Predicting Offspring Performance in Hop (*Humulus lupulus* L.) Using AFLP Markers

J. H. Henning,[1] *USDA-ARS-FSRC, Corvallis, OR;* **M. S. Townsend,** *Department of Crop and Soil Sciences, Oregon State University, Corvallis, OR;* **and P. Matthews,** *S.S. Steiner, Yakima, WA*

ABSTRACT

J. Am. Soc. Brew. Chem. 68(3):125-131, 2010

Genetic information for male and female hop accessions is limited, hampering parental selection to maximize offspring productivity. Our objective was to determine whether amplified fragment length polymorphism (AFLP)-derived genetic distance (GD_{mm}) estimates and coancestry estimates from pedigrees (GS_{ped}) between parental pairs could be used to predict offspring performance. GD_{mm} estimates among 16 cultivars and 31 male accessions were used to create breeding pairs that were either distantly related (HGD) or closely related (LGD). Families were grown under field conditions in a randomized complete block design with three replicates; yield and vigor were recorded in 2006 and 2007. The HGD families had nearly significantly higher yields (LS means = 1.56 kg per plant versus 1.21 kg per plant; $P = 0.056$) and significantly higher vigor (LS means = 7.29 [ordinal scale 1–10] versus 6.54; $P = 0.0001$) than LGD families. GD_{mm} estimates were significantly correlated with yield ($r = 0.2438$; $P = 0.023$) and vigor ($r = 0.2435$; $P = 0.024$). GD_{mm} estimates were also correlated with midparent heterosis (MPH) for vigor ($r = 0.236$; $P = 0.03$) but were not correlated with yield-based MPH or specific combining ability estimates based on yield or vigor. GS_{ped} estimates were negatively correlated with vigor ($r = –0.312$; $P = 0.018$) and vigor-based estimates of MPH ($r = –0.291$; $P = 0.01$). The information from this study suggests that molecular-based GD_{mm} estimates and, in some cases, GS_{ped} estimates can predict superior offspring in a known set of hop accessions.

Keywords: AFLP, Coancestry, Genetic distance, Heterosis, *Humulus*

RESUMEN

La información genética para accesiones de cultivares de los lúpulos masculinos y femeninos es limitada, que dificulta la selección de los padres para maximizar la productividad de las descendencias. Nuestro objetivo fue determinar si el polimorfismo de longitud de fragmentos amplificados (AFLP)-derivados distancia genética (GD_{mm}) las previsiones y estimaciones a partir de pedigríes coancestría (GS_{ped}) entre pares de los padres podría ser utilizado para predecir el rendimiento descendencia. GD_{mm} estimaciones entre 16 y 31 accesiones de cultivares masculinos fueron utilizados para crear parejas reproductoras que eran parientes lejanos (HDG) o están estrechamente relacionadas (LGD). Las familias fueron cultivadas bajo condiciones de campo en un diseño de bloques completos al azar con tres repeticiones, rendimiento y vigor se registraron en 2006 y 2007. Las familias presentaron mayor producción de HDG casi significativa (LS medio = 1.56 kg por planta en comparación con 1.21 kg por planta; $P = 0.056$) y el vigor significativamente superior (LS = 7.29 significa [escala ordinal 1–10] frente a 6.54; $P = 0.0001$) que hizo familias LGD. Estimaciones de GD_{mm} se correlacionaron significativamente con el rendimiento ($r = 0.2438$; $P = 0.023$) y vigor ($r = 0.2435$; $P = 0.024$). Estimaciones de GD_{mm} se puede correlacionar con la heterosis mediopadre (MPH) en vigor ($r = 0.236$; $P = 0.03$) pero no se correlaciona con el rendimiento basado en MPH o específica capacidad de combinarse las estimaciones basadas en el rendimiento o el vigor. Estimaciones de GS_{ped} se correlacionaron negativamente con el vigor ($r = –0.312$; $P = 0.018$) y estimaciones basadas en el vigor de MPH ($r = –0.291$; $P = 0.01$). La información de este estudio sugiere que las estimaciones de base molecular de GD_{mm} y, en algunos casos, las estimaciones GS_{ped} puede predecir descendencia superior en un conjunto conocido de accesiones de lúpulo.

Palabras claves: AFLP, Coancestría, Distancia genética, Heterosis, *Humulus*

Hop is a dioecious perennial climbing vine grown worldwide in regions north and south of the 35th parallel. It is primarily used in the bittering and flavoring of beer, although recent information indicates it has potential as a replacement for antibiotics in livestock feed, particularly for poultry (5), a general antimicrobial agent in sugar processing (24), and a pharmaceutical (28,35). Most hop production in the United States occurs within the states of Washington, Oregon, and Idaho. Hop is typically grown in fields with 4.5 × 1 m spacing on a trellis form that is 6 m tall. New and emerging diseases coupled with continued pressures from established fungal and insect pests drive the need for new and superior hop germplasm and cultivars.

Season-long horticultural practices, fungal pathogens, and insect pressure make hop a high-input, labor-intensive crop. Thus, screening for superior offspring in breeding programs is not a trivial matter; finding new ways to reduce costs and eliminate underperforming offspring and crosses early in the selection process is a high priority. Selection of male parents for yield, morphological, or brewing qualities is difficult due to disparate growth patterns and obvious floral morphology differences between the sexes. Hop breeders generally resort to genetic tests to determine breeding values for male genotypes. The expense, time, and space required for these tests limit the number of male genotypes typically used in breeding programs, and some other means of predicting and identifying superior male parents would benefit breeding efforts. At the same time, avoidance of inbreeding through the choice of unrelated parents is critical when selecting for yield, physiological, and morphological characteristics. Molecular markers offer the most promising means of meeting these hop breeding needs, and prescreening male and female hop accessions for potential additive gene effects and dominance or heterosis before performing a cross could increase the probability of obtaining superior offspring.

Two measures of heterosis are usually found in literature describing the prediction of offspring superiority using molecular markers. The most widely used method, specific combining ability (SCA), is defined as the deviation from the expected value arising from the sum of the general combining abilities (GCA) of both parents (6). The expectation is that offspring performance should equal the sum of each parent's GCA or additive effects. When the offspring from a cross exhibits phenotypic values exceeding the sum of both parents' GCA, this value is termed the SCA for a cross and is equivalent to the dominance effect for a trait. The other measure of heterosis is midparent heterosis (MPH). This value is defined as the phenotypic deviation in offspring from the phenotypic average of both parents (6). In a dioecious crop, with yield based on the female-borne hop cone, the only possible measure of parental value is based on a genotypic value rather than a phenotypic value. Thus, in hop, MPH is calculated as

$$\text{MPH} = \Sigma Xij/nij - [(\Sigma Xi./ni. + \Sigma X.j/n.j)/2] \quad (1)$$

[1] Corresponding author. USDA-ARS-FSRC, 3450 SW Campus Way, Corvallis, OR; E-mail: John.Henning@ars.usda.gov.

doi:10.1094/ASBCJ-2010-0706-01

where $\Sigma Xij/nij$ is the average value of the offspring from cross *ij*; $\Sigma Xi./ni.$ is the average value of offspring from female parent *i*; and $X.j/n.j$ is the average of offspring from male parent *j*.

Quantitative genetic theory suggests that crosses between unrelated individuals should result in superior offspring or high phenotypic values expressed as heterosis (6). Estimating genetic distance (GD) between individuals is accomplished by three methods: 1) theoretical, which is based on numerical pedigree analysis derived from Malecot's (19) coefficient of coancestry (numerically a genetic similarity estimate herein referred to as GS_{ped}); 2) morphological (GD_{mor}), which is based on a set of morphological or physiological traits; and 3) estimate from molecular marker data (GD_{mm}). Recent results from several studies of different crops using these techniques were mixed, with some studies reporting significant correlations between GD measurements and heterosis (9,17,18,30,36), while others reported correlation values that were either low but significant or nonsignificant (1,4,7,26,33).

Several points of interest were noted in these studies. First, the choice of molecular marker technology used to estimate GD appeared to have little influence on prediction of heterosis. However, the use of multiple marker systems increased the likelihood of success (6) by providing greater accuracy in estimates of GD between parents. Furthermore, estimates of GD_{mor} and GS_{ped} were generally unacceptable as methods of predicting heterosis (4,9,18), although some authors did report correlations between GD_{mor} and heterosis (30,34). Estimates based on GS_{ped}, in most cases, were unsuccessful in predicting heterosis, even though they were correlated with GD_{mm} (4,18). In addition, estimates of GS_{ped} underestimated similarity between genotypes compared with GD_{mm} and GD_{mor} estimates and proved inefficient at delineating diversity groups. Finally, many of these studies advanced the theory (2,3) that successful prediction of heterosis is primarily associated with the presence of molecular markers linked to quantitative trait loci (QTLs) controlling the trait in question (1,4,33,36).

While several groups have reported on hop diversity (11,14,15, 20,21,23,25,27,29,31), most of these studies focused on diversity among female cultivars, except for Townsend and Henning (31), who reported diversity among numerous male and female acces-

TABLE I
Hop Cultivars (Female Lines) and Male Accessions Used for Specific Crosses[a]

Cultivar	Male Accession	PDG	GD_{mm}	GS_{ped}	Avg. Yield ± SD (kg/plant)	Yield		Vigor ± SD	Vigor	
						SCA	MPH		SCA	MPH
Brewers Gold	M21488	High	0.38	0.00	1.02 ± 0.50	0.00	–19.17	7.42 ± 1.67	0.29	5.42
Brewers Gold	M64035	High	0.39	0.00	1.62 ± 0.73	0.37	17.61	6.71 ± 2.28	0.08	–1.14
Brewers Gold	M64101	High	0.39	0.00	1.06 ± 0.66	–0.06	–19.36	5.96 ± 1.63	–0.03	–7.88
Brewers Gold	M21300	Low	0.23	0.13	1.35 ± 0.59	–0.02	–6.11	6.33 ± 1.16	–0.32	–6.86
Brewers Gold	M21420	Low	0.20	0.11	0.93 ± 0.55	0.31	–12.31	5.08 ± 2.74	0.64	–10.69
Cascade	M21072	High	0.36	0.00	0.97 ± 0.74	–0.08	–24.16	8.17 ± 0.61	–0.27	6.23
Cascade	M64101	High	0.36	0.00	2.64 ± 0.99	1.13	75.10	7.75 ± 2.26	0.85	11.96
Cascade	M21426	Low	0.24	0.32	1.89 ± 0.84	–0.08	8.63	7.92 ± 1.10	–0.27	4.68
Cascade	M21427	Low	0.25	0.28	1.12 ± 0.67	–0.08	–17.27	6.54 ± 1.22	–0.27	–4.85
Cascade	M21428	Low	0.19	0.30	1.32 ± 0.39	–0.08	–9.16	5.67 ± 2.62	–0.27	–11.97
Challenger	M21435	High	0.39	0.00	1.95 ± 1.01	0.16	18.31	7.88 ± 2.26	–0.64	1.90
Challenger	M21488	High	0.37	0.00	1.66 ± 0.47	0.12	9.05	8.50 ± 1.13	–0.13	9.14
Challenger	M64102	High	0.38	0.00	2.23 ± 1.11	–0.17	14.34	8.63 ± 0.72	0.21	12.31
Challenger	M21268	Low	0.24	0.07	1.03 ± 0.59	–0.48	–31.63	7.92 ± 1.06	0.02	6.68
Challenger	M21335	Low	0.25	0.06	2.06 ± 0.98	–0.13	11.53	7.17 ± 2.12	–0.28	–0.39
Challenger	M21336	Low	0.22	0.06	1.29 ± 0.75	–0.19	13.62	6.75 ± 1.15	–0.96	–7.86
Comet	M21089	High	0.43	0.00	1.87 ± 0.52	–0.06	9.09	6.75 ± 1.86	–0.10	–2.13
Comet	M21488	High	0.37	0.00	1.53 ± 0.89	0.41	16.38	7.58 ± 1.41	0.06	4.85
Comet	M64101	High	0.39	0.00	0.89 ± 0.59	–0.32	–34.17	7.00 ± 0.67	0.61	5.03
Comet	M21313	Low	0.23	0.25	0.43 ± 0.17	0.22	–50.17	6.08 ± 1.86	0.25	–4.78
Comet	M21417	Low	0.24	0.19	1.28 ± 0.85	0.22	–0.39	7.25 ± 2.32	0.25	3.98
Comet	M21465	Low	0.24	0.25	1.74 ± 0.87	0.22	14.73	5.50 ± 1.85	0.25	–9.80
Galena	M21087	High	0.38	0.00	0.97 ± 0.84	0.35	–8.82	6.79 ± 1.70	0.62	3.60
Galena	M64035	High	0.41	0.00	1.49 ± 1.15	0.42	15.96	7.83 ± 2.31	1.10	14.58
Galena	M64101	High	0.39	0.00	1.12 ± 0.69	0.18	–8.50	5.83 ± 0.58	–0.26	–10.52
Galena	M21110	Low	0.28	0.03	0.90 ± 0.41	0.12	–21.19	7.04 ± 1.02	0.73	6.23
Galena	M21345	Low	0.28	0.06	0.80 ± 0.48	0.49	–12.15	5.13 ± 2.83	0.54	–11.08
Galena	M63015	Low	0.29	0.19	0.83 ± 0.64	0.49	–10.37	5.79 ± 2.07	0.54	–5.01
Magnum	M21089	High	0.43	0.00	2.58 ± 0.93	0.06	28.00	7.75 ± 0.99	–0.22	3.94
Magnum	M21488	High	0.38	0.00	1.13 ± 0.35	–0.58	–29.48	7.58 ± 0.52	–1.06	–2.67
Magnum	M64102	High	0.38	0.00	2.06 ± 0.51	–0.52	0.93	8.38 ± 1.19	–0.05	9.01
Magnum	M21110	Low	0.26	0.03	1.64 ± 0.53	–0.01	3.91	6.67 ± 1.94	–1.06	–9.09
Magnum	M21300	Low	0.26	0.05	2.10 ± 0.85	0.05	18.02	9.04 ± 0.66	0.87	19.66
Magnum	M21415	Low	0.24	0.03	1.79 ± 1.02	–0.28	0.10	7.46 ± 1.76	–0.90	–2.50
Mittelfrueh	M19009	High	0.41	0.00	1.51 ± 0.82	0.37	13.94	8.58 ± 0.47	1.17	19.57
Mittelfrueh	M21435	High	0.43	0.00	1.52 ± 1.10	0.16	6.05	7.71 ± 0.73	–0.71	0.37
Mittelfrueh	M21437	High	0.39	0.00	1.11 ± 0.71	0.23	–6.93	6.46 ± 1.91	–0.77	–8.82
Mittelfrueh	M21268	Low	0.20	0.03	1.12 ± 0.86	0.04	–13.40	9.13 ± 0.77	1.32	23.77
Mittelfrueh	M21336	Low	0.20	0.03	0.99 ± 0.65	–0.06	–22.36	6.75 ± 1.30	–0.86	–7.24
Mittelfrueh	M64036	Low	0.21	0.13	1.38 ± 0.71	0.23	4.15	7.63 ± 1.39	–0.77	–0.54
Newport	M21089	High	0.40	0.00	2.23 ± 1.11	–0.68	1.13	6.83 ± 2.27	0.08	–0.24
Newport	M64102	High	0.39	0.00	3.17 ± 0.69	0.21	42.06	8.79 ± 0.60	1.58	24.23
Newport	M21272	Low	0.29	0.02	2.39 ± 1.24	–0.76	2.68	5.21 ± 1.53	0.34	–11.79

(continued on next page)

[a] Plant material used for crosses between specific parent pairs included parent genetic distance groups (PDG: high versus low genetic distance groups) classified based on previously reported (17) genetic distance (GD_{mm}) estimates and coefficients of coancestry (GS_{ped}; as defined by Malecot [19]) derived using amplified fragment length polymorphism. SCA = specific combining ability; MPH = midparent heterosis.

sions. In general, three primary diversity groups have been identified: European, wild American, and hybrids between wild American and European. One recent study (27) identified a fourth diversity group of wild European hops, while another delineated subspecies within wild American germplasm based on molecular marker data (32).

There are no reports on the efficacy of molecular markers, morphological measurements, or coancestry estimates to predict heterosis or offspring performance in hop. Previous work by Henning et al (13) documented genetic diversity between specific male and female pairs, hypothesizing that crosses between genetically diverse hop parents could potentially result in heterotic responses in offspring. The authors determined that estimates of GD_{mm} (in this case amplified fragment length polymorphism [AFLP] markers) were significantly but negatively correlated with GS_{ped}. The report also discussed possible reasons for underperforming offspring from crosses between genetically similar parents and hypothesized that GD_{mm} estimates could be used to predict heterosis in hop.

The objective of this study was to test the hypothesis proposed by Henning et al (13) that GD_{mm} estimates derived from AFLP markers as well as coancestry estimates (GS_{ped}) may be used to predict superior performance in yield in the offspring from specific male and female combinations in hop.

EXPERIMENTAL

Plant Material

Estimates of genetic distance between 19 hop cultivars and 82 male accessions were determined previously using AFLP (13). Based on these estimates, each of 16 hop cultivars were paired with 2 or 3 genetically similar and 2 or 3 genetically distant male accessions (Table I). Each female cultivar was mated with 4–6 male accessions. Male parental accessions were maintained in the field at the USDA-ARS Hop Research facility located near Corvallis, OR. Soil type and growing conditions for this facility were as reported in Henning and Townsend (12). Female cultivars were grown under isolation at the USDA-NCGR North Farm (Corvallis, OR). Soils for this location were predominantly Wapato silty clay loam, and cultural management practices were identical to those previously

TABLE I
(*continued from previous page*)

Cultivar	Male Accession	PDG	GD_{mm}	GS_{ped}	Avg. Yield ± SD (kg/plant)	Yield		Vigor ± SD	Vigor	
						SCA	MPH		SCA	MPH
Newport	M21335	Low	0.28	0.02	1.92 ± 0.78	–0.83	–9.88	6.00 ± 1.23	–0.24	–8.98
Newport	M21336	Low	0.29	0.02	1.62 ± 0.45	–0.43	–8.63	6.17 ± 0.88	–0.34	–8.27
Northern Brewer	M19009	High	0.41	0.00	0.97 ± 0.40	–0.41	–32.89	3.79 ± 1.09	–1.45	–37.76
Northern Brewer	M21435	High	0.41	0.00	2.22 ± 0.48	0.62	43.23	7.83 ± 1.63	1.59	18.81
Northern Brewer	M64102	High	0.38	0.00	1.65 ± 0.34	–0.56	–11.20	5.17 ± 2.64	–0.98	–21.05
Northern Brewer	M21268	Low	0.18	0.27	1.74 ± 0.57	0.42	23.37	4.88 ± 2.37	–0.75	–22.44
Northern Brewer	M21415	Low	0.23	0.03	1.28 ± 0.76	–0.42	–20.25	7.04 ± 2.16	0.96	8.16
Northern Brewer	M21268	Low	0.18	0.27	1.74 ± 0.57	0.42	23.37	4.88 ± 2.37	–0.75	–22.44
Northern Brewer	M21415	Low	0.23	0.03	1.28 ± 0.76	–0.42	–20.25	7.04 ± 2.16	0.96	8.16
Northern Brewer	M21446	Low	0.22	0.30	1.21 ± 0.53	0.31	0.43	4.50 ± 2.10	0.94	-14.34
Nugget	M19009	High	0.37	0.00	1.92 ± 0.97	0.16	17.55	5.50 ± 0.89	–0.90	–17.56
Nugget	M21089	High	0.41	0.00	1.96 ± 0.66	–0.56	–2.58	8.00 ± 1.86	1.15	15.99
Nugget	M21435	High	0.37	0.00	1.61 ± 0.89	–0.36	–7.26	6.18 ± 1.27	–1.22	–13.84
Nugget	M21300	Low	0.23	0.17	1.94 ± 0.50	–0.12	8.66	5.83 ± 1.07	–1.22	–16.63
Nugget	M21415	Low	0.20	0.17	2.01 ± 0.82	–0.07	12.19	7.96 ± 1.53	0.72	12.24
Orion	M19009	High	0.40	0.00	1.47 ± 0.15	0.07	1.18	6.75 ± 0.84	0.32	0.97
Orion	M21435	High	0.40	0.00	1.61 ± 0.55	–0.01	2.86	7.88 ± 1.13	0.44	9.58
Orion	M64102	High	0.37	0.00	1.80 ± 0.85	–0.42	–3.25	7.08 ± 2.36	–0.25	–0.76
Orion	M21087	Low	0.22	0.00	1.25 ± 0.77	0.11	–5.21	6.63 ± 1.77	0.14	–1.34
Orion	M21268	Low	0.21	0.07	1.39 ± 0.75	0.04	–2.61	6.21 ± 1.55	–0.61	–9.75
Orion	M58111	Low	0.22	0.00	1.67 ± 0.79	–0.03	4.43	5.79 ± 2.20	0.22	–7.44
Perle	M21435	High	0.45	0.00	1.36 ± 0.70	–0.09	–7.67	7.88 ± 2.04	0.41	9.30
Perle	M21461	High	0.43	0.01	1.40 ± 0.88	0.14	–17.43	8.75 ± 1.02	0.18	12.84
Perle	M64102	High	0.43	0.00	1.61 ± 0.98	–0.44	–9.31	7.21 ± 2.26	–0.16	0.73
Perle	M21268	Low	0.25	0.13	1.50 ± 0.61	0.33	12.15	6.08 ± 1.42	–0.77	–11.80
Perle	M21446	Low	0.25	0.15	0.93 ± 0.84	0.18	–17.43	3.88 ± 0.65	–0.91	–33.93
Saxon	M19009	High	0.39	0.00	1.34 ± 0.81	0.20	1.55	6.79 ± 0.86	–0.38	–3.78
Saxon	M21435	High	0.41	0.00	0.99 ± 0.31	–0.37	–30.60	7.92 ± 1.00	–0.26	4.72
Saxon	M21268	Low	0.22	0.02	1.51 ± 0.70	0.43	16.85	7.04 ± 0.60	–0.52	–2.91
Saxon	M21336	Low	0.19	0.02	1.24 ± 0.58	0.18	–3.21	8.13 ± 0.82	0.75	13.53
Target	M19007	High	0.37	0.00	1.43 ± 0.48	–0.16	–7.76	5.08 ± 2.05	1.07	–7.22
Target	M21089	High	0.43	0.00	2.08 ± 1.37	–0.23	8.94	6.17 ± 1.58	0.13	–4.95
Target	M21435	High	0.37	0.00	2.28 ± 0.74	0.52	39.77	6.63 ± 1.35	0.04	–2.04
Target	M21300	Low	0.24	0.01	1.30 ± 0.71	–0.54	–22.24	6.50 ± 1.98	0.27	–1.33
Target	M21336	Low	0.25	0.06	1.26 ± 0.37	–0.20	–15.03	5.00 ± 2.35	2.47	29.72
Viking	M19009	High	0.41	0.00	0.80 ± 0.35	0.06	–28.74	8.13 ± 0.59	0.52	11.69
Viking	M21435	High	0.43	0.00	1.29 ± 0.33	0.33	4.68	7.92 ± 0.72	–0.69	1.81
Viking	M21268	Low	0.22	0.02	0.73 ± 0.57	0.06	–32.90	8.67 ± 0.66	0.67	16.05
Viking	M21336	Low	0.22	0.02	0.98 ± 0.53	0.33	–9.31	8.25 ± 0.45	0.45	11.90
Viking	M21446	Low	0.22	0.02	0.54 ± 0.37	0.29	–38.47	6.54 ± 1.05	0.61	1.64
Yeoman	M19009	High	0.39	0.00	1.62 ± 0.54	0.02	4.54	7.00 ± 1.51	–0.27	–1.49
Yeoman	M21435	High	0.40	0.00	1.08 ± 0.69	–0.74	–34.88	8.71 ± 0.66	0.44	14.48
Yeoman	M64102	High	0.38	0.00	2.85 ± 1.36	0.43	45.23	7.63 ± 0.82	–0.55	0.89
Yeoman	M21268	Low	0.25	0.02	1.49 ± 0.71	–0.05	–2.04	6.38 ± 2.36	–1.28	–12.67
Yeoman	M21300	Low	0.25	0.00	1.70 ± 0.89	–0.20	–0.18	8.79 ± 0.80	0.87	18.31
Yeoman	M21336	Low	0.23	0.02	1.63 ± 0.75	0.12	8.32	6.88 ± 1.08	–0.59	–4.57
Average					1.50 ± 0.70	0.00	–1.89	6.94 ± 1.44	0.04	0.07

reported (12). Controlled crosses between select male and female pairs were made in July 2003 using the technique described by Henning and Townsend (12). Seedlings from these crosses were planted at the USDA-ARS hop yard located near Corvallis, OR, on June 14, 2004, in a randomized complete block design, with three replicates and four genotypes per plot within replicates. Data were collected during 2005 and 2006, with years representing environments. All plants within a plot were harvested for whole plant yield using a stationary picker (Type I, Wolf Anglagen-Technik). In addition to yield, qualitative estimates of overall plant vigor using an ordinal score of 1–10 (1 = plant was dead; 10 = plant had several vines reaching the top of the trellis with vigorous side arm development) were recorded for each plant. Due to a variable number of female offspring within each plot, average cone yields (kilograms per plant) and average vigor per female genotype per plot were calculated and used for data analysis.

Statistical Analyses

Statistical analyses consisted of tests for differences among treatments, as well as correlation analysis between parental genetic diversity estimates and corresponding responses in offspring. A mixed model analysis (PROC MIXED procedure for Windows v.9.1.3, SAS Institute) was used to test for differences between treatments. Spearman's rank order correlation was used to test relationships among genetic diversity estimates (GD_{mm} and GS_{ped}) and the phenotypic response in offspring for yield and vigor, as well as corresponding measures of heterosis (MPH and SCA) for these two traits. The covariance parameter estimates of random effects were tested by Wald *Z* tests. Restricted maximum likelihood was used for mixed model analysis of fixed effects, while the degrees of freedom for fixed effects were determined by the Kenward-Rogers method (16). Previously determined GD_{mm} estimates between male and female parents (13) were used to separate crosses into two parent genetic distance groups (PDG): a high genetic distance group (HGD) and a low genetic distance group (LGD). Blocks and years were considered random effects, while females, males (nested within female × PDG), and PDG were considered fixed effects in the mixed model analysis. Males were nested within the interaction between female × PDG due to an unbalanced design between males and females within PDG. Average yield per plot (kilograms per plant), as well as vigor,

TABLE II
Mixed Model Analysis of Fixed-Effect Variables for Average Yield and Vigor[a]

Effect	Numerator (df)	Denominator (df)	χ^2	*F* Value	$P > \chi^2$	$P > F$
Average yield						
Female	15	30	97.51	6.50	<0.0001	<0.0001
PDG	1	2.93	9.42	9.42	0.0021	0.0562
Female × PDG	15	30.8	15.03	1.00	0.4496	0.4779
Male (female × PGD)	55	368	151.05	2.75	<0.0001	<0.0001
Average vigor						
Female	15	29.9	60.67	4.04	<0.0001	0.0006
PDG	1	32.3	15.75	15.75	<0.0001	0.0004
Female × PDG	15	32.2	22.01	1.47	0.1076	0.1763
Male (female × PDG)	55	367	166.61	3.03	<0.0001	<0.0001

[a] Variables analyzed (PROC MIXED, SAS) included female, parent distance groups (PDG: high versus low genetic distance groups), and male (males nested within female × PDG interaction; males were nested within the female × PDG interaction due to an unbalanced design between males and females in PDG).

TABLE III
Random Effects Tests for Covariance Parameters[a]

Covariance Parameter	Estimate	STD Error	*Z* Value	*P* (*Z*)
Average yield				
Block	0	–	–	–
Female × block	0.04616	0.1039	0.44	0.3284
PDG × block	0.06253	0.07959	0.79	0.2160
Female × PDG × block	0.1405	0.1368	1.03	0.1523
Year	1.4034	2.0862	0.67	0.2506
Block × year	0.1901	0.1576	1.21	0.1138
Female × year	3.02E-19	–	–	–
Female × block × year	0	–	–	–
PDG × block × year	0	–	–	–
Female × PDG × block × year	0	–	–	–
Residual	2.0665	0.1523	13.57	<0.0001
Average vigor				
Block	0.07632	0.1024	0.75	0.2281
Female × block	0.03692	0.1393	0.27	0.3955
PDG × block	0	–	–	–
Female × PDG × block	0.3778	0.1901	1.99	0.0235
Year	0.1688	0.2496	0.68	0.2494
Block × year	0	–	–	–
Female × year	0	–	–	–
Female × block × year	0	–	–	–
PDG × block × year	5.6E-19	–	–	–
Female × PDG × block × year	0	–	–	–
Residual	2.0030	0.1478	13.55	<0.0001

[a] Random effects test (PROC MIXED, SAS) for parameters significantly different from zero (blocks and years) and interactions for average yield (kg/plant) and vigor (ordinal scale of 1–10).

were used as dependent variables. GD_{mm} estimates for crosses placed within the HGD group ranged from 0.355 to 0.447, while GD_{mm} estimates for crosses within the LGD group ranged from 0.183 to 0.291 (Table I). Estimates of GS_{ped} and the pedigrees used to estimate these values for all crosses were previously reported by Henning et al (13). Estimates of GS_{ped} for all crosses within the HGD group were equal to zero, with the exception of one cross (Perle × M21461), which exhibited a GS_{ped} equal to 0.0098. Estimates of GS_{ped} for crosses within the LGD ranged from 0.003 to 0.32. Midparent heterosis for each cross was calculated from the yield and vigor data averaged across blocks and years as described by Benchimol et al (1), while SCA estimates were calculated using the same yield and vigor data and were based on methods reported by Falconer and Mackay (6). Spearman's rank correlations among average yield, average vigor, GD_{mm}, GS_{ped}, SCA, and MPH values were analyzed using Statistica (release 6.1, StatSoft).

RESULTS AND DISCUSSION

Mixed Model Analysis Results

We observed highly significant ($P \leq 0.01$) differences for yield in the fixed-effect variables "female" and "male" (males nested within female × PDG). Differences between PDG were nearly significant ($P = 0.056$), while the interaction between female × PDG was not statistically significant ($P = 0.4779$) (Table II). Average yield for the HGD group was 1.74 kg/plant, while the average yield for the LGD group was 1.39 kg/plant. The experiment-wide average yield during 2006 (1.89 kg/plant) was not significantly different from the average yield in 2007 (1.13 kg/plant) (Table III). Higher numerical yields in 2006 than in 2007 were most likely due to higher levels of downy mildew (*Psuedoperonospora humuli* Miyabe and Tak. (Wil.)) in 2007, as much of the germplasm used in this study was susceptible, and efforts at controlling fungal pathogens were minimal. No significant interactions between year and other factors for average yield were observed (Table III).

The *F* test for differences in average vigor per female between HGD and LGD was highly significant ($P = 0.0004$) (Table II). We also observed highly significant differences in average vigor among female lines ($P < 0.0001$) as well as among male lines ($P < 0.0001$). None of the variance parameter estimates for random effects based on average plant vigor were significantly different from zero, with the exception of the interaction between female × PDG × block ($P = 0.0235$) (Table III).

Some individual crosses were noteworthy because of high yield and vigor scores, as well as high MPH values, for those traits (Table I). The Cascade × M64101 cross exhibited a MPH value of 75.09, with an average yield of 2.641 ± 0.987 kg/plant (overall experimental average yield = 1.504 ± 1.20 kg/plant) and an average vigor score of 7.75 ± 2.26, with an MPH_{vigor} of 11.96. Another cross, Newport × M64102, had twice the overall average yield (3.168 ± 0.690 kg/plant), with an MPH_{yield} of 42.06. This cross also exhibited superior plant vigor (score = 8.79 ± 0.60), with an MPH_{vigor} of 24.23. Certainly, several of the crosses in this study with excellent phenotypic values in offspring and high levels of heterosis would be worthy of future evaluation as breeding stock.

We did not observe a significant genetic × environment (G × E) interaction in the mixed model analyses (Tables II and III), which might be due to the limited number of environments assayed. Additional analyses under a greater number of environments (locations and years) could have resulted in a significant G × E interaction for yield. Wu et al (34) observed significant G × E interactions in rice across a number of traits, including yield. Lee et al (18) observed highly significant G × E interaction for several traits in maize. As single-plant spaced-hop nurseries are expensive to establish and maintain, we suggest that data be collected for more than two years at a minimal set of locations in future studies for phenotype determination when developing new selectable molecular markers.

Correlation Between Genetic Distance and Performance Measurements

Spearman's rank correlation coefficients (Table IV) between average yield/plant and GD_{mm} were statistically significant ($P = 0.023$), suggesting that estimates of GD_{mm} were partially predictive of male and female pairs that would, on average, produce superior offspring. However, GD_{mm} was not statistically associated with traditional measures of heterosis such as SCA ($P > 0.05$) or MPH ($P > 0.05$). Estimates of GS_{ped} derived from pedigree analysis were negatively correlated with GD_{mm} ($r = –0.586$; $P < 0.01$) but were not significantly ($P > 0.05$) associated with average yield ($r = –0.154$), SCA ($r = 0.163$), or MPH ($r = –0.053$). Finally, average yield was not correlated with SCA ($r = 0.0735$) but was correlated with MPH ($P < 0.01$).

Average plant vigor scores were highly correlated with average yield ($r = 0.547$; $P < 0.01$), suggesting that plant vigor might prove useful as an estimator of yield potential for parent combinations used in our study. In contrast to the results discussed above for yield, average plant vigor was highly correlated ($P < 0.01$) with SCA ($r = 0.307$), MPH ($r = 0.863$), and GS_{ped} (–0.312) and was significantly correlated with GD_{mm} ($r = 0.243$; $P < 0.05$) (Table V). GD_{mm} estimates were also correlated with MPH values ($r = 0.236$; $P < 0.05$). Interestingly, GS_{ped} estimates were more closely related to average plant vigor and MPH than were GD_{mm} estimates (Table V).

A statistically significant correlation between GD_{mm} and yield existed, while GD_{mm} was not correlated with MPH or SCA. This suggests that the AFLP markers used in this study were linked to QTLs associated with yield, but these QTLs appeared to have additive effects rather than dominance or epistatic effects. We would have expected a stronger correlation between GD_{mm} and MPH or SCA if markers were linked to QTLs possessing dominance at a locus or epistasis between loci, as was proposed by Wu et al (34). In contrast, GD_{mm} and GS_{ped} were both correlated with plant vigor and MPH, suggesting that the AFLP markers used in our study were par-

TABLE IV
Spearman's Rank Correlation Coefficients Between Vectors for Yield[a]

	Yield	SCA	MPH	GD_{mm}	GS_{ped}
Vigor	0.547**				
SCA	0.0735	1.00			
MPH	0.831***	0.555**	1.00		
GD_{mm}	0.244*	–0.022	0.156	1.00	
GS_{ped}	–0.154	0.163	–0.053	–0.586**	1.00

[a] Vectors: average yield, average vigor, specific combining ability (SCA), midparent heterosis (MPH), and genetic distance estimates based on molecular markers (GD_{mm}) and pedigree analysis (GS_{ped}). *, **, and *** indicate value is statistically significant at $P < 0.05$, 0.01, and 0.001, respectively. $N = 87$.

TABLE V
Spearman's Rank Correlation Coefficients Between Vectors for Vigor[a]

	Vigor	SCA	MPH	GD_{mm}	GS_{ped}
Yield	0.547**				
SCA	0.307**	1.00			
MPH	0.863***	0.654***	1.00		
GD_{mm}	0.243*	0.032	0.236*	1.00	
GS_{ped}	–0.312**	–0.055	–0.291**	–0.782***	1.00

[a] Vectors: average vigor, average yield, specific combining ability (SCA), midparent heterosis (MPH), and genetic distance estimates based on molecular markers (GD_{mm}) and pedigree analysis (GS_{ped}). *, **, and *** indicate value is statistically significant at $P < 0.05$, 0.01, and 0.001, respectively. $N = 87$.

tially linked to QTLs possessing both additive and dominance effects over expression of this trait.

There is some question as to the predictive quality of the number of AFLP markers used in our study with respect to yield in offspring. Even though the correlation between GD_{mm} and yield and between GD_{mm} and vigor were both statistically significant, whether these correlations are biologically significant is debatable. Several publications have stressed the importance of sufficient genomic coverage, as well as the inclusion of multiple marker types to accurately predict which parents will have the highest potential for heterotic offspring (2,8). While our study used 490 polymorphic markers, these markers all resulted from *Eco*RI/*Mse*I primer pair combinations. It is possible that these markers did not adequately cover the hop genome (8). Perhaps using *Pst*I/*Mse*I primer pair combinations for AFLP, as well as other molecular marker systems, would have increased the predictive nature of molecular markers to select superior combining parents.

Traditional measures of heterosis (SCA and MPH) are particularly important for development of hybrid breeding programs, as these measures assist in determining parent combinations between inbred lines that may result in offspring exceeding parent production levels. Estimates of SCA and MPH are of great importance in many monoecious field crops, such as maize and rice, but their importance in dioecious crops is relatively untested due to a lack of inbred line development in many of these crops. Hop cultivars have been developed primarily through phenotypic selection from single crosses (10,22). No published work exists on the development of inbred lines for hybrids.

Hop breeding programs require the development of new germplasm sources that are superior to cultivars currently grown to ensure stable production in changing environments. Heterosis, as measured by MPH or SCA, is of little value unless offspring surpass current cultivars already in production. Hale et al (9) made a distinction between the importance of heterosis per se and what they term "absolute trait values." This group observed scenarios where two underperforming parents were crossed, and the resulting offspring showed significant heterosis, as measured by MPH. Nevertheless, the offspring from these crosses underperformed relative to other families. In contrast, Hale et al (9) also observed crosses between two highly productive parents in which the offspring exhibited low levels of heterosis, as measured by MPH; the offspring from these crosses exhibited superior yield characteristics compared with other families. The presence of numerically high MPH or SCA values in our data did not always result in offspring expressing yield superior to those of other crosses. This scenario was illustrated by the crosses Northern Brewer × M21268 (MPH = 23.37, yield = 1.74 kg/plant) and Saxon × M21268 (MPH = 16.85, yield = 1.51 kg/plant). In both cases, the resulting offspring were only slightly better than the experiment-wide average (1.50 kg/plant) but were lower in yield than Target × M21089 (MPH = 8.94, yield = 2.076 kg/plant).

The prediction of superior mating pairs in the hop accessions used in our study, using GD_{mm} estimates, would presumably be most effective when parental pairs have a $GD_{mm} > 0.35$; this was the cutoff value for the HGD group. However, we did not determine an optimum GD_{mm} value for greatest precision; additional work in hops is needed to clarify this value. In addition to greater marker saturation and coverage of the hop genome, additional testing of environments would likely improve the predictive capability of this technique. The potentially limiting coverage of the genome by the AFLP markers used in this study, coupled with reduced environments, resulted in a situation where 19 of 44 crosses assigned to the HGD group had average yields that were numerically lower than the experiment-wide average, while 16 of 43 crosses within the LGD group exhibited yields that were numerically higher than the average. Thus, better predictive characterization of the male and female genotypes studied might be enhanced by saturating the hop genome with molecular markers and by more robust phenotypic trait data.

The ratio of additive genetic effects to nonadditive effects (dominance/epistasis) for yield in hop was high in previous work by Henning and Townsend (12). In the present study, using AFLP to predict offspring performance for yield controlled by dominance effects was not possible with the male and female genotypes studied, but prediction of offspring performance controlled by additive effects for yield appears to hold promise. This supposition is based on the observation that GD_{mm} and yield were correlated. As previously discussed, a correlation value of 0.24 is questionable for breeding purposes, even though this value is statistically significant (P = 0.023). Nonetheless, given that hop breeding is in its infancy compared with other crops, molecular markers show some promise as a means of predicting offspring performance in the set of male and female lines used in our study.

Because hop breeding currently focuses on identification and use of additive gene effects using mass selection and does not use hybrid breeding in germplasm development, use of AFLP to predict offspring performance for yield and vigor shows promise within the group of males and females used in our study. The key to successful implementation depends on the inclusion of markers linked to QTL regions controlling the expression of desired traits. Additional AFLP primer pair combinations beyond what was used in our study should increase the genomic coverage and saturation required to better select parental pairs producing offspring with above average yields. A combination of marker systems may also enhance the level of predictability, as proposed by Hale et al (8). Finally, greater precision in defining phenotypes for offspring through the use of multiple environments beyond the use of years would most likely increase the predictive quality of molecular markers. Ultimately, this would result in greater precision in predicting parental pairs of hop accessions exhibiting superior trait expression in their offspring.

LITERATURE CITED

1. Benchimol, L. L., De Souza, C. L., Jr., Garcia, A. A. F., Kono, P. M. S., Mangolin, C. A., Barbosa, A. M. M., Coelho, A. S. G., and De Souza, A. P. Genetic diversity in tropical maize inbred lines: Heterotic group assignment and hybrid performance determined by RFLP markers. *Plant Breed.* 119:491-496, 2000.
2. Bernardo, R. Relationship between single-cross performance and molecular marker heterozygosity. *Theor. Appl. Genet.* 83:628-634, 1992.
3. Charcosset, A., and Essioux, L. The effect of population-structure on the relationship between heterosis and heterozygosity at marker loci. *Theor. Appl. Genet.* 89:336-343, 1994.
4. Corbellini, M., Perenzin, M., Accerbi, M., Vaccino, P., and Borghi, B. Genetic diversity in bread wheat, as revealed by coefficient of parentage and molecular markers, and its relationship to hybrid performance. *Euphytica* 123:273-285, 2002.
5. Cornelison, J. M., Yan, F., Watkins, S. E., Rigby, L., Segal, J. B., and Waldroup, P. W. Evaluation of hops (*Humulus lupulus*) as an antimicrobial in broiler diets. *Int. J. Poultry Sci.* 5:134-136, 2006.
6. Falconer, D. S., and Mackay, T. F. C. *Introduction to Quantitative Genetics,* 4th ed. Addison Wesley Longman, Harlow, UK, 1996.
7. Gelata, L. F., Labuschagne, M. T., and Viljoen, C. D. Relationship between heterosis and genetic distance based on morphological traits and AFLP markers in pepper. *Plant Breed.* 123:467-473, 2004.
8. Hale, A. L., Farnham, M. W., and Menz, M. A. Use of PCR-based markers for differentiating elite broccoli inbreds. *J. Am. Soc. Hortic. Sci.* 131:418-423, 2006.
9. Hale, A. L., Farnham, M. W., Nzaramba, M. N., and Kimbeng, C. A. Heterosis for horticultural traits in broccoli. *Theor. Appl. Genet.* 115: 351-360, 2007.
10. Henning. J. The breeding of hop. In: *Brewing.* C. W. Bamforth, ed. CRC Press, Boca Raton, FL. Pp. 102-122, 2006.
11. Henning, J. A., Steiner, J. J., and Hummer, K. E. Genetic diversity

among world hop accessions grown in the USA. *Crop Sci.* 44:411-417, 2004.
12. Henning, J. A., and Townsend, M. S. Field-based estimates of heritability and genetic correlations in hop. *Crop Sci.* 45:1469-1475, 2005.
13. Henning, J. A., Townsend, M. S., and Kenny, S. Potential heterotic crosses in hop as estimated by AFLP-based genetic diversity and coefficient of coancestry. *J. Am. Soc. Brew. Chem.* 62:63-70, 2004.
14. Jakse, J., Kindlhofer, K., and Javornik, B. Assessment of genetic variation and differentiation of hop genotypes by microsatellite and AFLP markers. *Genome* 44:773-782, 2001.
15. Jakse, J., Satovic, Z., and Javornik, B. Microsatellite variability among wild and cultivated hops (*Humulus lupulus* L.). *Genome* 47:889-899, 2004.
16. Kenward, M. G., and Roger, J. H. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983-997, 1997.
17. Kolliker, R., Boller, B., and Widmer, F. Marker assisted polycross-breeding to increase diversity and yield in perennial ryegrass (*Lolium perenne* L.). *Euphytica* 146:55-65, 2005.
18. Lee, E. A., Ash, M. J., and Good, B. Re-examining the relationship between degree of relatedness, genetic effects and heterosis in maize. *Crop Sci.* 47:629-635, 2007.
19. Malécot, G. Les mathématiques de l'hérédité. Masson & Cie, Paris, 1948.
20. Murakami, A., Darby, P., Javornik, B., Pais, M. S. S., Seigner, E., Lutz, A., and Svoboda, P. Microsatellite DNA analysis of wild hops, *Humulus lupulus* L. *Gen. Res. Crop Evol.* 53:1553-1562, 2006.
21. Murakami, A., Darby, P., Javornik, B., Pais, M. S. S., Seigner, E., Lutz, A., and Svoboda, P. Molecular phylogeny of wild hops, *Humulus lupulus* L. *Heredity* 97:66-74, 2006.
22. Neve, R. A. *Hops.* Chapman and Hall, London, 1991.
23. Patzak, J. Characterization of Czech hop (*Humulus lupulus* L.) genotypes by molecular methods. *Rostlinná Výroba* 48:343-350, 2002.
24. Pollach, G., Hein, W., and Hollaus, F. Use of hop products as bacteriostatic agents in the sugar industry. (In German) *Zuckerindustrie* 121: 919-926, 1996.
25. Seefelder, S., Ehrmaier, H., Schweizer, G., and Seigner, E. Genetic diversity and phylogenetic relationships among accessions of hop, *Humulus lupulus*, as determined by amplified fragment length polymorphism and fingerprinting compared with pedigree data. *Plant Breed.* 119:257-263, 2000.
26. Solomon, K. F., Labuschagne, M. T., and Viljoen, C. D. Estimates of heterosis and association of genetic distance with heterosis in durum wheat under different moisture regimes. *J. Agric. Sci.* 145:239-248, 2007.
27. Stajner, N., Satovic, Z., Cerenak, A., and Javornik, B. Genetic structure and differentiation in hop (*Humulus lupulus* L.) as inferred from microsatellites. *Euphytica* 161:301-311, 2008.
28. Stevens, J. F., and Page, J. E. Xanthohumol and related prenylflavonoids from hops and beer: To your good health! *Phytochemistry* 65:1317-1330, 2004.
29. Stevens, J. F., Taylor, A. W., Nickerson, G. B., Ivancic, M., Henning, J., Haunold, A., and Deinzer, M. Prenylflavonoid variation in *Humulus lupulus*: Distribution and taxonomic significance of xanthogalenol and 4′-*O*-methylxanthohumol. *Phytochemistry* 53:759-775, 2000.
30. Teklewold, A., and Becker, H. C. Comparison of phenotypic and molecular distance to predict heterosis and F1 performance in Ethiopian mustard (*Brassica carinata* A. Braun). *Theor. Appl. Genet.* 112:752-759, 2006.
31. Townsend, M. S., and Henning, J. A. Potential heterotic groups in hop as determined by AFLP analysis. *Crop Sci.* 45:1901-1907, 2005.
32. Townsend, M. S., and Henning. J. A. AFLP discrimination of native North American and cultivated hop. *Crop Sci.* 49:600-607, 2009.
33. Wald, A. Tests of statistical hypotheses concerning several parameters when the number of observations is large. *Trans. Am. Math. Soc.* 54:426-482, 1943.
34. Wu, W., Virmani, S. S., Hernandez, J. E., Sebastion, L. S., Redona, E. D., and Li, Z. Genetic diversity in the parental lines and heterosis of the tropical rice hybrids. *Euphytica* 127:139-148, 2002.
35. Zanoli, P., and Zavatti, M. Pharmacognistic and pharmacological profile of *Humulus lupulus* L. *J. Ethno-Pharm.* 116:383-396, 2008.
36. Zhang, X. Q., Wang, X. D., Jiang, P. D., Hua, S. J., Zhang, H. P., and Dutt, Y. Relationship between molecular marker heterozygosity and hybrid performance in intra- and interspecific hybrids of cotton. *Plant Breed.* 126:385-391, 2007.