Effects of Barley (*Hordeum vulgare* L.) Variety and Growing Environment on Beer Flavor

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ABSTRACT

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This research tested the hypothesis that barley genotype can affect beer flavor and assessed the relative contributions of genotype and location to beer sensory descriptors. Golden Promise, Full Pint, 34 of their doubled haploid progeny, and CDC Copeland were grown at three locations in Oregon, U.S.A. Grain from these trials was micromalted and the resulting malts used for nano-brewing. Sensory evaluations were conducted on the nano-brews. Barley genotype had significant effects on many sensory descriptors. The most significant sensory descriptors—when comparing barley genotypes—were cereal, color, floral, fruity, grassy, honey, malty, toasted, toffee, and sweet. Golden Promise was significantly higher in fruity, floral, and grassy flavors, whereas Full Pint was significantly higher in malty, toffee, and toasted flavors. CDC Copeland was closest to neutral for most flavor traits. There were notable differences for some descriptors between locations. New combinations of parental flavor attributes were observed in the progeny. Multitrait analysis revealed regions of the barley genome with significant effects on malting quality and flavor traits. These findings are, of course, applicable only to the barley germplasm tested, the environment sampled, and the protocols used for micromalting and brewing. The necessary larger-scale experiments involving optimized malts and larger volumes of beer are in process.

Keywords: Barley, Beer, Flavor, Genetics, Malt, Terroir, Variety

Barley, in its malted form, is the principal source of fermentable sugars for most beers and some spirits. Because barley malt, rather than barley grain, is used in these applications, flavor contributions to beer are usually ascribed to the malt rather than to the variety. Indeed, the range of malts available from base (e.g., pilsen and pale) to specialty (e.g., Vienna, Munich, crystal, and caramel) to roasted (e.g., chocolate, coffee, and roasted grains) gives the brewer a palette of potential flavors (10,26). Fundamental contributors to malt flavor are Maillard reaction and Strecker degradation products, such as melanoidins, formed during the kilning and roasting stages of malting (10). Understanding differences in malt flavor is the subject of ongoing research (http://blog.brewingwithbriess.com/wp-content/uploads/2016/09/Briess_Whitepaper_ASBCHotSteepMethod.pdf). Because the same variety can be used to make the full spectrum of malts, most emphasis is placed on the suitability of barley cultivars for malting, rather than on the potential favorable contributions of the variety per se.

New barley varieties are rigorously tested for malting suitability through programs set by bodies such as the American Malting Barley Association (AMBA) (www.ambainc.org), the Brewing and Malting Barley Research Institute (www.bmbri.ca), Barley Australia (www.barleyaustralia.com.au), the Canadian Malting Barley Technical Centre (www.cmbtc.com), the European Brewery Convention (EBC) (www.analytica-ebc.com), and agencies within individual countries (e.g., Institute of Brewing and Distilling in the United Kingdom). In addition to malting quality specifications, varieties must meet a comprehensive list of agronomic and brewing (and in some cases distilling) metrics prior to release. The approval process, in its final steps, involves elimination of potential varieties based on negative contributions to beer flavor. Examples of such negative flavors include, but are not limited to, excessive dimethyl sulfide (DMS), diacetyl, and aldehydes resulting from lipoxygenase activity (19,31,41).

Based on the assumption that the malt (or malts) used in a particular brew provide all the desired flavors, aromas, and other sensory attributes from the barley, brewers have explored the major contributions of hops, water, yeast, and adjuncts to beer flavor. The impacts of these materials can be considerable and account for some obvious flavor differences between different beer styles (e.g., between a standard American lager, a saison, and an India pale ale). Therefore, it is not surprising that the barley variety contribution to beer flavor has not been a high research priority. Nonetheless, certain barley varieties are acknowledged by some brewers to have notable flavor attributes. These attributes have been sufficient to ensure the continued production of specific varieties even when newer varieties have superior agronomic characteristics, malting performance, or both (27). Notable examples are Golden Promise, Klages, and Maris Otter. Occasionally, newer varieties, such as Full Pint, attract the interest of the craft malting, brewing, and distilling industries based on their perceived unique contributions to product flavor (26).

Barley production is largely driven by a desire to maximize productivity, consistency, and profitability across as extensive a geographic area as possible (10,23,26). This approach contrasts with the terroir concept, in which growing environment is of paramount importance. Considerable progress has been made in characterizing contributors to terroir in terms of viticulture practices, wine-making practices (6,24,32), and environmental factors (6,28, 33). However, terroir remains elusive in the beer industry; only recently has the term appeared with reference to cereal grains (www.modernfarmer.com/2016/07/wheat-terroir), and it is now

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starting to impact the Scotch whisky (www.bruichladdich.com) and Irish whiskey (www.waterforddistillery.ie) markets.

To test the hypothesis that barley variety contributes to beer flavor, varieties reported to contribute unique flavors to beer (Golden Promise and Full Pint) were selected as parents, the parents were crossed, and 200 doubled haploid progeny were made from the cross. Doubled haploid production is a process, widely used in contemporary barley breeding, that accelerates the breeding process by creating true-breeding (completely homozygous) genotypes (12). For the research described in this report, contributions to beer flavor were based on sensory assessment of nano-beers made from micromalts of the parental varieties, a sample (n = 34) of their doubled haploid progeny, and a malting variety standard (CDC Copeland) grown at three locations in Oregon, U.S.A. (Corvallis, Lebanon, and Madras). The multilocation assessment allowed for comparison of the relative importance of genotype and location on sensory descriptors.

EXPERIMENTAL

Germplasm

Two hundred doubled haploid lines were derived from the F1 generation of the cross of Golden Promise × Full Pint following the anther culture protocol described by Cistué et al. (12). The 200 doubled haploids, referred to as the "Oregon Promise" population, are available to the research community; instructions for accessing seed and data are available at www.barleyworld.org. The doubled haploids were produced in the L. Cistué lab at Estacion Experimental de Aula Dei, Consejo Superior de Investigaciones Cientificas in Aula Dei, Spain, and in the P. Hayes lab at Oregon State University in Corvallis, OR, U.S.A. For this study, a subset of 34 doubled haploids was selected from the full population to ensure adequate levels of agronomic performance: these data are provided in Supplementary Tables I–III.

Field Trials

The 34 doubled haploids, the two parents, and CDC Copeland were grown, using two-replicate randomized complete block designs, at one location in 2014 (Corvallis) and three locations (Corvallis, Lebanon, and Madras) in 2015. All locations are in the state of Oregon, U.S.A. Corvallis and Lebanon are in the high-rainfall Willamette Valley with 110.9 and 114.3 cm of average annual precipitation, respectively. Therefore, no supplemental irrigation was used at these sites. The Corvallis site is located at 44.56°N, 123.26°W, and the Lebanon site is located at 44.53°N, 122.90°W. Madras (at 44.63°N, 121.12°W) was irrigated, because it is located in a 25.4 cm average annual precipitation zone in central Oregon. Plots (9.2 m^2) were sown with a Wintersteiger Plot Seeder XL grain drill and harvested with a Wintersteiger Classic plot combine. Crop management practices (seeding rate, fertilization, and weed control) were in accordance with local best practices and are detailed in Supplementary Table IV.

Micromalting and Malt Quality

Samples from the 2014 experiment were micromalted by the USDA Cereal Crop Research Unit (Madison, WI, U.S.A.) using the procedures described by Mohammadi et al. (27). Samples (250 g each) of the parents from the 2014 trial, and of all entries from the 2015 trials, were micromalted by Rahr Malting Co. (Shakopee, MN, U.S.A.) using a Joe White Malting unit. Each entry from each experiment was malted once. A proprietary internal standard was used to track malting consistency in each of four runs for the 2015 samples. The 2014 samples (parents only) were malted in a separate run. The malting protocol was as follows: steeping, 9 h wet at 11°C, 9 h couch at 11°C with 30% air flow, 8 h wet at 11°C, 17 h couch at 11°C with 30% air flow; germination, 24 h at 15°C with 30% air flow and 50% recirculation and a temperature ramp of 1°C per 24 h until 17°C and then held for 48 h; and kilning, 3 h at 57°C with 50% air flow, 3 h at 60°C with 50% air flow, 6 h at 63°C with 50% air flow, 4 h at 68°C with 50% air flow and 40% recirculation, and 3 h at 71°C with 50% air flow and 40% recirculation. Malt quality analyses were performed following ASBC standard methods (2). Malt quality data are shown in Supplementary Tables V–VII.

Nano-brewing

Nano-beers were brewed using 1) a "commercial" beer recipe developed by New Glarus Brewing Co. (New Glarus, WI, U.S.A.) and 2) a "research" recipe developed by Rahr Malting Co. The commercial recipe was prepared as follows: 116 g of malt (Corvallis, 2014) was milled to approximately 2 mm using a Captain Crush grain mill (41212, Northern Brewer, Roseville, MN, U.S.A.). Brewery water (925 mL) was added to 1.2 L stainless steel beakers and maintained at 68°C (strike water). To each beaker, 115 g of malt grist was added, and the mash temperature was held at 65°C for 30 min. Mash temperature was increased to 70°C at a rate of 1°C/min and held for 60 min. The mash was then cooled from 70°C to room temperature. Mash samples were lautered using high-density polyethylene funnels (10-349A, Fisher Scientific, Hampton, NH, U.S.A.) lined with Ahlstrom 562 fluted filter paper (15 µm particle size) (09-901E, Fisher). Filtration was done over 2.5 h, without sparging, with one stir of the lauter bed at 2 h to improve run-off. Approximately 800 mL of clean wort per sample was collected and standardized to a total volume of 1,100 mL with brewery water. The resultant wort was boiled in 4 L beakers for 1 h to separate denatured proteins, tannins, and fatty acids (trub) from the wort and removed through secondary filtration to achieve a sample volume of 500 mL with a finished alcohol by volume target of 5%. Cascade 45-pelletized hops were added 30 min into the boil at a hop rate of 0.5 g/L for a target bitterness of 12 IBU, assuming 20% utilization. Each wort was standardized to a volume of 650 mL with brewery water, filtered into a 1 L sterile fermentation flask, and cooled to room temperature. Wort samples were pitched at a rate of 14×10^6 cells/mL with New Glarus Brewing ale yeast. After pitching, wort samples were swirled vigorously for 10 revolutions to aerate and mix the yeast. Samples were fermented at room temperature, using air locks with silicone stoppers to avoid excessive oxidation until fully attenuated. The samples were swirled daily during fermentation to prevent foaming during the bottling phase. After 6 days of fermentation, the flasks were cooled for 2 h at 4°C to reduce foaming. Precisely 2.2 g of sugar was added to empty bottles, which were purged with CO_2 before filling with green beer. The bottles were then crowned, labeled, and stored at room temperature to condition for 14 days before sensory analysis.

The research recipe was prepared as follows: 80 g of malt was coarse-milled with a Monster Mill MM3 three-roller mill (40331, Northern Brewer), and 75 g was sieved to a 2 mm particle size into mashing cans, to which 400 mL of distilled water was added. The mash cans were placed in a 25-sample CT4 mash bath (Canongate Technology Limited, Loanhead, U.K.). Two mash cans were prepared per malt sample to obtain the target minimum beer volume of 700 mL. The mashing protocol followed a modified EBC standard method: 1) heat to 45°C and hold to 30 min, 2) ramp temperature from 45 to 70°C at a rate of 1°C per min, 3) hold at 70°C for 1 h, 4) cool from 70 to 22.5°C, and 5) remove samples. The volume of each mash was standardized to 450 mL with distilled water and poured over 30 µm pore size Ahlstrom 562 fluted filter paper (15 µm particle size) (09-901E, Fisher) to separate the clear wort from the grist. The first 100 mL of filtered wort was poured over the grain bed to increase clarity. Each sample was sparged with 100 mL of 78°C distilled water to wash out residual

sugars in the grain bed. Once the collected wort volume reached 500 mL, the two mash cans per sample were combined and dosed with 10 µL of Isohop 30% iso-α-acid concentrate (Barth-Haas Group, Nuremberg, Germany), pipetted for a target of 10–12 IBU assuming 80% utilization. The combined wort samples were boiled for 35 min on a hot plate. The sample volume at the end of boiling ranged from 700 to 800 mL. Therefore, samples were standardized to a target specific gravity of 1.032 by the addition of distilled water. The wort samples were filtered into 1 L media bottles (10754-820, VWR International, Radnor, PA, U.S.A.) to remove trub precipitated during the boil and then placed in a water bath to reduce temperature to 20°C for pitching yeast. WhiteLabs (www.whitelabs.com) Czech Budejovice flavor inert ale yeast was propagated from medium to a slurry 48 h prior to pitching. The wort samples were transferred to ASBC fermentation tubes and pitched with 2.0–3.5 mL of yeast slurry at a rate of 1.0×10^7 cells/mL into wort with a target specific gravity 1.032. The fermentation tubes were placed in a fermentation chamber at 12°C and fermented for 14 days. After fermentation, the green beer was transferred to centrifuge bottles (75006443, Thermo Scientific, Waltham, MA, U.S.A.) and centrifuged at 4,000 rpm in an Eppendorf 5810 R centrifuge to separate sediment from the green beer. Samples were then vacuum sterile-filtered using 0.45 µm vacuum filtration cups (10040-474, VWR) to remove residual yeast before being bottled and carbonated to approximately 10 psi. The bottles were boxed and placed in a refrigerator at 16°C to condition for three weeks. All beers were brewed over 12 days: beers from 11 unreplicated samples from each location (two parents, 34 doubled haploid progeny, and CDC Copeland) were brewed on each of 10 days, and 11 beers of each of the parents were brewed on two days. All brewing sessions included an internal control (Rahr Pils), a light ale made with pilsner and crystal malts and Amarillo and Cascade hops. In total, 145 beers were brewed.

Sensory Evaluation

Separate sensory assessments were conducted at New Glarus Brewing and Rahr Malting. At New Glarus Brewing, the two sensory experts used free-choice descriptions during several blind tastings. Nano-beers were randomized, and each beer was assessed twice. Owing to the subjective nature of these sensory assessments, they could not be used for the statistical and genetic analyses that comprise most of this report. They are, however, of tremendous value for assessing expert opinion and commercializing potential of intrinsic barley flavors. A subset of the New Glarus data are presented in the Discussion section of this report, and the full data are presented in Supplementary Table VIII.

At Rahr Malting, the sensory assessment was based on comparison-to-reference descriptive analysis. Each sample was compared with a reference beer (Miller High Life) to characterize flavors and to estimate variability between samples. Miller High Life was used as the reference because it is a commercially available beer exemplary of an American lager with a consistent flavor profile. The flavor scores ranged from 0 to 8, where 0–3 was less than the reference, 4 was no different from the reference, and 5–8 was greater than the reference. The ballot consisted of 17 sensory descriptors (Supplementary Fig. 1). The full ballot was used for all beers except those from Corvallis, for which the descriptor "color" was not used. Twelve panelists participated in the sensory assessments. A pool of panelists was selected based on prior experience in beer sensory evaluation and availability for the duration of the sensory evaluation process. Panelists were further screened to determine their ability to identify specific compounds using triangle tests of diluted solutions of FlavorActiV samples for basic tastes (sour, sweet, bitter, and salty), DMS, and diacetyl. Selected panelists were trained on the reference beer and FlavorActiV samples to identify ballot descriptors. Panelist performance was assessed after each tasting session to determine consistency.

Owing to the large number of beers involved in this experiment, full replication of sensory assessments was not possible. Therefore, an augmented design (27) was used in which sensory assessments were structured so that in each session there were, first, reference (Miller High Life), parent, (Golden Promise and Full Pint), and check (Rahr Pils) beers and, second, unreplicated parent, doubled haploid, and CDC Copeland beers. Each tasting session included Miller High Life, Golden Promise, Full Pint, Rahr Pils, and 11 unreplicated beers. Tasting samples were approximately 60 mL. A total of 150 beers were tasted over 10 tasting sessions.

Statistical Analysis

Statistical analyses were conducted with JMP Pro statistical software (version 12, SAS Institute, Cary, NC, U.S.A.). A mixed linear model approach was used to analyze the sensory data. First, analyses of variance (ANOVAs) were performed for the sensory descriptors of replicated sensory checks. Student's *t* test was used to calculate *F*-protected least significant differences for mean separation, and the Bonferroni correction was applied to adjust the critical *P* values to reduce the incidence of false positives. The variance from the replicated beers within each session was used to adjust the unreplicated beers for the calculation of best linear unbiased predictors (BLUPs) using restricted maximum likelihood (REML). Panelists and checks were considered fixed effects. Sessions and unreplicated beers nested with sessions were considered random effects. Heritability (h^2) estimates were calculated as described by Comadran et al. (13): $h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_e^2)$, where σ_G^2 represents the genetic variance and σ_e^2 the residual variance. The genetic variance was estimated from the unreplicated doubled haploids and the residual variance from the replicated parents and checks. Principal component (PC) analysis was used to examine the relationships between the sensory descriptors and genotypes based on the correlation matrix formed from the BLUPs (within locations) and sensory checks (across locations). PC analysis was performed using nonrotated correlation matrices.

Genotyping, Linkage Map Construction, and Quantitative Trait Locus (QTL) Analysis

Genotyping of the full Oregon Promise population was performed using, first, a custom Illumina BeadExpress 384-plex array based on previously characterized single nucleotide polymorphisms (SNPs) with a high minor allele frequency across a representative sample of germplasm (15) and, second, Kompetitive Allele Specific PCR (KASP) markers developed from SNPs in the designs of the full Barley Oligo Pooled Arrays (13) and the Illumina iSelect 9K genotyping chip (15). JoinMap 4.1 software (35,39) was used to construct a genetic map that included 251 polymorphic markers, of which 206 were nonredundant. The total map length was 1,311 cM, using the Kosambi function, spread over eight linkage groups. Most marker intervals were <20 cM, except for four regions on chromosomes 3H, 6H, and 7H with intervals >20 cM. Chromosome 1H was composed of two unlinked groups with no polymorphic markers found to bridge the gap in the central portion of the chromosome. Marker orders are in agreement with the consensus genetic map of barley (15).

Individual and multitrait QTL analyses were performed for beer-flavor and malt variables on the average values across the three sites, using a mixed-model procedure implemented in Genstat software (18th edition) (5). Only variables that showed at least one significant QTL, when run independently using a conservative genome-wide significance level, were used for the multitrait QTL analysis, which was carried out simultaneously using malt and flavor traits. Given the exploratory nature of the QTL

analyses associated with the limited population size, in order to reach convergence in the REML algorithm, we used a restricted composite interval mapping strategy by excluding all cofactors on the same chromosome as the potential QTL. Similarly, only genetic predictors calculated at known marker positions were used.

RESULTS

The ANOVAs of sensory descriptor data confirmed that barley genotype had a significant effect on beer flavor and that some sensory descriptors were environment specific. The results described in this section are based on research malts and beers that would not be commercially acceptable. Therefore, the flavor differences detected, and the significance of these differences, might be different if malts were optimized for each genotype tested. Table I shows the sensory descriptors for which the genotype term was significant at one or more locations. Cereal, floral, fruit, grass, honey, malt, sweet, toasted, and toffee were significant at all locations, and color was significant at the two locations where it was assessed. Sulfur, chemical, roasted, and bitter were significant at two locations. Grain was significant at only one location. When data for significant descriptors were plotted across locations, there were changes in magnitude of response but not changes of rank.

The consistent effects of genotype across locations and the lesser, but still important, effects of locations for specific descriptors were apparent in the PC analysis of the nine descriptors with a significant genotype term at all locations (and for color at the two locations where this descriptor was assessed) (Fig. 1). At all locations, Golden Promise and a subset of progeny were associated with fruit and floral and Full Pint and another subset of progeny with toasted and toffee. CDC Copeland was most similar to Golden Promise at Corvallis and the least associated with specific descriptors at Lebanon and Madras. Descriptor relationships were similar across environments, with the highest associations among malt, toasted, and toffee versus floral and fruit. Location effects were most pronounced for grassy and honey. The biplot shown in Figure 1D supports the genotype effects observed for Golden Promise and Full Pint (Fig. 1A–C), using data from the replicated sensory checks. Golden Promise was associated with fruit and floral, and Full Pint was associated with with malt and toffee. Miller High Life was consistent and had no notable associations with specific descriptors. Rahr Pils performed as expected as the high flavor check (Fig. 1D).

The sensory descriptor associations observed in the PC analyses were mirrored by the significance and magnitude of mean differences between replicated sensory checks (Table II). Golden Promise was significantly higher for floral and fruit, whereas Full Pint was significantly higher for malt, sweet, toasted, and toffee. Miller High Life was neutral (with an average score of 3.71) and relatively consistent (with an average standard error of 0.30) across all sensory descriptors—evidence for its value as the reference beer for flavor difference assessments. Rahr Pils usually had the highest scores for all nine descriptors (with an average score of 4.53) and therefore established an "upper limit" for flavors in a beer brewed using base malt, specialty malts, and hops.

The adjusted unreplicated parent sensory values from each location were similar to the replicated sensory values, supporting the validity of the adjusted, unreplicated data on parents and progeny. Golden Promise was significantly higher than Full Pint for floral and fruity at all three locations. Full Pint was significantly higher than Golden Promise for malt and toasted at all three locations, for toffee at Corvallis, and for honey at Madras. CDC Copeland came closest to 4 on the 0–8 scale at each of the three locations (Table III). Within individual environments, Golden Promise and Full Pint had equally and significantly higher color ratings than CDC Copeland, whereas in the case of the replicated beers, Full Pint was substantially darker in color than Golden Promise.

There was significant variation for sensory descriptors among the doubled haploid progeny, and this variation was heritable (Table III). There were also differences for some sensory characteristics between locations. Most sensory descriptors had the highest values at Corvallis, with notable exceptions being cereal and sweet. Offspring with values higher or lower than the parents (transgressive segregants) were observed for all descriptors at Corvallis, for all descriptors except grass at Madras, and for all descriptors except cereal, grass, and honey at Lebanon. Minimum values for the doubled haploid progeny were significantly lower than the low parent for color, grass, honey, malt, sweet, toasted, and toffee. The maximum values were significantly greater than the high parent for cereal at Corvallis and for honey, toasted, and toffee at Madras. Data for each of the doubled haploid selections are shown in Supplementary Tables IX–XI. There were moderate to high (>30%) h^2 estimates for malt and toffee at all locations: these were highest at Lebanon (61.4 and 41.9%, respectively). Heritability estimates for color were high (64.2 and 45.3%) at the

TABLE I
Significance Levels of Key Terms in the ANOVAs of Beer Sensory Descriptor Data from the Assessment of 37 Barley Genotypes at Three Locations in Oregon, U.S.A. (Corvallis, Lebanon, and Madras)[a]

	Corvallis			Lebanon			Madras		
Source	**Panelist**	**Session**	**Genotype**	**Panelist**	**Session**	**Genotype**	**Panelist**	**Session**	**Genotype**
Color	NA	NA	NA	***		***	***		***
Sulfur	*		**	***	**	***	***		
Chemical	***		**	***	*	**	***		
Fruit	*		***	***		***	**		***
Floral	***	*	***	***		**	**		**
Grass	***		**	***		***	***		***
Grain	*			***		***	*	*	
Cereal	***		*	***	*	***	***	***	**
Malt			***	***		***	***	*	***
Toasted			***	***		***	**		***
Honey	***		**	***		*	***		**
Toffee	***		**	***		***	*		***
Roasted	***		**	***			***		***
Sweet	***	**	***	***		***	***		***
Bitter	***			***		**	**	**	**

[a] Genotypes: Golden Promise, Full Pint, 34 Golden Promise × Full Pint progeny, and CDC Copeland. *, **, and *** = $P < 0.05$, 0.01, and 0.001, respectively; blank cells = no significant difference. NA = not applicable; color was not assessed on samples from this location.

two locations where it was assessed. In contrast, h^2 estimates for honey were very low (≤3.2%). Other sensory descriptors had h^2 values falling between the two extremes.

The significant and heritable variation observed for some sensory traits could be assigned to 10 regions in the barley genome. As summarized in Table IV, the multitrait QTL analysis showed that in seven of 10 cases there were malting quality and sensory traits significantly associated with the same molecular marker. Most notable was a region on chromosome 5H, where there were significant associations for all traits, with Golden Promise contributing the higher value (and presumably favorable) alleles for all traits except wort β-glucan, for which Full Pint contributed the higher value (and presumably negative) allele. Golden Promise contributed greater numbers of higher value alleles (11 for malt-

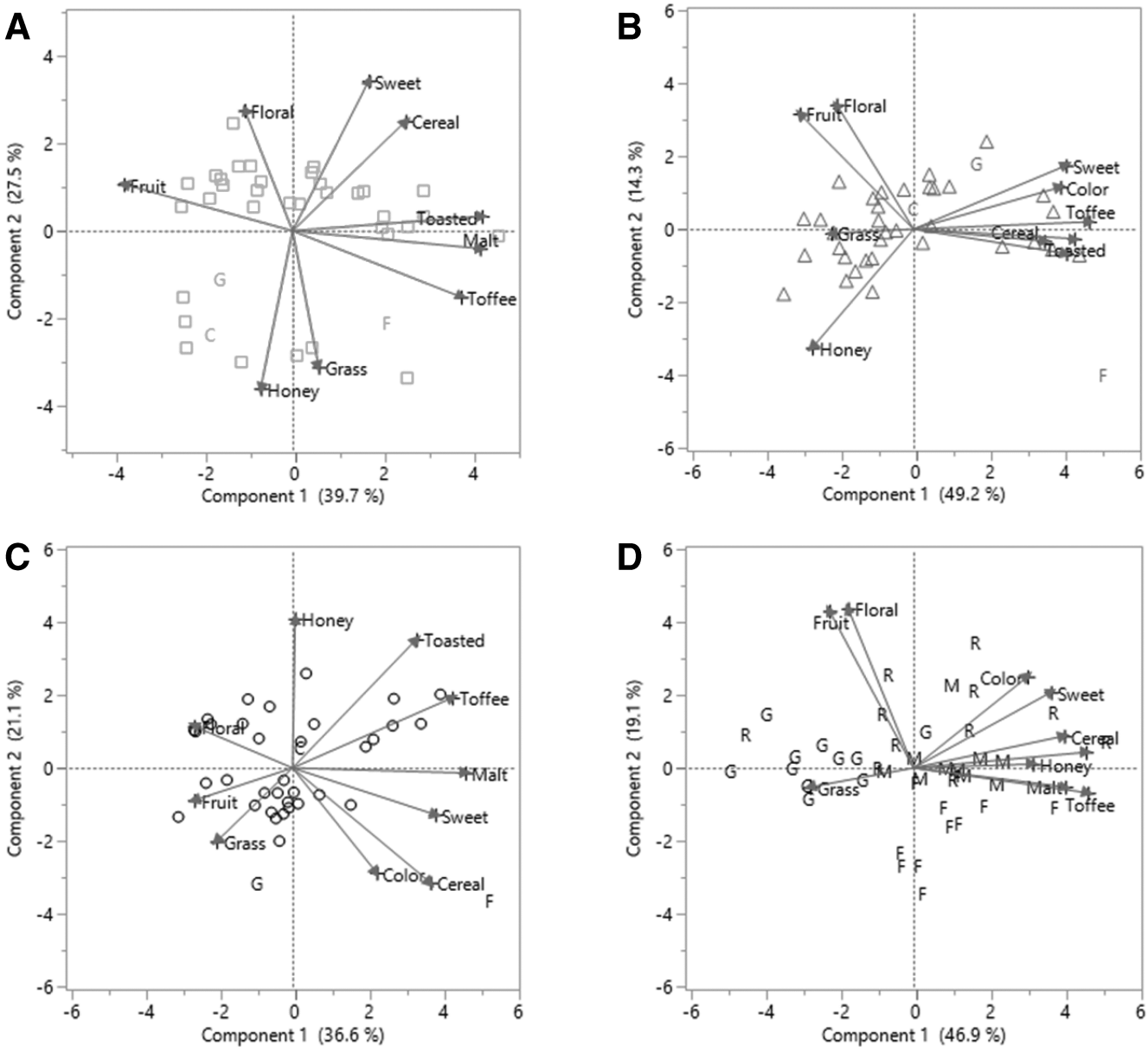

Fig. 1. Principal component analysis of flavors consistently significant across three production environments in Oregon, U.S.A. (Corvallis, Lebanon, and Madras), including parents and field check. Biplots: **A,** Corvallis (square) environment; **B,** Lebanon (triangle) environment; **C,** Madras (circle) environment; and **D,** replicated sensory checks. G = Golden Promise; F = Full Pint; C = CDC Copeland; R = Rahr Pils; and M = Miller High Life.

TABLE II
Means for Beer Sensory Traits Assessed on Replicated Beer Checks (Golden Promise, Full Pint, Rahr Pils, and Miller High Life)

Genotype	Cereal	Color	Floral	Fruit	Grass	Honey	Malt	Sweet	Toasted	Toffee
Golden Promise	3.5	1.8	4.6	5.1	4.7	3.6	2.7	3.7	3.2	2.7
Full Pint	3.8	2.4	3.5	3.5	4.5	3.6	5.1	3.8	4.5	4.3
Rahr Pils	4.1	4.2	4.6	5.2	4.5	4.4	5.1	4.6	4.3	4.0
Miller High Life	3.8	3.8	3.7	3.8	3.7	3.6	3.7	3.8	3.7	3.6
LSD (0.05)	0.6	1.3	0.9	1.1	0.8	0.8	1.3	0.8	0.9	1.0

TABLE III
Adjusted Means (Best Linear Unbiased Predictors) for Beer Sensory Traits Assessed on 37 Barley Genotypes Grown at Three Locations in Oregon, U.S.A. (Corvallis, Lebanon, and Madras) in 2015[a]

Location	Genotype	Cereal	Color	Floral	Fruit	Grass	Honey	Malt	Sweet	Toasted	Toffee
Corvallis	Golden Promise	3.6	…	4.7	5.2	4.7	4.5	3.6	3.6	4.1	3.1
	Full Pint	4.0	…	3.7	3.6	4.8	4.5	5.1	4.3	4.6	4.8
	CDC Copeland	3.7	…	4.0	4.9	4.7	4.5	3.0	2.7	3.2	3.7
	LSD (0.05)	0.9	…	0.8	0.9	0.8	0.9	1.2	1.1	1.3	1.2
	DH minimum	3.5	…	3.4	2.8	3.9	3.1	2.4	2.3	2.8	2.2
	DH maximum	5.1	…	4.7	5.2	4.8	4.5	5.9	4.8	5.6	5.9
	DH mean	4.2	…	4.2	4.3	4.4	3.9	4.1	3.8	4.1	3.6
	Standard error	0.1	…	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
	h^2 (%)	10.2	…	5.8	12	6.3	1.0	49.9	13.4	30	32.5
Lebanon	Golden Promise	3.8	4.6	4.2	5.0	4.4	3.8	3.8	4.6	3.8	3.7
	Full Pint	3.8	4.6	3.3	3.5	4.4	3.9	5.0	4.3	4.2	4.7
	CDC Copeland	3.5	2.9	4.3	4.8	4.4	3.9	4.0	4.4	3.9	3.5
	LSD (0.05)	0.8	0.9	0.6	0.9	0.8	0.9	1.2	1.2	1.1	1.2
	DH minimum	3.4	1.6	3.3	3.4	4.2	3.7	2.2	3.1	3.3	2.8
	DH maximum	3.9	4.4	4.3	5.3	4.7	4.0	5.8	5.0	4.5	4.9
	DH mean	3.6	2.8	4.2	4.5	4.5	3.9	3.9	4.2	3.8	3.5
	Standard error	0.1	0.3	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
	h^2 (%)	7.4	64.2	10.9	21.6	5.4	3.2	61.4	9.7	33.5	41.9
Madras	Golden Promise	3.9	4.5	4.2	4.5	4.6	3.0	3.5	4.2	3.3	2.6
	Full Pint	5.0	4.6	3.2	3.6	4.6	3.9	5.4	4.8	4.0	3.8
	CDC Copeland	3.5	3.5	4.3	4.6	4.6	3.9	3.8	3.9	3.9	4.0
	LSD (0.05)	1.0	0.7	0.8	0.9	0.8	0.7	1.2	1.1	1.2	1.3
	DH minimum	3.0	2.3	3.2	3.4	4.6	3.0	2.5	3.2	2.6	2.6
	DH maximum	5.0	4.6	4.4	5.3	4.6	4.7	5.8	4.9	5.4	5.3
	DH mean	3.6	2.9	4.3	4.2	4.6	3.9	4.0	4.1	3.9	3.7
	Standard error	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
	h^2 (%)	6.9	45.3	2.7	13.3	8.5	1.6	57	20.7	12.3	33.5

[a] Data shown are for the parents, the check, and 34 doubled haploid (DH) progeny. h^2 = heritability.

TABLE IV
Chromosome, Map Position in CentiMorgans, and Significant Marker for Malting Quality and Flavor Trait QTLs Detected in 34 Doubled Haploid Progeny of Golden Promise × Full Pint with *P* Values ≤ 0.05[a]

Chromosome	Position	Marker	Trait	GP	FP	*P* value	R^2
1Ha	67.9	1_0552	Soluble protein	0.31		0.013	9.5
1Ha	67.9	1_0552	Toasted	0.52		0	27.0
1Ha	67.9	1_0552	Toffee	0.41		0.008	16.9
2H	97.7	2_1242	α-Amylase	0.33		0	10.7
2H	97.7	2_1242	Kolbach index	0.36		0.012	13.2
2H	209.1	1_0791	Color		0.25	0.049	6.0
2H	209.1	1_0791	Malt extract	0.47		0.001	21.8
3H	32.5	SCRI_RS_153718	Color	0.37		0.007	13.8
3H	202.3	SCRI_RS_205957	β-Glucan	0.40	0.007	16.1	
3H	202.3	SCRI_RS_205957	Malt extract	0.31	0.026	9.9	
3H	202.3	SCRI_RS_205957	Sweet	0.33		0.038	11.1
5H	32.1	2_1324	α-Amylase	0.23	0.007	5.1	
5H	32.1	2_1324	β-Glucan	0.32		0.015	10.5
5H	32.1	2_1324	Color		0.25	0.028	6.3
5H	32.1	2_1324	Kolbach index	0.30	0.023	8.8	
5H	221.0	MC_46054_7684_F	α-Amylase	0.75		0	56.2
5H	221.0	MC_46054_7684_F	β-Glucan	0.37	0.011	13.3	
5H	221.0	MC_46054_7684_F	Color	0.72		0	52.3
5H	221.0	MC_46054_7684_F	Kolbach index	0.51		0	25.6
5H	221.0	MC_46054_7684_F	Malt extract	0.66		0	44.1
5H	221.0	MC_46054_7684_F	Soluble protein	0.83		0	68.0
5H	221.0	MC_46054_7684_F	Sweet	0.50		0.001	25.4
5H	221.0	MC_46054_7684_F	Toasted	0.46		0	20.8
5H	221.0	MC_46054_7684_F	Toffee	0.41		0.002	16.4
6H	0.0	2_0232	α-Amylase	0.23		0.013	5.2
6H	0.0	2_0232	Toasted		0.49	0	23.7
6H	0.0	2_0232	Toffee		0.35	0.009	12.5
7H	22.8	1_0841	Color	0.26		0.05	6.6
7H	22.8	1_0841	Soluble protein	0.33		0.004	11.1
7H	102.8	1_0431	Toasted		0.65	0	41.6
7H	102.8	1_0431	Toffee		0.74	0	55.1

[a] The additive allele effect is shown for the parent contributing the higher value allele (GP = Golden Promise and FP = Full Pint). R^2 refers to percentage of total phenotypic variance accounted for by the marker. QTLs = quantitative trait loci.

ing quality and nine for sensory traits) than Full Pint (five and six, respectively). Considering the individual allele effects for sensory descriptor traits, Golden Promise contributed more to sweetness (1.0), whereas Full Pint contributed slightly more to toasted (1.0 versus 1.1) and more to toffee (0.8 versus 1.1). Some of the associations accounted for a high percentage of phenotypic variation (e.g., 68% for soluble protein on chromosome 5H and 55% for toffee on chromosome 7H). Five of the QTL positions for malting quality traits detected in this study are in the same chromosomal regions as those recently summarized by Mohammadi et al. (27), an indirect validation of the multitrait QTL strategy employed with this small population size.

DISCUSSION

Multiple lines of evidence generated by this research indicate that there are significant differences in beer flavor owing to barley genotype and that these differences have a genetic basis. There is also evidence that location contributes to beer flavor. This evidence was generated using micromalting, nano-brewing, genetic marker data, and extensive sensory assessment.

Beer sensory experiments are typically conducted using trained panelists, a small number of test beers, and as many replications as possible (25,30). In our experiment, an alternative approach was necessary: we used trained panelists, but to accommodate the large number of samples required, we employed an augmented design that utilized replicated reference beers, checks, and 111 unreplicated beers brewed from malts made from 37 different barley varieties grown in three environments. A comparison of the ANOVA results from this experiment with the literature (34,40) indicates that our sensory approach was successful: significant effects were detected for multiple terms in the ANOVAs of beer flavors. The term "panelist" was significant in all but two of the 42 ANOVAs, which indicates that panelist perception was, in most cases, a significant source of variation. Recent papers involving hop contributions to beer flavor also report that panelist effect was often significant (34,40). Owing to the number of distinct beer samples and replicated checks, this experiment required separate tasting sessions to prevent incurring panelist fatigue. The results of the ANOVAs confirm the importance of partitioning "session" as a source of variation for some sensory descriptors. Most importantly, after accounting for other sources of variation, there were significant "genotype" effects for most of the sensory descriptors at each of the three locations. We focus on these descriptors in this report because there were significant genotype terms at each location, significant differences between replicated reference beers, significant and heritable differences between unreplicated beers, consistent patterns in the PC analyses, and significant QTLs for a subset of the descriptors.

The confirmation of flavor differences between nano-brews made from Golden Promise, Full Pint, and CDC Copeland malts provides crucial evidence that barley variety can contribute significantly to beer flavor. This finding supports the popular perception that Golden Promise can contribute unique flavors to beer and the accumulating evidence that Full Pint can also make unique and significant contributions (26,36). The associations of fruit and floral versus malt, toasted, and toffee are apparent in the PC analyses. These figures also underscore the environmentally dependent nature of flavor scores for honey, sweet, grass, and cereal. The clearest separation of these flavors was in beers made from malts produced from Madras grain: at this location, grass was associated with floral and fruity and Golden Promise; the other flavors were associated with Full Pint.

A comparison of the significance of sensory descriptors across locations suggests that environment can differentially affect the flavor contributions of a barley variety. Although these results are based on one year of data, this suggests that attributes of individual environments may promote specific flavors over others. These environmental factors include, but are not limited to, climate, soil type, irrigation, nutrients, pest control, and other management practices. Harvest and storage conditions (including moisture content, temperatures, and timing) can influence the germination capacity of barley and therefore its suitability for malting and brewing (16,22). Harvest and storage conditions were standardized for these experiments to reduce them as sources of variation. The association of environment and flavor—terroir—is used in the wine industry to differentiate quality attributes based on production region (7,38). Terroir has been used more broadly to describe a range of agricultural products, including meat and dairy (14,32), tea (11), coffee (3), and tequila (9). Additionally, Murphy et al. (29) reported on the development of technologies, such as the Irish single pot still, that may accentuate terroir-based attributes. Terroir in barley has not been reported. On one hand, terroir can be a marketing advantage for specialty malts and locally based maltsters. On the other hand, it can complicate efforts to produce uniform malts from large quantities of barley sourced from multiple environments, when the objective is to produce a consistent product. Whether or not the differences in sensory descriptors observed across locations in this study are intrinsic effects characteristic of these environments or are owing to seasonal variation cannot be answered with the available data. However, based on these preliminary results, a deeper characterization and analysis of the role of environment in barley contributions to beer flavor appears to be warranted.

Two lines of evidence support a genetic basis of barley contributions to beer flavor: heritability and molecular marker–trait associations (QTLs). Heritability estimates ranged from very low (indicating a large environmental effect, complex inheritance, and/or unaccounted for sources of variation in measuring the trait) to very high (indicative of simple inheritance, minimal environmental effects, and/or precise trait assessment). The sensory descriptors associated with the parents (floral and fruity with Golden Promise and malt, toasted, and toffee with Full Pint) showed contrasting patterns of heritability: those associated with Golden Promise were lower and those with Full Pint higher. Gains from phenotypic selection for specific flavors will be greater in high h^2 environments compared with lower h^2 environments.

QTLs for specific sensory descriptors provide an additional line of evidence for a genetic contribution of barley to beer flavor. The finding that both parents have favorable alleles for the same traits (e.g., toasted and toffee) provides a genetic explanation for the transgressive segregant progeny from the cross between them that exceeded the best parent. The QTL results reflect heritability estimates. For example, although floral and fruity were consistently associated with Golden Promise, heritabilities for these descriptors were low, and no QTLs were detected. Larger population sizes are required for detection of small-effect QTLs and more accurate estimates of allele effect and gene location (4). To this end, a mapping experiment is in progress using a larger sample of Oregon Promise doubled haploids. That larger and more robust data set can be used, together with the available barley genome sequence (21) and metabolomic data, to identify candidate genes and biochemical pathways for specific flavors, as described for tomato and cannabis (8,37).

Accurate QTL data can be used as a basis for implementing a range of molecular breeding strategies, from marker-assisted selection to genome editing, for specific flavor components. These marker- and/or gene-based approaches for developing barley varieties with specific attributes are feasible but not straightforward. Bernardo (4), for example, has pointed out the challenges of marker-assisted selection for complex traits, and application of CRISPR genome editing to barley is constrained by the trans-

TABLE V
Averaged Best Linear Unbiased Predictors for Beer Flavor Sensory Descriptors (Rahr Malting) and Free-Choice Descriptors (New Glarus Brewing) on Selected Doubled Haploid Lines from the Oregon Promise Subset Compared with Golden Promise and Full Pint

Genotype	Cereal	Color	Floral	Fruit	Grass	Honey	Malt	Sweet	Toasted	Toffee	Free-choice descriptions
120520	3.7	2.8	5.2	5.3	4.3	4.0	5.3	4.6	4.7	4.4	Malty, sweet
120521	3.6	2.8	4.9	5.2	4.4	4.0	3.0	4.3	4.0	2.4	Bitter, bland, clean, hoppy, lean, malty, neutral
120156	4.0	2.8	4.3	2.8	4.6	3.7	5.9	4.3	5.6	5.9	American, astringent, balanced, crisp, sweet, thin
120166	3.1	2.8	2.7	2.9	4.6	3.6	2.9	3.8	3.2	2.9	Middle of the road
Golden Promise	3.8	2.9	4.5	5.0	4.6	3.8	3.4	4.3	3.7	3.1	Clean, grainy, harsh bitterness, lean, sweet
Full Pint	4.3	2.8	3.4	3.2	4.6	4.1	5.3	4.7	4.6	4.8	European, full, grainy, malty, nice foam, sulfur
LSD (0.05)	0.5	0.2	0.6	0.8	0.3	0.5	0.9	0.7	0.5	0.7	…

formability of barley genotypes. In this context, Golden Promise is one of the most transformation-amenable varieties known, and doubled haploids from the Oregon Promise population have been identified with Golden Promise levels of transformability (20).

The coincidence of associations for malting quality and sensory traits at the same genomic regions suggests that flavor is not an intrinsic variety characteristic independent of malting but rather that flavor develops during malting. However, there does not appear to be a direct causal relationship between malting quality parameters and sensory descriptors. For example, CDC Copeland had the "best" overall malting profile and yet was "flavor neutral." The Golden Promise and Full Pint malting quality profiles did not meet AMBA specifications, and yet these varieties had unique and distinct flavor attributes. A working hypothesis is that the genetic variation observed for differences in flavor contributions to beer is owing to the quantity and composition of substrates, generated during steeping and germination, for the flavor-producing reactions that occur during kilning. Differences in degree of modification are reported to affect malt and beer flavor (1,10,17). The effects of degree of malt modification on beer flavor, under research conditions, are discussed in the companion article that appears in the same issue (18).

Of immediate interest are Oregon Promise doubled haploids with unique flavors and commercial potential. In Table V, the average sensory descriptor values across the three locations are shown for four doubled haploid selections, the two parents, and the CDC Copeland check. DH120156 was high for the flavors characteristic of Full Pint (malt, toasted, and toffee) and low for the Golden Promise flavors (fruity and floral), whereas DH120521 was high for the Golden Promise flavors and low for the Full Pint flavors; DH120166 was a low transgressive segregant for both Golden Promise and Full Pint flavors, and DH120520 was a high transgressive segregant for both Golden Promise and Full Pint flavors. These selections were also among those nano-brewed by New Glarus Brewing Co. to specifications typical of commercial beer and subjected to free-choice flavor profiling. Interestingly, the Rahr and New Glarus assessments coincided for Full Pint and DH120520, but there was no obvious relationship between the two sensory approaches for the other three doubled haploid selections.

A genetic dissection of flavor attributes requires objective sensory assessments, such as those used for the nano-beers brewed and tested at Rahr Malting. However, individual brewers, such as those at New Glarus, may use free-choice descriptors to make critical malt-choice and beer-formulation decisions. It is important to note that in the case of flavor, "positive" terms such as malty are not always a case of more is better, and "negative" terms such as astringency are not always best when absent. Rather, a balance of flavors may be the goal. Experiments are underway engaging maltsters and brewers in larger scale pilot malting and brewing trials to 1) validate the nano-brew results and 2) assess the flavor attributes of selected Oregon Promise doubled haploids in commercial beers.

CONCLUSIONS

This multifaceted and exploratory study implemented high-throughput small-scale malting and brewing coupled with sensory assessment of a large number of beers and provided evidence that 1) barley varieties can make different contributions to beer flavor, 2) growing environment of the barley can have an effect on beer favor, 3) variety contributions to beer flavor have a genetic basis, and 4) variety contributions to beer flavor develop during malting. However, the effects of malt modification may further impact these contributions on beer flavor and are discussed in the companion article (18).

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