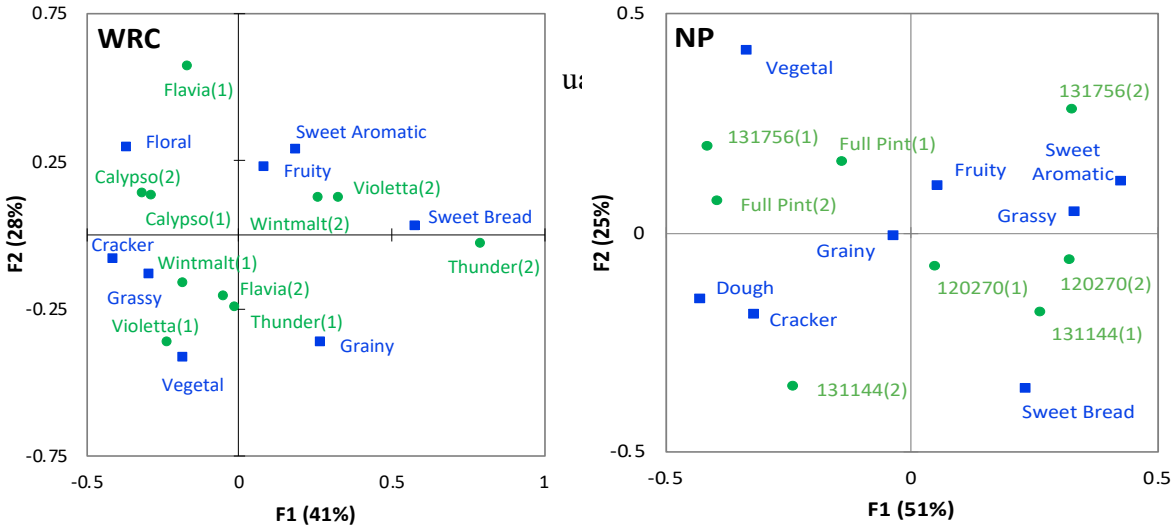


Figure 2: Correspondence Analysis of top 8 most used aroma attributes from beer Projective Mapping with Laboratory Panel (left pane: Western Rivers Conservancy beers; right pane: Next Pint beers) Blue squares indicate aroma attributes, green circles indicate beer samples. 1 and 2 designates duplicate observations of the same samples with different blind codes.
Figure 3: Pie charts of classes

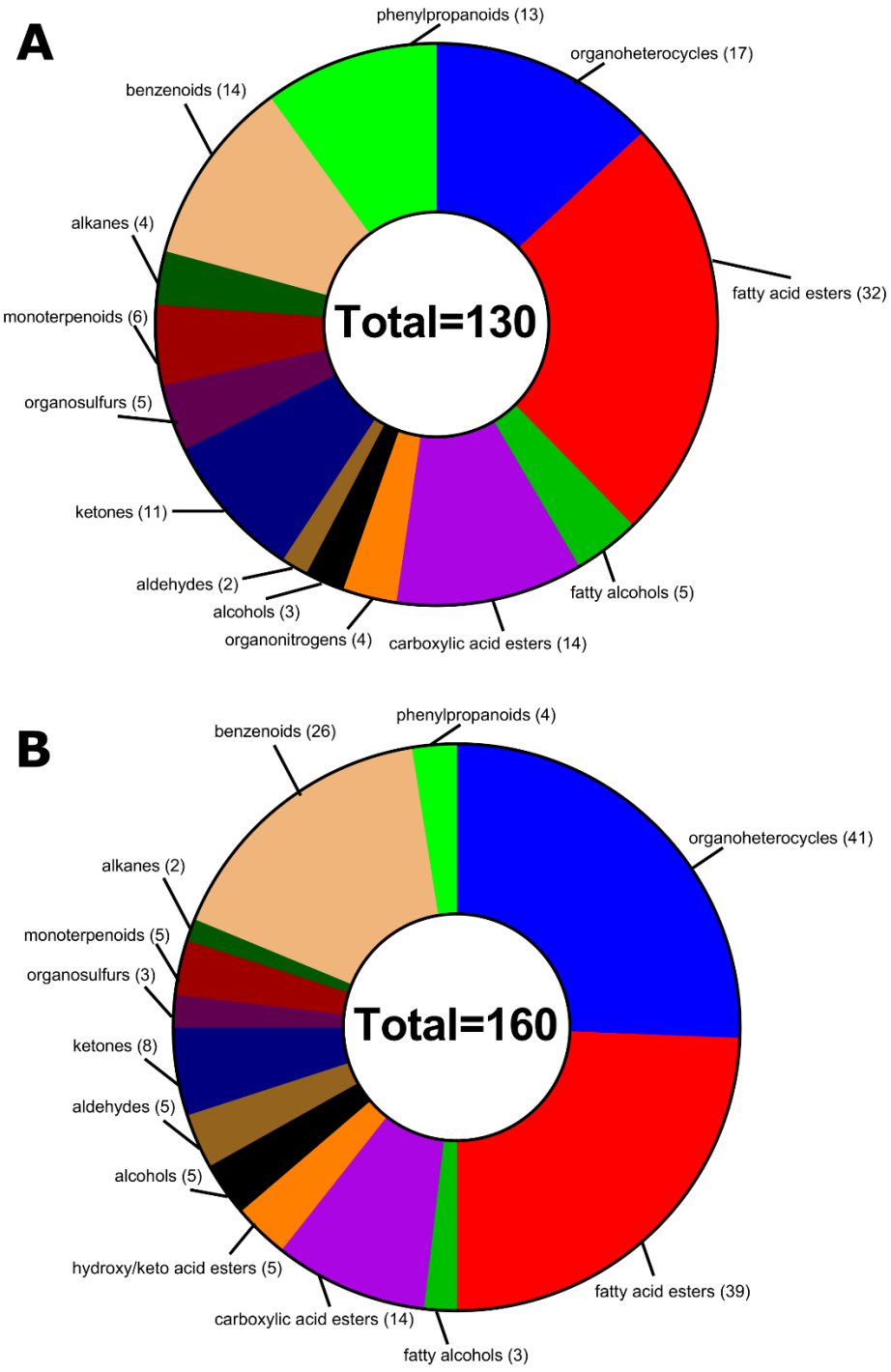


Fig. 3. Annotated beer metabolites and the corresponding chemical classes for WRC and NP datasets. A total of 130 and 160 metabolites were annotated for (A) WRC and (B) NP, respectively. Pie charts display metabolites, by broad class (black text).

Figure 4. PCA scores and loadings plots

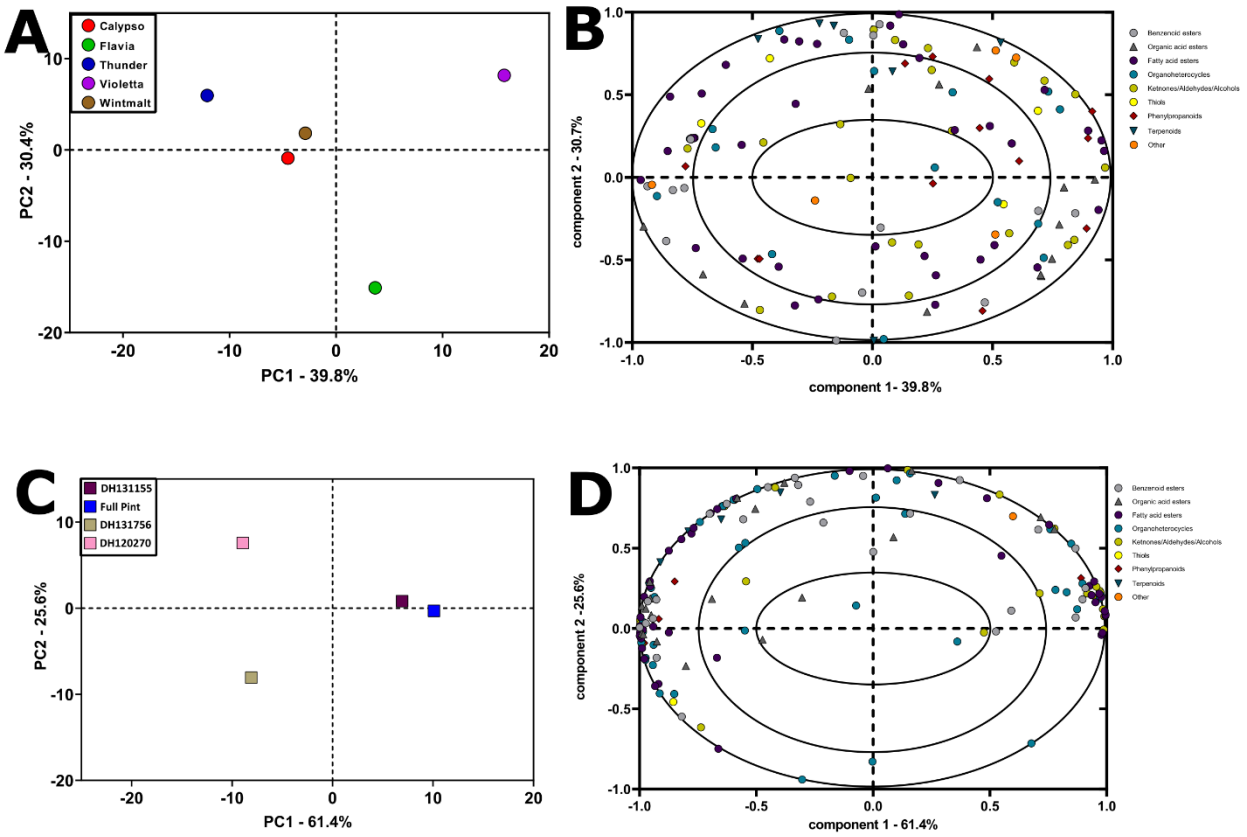


Fig. 4. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, performed on the annotated metabolites for those datasets. PCA scores plots were produced based analysis of the 130 and 160 volatile metabolites, respectively (A) PC1 and PC2 for WRC and (B) corresponding correlation-scaled loadings plot, (C) PC1 and PC2 for NP and (D) corresponding correlation-scaled loadings plot. Loadings were colored according to broad sensory trait.

Figure 5. O2PLS Plots

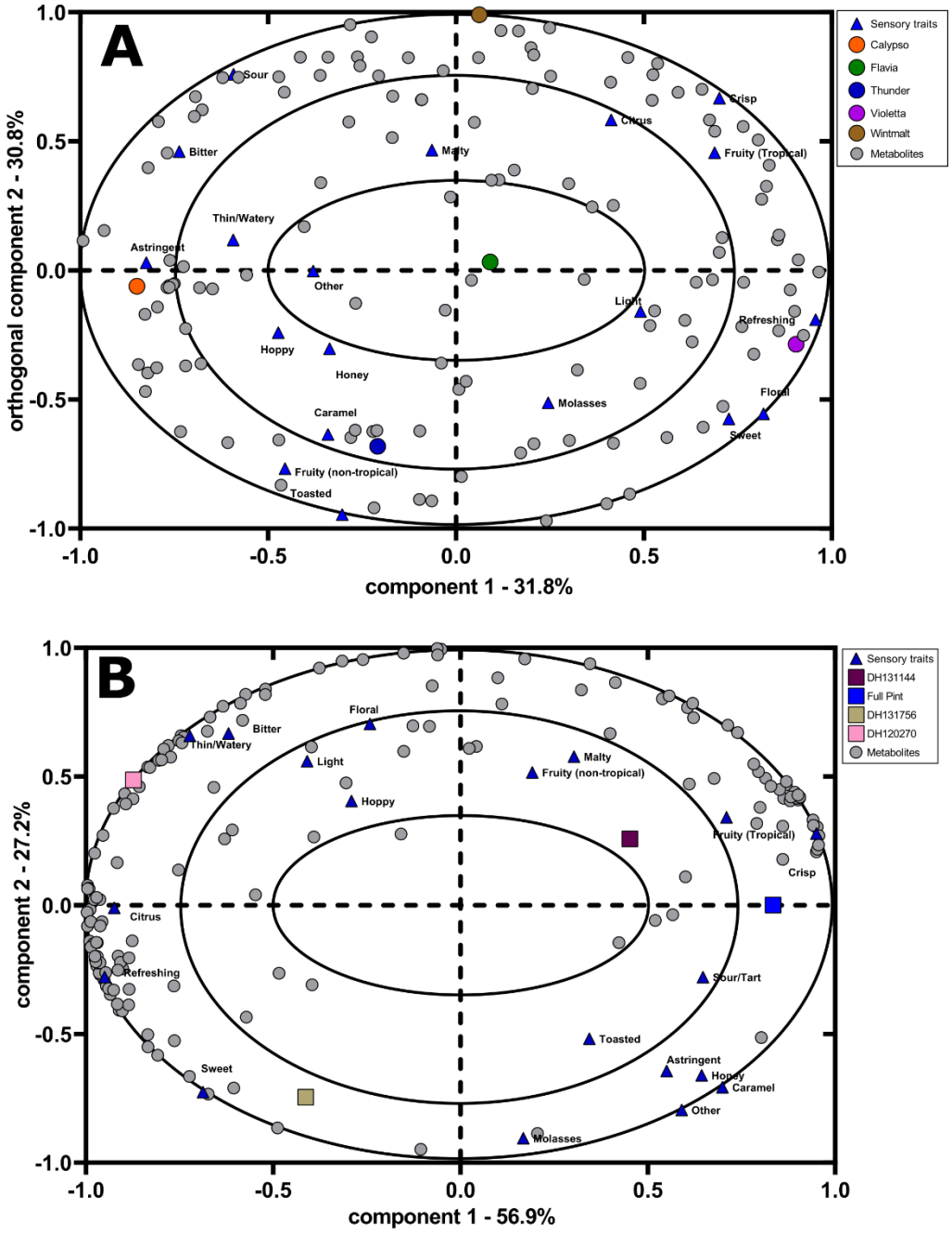


Fig 5. Multivariate association of beer metabolites with consumer panel sensory traits. The association between beer metabolites and consumer panel sensory traits was evaluated with two-way orthogonal partial least squares (O2PLS) and performed on 130 and 160 volatile metabolites, respectively and 20 sensory traits (A) O2PLS scores and loadings plot, for the WRC dataset, of the metabolites (gray circles) and sensory attributes (blue triangles); (B) O2PLS scores and loadings plot, for the NP dataset.

Figure 6. Heatmaps

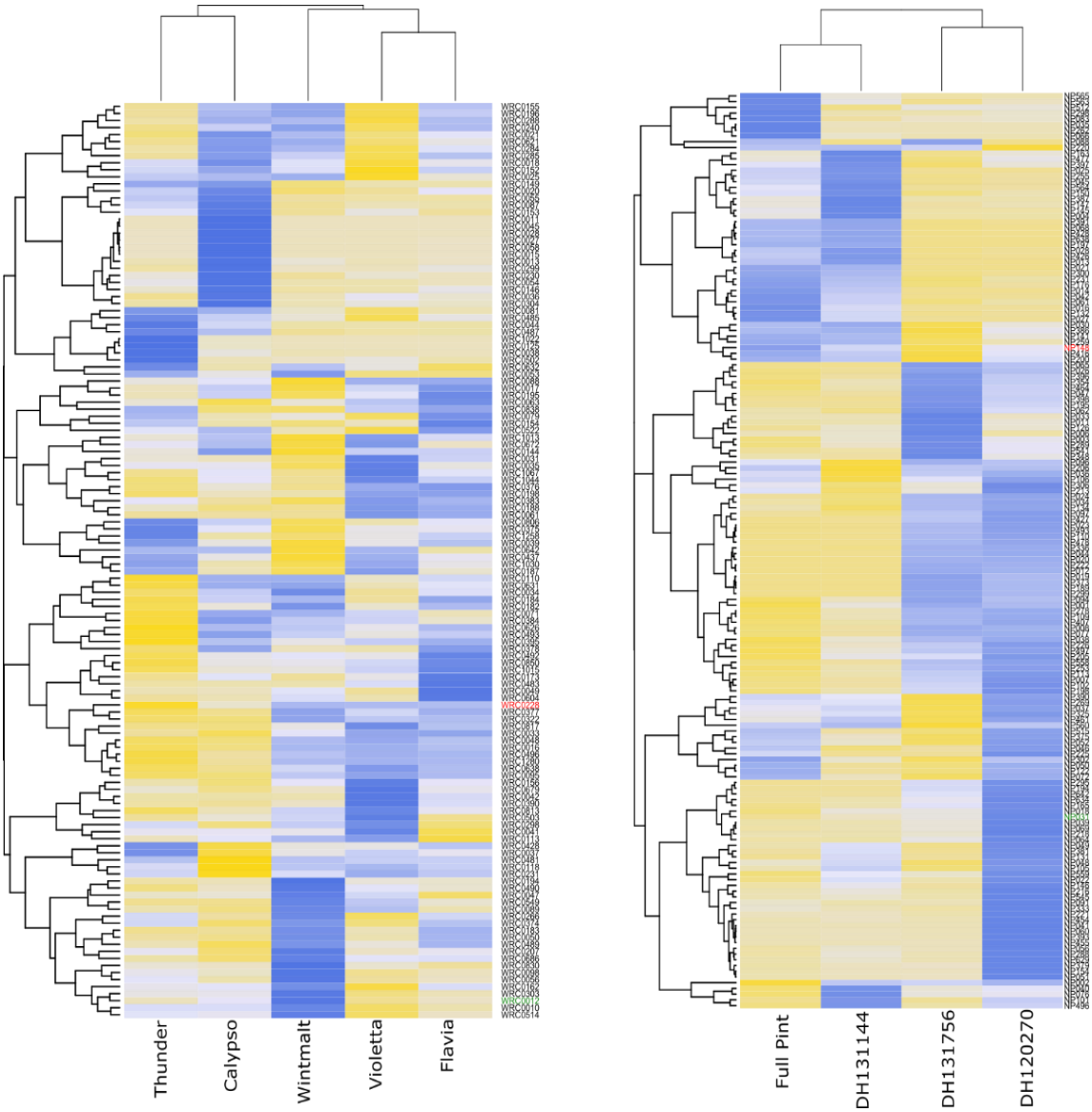


Fig 6. Univariate analysis of volatile metabolite variation among the 9 beers. Prior to heatmapping, volatile metabolite data were normalized within each variety via z-transformation normalized peak area - mean/standard deviation of total peak area of each metabolite).The resulting z-scores were converted into colors and grouped using hierarchical clustering on the Spearman's rank correlation (r^s) between metabolite and sensory trait values. Heat maps with hierarchical clustering were built within for (A) WRC dataset (B) NP dataset. The color in each cell represents the z-transformed abundances of the averaged replicates (n = 2) per beer sample. Z-transformation was based on the mean abundance and standard deviation of the metabolite across all samples. Metabolites in heatmaps are cross-referenced in Tables 3, 4, and Supplemental Tables.

Figure 7. PCA of WRC and NP

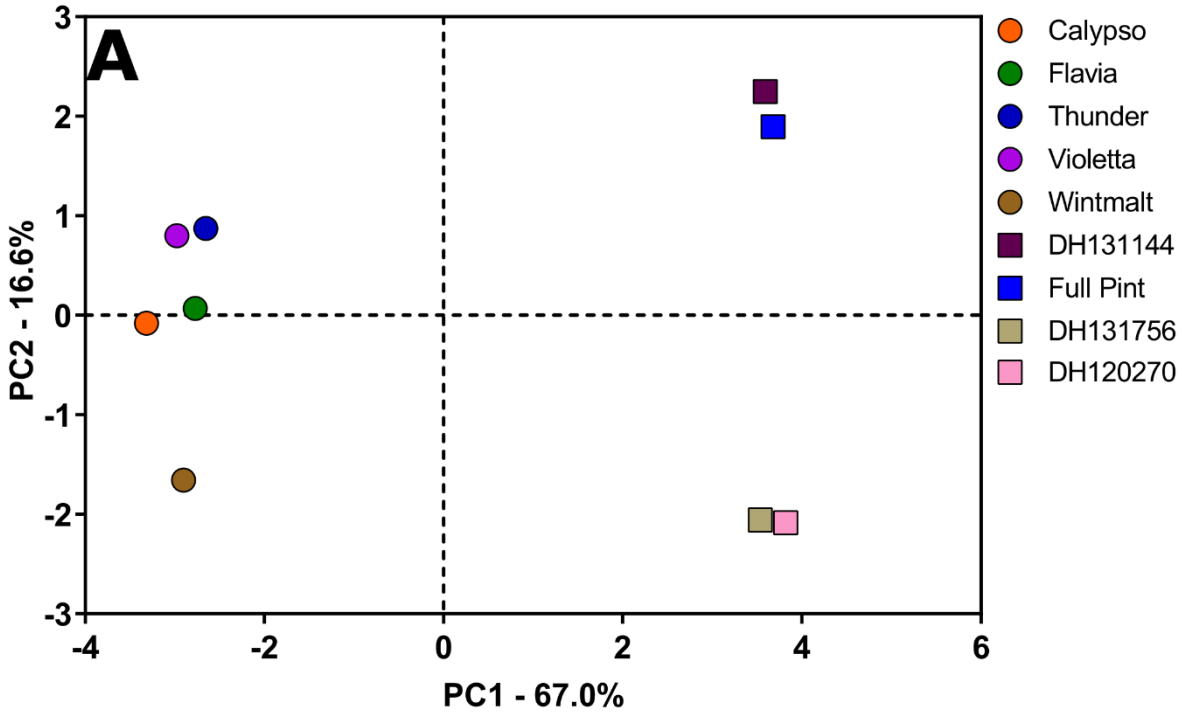


Fig. 7. Principal component analysis (PCA) of beer metabolites of the 9 beers from WRC and NP, combined, performed on the annotated metabolites for those datasets. PCA scores plots were produced based on analysis of 290 metabolites.

Chapter 5: General Conclusions

Barley breeders face the continual challenge of breeding varieties that will not only meet the stringent standards for malting while remaining competitive agronomically for a number of years. Genetic tools can significantly improve this process by pinpointing which genes contribute directly or indirectly to phenotypes of interest. While the barley genome has been sequenced and many genes have been found to contribute to vital agronomic traits (i.e. disease resistance, dwarfism, naked caryopsis), there are still complex characteristics that have yet to be traced back to their genetic origins. Two examples are perennial growth habit and the genetic contributions to beer flavor. Malting barley with a perennial growth habit, defined as a plant life cycle of more than two years, may assist the barley/beer industry to combat climate change while providing beneficial ecosystem services. Recent research exploring the relationship between barley genetic variation and beer flavor has shown that genotype does impact beer flavor. Unraveling the complexities of genotype on phenotypic expression of these quantitative traits offers an additional tool for breeders to develop new varieties and could lead to valuable early variety selection. However, continued research is still necessary to explore the genetic bases of these complex traits.

The economic and environmental sustainability of malting barley production is becoming increasingly fragile in the face of climate change. Breeding a malting barley for perennial growth habit could reduce the environmental impact of barley production. A perennial crop offers deep roots to tap into water sources, stable ground cover to reduce soil erosion, and long-term, undisturbed fields to support a vibrant soil microbiome. These benefits may assist in combating the uncertainties of a variable climate while introducing a sustainable alternative to current agricultural management practices. However, the genetic determinants of perennial growth habit are unknown, making the prospect of breeding a perennial malting barley not only difficult but unlikely to happen within the foreseeable future. In this thesis, we reviewed the incentive for developing perennial malting barley and identified possible

paths towards accomplishing the goal via *i*) direct domestication of a wild perennial relative, *ii*) hybridization between annual and wild perennial barley, *iii*) manipulating genetics of growth habit within the *Hordeum* genome, and/or *iv*) comparative genomic analysis between *Lolium* and *Hordeum*. We concluded that extensive research would be required with a dedicated team, resources, and time in order to achieve a product comparable to annual malting barley. In the meantime, in order to improve the sustainability of current annual malting barley production, alternative agricultural management practices could be implemented. No-till farming and double-cropping have both been shown to decrease soil and water erosion with continual ground cover providing a healthy environment for soil microbiota. Breeding efforts focusing on facultative and winter growth habit barley could also prove beneficial. Taking advantage of winter precipitation during crucial growth stages, winter and facultative varieties may require less water input from growers through the warmer growing season. Using current and upcoming annual malting barley varieties with sustainable available management practices will be more efficient for the barley/beer production supply chain than adapting to the potential disruptions introduced by a perennial alternative.

Contemporary varieties of malting barley are bred to meet exacting standards for malting and brewing. Sufficient agronomic, malting, and brewing performance are all vital criteria for the development of a successful malting barley variety. The contributions of barley varieties to flavor characteristics of beer is currently determined near the end of the variety development process. Sensory assessments, usually of finished beer, identify potential flavor defects with final variety recommendations based on zero defects. Positive selection for flavor, derived from the malted barley grain, and the discovery of new flavor components provide new opportunities. Recently, research has indicated that barley genomic makeup plays an important role in the finished flavor profile of beer. Expanding on this foundation, in this research contemporary barley varieties were found to have distinct, subtle differences that contributed to nuanced flavor profiles of both hot steeps and finished lager beer. This confirms previous findings. Metabolomics provided insights into the

chemical basis of specific sensory descriptors and consumer preferences of beer. Distinct metabolomic profiles attributable to barley variety were found along with covariation of metabolomic profiles and sensory attributes identified by sensory panels. These observations lead to the conclusion that the observed variable metabolites are a direct result of differing genomes that lead to differential responses within the malting and brewing processes. Although none of these measures and procedures were directly predictive of one another, they provide valuable guides for decision-making and variety selection. Brewing and sensory assessment could be utilized earlier during the barley variety development process. In addition, maltsters and brewers could use sensory analysis of beer derived from different barley varieties for the development of new flavor opportunities.

The research results reported in this thesis may assist in supporting the rapidly expanding interest in locally sourced agriculture, industries, and businesses that is mirrored in the craft beer industry. The demand for unique, local flavor with regional ingredients has increased significantly and there is interest in distinctive, even unconventional, barley varieties. Parallel with the local movement is the push for sustainable practices for growing malting barley, such as organic, no-till, and double-cropping, that provide beneficial ecosystem services. One such solution, the conversion of barley from annual to perennial growth habit, has been proposed as a promising strategy to mitigate the negative impacts of agricultural production on the environment. In this thesis, we conclude that there may be other, more cost-effective solutions to achieving greater sustainability in barley production.

The research results reported in this thesis, combined with results from previous studies, indicates that even the subtle differences in genomes between contemporary malting varieties can lead to detectable differences in beer flavor. This leads to new questions: could radical changes resulting from breeding for resilience to climate change alter the genotype of the barley to the extent that the flavor profile of beer is substantially altered? Could alternative management practices also lead to changes in

beer flavor? Barley plays an integral part in the making of beer – and in the flavor stability in the finished product. As barley breeders continually strive to supply superior varieties to growers and the industry that meet agronomic, malting and brewing expectations, consideration must be given to the impact of barley genetics and management on the flavor characteristics of beer.

Bibliography

Alaux, M., J. Rogers, T. Letellier, R. Flores, F. Alfama, C. Pommier, et al. 2018. Linking the International Wheat Genome Sequencing Consortium bread wheat reference genome sequence to wheat genetic and phenomic data. Genome Biol. 19:111. doi:10.1186/s13059-018-1491-4.

Alvarez Prado, S., J.M. Gallardo, R.A. Serrago, B.C. Kruk, and D.J. Miralles. 2013. Comparative behavior of wheat and barley associated with field release and grain weight determination. Field Crops Res. 144:28-33. doi:10.1016/j.fcr.2012.12.018.

AMBA (American Malting Barley Association, Inc.). Guidlines for malting barley breeders. Malting barley breeding guidelines, ideal commercial malt criteria. https://ambainc.org/amba-publications/guidelines-for-malting-barley-breeders/. (accessed February 2020).American Malting Barley Association. 2019. AMBA publications. Am. Malting Barley Assoc., Milwaukee, WI. http://ambainc.org/content/6/amba-publications (accessed June 2019).

Armstrong, J.M., and T.M. Stevenson. 1947. The effects of continuous line selection in *Triticum-Agropyron* hybrids. Emp. J. Exp. Agric. 15:51-66.

ASA, CSSA, and SSSA. 2011. Position statement on climate change. ASA, CSSA, and SSSA, Madison, WI. https://www.crops.org/files/science-policy/asa-cssa-sssa-climate-change-policy-statement.pdf (accessed June 2019).

ASBC Methods of Analysis, online. Sensory Analysis 14. Hot Steep Malt Sensory Evaluation Method. Approved 2017. American Society of Brewing Chemists, St. Paul, MN, U.S.A. DOI:10.1094/ASBCMOA-Sensory Analysis-14.

Bailey, K.L., B.D. Gossen, G.P. Lafond, P.R. Watson, and D.A. Derksen. 2001. Effect of tillage and crop rotation on root and foliar diseases of wheat and pea in Saskatchewan from 1991 to 1998: Univariate and multivariate analyses. Can. J. Plant Sci. 81:789-803. doi:10.4141/P00-152.

Beier, S., A. Himmelbach, C. Colmsee, X. Zhang, R.A. Barrero, Q. Zhang, et al. 2017. Construction of a map-based reference genome sequence for barley, *Hordeum vulgare* L. Sci. Data 4:170044. doi:10.1038/sdata.2017.44.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. B. 57(1):289-300. PubMed PMID: WOS:A1995QE45300017.

Bettenhausen, H.M., A. Benson, S. Fisk, D. Herb, J. Hernandez, J. Lim, et al. 2020. Variation in Sensory Attributes and Volatile Compounds in Beers Brewed from Genetically Distinct Malts: An Integrated Sensory and Non-Targeted Metabolomics Approach. J. Am. Soc. Brew. Chem.1-17. doi: 10.1080/03610470.2019.1706037.

Bettenhausen, H.M., L. Barr, C.D. Broeckling, J.M. Chaparro, C. Holbrook, D. Sedin, A.L. Heuberger. 2018. Influence of malt source on beer chemistry, flavor, and flavor stability. Food Res. Int. 113:487-504. doi: 10.1016/j.foodres.2018.07.024.

Biotech SS. 2017. User Guide to SIMCA 15 2017.

Blattner, F.R. 2009. Progress in phylogenetic analysis and a new infrageneric classification of the barley genus *Hordeum* (*Poaceae*: *Triticeae*). Breed. Sci. 59:471-480. doi:10.1270/jsbbs.59.471.

Brewers Association. 2019. National beer sales & production data. Brewers Assoc. https://www.brewersassociation.org/statistics/national-beer-sales-production-data/ (accessed June 2019).

Briggs, D.E. 1998. Malts and Malting. Blackie Academic and Professional.

Broeckling, C.D., and J.E. Prenni. 2018. Stacked Injections of Biphasic Extractions for Improved Metabolomic Coverage and Sample Throughput. Anal. Chem. 90(2):1147-53. doi: 10.1021/acs.analchem.7b03654.

Broeckling, C.D., A. Ganna, M. Layer, K. Brown, B. Sutton, E. Ingelsson, et al. 2016. Enabling Efficient and Confident Annotation of LC−MS Metabolomics Data through MS1 Spectrum and Time Prediction. Anal. Chem. 88(18):9226-34. doi: 10.1021/acs.analchem.6b02479.

Broeckling, C.D., F.A. Afsar, S. Neumann, A. Ben-Hur, J.E. Prenni. 2014. RAMClust: a novel feature clustering method enables spectral-matching-based annotation for metabolomics data. Anal. Chem. 86(14):6812-7. Epub 2014/06/14. doi: 10.1021/ac501530d. PubMed PMID: 24927477.

Byrne, S.L., I. Nagy, M. Pfeifer, I. Armstead, and S. Swain. 2015. A synteny-based draft genome sequence of the forage grass *Lolium perenne*. Plant J. 84:816–826. doi:10.1111/tpj.13037.

Camper, H.M., C.F. Genter, and K.E. Loope. 1972. Double cropping following winter barley harvest in eastern Virginia. Agron. J. 64:1–3. doi:10.2134/agronj1972.00021962006400010001x.

Chambers, E., and K. Koppel. 2013. Associations of Volatile Compounds with Sensory Aroma and Flavor: The Complex Nature of Flavor. Molecules. 18(5):4887. PubMed PMID: doi:10.3390/molecules18054887.

Chauhan, B.S., G.S. Gill, and C. Preston. 2006. Tillage system effects on weed ecology, herbicide activity and persistence: A review. Aust. J. Exp. Agric. 46:1557-1570. doi:10.1071/EA05291.

Coghe, S., E. Martens, H. D'Hollander, P.J. Dirinck, F.R. Delvaux. 2004. Sensory and Instrumental Flavour Analysis of Wort Brewed with Dark Specialty Malts. J. Inst. Brew. 110(2):94-103. DOI:10.1002/j.2050-0416.2004.tb00188.x.

Cox, C.M., D.L. Van Tassel, J.D. Glover, L.R. DeHaan, and T.S. Cox. 2006. Prospects for developing perennial grain crops. Bioscience 56:649-659. doi:10.1641/0006-3568(2006)56[649:PFDPGC]2.0.CO;2.

Crews, T.E., and D.J. Cattani. 2018. Strategies, advances, and challenges in breeding perennial grain crops. Sustainability 10:2192. doi:10.3390/su10072192.

Cuesta-Marcos, A., M. Muñoz-Amatriaín, T. Filichkin, I. Karsai, B. Trevaskis, S. Yasuda, et al. 2015. The relationships between development and low temperature tolerance in barley near isogenic lines differing for flowering behavior. Plant Cell Physiol. 56:2312-2324. doi:10.1093/pcp/pcv147.

Curwen-McAdams, C., and S.S. Jones. 2017b. Breeding perennial grain crops based on wheat. Crop Sci. 57:1172-1188. doi:10.2135/cropsci2016.10.0869.

Curwen-McAdams, C., M. Arterburn, K. Murphy, X. Cai, and S.S. Jones. 2017a. Toward a taxonomic definition of perennial wheat: a new species x Tritipyrum aaseae described. Genet. Resour. Crop Evol. 7:1651-1659. doi.org/10.1007/s10722-016-0463-3.

DeHaan, L., S. Wang, S. Larson, D. Cattani, Z. Zhang, and T. Kantarski. 2014. Current efforts to develop perennial wheat and domesticate *Thinopyrum intermedium* as a perennial grain. In: C. Batello, et al., editors, Perennial crops for food security. FAO, Rome. p. 72–89.

Ding, J., J. Shen, H. Mao, W. Xie, X. Li, and Q. Zhang. 2012. RNA-directed DNA methylation is involved in regulating photoperiod-sensitive male sterility in rice. Mol. Plant 5:1210-1216. doi:10.1093/mp/sss095.

Djoumbou Feunang, Y., R. Eisner, C. Knox, L. Chepelev, J. Hastings, G. Owen, et al. 2016. ClassyFire: automated chemical classification with a comprehensive, computable taxonomy. J. Cheminform. 8:61-. doi: 10.1186/s13321-016-0174-y. PubMed PMID: 27867422.

Doebley, J.F., B.S. Gaut, and B.D. Smith. 2006. The molecular genetics of crop domestication. Cell 127:1309-1321. doi:10.1016/j.cell.2006.12.006

Eagles, H.A., K. Cane, and N. Vallance. 2009. The flow of alleles of important photoperiod and vernalisation genes through Australian wheat. Crop Pasture Sci. 60:646–657. doi:10.1071/CP09014.

FAOSTAT. 2019. Data. FAO, Rome. http://www.fao.org/faostat/en/#data (accessed June 2019).

FAS. 2011. U.S. regulation of genetically modified crops. Case studies in agricultural biosecurity. Fed. Am. Sci. https://fas.org/biosecurity/education/dualuse-agriculture/2.-agricultural-biotechnology/us-regulation-of-genetically-engineered-crops.html (accessed June 2019).

FDA. 2019. Foods derived from plants produced using genome editing. US Food Drug Admin. https://www.fda.gov/food/food-new-plant-varieties/foods-derived-plants-produced-using-genome-editing (accessed June 2019).

Feuillet, C., P. Langridge, and R. Waugh, 2008. Cereal breeding takes a walk on the wild side. Trends in Genetics 24:24-32. doi:10.1016/j.tig.2007.11.001.

FooDB. 2017. FooDB Version 1.0:[Available from: http://foodb.ca/compounds.

Gasparis, S., M. Kała, M. Przyborowski, L.A. Łyżnik, W. Orczyk, and A. Nadolska-Orczyk. 2018. A simple and efficient CRISPR/Cas9 platform for induction of single and multiple, heritable mutations in barley (*Hordeum vulgare* L.). Plant Methods 14:111. doi:10.1186/s13007-018-0382-8.

Gazza, L., E. Galassi, R. Ciccoritti, P. Cacciatori, and N.E. Pogna. 2016. Qualitative traits of perennial wheat lines derived from different *Thinopyrum* species. Genet. Resour. Crop Evol. 63:209-219. doi:10.1007/s10722-015-0240-8.

Glidewell, C. 2010. Annual ryegrass: A double-edged sword. Noble Res. Inst. https://www.noble.org/news/publications/ag-news-and-views/2010/may/annual-ryegrass-a-double-edged-sword/ (accessed June 2019).

Hajjar, R., and T. Hodgkin. 2007. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. Euphytica 156:1-13. doi:10.1007/s10681-007-9363-0.

Hannaway, D., S. Fransen, J. Cropper, M. Teel, M. Chaney, T. Griggs, et al. 1999. Perennial ryegrass (*Lolium perenne* L.). PNW 503. Oregon State Univ. Ext. Serv., Corvallis.

Hayes, R.C., M.T. Newell, L.R. DeHaan, K.M. Murphy, S. Crane, M.R. Norton et al. 2012. Perennial cereal crops: An initial evaluation of wheat derivatives. Field Crops. Res. 133:68-89. doi: org/10.1016/j.fcr.2012.03.014.

Heidel, A.J., C. Kiefer, G. Coupland, and L.E. Rose. 2016. Pinpointing genes underlying annual/perennial transitions with comparative genomics. BMC Genomics 17:921. doi:10.1186/s12864-016-3274-1.

Herb, D., T. Filichkin, S. Fisk, L. Helgerson, P. Hayes, A. Benson, V. Vega, D. Carey, R. Thiel, L. Cistue, et al. 2017. Malt Modification and Its Effects on the Contributions of Barley Genotype to Beer Flavor. J. Am. Soc. Brew. Chem. 75:354–362. DOI: 10.1094/ASBCJ-2017-4976-01.

Herb, D., T. Filichkin, S. Fisk, L. Helgerson, P. Hayes, B. Meints, R. Jennings, R. Monsour, S. Tynan, K. Vinkemeier, et al. 2017a. Effects of Barley (Hordeum vulgare L.) Variety and Growing Environment on Beer Flavor. J. Am. Soc. Brew. Chem. 75:345–353. DOI: 10.1094/ASBCJ-2017-4860-01.

Herb, D.W., B.M. Meints, R. Jennings, I. Romagosa, M. Moscou, D. Carey, et al. 2017b. Effects of Barley (*Hordeum vulgare* L.) variety and growing environment on beer flavor. J. Amer. Soc. Brew. Chem. 75:345-353.

Heuberger, A.L., C.D. Broeckling, D. Sedin, C. Holbrook, L. Barr, K. Kirkpatrick, et al. 2016. Evaluation of non-volatile metabolites in beer stored at high temperature and utility as an accelerated method to predict flavour stability. Food Chem. 200:301-7. Epub 2016/02/03. doi: 10.1016/j.foodchem.2016.01.022. PubMed PMID: 26830592.

Hisano, H., and K. Sato. 2016. Genomic regions responsible for amenability to *Agrobacterium*-mediated transformation in barley. Sci. Rep. 6:37505. doi:10.1038/srep37505.

Hopworks Urban Brewery. 2016. Long Root Ale: A partnership between Patagonia Provisions and Hopworks. Hopworks Urban Brewery. https://hopworksbeer.com/2016/10/03/long-root-ale-a-partnership-between-patagonia-provisions-and-hopworks/ (accessed June 2019).

Hummel, J., N. Strehmel, C. Bölling, S. Schmidt, D. Walther, J. Kopka. 2013. Mass Spectral Search and Analysis Using the Golm Metabolome Database. The Handbook of Plant Metabolomics: Wiley-VCH Verlag GmbH & Co. KGaA; p. 321-43.

Hyun, Y., C. Vincent, V. Tilmes, S. Bergonzi, C. Kiefer, R. Richter, et al. 2019. A regulatory circuit conferring varied flowering responses to cold in annual and perennial plants. Science 363:409–412. doi:10.1126/science.aau8197.

IBI (International Brachypodium Initiative). 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. Nature 463:763-768. doi:10.1038/nature08747.

Jaikumar, N.S., S.S. Snapp, K. Murphy, and S.S. Jones. 2012. Agronomic assessment of perennial wheat and perennial rye as cereal crops. Agron. J. 104:1716-1726. doi:10.2134/agronj2012.0291.

Johnston, P.A., G.M. Timmerman-Vaughan, K.J. Farnden, and R. Pickering. 2009. Marker development and characterisation of *Hordeum bulbosum* introgression lines: A resource for barley improvement. Theor. Appl. Genet. 118:1429-1437. doi:10.1007/s00122-009-0992-7.

Jones, B.L., 2005. Endoproteases of barley and malt. Journal of Cereal Science Volume 42, Issue 2, September 2005, Pages 139-156.

Jungers, J.M., L.H. DeHaan, D.J. Mulla, C.C. Sheaffer, and D.L. Wyse. 2019. Reduced nitrate leaching in a perennial grain crop compared to maize in the Upper Midwest, USA. Agric. Ecosyst. Environ. 272:63–73. doi:10.1016/j.agee.2018.11.007.

Kasha, K.J., and R.S. Sadasivaiah. 1971. Genome relationships between *Hordeum vulgare* L. and *H. bulbosum* L. Chromosoma 35:264–287. doi:10.1007/BF00326278.

Kaspar, T.C., J.K. Radke, and J.M. Laflen. 2001. Small grain cover crops and wheel traffic effects on infiltration, runoff, and erosion. J. Soil Water Conserv. 56:160–164.

Kiefer, C., E. Severing, R. Karl, S. Bergonzi, M. Koch, A. Tresch, et al. 2017. Divergence of annual and perennial species in the *Brassicaceae* and the contribution of cis-acting variation at FLC orthologues. Mol. Ecol. 26:3437–3457. doi:10.1111/mec.14084.

Kim, D.H., and S. Sung. 2014. Genetic and epigenetic mechanisms underlying vernalization. Arabidopsis Book 12:e0171. doi:10.1199/tab.0171.

Kishimoto, T., A. Wanikawa, K. Kono, and K. Shibata. 2006. Comparison of odor active compounds in unhopped beer and beers hopped with different hop varieties. J. Agric. Food Chem. 54:8855-886. DOI: 10.1021/jf061342c.

Lai, Z., H. Tsugawa, G. Wohlgemuth, S. Mehta, M. Mueller, Y. Zheng, et al. 2017. Identifying metabolites by integrating metabolome databases with mass spectrometry cheminformatics. Nat. Methods. 15:53. doi: 10.1038/nmeth.4512.

Lammer, D., X. Cai, M. Arterburn, J. Chatleain, T. Murray, and S. Jones. 2004. A single chromosome addition from *Thinopyrum elongatum* confers a polycarpic, perennial habit to annual wheat. J. Exp. Bot. 55: 1715-1720. doi: 10.1093/jxb/erh209.

Liscomb, C. 2016. The hot steep sensory method: a rapid and standardized sensory evaluation method for malt flavor. World Brewing Congress.

Lisec, J. 2017. Package "InterpretMSSpectrum". Interpreting High Resolution Mas Spectra2017.

Mascher, M., H. Gundlach, A. Himmelbach, S. Beier, S.O. Twardziok, T. Wicker, et al. 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature 544:427–433. doi:10.1038/nature22043.

Mottram, D.S. 1993. Flavor compounds formed during the Maillard reaction. Thermally generated flavors. ACS Symposium Series. 543: American Chemical Society. p. 104-26.

Nursten, H.E. 2007. The Maillard Reaction. Cambridge: Royal Soc Chemistry. p. 1-214.

Nyborg, M., E.D. Solberg, R.C. Izaurralde, S.S. Malhi, and M. Molina-Ayala. 1995. Influence of long-term tillage, straw and N fertilizer on barley yield, plant-N uptake and soil-N balance. Soil Tillage Res. 36:165-174. doi:10.1016/0167-1987(95)00502-1.

O'Donovan, J.T., and D.W. McAndrew. 2000. Effect of tillage on weed populations in continuous barley (*Hordeum vulgare*). Weed Technol. 14:726-733. doi:10.1614/0890-037X(2000)014[0726:EOTOWP]2.0.CO;2.

Ofir, M., and D. Koller. 1974. Relationship between thermoinduction and photoinduction of flowering and dormancy in *Hordeum bulbosum* L., a perennial grass. Aust. J. Plant Physiol. 1:259-270.

Olaniran, A.O., L. Hiralal, M.P. Mokoena, B. Pillay. 2017. Flavour-active volatile compounds in beer: production, regulation and control. J. Inst. Brew. 123(1):13-23. doi: 10.1002/jib.389.

Oliver, S.N., E.J. Finnegan, E.S. Dennis, W.J. Peacock, and B. Trevaskis. 2009. Vernalization-induced flowering in cereals is associated with changes in histone methylation at the *VERNALIZATION1* gene. Proc. Natl. Acad. Sci. USA 106:8386-8391. doi:10.1073/pnas.0903566106.

Penrose, L.D.J., M. Mosaad, T.S. Payne, G. Ortiz-Ferrara, and H.J. Braun. 1996. Comparison of controls on development in breeding lines from Australian and CIMMYT/ICARDA winter and facultative wheat breeding programs. Aust. J. Agric. Res. 47:1–15. doi:10.1071/AR9960001.

Perrin, L., and J. Pages. 2009. Construction of a product space from the ultra-flash profiling method: application to 10 red wines from the Loire Valley. J. Sens. Stud. 24:372–395. DOI:10.1111/j.1745-459X.2009.00216.x.

Petersen, G., and. O. Seberg. 1998. Molecular studies on the phylogeny of the genus barley (*Hordeum*; *Poaceae*). In: Karp, A. Isaac, P.G., and D.S. Ingram (eds.) Molecular tools for screening biodiversity. Springer, Dordrecht. 437-440. doi: 10.1007/978-94-009-0019-6_78.

Pickering, R.A., A.M. Hill, M. Michel, and G.M. Timmerman-Vaughan. 1995. The transfer of a powdery mildew resistance gene from *Hordeum bulbosum* L. to barley (*H. vulgare* L.) chromosome 2 (2I). Theor. Appl. Genet. 91:1288-1292. doi:10.1007/BF00220943.

Pickering, R.A., S. Malyshev, G. Künzel, P.A. Johnston, V. Korzun, M. Menke, and I. Schubert. 2000. Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor. Appl. Genet. 100:27–31. doi:10.1007/PL00002904.

Pimentel, D., D. Cerasale, R.C. Stanley, R. Perlman, E.M. Newman, L.C. Brent, et al. 2012. Annual vs. perennial grain production. Agric. Ecosyst. Environ. 161:1-9. doi:10.1016/j.agee.2012.05.025.

Pittelkow, C.M., B.A. Linquist, M.E. Lundy, X. Liang, K.J. van Groenigen, J. Lee, et al. 2015. When does no-till yield more? A global meta-analysis. Field Crops Res. 183:156-168. doi:10.1016/j.fcr.2015.07.020.

Renny-Byfiled, X., and J. Wendel. 2014. Doubling down on genomes: polyploidy and crop plants. Amer. J. Bot. 101:1711-1725. doi/abs/10.3732/ajb.1400119.

Risvik, E., J. McEwan, J. Colwill, R. Rogers, and D. Lyon. 1994. Projective mapping: a tool for sensory analysis and consumer research. Food Quality and Preference. 5:263-269. DOI: 10.1016/0950-3293(94)90051-5.

Roth, M., M. Meiringer, H. Kollmannsberger, M. Zarnkow, M. Jekle, and T. Becker. 2014. Characterization of key aroma compounds in distiller's grains from wheat as a basis for utilization in the food industry. J. Agric. Food Chem. 62(45):10873-80. doi: 10.1021/jf503281x. PubMed PMID: 25243324.

Ryan, M.R., T.E. Crews, S.W. Culman, L.R. DeHaan, R.C. Hayes, J.M. Jungers, et al. 2018. Managing for multifunctionality in perennial grain crops. Bioscience 68:294-304. doi:10.1093/biosci/biy014.

Saerens, S.M., F.R. Delvaux, K.J. Verstrepen, and J.M. Thevelein. 2010. Production and biological function of volatile esters in Saccharomyces cerevisiae. Microb. Biotechnol. 3(2):165-77. Epub 2011/01/25. doi: 10.1111/j.1751-7915.2009.00106.x. PubMed PMID: 21255318; PubMed Central PMCID: PMCPMC3836583.

Schinkel, H., and S. Schillberg. 2016. Genome editing: Intellectual property and product development in plant biotechnology. Plant Cell Rep. 35:1487–1491. doi:10.1007/s00299-016-1988-9.

Shewry, P.R., and S.E. Ullrich. 2014. Barley: Chemistry and Technology, Second Edition. AACC International, Inc.

Smith, C.A., E.J. Want, G. O'Maille, R. Abagyan, and G. Siuzdak. 2006. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and

identification. Anal Chem. 78(3):779-87. Epub 2006/02/02. doi: 10.1021/ac051437y. PubMed PMID: 16448051.

Song, X., and X. Cao. 2017. Transposon-mediated epigenetic regulation contributes to phenotypic diversity and environmental adaptation in rice. Curr. Opin. Plant Biol. 36:111–118. doi:10.1016/j.pbi.2017.02.004.

Sprague, S.J., J.A. Kirkegaard, L.W. Bell, M. Seymour, J. Graham, and M. Ryan. 2018. Dual-purpose cereals offer increased productivity across diverse regions of Australia's high rainfall zone. Field Crops Res. 227:119–131. doi:10.1016/j.fcr.2018.08.008.

Stace, C.A. 1987. Triticale: A case of nomenclatural mistreatment. Taxon: 36: 445-452. doi: 10.2307/1221447.

Steinwand, M.A., H.A. Young, J.N. Bragg, C.M. Tobias, and J.P. Vogel. 2013. *Brachypodium sylvaticum*, a model for perennial grasses: Transformation and inbred line development. PLoS ONE 8:e75180. doi:10.1371/journal.pone.0075180.

Tautenhahn, R., K. Cho, W. Uritboonthai, Z.J. Zhu, G.J. Patti, G. Siuzdak. 2012. An accelerated workflow for untargeted metabolomics using the METLIN database. Nat. Biotechnol. 30(9):826-8. doi: 10.1038/nbt.2348. PubMed PMID: WOS:000308705700012.

Team, R.C. 2014. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2014.

Thomas, H. 2013. Senescence, ageing and death of the whole plant. New Phytol. 197:696–711. doi:10.1111/nph.12047.

Thomas, H., H.M. Thomas, and H. Ougham. 2000. Annuality, perenniality and cell death. J. Exp. Bot. 51:1781–1788. doi:10.1093/jexbot/51.352.1781.

Thurston, P.A., D.E. Quain, and R.S. Tubb. 1982. Lipid metabolism and the regulation of volatile ester synthesis in saccharomyces cerevisiae. J. Inst. Brew. 88(2):90-4. doi: 10.1002/j.2050-0416.1982.tb04078.x.

Tsugawa, H., T. Kind, R. Nakabayashi, D. Yukihira, W. Tanaka, T. Cajka, et al. 2016. Hydrogen rearrangement rules: Computational ms/ms fragmentation and structure elucidation using MS-FINDER Software. Anal. Chem. 88(16):7946-58. doi: 10.1021/acs.analchem.6b00770.

Ullrich, S.E. 2011. Barley: Production, improvement, and uses. 1st ed. Blackwell Publ., West Sussex, UK.

Umetrics. MVA FAQ SIMCA –P and Multivariate Analysis Frequently Asked Questions. 2015.

University of Minnesota. 2019. Winter barley. Forever green. Univ. Minnesota. https://www.forevergreen.umn.edu/crops-systems/winter-annual-grains-oilseeds/winter-barley (accessed June 2019).

USDA-NASS. Small grains 2017 summary. USDA Natl. Agric. Stat. Serv. https://usda.library.cornell.edu/concern/publications/5t34sj573 (accessed August 2018).

USDA. 2020. Grain: World Markets and Trade. United States Department of Agriculture: Foreign Agricultural Service. (accessed February 2020). https://apps.fas.usda.gov/psdonline/circulars/grain.pdf

van Ooijen, J.W. 1999. LOD significance thresholds for QTL analysis in experimental populations of diploid species. Heredity 83:613–624. doi:10.1038/sj.hdy.6886230.

Vanderhaegen, B., F. Delvaux, L. Daenen, H. Verachtert, and F.R. Delvaux. 2006. The chemistry of beer aging – a critical review. Food Chem. 95(3):357-81. doi: 10.1016/j.foodchem.2005.01.006.

Vanderhaegen, B.D. and Luk Filip Daenen. 2007. Aging characteristics of different beer types. Food Chem. 103(2):404-12. doi: 10.1016/j.foodchem.2006.07.062.

Verstrepen, K.J., G. Derdelinckx, J.-P. Dufour, J. Winderickx, J.M. Thevelein, I.S. Pretorius, and F.R. Delvaux. 2003. Flavor active esters: Adding fruitiness to beer. J. Biosci. Bioeng. 96(2):110-118. DOI: 10.1016/S1389-1723(03)90112-5.

Vico, G., S. Manzoni, L. Nkurunziza, K. Murphy, and M. Weih. 2016. Trade-offs between seed output and life span- a quantitative comparison of traits between annual and perennial congeneric species. New Phytol. 209:104–114. doi:10.1111/nph.13574.

Vilela, A., L. González-Paleo, K. Turner, K. Peterson, D. Ravetta, T.E. Crews, et al. 2018. Progress and bottlenecks in the early domestication of the perennial oilseed *Silphium integrifolium*, a sunflower substitute. Sustainability 10:638. doi:10.3390/su10030638.

von Zitzewitz, J., A. Cuesta-Marcos, F. Condon, A.J. Castro, S. Chao, A. Corey, et al. 2011. The genetics of winterhardiness in barley: Perspectives from genome-wide association mapping. Plant Genome 4:76–91. doi:10.3835/plantgenome2010.12.0030.

Warnke, S.E., R.E. Barker, G. Jung, S.C. Sim, M.A. Rouf Mian, M.C. Saha, et al. 2004. Genetic linkage mapping of an annual × perennial ryegrass population. Theor. Appl. Genet. 109:294–304. doi:10.1007/s00122-004-1647-3.

Watson, S.J., G.W. Luck, P.G. Spooner, and D.M. Watson. 2014. Land-use change: Incorporating the frequency, sequence, time span, and magnitude of changes into ecological research. Front. Ecol. Environ. 12:241–249. doi:10.1890/130097.

Wendler, N., M. Mascher, A. Himmelbach, F. Bini, J. Kumlehn, and N. Stein. 2017. A high-density, sequence-enriched genetic map of *Hordeum bulbosum* and its collinearity to *H. vulgare*. Plant Genome 10(3). doi:10.3835/plantgenome2017.06.0049.

Wendler, N., M. Mascher, A. Himmelbach, P. Johnston, R. Pickering, and N. Stein. 2015. Bulbosum to go: A toolbox to utilize *Hordeum vulgare/bulbosum* introgressions for breeding and beyond. Mol. Plant 8:1507–1519. doi:10.1016/j.molp.2015.05.004.

Westerbergh, A., E. Lerceteau-Köhler, M. Sameri, G. Bedada, and P.-O. Lundquist. 2018. Towards the development of perennial barley for cold temperate climates: Evaluation of wild barley relatives as genetic resources. Sustainability 10:1969. doi:10.3390/su10061969.

Wishart, D.S., T. Jewison, A.C. Guo, M. Wilson, C. Knox, Y. Liu, et al. 2013. HMDB 3.0--The Human Metabolome Database in 2013. Nucleic Acids Res. 41(Database issue):D801-7. Epub 2012/11/20. doi: 10.1093/nar/gks1065. PubMed PMID: 23161693; PubMed Central PMCID: PMCPMC3531200.

Yu, X., H.Y. Kong, V. Meiyalaghan, S. Casonato, S. Chng, and E.E. Jones. 2018. Genetic mapping of a barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*. Theor. Appl. Genet. 131:2567–2580. doi:10.1007/s00122-018-3173-8.

Zar, J.H. 1972. Significance testing of the Spearman rank correlation coefficient. J. Am. Stat. Assoc. 67(339):578-80. doi: 10.1080/01621459.1972.10481251.

Zhang, S., J. Hu, C. Yang, H. Liu, F. Yang, J. Zhou, et al. 2017. Genotype by environment interactions for grain yield of perennial rice derivatives (*Oryza sativa* L./*Oryza longistaminata*) in southern China and Laos. Field Crops Res. 207:62–70. doi:10.1016/j.fcr.2017.03.007