Potential Heterotic Crosses in Hops as Estimated by AFLP-Based Genetic Diversity and Coefficient of Coancestry

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ABSTRACT

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Hop is a dioecious perennial with female plants grown commercially for brewing purposes. Parent selection in hop breeding on the basis of heterotic potential has not been reported in literature even though dominance has been reported in hops for several economically important traits. The objectives of this study were to determine if amplified fragment length polymorphism (AFLP)-based genetic distance among male and female accessions accurately reflect pedigree relationships and present information on potential heterotic crosses in hop. Nineteen cultivars were analyzed for genetic distance to 82 male accessions representing the assumed diversity of U.S. hops. Genetic distances (GD) between male/female pairs were estimated using AFLP (490 polymorphic bands). Distance estimates comparing males with females ranged from 0.169 to 0.62 with an overall average of 0.306. For each hop female, the 10 most genetically diverse and 10 most genetically similar males were identified and grouped. Coefficients of coancestry (COA) for each male/female pair within these groups were calculated using pedigree analysis. Values of COA for the genetically similar pairs (COA_{avg} = 0.046) were significantly higher than the COA for the diverse pairs (COA_{avg} = 0.013), suggesting that choosing male/female pairs on the basis of AFLP-based genetic distance may predict heterotic potential in crosses when GD >0.36.

Keywords: Heterosis, *Humulus lupulus*, Inbreeding depression

RESUMEN

Cruces Heteróticas Potenciales en Lúpulo Según Eestimados por Diversidad Genética Base-AFLP y Coeficiente de Coascendencia

El lúpulo es un "perennial dioecious" con plantas femeninas cultivadas comercialmente en propósitos de elaboración de cerveza. La selección de padres en el cruce de lúpulo en base de potencial heterótico no se ha reportado en literatura aunque la dominación se ha reportado en lúpulos por varios rasgos económicamente importantes. Los objetivos de este estudio eran determinar si la distancia genética en base de polimorfismo de longitudes de fragmentos amplificados (AFLP) entre las accesiones masculinas y femeninas reflejan exactamente las relaciones pedigríes y información actual sobre cruces heteróticas potenciales en lúpulo. Diecinueve cultivos fueron analizados en distancia genética a 82 accesiones masculinas que representan la diversidad asumida de lúpulo EE.UU. Distancias genéticas (GD) entre pares masculinos/femeninos fueron estimadas usando AFLP (490 bandas polimórficas). Estimaciones de comparación de distancia masculina a femenina se extendieron a partir de 0.169 a 0.62 con un promedio total de 0.306. Para cada lúpulo femenino, se identificaron y agruparon los 10 masculinos más genéticamente diversos y los 10 más genéticamente similares. Coeficientes de coascendencia (COA) para cada par masculino/femenino dentro de estos grupos fueron calculados usando análisis de pedigrí. Los valores COA de los pares genético similares (COA_{avg} = 0.046) eran perceptiblemente más altos que el COA de los pares diversos (COA_{avg} = 0.013), sugiriendo que elegir pares masculinos/femeninos en base de distancia genética AFLP pueden predecir potencial heterótico en cruces cuando GD >0.36.

Palabras claves: Heterosis, *Humulus Lupulus*, Depresión de endogamia

Hop (*Humulus lupulus* L.) is a dioecious perennial crop species grown primarily in the U.S. Pacific Northwest and selected regions of Europe. Variety distribution is accomplished via rhizome cuttings that are mass produced and transplanted into monoculture hop yards. The unfertilized mature female floral structure, strobulis or hop cone, is harvested, cured, and processed for the beer brewing industry as a flavoring or bittering agent. The history of hop production, curing, and use in beer brewing suggest that much of early germ plasm development consisted of clonal selection and mass distribution of cuttings (21).

Current hop breeding techniques usually involve simple breeding schemes with controlled crosses between several males and a single female variety or experimental line. Seeds are collected, germinated, and grown in large-scale nurseries where one or several female genotypes are selected for large-scale commercial growth. Typically, one of these selected females will be released as a new variety. Some of the male offspring from these crosses are subsequently evaluated for phenotypic expression of various traits. The determination of whether to use a male for breeding purposes is almost always on the basis of phenotypic data rather than genotypic tests.

Many traits of economic importance in hops are controlled by a combination of additive and dominance genetic effects (10,11). Significant levels ($P < 0.01$) of specific combining ability were observed for yield, alpha acids levels, beta acid levels, cohumulone content, and hop storability (10). Recently completed field studies have validated these findings (J. A. Henning, *unpublished data*). Certainly, much of the varietal work in hops effectively utilizes additive effects in selection. Nevertheless, a large portion of genetic expression, because of dominance or heterosis, probably remains untapped in the development of hop cultivars. This is interesting since most hop varieties are made from single crosses rather than recurrent mating schemes or genotypic testing schemes. Because of this varietal development scheme, it would behoove hop breeders to maximize heterozygosity when developing new varieties.

One commonly accepted genetic theory suggests that heterosis or transgressive segregation is the result of cumulative effects of heterozygous combinations at multiple loci (8). Falconer (8) describes this as:

$$H_{F1} = \Sigma d_i y_i^2$$

with heterosis in the F1 generation (H_{F1}) equivalent to the sum (across all loci) of the products between the dominance difference for locus i (d_i) and the square of the genetic divergence for locus i (y_i^2). Thus, the more genetically diverse two genotypes are, the greater the likelihood that heterosis or transgressive segregation

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will occur in the offspring. Numerous attempts to corroborate this theory in other crop species with coefficient of coancestry, molecular markers, or morphological data have been made (3–7,14,18,22,30,32). Some researchers have reported positive correlations between offspring heterosis and parental diversity (30,32). In most cases, a direct correlation between molecular distance values and resulting heterosis of offspring was not obtained. Nevertheless, many reports described a tendency for greater heterosis among intraspecific parental lines with the highest genetic distance between parents (3,5–7,14,18,22).

There are no reports on heterosis in hop or any published work on crossing schemes to document or determine heterosis in hop. It would be impossible to determine the accuracy of heterosis prediction with molecular estimates without empirical data. However, before embarking on a crossing scheme to determine the accuracy of heterosis prediction with molecular data, it would be prudent to determine if molecular estimates of genetic distance agree with genetic distance levels as determined by pedigree analysis using males and females with known genetic backgrounds. Burkhammer et al (3) and Cheres et al (6) analyzed the correlation between genetic distance estimated by molecular data and genetic distance measured by pedigree analysis using the coefficient of coancestry (COA). COA measures the probability of alleles in two genotypes being identical by descent and has been used to indirectly measure germ plasm pool diversity—the higher the COA value, the more genetically similar two individuals are thought to be. In the aforementioned reports, direct correlation between molecular data and pedigree analysis was obtained. Furthermore, in both reports, there was a strong tendency for individual pairs with low COA and high genetic distance to produce offspring with higher than average yields and other agronomic traits.

TABLE I
Female and Male Accessions with Their Respective Pedigrees (If Known)[a]

Accession No. or Variety Name	Pedigree
Females	
Brewers Gold	Wye BB1 × OP
Cascade	[Fuggle × (Serebrianka × Fuggle-seedling)] × OP
Challenger	Wye 17/54/2 × Wye 1/61/57
Comet	(Sunshine × OP) × Wild American (#524-2)
East Kent Golding	Clonal selection from Golding field
Fuggle N	Clonal selection from Fuggle field
Galena	Brewers Gold × OP
H. Mittelfrue	Old German landrace
Magnum	Galena × German (75/5/3)
Newport	Magnum × 58111M
Northern Brewer	Canterbury Golding × (Brewers Gold × OY1)
Nugget	Brewers Gold, Early Green, Golding, and Bavarian
Omega	Challenger × unknown male
Orion	Perle x German (70/10/15)[b]
Perle	Northern Brewer ×German (63/5/27)
Saxon	Swalof (Swedish Variety) × Wye 14/66/136
Target	Northern Brewer, Golding, German male, Wild American
Viking	Swalof (Swedish variety) × Wye 14/66/136
Yeoman	Wye 43/69/17 × Wye 25/68/173[c,d]
Males	
19005M	Late Cluster × OP
19007M	Brewers Favorite × OP
19009M	(Fuggle x OP) × OP
19036M	Late Cluster × (Fuggle × OP)
19037M	(Fuggle × OP) × (Fuggle × OP)
19041M	Early Green × OP
19046M	(Fuggle × OP) × (Late Cluster × OP)
19047M	Elsasser × Fuggle seedling
19060M	East Kent Golding × (Bavarian × OP)
19172M	Cat's Tail × [Fuggle × (Fuggle × OP)]
21009M	Sunshine, Early Green, Wild American
21072M	Brewers Gold × Wild American
21076M	Comet × [Brewers Gold × (Fuggle × Wild American)]
21087M	Yugoslavian wild female × OP
21089M	Wild Yugoslavian × OP
21090M	Wild Yugoslavian male
21110M	Bullion × 64035M
21129M	Wild American, Brewers Gold, Fuggle, Late Grape
21132M	Yakima Cluster × [(H. Mittelfrue x OP) x OP]
21135M	Early Green, Brewers Gold, H. Mittelfrue
21184M	Comet × OP
21268M	Northern Brewer, Brewers Gold, Early Green, H. Mittelfrue
21272M	Northern Brewer × 21110M
21273M	Early Green, H. Mittelfrue, Brewers Gold, Comet, Fuggle, Wild American
Males (*continued*)	
21300M	[Brewers Gold × (Early Green × OP)] × 64035M
21303M	(Bullion × OP) × 64035M
21306M	H. Mittelfrue, Bullion, Comet, Fuggle, Brewers Gold, Wild American
21313M	Comet, Early Green, Brewers Gold, H. Mittelfrue
21335M	Northern Brewer × 21110M
21336M	Northern Brewer × (Bullion × 64035M)
21345M	Early Green, H. Mittelfrue, Brewers Gold, Comet, Fuggle, Wild American
21351M	Bullion, H. Mittelfrue, Fuggle, Sunshine, Wild American
21398M	Yugoslavian (01P14) × OP
21400M	Yugoslavian (2OP09) × OP
21415M	Early Green, Brewers Gold, Fuggle, Late Cluster
21416M	Bullion × 64035M
21417M	Bullion, Comet, H. Mittelfrue, Early Green, Brewers Gold, Fuggle, Wild American
21420M	Northern Brewer, Bullion, H. Mittelfrue, Fuggle, Comet, Wild American, Early Green
21424M	Cascade × (Late Cluster × OP)
21428M	Cascade × (Serebrianka × Fuggle seedling)
21435M	Cascade × Wild American
21437M	Fuggle × OP
21444M	Comet, Early Green, Brewers Gold, H. Mittelfrue
21446M	Northern Brewer, Golding, Brewers Gold, Bavarian
21448M	Cascade, Early Green, Brewers Gold, H. Mittelfrue
21461M	Bullion, Comet, Brewers Gold, Fuggle, Wild American, Early Green
21463M	Cascade × (Yugoslavian wild female × OP)
21465M	Comet, Early Green, Brewers Gold, H. Mittelfrue
21487M	Cascade × (East Kent Golding × Bavarian sSeedling)
21488M	Cascade × (East Kent Golding × Bavarian seedling)
21603M	Cascade × (Semensch seedling × OP)
21692M	Late Cluster, Late Grape, Fuggle
52042M	(Late Grape × Fuggle seedling) × (Late Grape × Fuggle seedling)
52047M	(Strisselspalt × Late Cluster seedling) × (Strisselspalt × Early Green seedling)
63011M	(Early Green × OP) × (Late Grape × Fuggle seedling)
63015M	[Brewers Gold × (East Kent Golding × Bavarian seedling)] × OP
64033M	(H. Mittelfrue × OP) × OP
64034M	(H. Mittelfrue × OP) × OP
64035M	(H. Mittelfrue × OP) × OP
64036M	(H. Mittelfrue × OP) × OP
64037M	(H. Mittelfrue × OP) × OP
64101M	Unknown pedigree obtained from Germany
64102M	Wild American × OP

[a] Simple pedigrees are written in full while more complex pedigrees have only the primary ancestral females listed. Eighteen males are not listed since they were intermediate in their genetic similarity and distance with the females in our study.
[b] German = coming from the Huell Breeding program, Friesing, Germany.
[c] Wye = coming from the Wye Breeding program, Wye College, England.
[d] Complex pedigree including many "Golding" types, Brewers Gold, Wild American, and parental material from Huell Germany.

Prior to assessing the value of heterosis prediction on the basis of molecular data, the genetic distance among a wide range of male and female hop accessions needs to be obtained. There are several reports of diversity in hops using morphological chemical or molecular means of estimation (12,19,20,23–27). Nonetheless, only one report (23) includes males in the analysis, and in this report, approximately four males were assayed. Furthermore, the males assayed in this study were experimental lines not generally available to the public.

There are no reports on the genetic distance between pairs of male and female hop accessions. Nor are there any reports on the potential for heterosis in hops using morphological, chemical, or molecular data. The objectives of this study were to: 1) determine if genetic distance among male and female accessions, as measured by amplified fragment length polymorphism (AFLP), accurately reflect estimated relatedness as determined by pedigree analysis; and 2) report on potential heterotic crosses between 19 common hop varieties and 82 male accessions.

TABLE II
Amplified Fragment Length Polymorphism Analysis

Primer Pair[a]	No. of Monomorphic Bands	No. of Polymorphic Bands	No. of Total Bands	Polymorphic Bands (%)
eAAC/mCAC	16	56	72	77.8
eAAC/mCAG	9	86	95	90.5
eAAC/mCTC	11	99	110	90.0
eACC/mCAC	13	71	84	84.5
eAGC/mCAG	1	97	98	99.0
eAGC/mCTC	10	81	91	89.0
Total	60	490	550	
Mean (per primer)	10	88.2	91.7	89.1

[a] Primer pair combinations were previously identified as having the highest level of polymorphism in hop.

TABLE III
Average Coefficient of Coancestry (COA) for Male/Female Pairs with Low (Low) and High (High) Levels of Genetic Distance

	COA	
Female Variety	Low	High
Brewers Gold	0.0938	0.0125
Cascade	0.1912	0.0256
Challenger	0.2871	0.0013
Comet	0.1125	0.0000
East Kent Golding[a]	0.0000	0.0250
Fuggle N[b]	0.0219	0.1078
Galena	0.0461	0.0063
Hallertau Mittelfrue	0.0313	0.0031
Magnum	0.0270	0.0138
Newport	0.0240	0.0121
Northern Brewer	0.0895	0.0051
Nugget	0.0949	0.0182
Omega	0.0170	0.0010
Orion	0.0135	0.0013
Perle	0.0438	0.0041
Saxon	0.0034	0.0003
Target	0.0188	0.0020
Viking	0.0053	0.0003
Yeoman	0.0058	0.0005
Average	0.0457	0.0126

[a] Instance where male/female pairs with low genetic distance (most genetically similar) had higher average COA.
[b] Instance where male/female pairs with low genetic distance (most genetically similar) had higher average COA.

EXPERIMENTAL

Leaf tissue from 19 female and 82 male hop accessions were obtained from the USDA-ARS hop germ plasm collection located at Corvallis, OR and the Washington State University hop collection located at Prosser, WA (Table I). Leaf tissue for all female accessions was obtained from plants maintained in the greenhouse, while tissue for all male accessions was obtained from field-grown plants. Young leaves were collected, rinsed with deionized water, and stored at –86°C for several days. Leaf tissue was then freeze-dried at –40°C for 24–26 hr, at –20°C for an additional 24–26 hr, and finally stored at –20°C until processed. We observed that freeze-drying hop leaf tissue prior to DNA extraction resulted in a larger volume of DNA for analysis. Approximately 100–600 g of freeze-dried tissue was used in the extraction of DNA, following the protocol as published by Kidwell and Osborn (13). AFLP analysis was performed with six primer pair combinations (Table II) that were previously identified as having the highest level of polymorphism in hop (29). The resulting AFLP fragments were separated with an Applied Biosystems ABI 377 (Applied Biosystems, Sunnyvale, CA) with the raw trace files exported for band analysis. Reproducibility was tested by repeating the DNA extraction and AFLP analysis from six assumed genetically divergent accessions (representing the range of diversity in the collection).

Trace files were imported into the gel imaging analysis software, "Genographer" (1) for band scoring and the subsequent generation of binary data files for estimation of genetic distance. Analysis of band scoring was performed as reported by Townsend et al (29). Binary data files were analyzed for genetic similarity on SYSTAT Version 8.0 (SPSS Incorporated, Chicago, IL) using Jaccard's similarity estimate as follows:

$$GS_{ij} = N_{ij}/(N_t - N_0)$$

where GS_{ij} = the genetic similarity value for male/female pair ij, N_{ij} = the total number of bands shared between pair ij, N_t = the total number of loci, and N_0 = the total number of loci where both i and j did not express the band. Genetic distance (GD_{ij}) was calculated as $GD_{ij} = 1 - GS_{ij}$.

For pedigree analysis we calculated the COA as reported by Falconer (7).

Each of the 19 female accessions was grouped with males having the top 10 highest genetic distance (HGD) values and the top 10 lowest genetic distance (LGD) values. The COA values for each of these 20 pairs were subsequently calculated. Thus, COA values for 19 × 20, or 380, female × male pairs were calculated on the basis of known ancestral background. If all the ancestors for a particular accession were unknown, as was the case for male 64101M, the COA value with any other female was assumed to be equal to zero. This was done only in the case of pairs between male 64101M and six females. An analysis of variance comparing the COA values between the 10 HGD pairs within a female (as determined by AFLP analyses) and the 10 LGD pairs within a female was analyzed using SYSTAT.

RESULTS AND DISCUSSION

We observed 550 bands (loci) of which 490, or 89.1%, were polymorphic (Table II). Several loci (77) that were consistently present in most genotypes but were not observed in four or less individuals were considered as polymorphic for purposes of this study. If these loci were considered as false negatives, then the percent polymorphism declines to 77.6%. These values for both total number of bands and percent polymorphism are higher than those observed in other hop studies using AFLP (12,23). Reasons for the discrepancy may include: 1) greater number of genotypes

TABLE IV
High Genetically Diverse Male/Female Pairs for 19 Hop Varieties with Genetic Distance (GD) Values and Coefficient of Coancestry (COA) for Each Pair[a]

Hop Variety	GD	COA	Hop Variety	GD	COA	Hop Variety	GD	COA
Comet			Newport (*continued*)			Omega (*continued*)		
21089M	0.431	0	64035M	0.392	0.0156	21129M	0.383	0.0039
64101M	0.393	0	64102M	0.389	0	21089M	0.375	0
64035M	0.389	0	19009M	0.386	0.0234	East Kent Golding		
21488M	0.365	0	19041M	0.382	0	19009M	0.418	0
19007M	0.364	0	21435M	0.375	0.0039	21435M	0.406	0
21435M	0.353	0	21076M	0.371	0.0254	64102M	0.392	0
63011M	0.347	0	Magnum			21461M	0.384	0
21487M	0.346	0	21089M	0.428	0	21488M	0.384	0.1250
21072M	0.345	0	21072M	0.425	0.0625	21089M	0.378	0
19047M	0.342	0	64035M	0.392	0.0313	21437M	0.376	0
Orion			21462M	0.383	0	21487M	0.374	0.1250
21435M	0.402	0	21129M	0.379	0.0313	21129M	0.373	0
19009M	0.398	0	21488M	0.377	0	21465M	0.363	0
21461M	0.385	0.0049	64101M	0.377	0	Saxon		
19005M	0.383	0	64102M	0.377	0	21435M	0.413	0
21129M	0.374	0.0078	21435M	0.375	0	21461M	0.392	0.0012
64102M	0.373	0	19046M	0.370	0	19009M	0.388	0
21437M	0.365	0	Viking			19005M	0.386	0
21089M	0.361	0	21435M	0.428	0	21437M	0.383	0
19037M	0.360	0	19009M	0.408	0	64102M	0.375	0
19041M	0.352	0	21461M	0.407	0.0012	21089M	0.373	0
Yeoman			21129M	0.396	0.0020	21488M	0.370	0
21435M	0.398	0	19005M	0.390	0	21129M	0.369	0.0020
21089M	0.396	0	21437M	0.386	0	21424M	0.364	0
19009M	0.385	0	64102M	0.383	0	Galena		
64102M	0.380	0	21487M	0.377	0	64035M	0.413	0
64035M	0.375	0	19172M	0.371	0	64101M	0.390	0
19036M	0.370	0	19041M	0.367	0	19037M	0.375	0
19005M	0.369	0	Nugget			21087M	0.375	0
21437M	0.368	0	21089M	0.410	0	19007M	0.372	0
21461M	0.368	0.0018	21435M	0.374	0	21268M	0.370	0.0625
21129M	0.365	0.0029	21487M	0.371	0.0312	64034M	0.369	0
Perle			19005M	0.370	0	21488M	0.366	0
21435M	0.447	0	19009M	0.365	0	19060M	0.365	0
21461M	0.426	0.0098	64035M	0.359	0	19046M	0.363	0
64102M	0.425	0	21129M	0.358	0.0781	Northern Brewer		
21488M	0.420	0	21437M	0.356	0	19009M	0.413	0
21129M	0.419	0.0156	19036M	0.349	0	21435M	0.405	0
21437M	0.417	0	21461M	0.348	0.0723	21461M	0.388	0.0195
19009M	0.415	0	Target			19005M	0.382	0
21487M	0.400	0	21089M	0.429	0	64102M	0.376	0
19005M	0.388	0	21072M	0.410	0.0156	21437M	0.372	0
21076M	0.383	0.0156	64035M	0.378	0	21089M	0.364	0
Brewers Gold			21435M	0.372	0	21487M	0.362	0
64101M	0.390	0	19007M	0.368	0	21129M	0.361	0.0313
64035M	0.386	0	21488M	0.365	0	19037M	0.359	0
21488M	0.383	0	64101M	0.365	0	Fuggle N		
19007M	0.362	0	21076M	0.352	0.0078	19009M	0.411	0.3750
21268M	0.355	0.1250	64102M	0.347	0	21435M	0.407	0.0781
21087M	0.348	0	21437M	0.346	0	64102M	0.385	0
21400M	0.345	0	Challenger			21461M	0.377	0.0156
21090M	0.341	0	21435M	0.386	0	19005M	0.375	0
63011M	0.340	0	64102M	0.381	0	21488M	0.359	0.0781
64036M	0.340	0	64035M	0.376	0	21437M	0.357	0.2500
Cascade			21488M	0.372	0	19172M	0.356	0.1875
21089M	0.451	0	19005M	0.370	0	21129M	0.354	0.0938
64035M	0.384	0	19009M	0.369	0	19041M	0.352	0
64101M	0.356	0	21089M	0.366	0	H. Mittelfrue		
21072M	0.355	0	19041M	0.361	0	21435M	0.428	0
19007M	0.351	0	21129M	0.359	0.0078	19009M	0.412	0
63011M	0.345	0.0313	21461M	0.357	0.0049	19005M	0.393	0
64037M	0.345	0	Omega			21437M	0.390	0
21488M	0.342	0.2500	21435M	0.408	0	21461M	0.390	0.0313
64033M	0.335	0	21461M	0.406	0.0024	64102M	0.382	0
64034M	0.335	0	19009M	0.403	0	21488M	0.373	0
Newport			64102M	0.401	0	21424M	0.363	0
19005M	0.404	0	19005M	0.392	0	19036M	0.359	0
21089M	0.402	0	21487M	0.388	0	19172M	0.352	0
21129M	0.398	0.0332	21437M	0.385	0			
21461M	0.397	0.0190	21076M	0.383	0.0039			

[a] Male/female pairs with high GD and low or zero COA would presumably have a greater probability of heterosis in offspring.

TABLE V
Low Genetically Diverse Male/Female Pairs for 19 Hop Varieties with Genetic Distance (GD) Values and Coefficient of Coancestry (COA) for Each Pair[a]

Hop Variety	GD	COA
Comet		
21351M	0.258	0.1250
21135M	0.252	0
21428M	0.245	0
21417M	0.242	0.1875
21465M	0.242	0.2500
21110M	0.239	0
21444M	0.239	0.2500
21448M	0.239	0
21313M	0.232	0.2500
21420M	0.228	0.0625
Orion		
52042M	0.234	0
21009M	0.232	0
21090M	0.226	0
21132M	0.225	0
21087M	0.218	0
58111M	0.215	0.0039
21400M	0.213	0
21268M	0.21	0.0665
21336M	0.196	0.0645
21398M	0.196	0
Yeoman		
21268M	0.249	0.0249
21300M	0.249	0.0030
21400M	0.249	0
58111M	0.249	0.0015
21415M	0.245	0.0030
21090M	0.240	0
21087M	0.238	0
21417M	0.236	0.0018
21398M	0.232	0
21336M	0.230	0.0242
Perle		
52042M	0.271	0
58111M	0.270	0.0078
21090M	0.267	0
21400M	0.256	0
21415M	0.256	0.0156
21087M	0.255	0
21268M	0.246	0.1328
21446M	0.246	0.1523
21336M	0.242	0.1289
21398M	0.239	0
Brewers Gold		
21416M	0.236	0.0625
21300M	0.234	0.1250
21306M	0.234	0.0781
21444M	0.232	0.0625
21428M	0.217	0
21424M	0.214	0
21110M	0.211	0.0625
63015M	0.204	0.3750
21448M	0.197	0.0625
21420M	0.195	0.1094
Cascade		
21444M	0.247	0
21463M	0.247	0.2500
21603M	0.246	0.2500
21427M	0.245	0.2813
21420M	0.242	0.0049
21417M	0.241	0.0049
21426M	0.237	0.3203
21424M	0.220	0.2500
21448M	0.197	0.2500
21428M	0.194	0.3008
Newport		
64033M	0.302	0.0156
21268M	0.299	0.0273
21420M	0.298	0.0249
21446M	0.294	0.0527
Newport (*continued*)		
21110M	0.291	0.0195
21272M	0.291	0.0215
21444M	0.287	0.0156
21336M	0.286	0.0215
21335M	0.278	0.0215
21448M	0.273	0.0195
Magnum		
21428M	0.278	0
21692M	0.274	0
21420M	0.266	0.0352
21303M	0.265	0.0313
21110M	0.263	0.0313
21300M	0.260	0.0469
21135M	0.249	0.0469
21448M	0.249	0.0234
21444M	0.244	0.0234
21415M	0.237	0.0313
Viking		
21400M	0.248	0
21692M	0.248	0
21009M	0.245	0
21087M	0.237	0
21090M	0.229	0
21398M	0.226	0
21446M	0.224	0.0190
21268M	0.223	0.0166
21336M	0.220	0.0161
58111M	0.209	0.0010
Nugget		
21273M	0.251	0.1250
21420M	0.251	0.0918
21336M	0.246	0.0586
21135M	0.243	0.1719
21444M	0.239	0.0859
21417M	0.237	0.0723
21428M	0.236	0
21692M	0.231	0
21300M	0.226	0.1719
21415M	0.197	0.1719
Target		
21444M	0.251	0.0039
21272M	0.249	0.0645
21336M	0.245	0.0645
21300M	0.244	0.0078
21110M	0.238	0.0039
21448M	0.238	0.0039
21428M	0.235	0
21463M	0.232	0
21313M	0.221	0.0039
21420M	0.218	0.0361
Challenger		
21444M	0.250	0.0039
21087M	0.247	0
21335M	0.245	0.0645
21268M	0.243	0.0665
21415M	0.239	0.0078
21400M	0.232	0
21398M	0.231	0
58111M	0.228	0.0039
21336M	0.224	0.0645
21446M	0.224	0.0762
Omega		
21272M	0.241	0.0322
21446M	0.241	0.0381
58111M	0.241	0.0020
21132M	0.236	0
21268M	0.236	0.0332
21336M	0.227	0.0322
21400M	0.225	0
21087M	0.224	0
Omega (*continued*)		
21335M	0.223	0.0322
21398M	0.213	0
East Kent Golding		
21090M	0.247	0
21415M	0.247	0
64036M	0.243	0
21336M	0.242	0
21132M	0.241	0
64033M	0.237	0
21268M	0.236	0
21400M	0.235	0
21398M	0.224	0
21087M	0.214	0
Saxon		
21400M	0.242	0
21090M	0.239	0
21132M	0.238	0
52042M	0.237	0
21087M	0.236	0
63011M	0.235	0
21268M	0.218	0.0166
58111M	0.218	0.0010
21398M	0.215	0
21336M	0.189	0.0161
Galena		
21415M	0.285	0.0625
63015M	0.285	0.1875
21110M	0.282	0.0313
21424M	0.276	0
21345M	0.275	0.0625
21184M	0.274	0
21428M	0.274	0
21420M	0.267	0.0547
21444M	0.246	0.0313
21448M	0.246	0.0313
Northern Brewer		
21417M	0.226	0.0195
21415M	0.225	0.0313
21400M	0.218	0
21446M	0.215	0.3047
58111M	0.215	0.0156
21087M	0.213	0
21090M	0.210	0
21398M	0.201	0
21336M	0.196	0.2578
21268M	0.183	0.2656
Fuggle N		
52042M	0.213	0.1250
63011M	0.212	0.0625
52047M	0.204	0
58111M	0.199	0.0313
21268M	0.198	0
21400M	0.196	0
21336M	0.190	0
21090M	0.183	0
21398M	0.179	0
21087M	0.170	0
H. Mittelfrue		
52042M	0.219	0
64037M	0.218	0.1250
64036M	0.213	0.1250
21268M	0.204	0.0313
21087M	0.202	0
21400M	0.197	0
21336M	0.195	0.0313
58111M	0.190	0
21090M	0.183	0
21398M	0.169	0

[a] Male/female pairs with low GD and high COA would presumably have a greater probability of inbreeding depression in offspring.

with a significantly broader genetic background represented, 2) different primer pairs used, 3) a broader range of fragment sizes included in our study (50 to 500 bp in our study versus 100 to 450 bp (23) and 50 to 350 bp (12), and 4) the presence of a large number of male accessions. This last reason may prove important, since all male accessions in hops have arisen via recombination rather than point mutations. The USDA-ARS hop genetics and breeding program has historically utilized a wide variety of wild American hops, both male and female, in the development of new germ plasm. Thus, the males in the USDA-ARS collection represent a broad range of the potential gene pool present in wild American hops. In contrast, many of the older European varieties were developed via clonal selection and most likely would have lesser amounts of detectable polymorphism among them. As a result, the male and female genotypes selected within in the European collection may have arisen from a narrower genetic base than their counterparts in the USDA-ARS collection and, therefore represent a more homogeneous gene pool. In turn, crosses among relatively narrow-based parents should result in offspring that are less genetically diverse than offspring based on wild American germ plasm.

Initial investigations (data not included) did not support a hypothesis of significant $GD_{ij} - COA_{ij}$ correlation when all male/female pairs were included. However, when comparing GD with COA of pairs with extreme GD values, HGD or LGD, we observed significant trends of association, indicating that AFLP estimates of GD agree with pedigree analysis when divergent or closely related pairs are examined. This was demonstrated by the significant differences ($P = 0.016$) in COA values between HGD male/female pairs (average COA = 0.0126) and LGD male-female pairs (average COA = 0.0457) (Table III).

Some differences between HGD and LGD appear to be narrower than those in the rest of the comparisons (Table III). These narrow differences arise principally with the male/female pairs between English-derived cultivars and the males in the U.S. hop collection. The English cultivars, Omega, Saxon, Target, Viking, and Yeoman have not been used to any great extent in the U.S. hop-breeding program. Additionally, the direct parents for these cultivars have generally not been available to the U.S. hop-breeding program. As a result, reduced COA values near zero should be expected for all male/female pairs regardless of how genetically diverse the male/female pair register as determined by AFLP. In all other cases, the differences in COA between HGD pairs and LGD pairs were apparent and explainable because of the unrelated ancestors for most of the male/female pairs.

Two varieties, East Kent Golding (high = 0.0250 and low = 0.0000) and Fuggle N (high = 0.1078 and low = 0.0219), had COA values that were higher for the HGD group than those for the LGD group. In the East Kent Golding case, little is known about the actual ancestry and it has been used infrequently in USDA-ARS crosses. In our study, only two of the males had known coancestry with East Kent Golding and both of these were part of the HGD group. Whether members of the LGD group are related to East Kent Golding is unknown. In the case of Fuggle N, a number of the HGD group exhibited high coancestry with Fuggle N. Male 19009M (COA = 0.375), originating from a mother/son cross, represents one generation of backcrossing. Another male, 21437M, is a son of Fuggle with unknown paternal heritage. Why high levels of genetic distance would be observed between these two mother/son pairs is unknown. It is possible that sample DNA for Fuggle N was degraded or not adequately purified resulting in false positives, missing bands, or both. However, an examination of the banding patterns for this variety did not reveal any noticeable incongruities with other accessions, and the AFLP fingerprint for this unknown genotype is most likely accurate. A more likely explanation is that the genotype examined in this study, labeled as "Fuggle N" in the greenhouse is actually not Fuggle N, but some other unrelated genotype that was mislabeled. Further research is underway to ascertain the identity of this genotype.

We chose the top 10 most diverse male/female pairs (HGD pairs) and listed them in Table IV and also chose the bottom 10 or least diverse male/female pairs (LGD pairs) and listed these in Table V. A qualitative examination of the HGD male/female pairs reveals that of these pairs with GD values greater than 0.36, 90% had COA values less than 0.01 (Table IV) suggesting potential sources of heterosis based on molecular measures of diversity and ancestral records. Because AFLP-derived GD values greater than 0.36 appear to be associated with low potential of inbreeding and the potential for heterosis in hop, we recommend choosing male/female pairs with AFLP-derived GD values greater than 0.36 as a starting point when evaluating potential crosses.

Lists covering the HGD and LGD groups of males for each female reveal several points of interest for hop breeders. The listing of HGD pairs (Table IV) reveals numerous potential crosses between females and males that could potentially result in heterotic offspring on the basis of large GD values and low COA. On the other hand, several of the male/female pairs listed in the LGD table (Table V) appear to have little chance of expressing heterosis on the basis of pedigree (COA) and genetic distance (GD) estimates (Tables IV and V). The COA for any two individuals, i and j, is equivalent to the level of inbreeding in offspring k resulting from a cross between i and j, or $COA_{ij} = F_{ijk}$. Values for coefficients of coancestry in consanguineous relationships are as follows:

Parent–Offspring (PO) = 0.25
Full-Sib (FS) = 0.25
Half-Sib (HS) = 0.125
1st Cousins (FC) = 0.0625

Inbreeding depression in most diploid crops is observed in all of these consanguineous matings, and the severity of inbreeding depression is usually correlated with the COA value. Hop matings are no exception. Thus, any male/female pair that has low genetic distance and high COA should have a high probability of offspring exhibiting inbreeding depression. Six varieties studied here require special consideration.

The varieties, Cascade, Nugget, Brewers Gold, and Comet, have low GD and high COA with several males (Table V and Table IV for Fuggle N). In particular, of 10 LGD pairs listed for Cascade, seven had COA values equal or exceeding PO or FS matings. In Brewers Gold and Nugget, 8 of 10 LGD pairs listed had COA values equal or greater than that expected for FC, while Comet had 6 of 10. Certainly, crosses between parents with LGD and high COA should be avoided unless inbreeding and fixing of specific traits is desired.

Some other issues that we observed from this study were the numbers of offspring from specific varieties that are maintained in the USDA collection. Seventeen of the males included in this study are offspring of Cascade. Five of these 17 have notable inbreeding (21426M, 21427M, 21428M, 21432M, and 21462M). Another variety, Comet, has nine offspring included in this study with several other offspring in the collection but not included in this study. If inbreeding is to be avoided in cases such as Cascade and Comet, unrelated males should be used for crosses onto these two females. Several of these unrelated males are listed in Table VI.

To illustrate the effect of inbreeding in hop, consider the use of Nugget. Attempts to cross Nugget with many of the males maintained in the USDA collection, as well as the Washington State University collection, have repeatedly resulted in offspring that are inferior to Nugget (J. A. Henning and S. Kenny, data not shown).

The COA value for the parents of Nugget is equal to 0.1875 and it follows that the inbreeding value for Nugget is F = 0.1875. Along with the observed inbreeding in Nugget, we identified 20 males included in the study that are related to Nugget (six HSs [COA = 0.125], 11 nephews [COA = 0.0625], and 3 great-nephews [COA = 0.0325]). In addition, three ancestral males of Nugget (also part of the germ plasm collection) were included in this study with the father (63015M) having a COA value of 0.4065 with Nugget. The other ancestral males include one grandfather (19058M, COA = 0.125) and one great-grandfather (19062M, COA = 0.0625). Many of the males related to Nugget have been repeatedly used by breeders at the USDA and Washington State University for germ plasm development and crosses onto Nugget. If success in crossing with Nugget is desired, then breeders must choose male accessions that are unrelated to Nugget to ensure no inbreeding depression in the resulting offspring. As an example, Table IV reports several males (21089M, 21435M, 64035M are commonly used by the USDA-ARS for crossing purposes) with high levels of genetic distance from and no known genetic relationship to Nugget. Crosses onto these males using Nugget could potentially result in heterotic offspring while maintaining some of the desirable characteristics of Nugget.

Use of COA's for determining potential heterotic pairs has as an inherent risk the possibility that ancestors are not fully defined and that low or zero COA's between individuals may or may not be the true representation of relatedness. In the USDA-ARS hop collection, it is possible for two individuals thought to be genetically unrelated, to have an identical grandfather or great-grandfather because of the prevalent use of open pollination crosses prior to the 1950s. Records of the males present in the hopyard prior to the 1950s are no longer available and, therefore it is impossible to know the identity of the males that could have potentially contributed pollen to an open pollination cross. The benefits of using COA's or pedigree analysis is that breeders do not require the use of molecular tools and the expense associated with such procedures to actually obtain an estimate of genetic relatedness. Certainly this benefit is of great value for cash-strapped breeding companies and poorly funded research programs.

Contrarily, the use of molecular methods, particularly AFLP, for determining genetic relationships among individuals has as its benefit two factors in its favor. First, AFLP can be used to estimate GD among individuals of unknown pedigree, while COA estimation can only be accomplished when the ancestry of most if not all individuals is known. Second, it is a highly accurate method of estimating GD between individuals. Some research suggests that the percentage of genome that the molecular tool actually covers determines the ultimate accuracy of the GD value (the greater the coverage of the genome, the more accurate the GD value) (17). Studies by Virk et al (31) and Le Clerc et al (15,16) demonstrated that the use of mapped markers spanning the genome have not given greater precision than the use of random markers. Regardless, numerous studies (1,9,28) report a significant positive correlation between increased precision of GD estimates and the number of polymorphic markers used in the estimation. In our study, we utilized 490 polymorphic bands in the estimation of GD. This is significantly larger than the minimal optimum number of polymorphic loci (60 bands) reported by Le Clerc et al (16) as being necessary for precise estimation of GD.

The results of this study illustrate several important issues for hop breeders. First, choice of males for crossing to a particular female must include information on the relatedness of the male/female pair in addition to their respective characteristics for brewing and yield. The use of unrelated males must be pursued if inbreeding depression is to be avoided and potential heterosis maximized. Second, the germ plasm pool used by hop breeders has become relatively narrow and new germ plasm must be consistently incorporated to maintain genetic diversity and ultimately maximize heterosis. As female hop accessions are used for brewing and most brewers prefer using similar hop varieties in brewing, the greatest source of variability to introduce superior traits into specific hop lines is the use of new male genotypes. Thus, efforts should be made to not only focus on the development of superior female genotypes but also on superior, unique male lines that maximize heterosis in offspring.

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