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ORIGINAL PAPER

# High-throughput genotyping of hop (*Humulus lupulus* L.) utilising diversity arrays technology (DArT)

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**Abstract** Implementation of molecular methods in hop (*Humulus lupulus* L.) breeding is dependent on the availability of sizeable numbers of polymorphic markers and a comprehensive understanding of genetic variation. However, use of molecular marker technology is limited due to expense, time inefficiency, laborious methodology and dependence on DNA sequence information. Diversity arrays technology (DArT) is a high-throughput cost-effective method for the discovery of large numbers of quality polymorphic markers without reliance on DNA sequence information. This study is the first to utilise DArT for hop genotyping, identifying 730 polymorphic markers from 92 hop accessions. The marker quality was high and similar to the quality of DArT markers previously generated for other species; although percentage polymorphism and polymorphism information content (PIC) were lower than in previous studies deploying other marker systems in hop. Genetic relationships in hop illustrated by DArT in this study coincide with knowledge generated using alternate methods. Several statistical analyses separated the hop accessions into genetically differentiated North American and European groupings, with hybrids between the two groups clearly distinguishable. Levels of genetic diversity were similar in the North American and European groups, but higher in the hybrid group. The markers produced from this time and cost-efficient genotyping tool will be a valuable resource for numerous applications in hop breeding and genetics studies, such as mapping, marker-assisted selection, genetic identity testing, guidance in the maintenance of genetic diversity and the directed breeding of superior cultivars.

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## Introduction

Hop (*Humulus lupulus* L.) is a dioecious cone-bearing plant cultivated for use predominantly by the beer brewing industry (Neve 1991). Lupulin, a resin in the cones of the female hop plant, contains organic acids and essential oils which impart bitterness, flavour and preservation to beer (Goese et al. 1999; Mori 1961; Roberts and Stevens 1962; Tressl et al. 1983; Verzele 1986). Lupulin also contains other compounds with potential for the phytoceutical industry, including 8-prenylnaringenin, a potent phytoestrogen (Liu et al. 2001; Milligan et al. 2002), and xanthohumol, which exhibits possible anti-cancer properties (Milligan et al. 2002; Miranda et al. 1999).

*H. lupulus* has a native distribution throughout the Northern Hemisphere, between latitudes of approximately 35° and 70° North (Neve 1991). The species *H. lupulus* has been classified into five taxonomic varieties based on morphology and reflecting geographical distribution: var. *lupulus* from Europe and Western Asia (although it has been introduced widely); var. *cordifolius* from Eastern Asia; and var. *lupuloides*, var. *neomexicanus* and var. *pubescens* from East, West and midwest North America, respectively (Small 1978). Cultivated hops are derived from primarily var. *lupulus* ancestry, as it has traditionally been European landraces that have provided the flavour characteristics sought after by beer brewers (Neve 1991). In an attempt to expand the genetic variation of the hop breeding resource, North American wild hops have been hybridised with European cultivars (Moir 2000). The incorporation of North American germplasm has imparted several favourable qualities, including pest and disease resistance, higher yielding capacity and varying bittering potentials (Moir 2000; Neve 1991). Hops native to Asia are not commonly used in breeding programmes (Peredo et al. 2010).

Commercial hop cultivation occurs in many parts of the world, including Europe, North America, South Africa, Australia and New Zealand. Breeding programmes, operating largely independently, aspire to the development of new and improved cultivars, with a focus on yield, disease resistance and resin content and chemistry. Hop improvement relies on the effective utilisation of genetic diversity. Analysis of the world's major hop cultivars suggests limited genetic variability between them (Jakše et al. 2001), as despite a long cultivation history, current hop cultivars are derived from a narrow genetic source (Murakami et al. 2006b). This is indicative of restrictions of the current hop breeding varieties as sources for hop genetic improvement, verifying the need to understand the scope of genetic diversity available throughout the world.

Several studies have attempted to measure the genetic variation that exists in wild hops and to determine how much of this variation is captured in cultivated hop material (Bassil et al. 2008; Jakše et al. 2004; Murakami et al. 2006a, b; Patzak et al. 2010a, b; Peredo et al. 2010; Stajner et al. 2008; Townsend and Henning 2009). Genetic variation has also been used as a means of classification of hop germplasm, to assist hop breeders when making choices about which individuals to select as breeding parents, which individuals to retain to conserve the genetics of the hop collection and which new accessions to introduce to expand the genetics of the collection.

The earliest assessments of genetic variation in hop relied upon morphological studies (Davis 1957; Small 1980, 1981). The use of biochemical markers, such as essential oils and flavonoids, was later employed (Green 1986; Kammhuber 1997; Kralj et al. 1991; Nickerson et al. 1988; Stevens et al. 2000). In recent years, molecular marker technologies have been developed, allowing more directed and sophisticated investigation into hop variability and identity typing. Several different molecular markers have been utilised: random amplified polymorphic DNA (RAPD) (Brady et al. 1996; Patzak 2001; Šuštar-Vozlič and Javornik 1999); amplified fragment length polymorphisms (AFLP) (Hartl and Seefelder 1998; Henning et al. 2004; Jakše et al. 2001; Patzak 2001; Seefelder et al. 2000a; Townsend and Henning 2009); microsatellites (Bassil et al. 2008; Hadonou et al. 2004; Jakše et al. 2004; Murakami et al. 2006a; Patzak 2001; Patzak et al. 2010a, b; Peredo et al. 2010; Stajner et al. 2008); inter-simple sequence repeats (ISSR) (Danilova et al. 2003; Patzak 2001) and sequence tagged sites (STS) (Brady et al. 1996; Patzak 2001; Patzak et al. 2010b, 2007; Peredo et al. 2010). The majority of these studies have reached the consensus that there are two primary genetic groups: European (including wild and cultivated material) and North American (wild material only) (Bassil et al. 2008; Henning et al. 2004; Jakše et al. 2004; Murakami et al. 2006a; Patzak et al. 2010b; Peredo et al. 2010; Stajner et al. 2008; Šuštar-Vozlič and Javornik 1999). Some studies have been able to further resolve the European genetic group into smaller groups, corresponding to geographical origin (Bassil et al. 2008; Danilova et al. 2003; Henning et al. 2004; Jakše et al. 2004; Murakami 2000; Murakami et al. 2006a; Patzak et al. 2010b), breeding history (Murakami 2000; Stajner et al. 2008; Šuštar-Vozlič and Javornik 1999) and chemical content (Henning et al. 2004; Šuštar-Vozlič and Javornik 1999). Hybrids between the European and North American genetic groups have also been distinguished, and subgroups differentiated corresponding to geographical origin and pedigree data (Henning et al. 2004; Seefelder et al. 2000a).

While the use of the molecular markers discussed above has greatly expanded our understanding of genetic variation in hop, the cost of these marker technologies remains an obstacle to their utilization in breeding programmes for

the purpose of hop germplasm classification and selection of accessions. Use of the marker technologies is further constrained by their low throughput capacities, as a result of their dependence on gel electrophoresis or laborious and intensive DNA sequencing processes (Jaccoud et al. 2001; Kilian et al. 2005; Luikart et al. 2003; Wenzl et al. 2004).

Diversity arrays technology (DArT) is a relatively new DNA marker technology for genome profiling and genotyping of genetic variation that was invented to overcome limitations of other molecular marker technologies, including RFLP, AFLP and SSR (Jaccoud et al. 2001). It was developed for particular application to non-model species, mainly crop species for which limited resources may be available (Jaccoud et al. 2001; Kilian et al. 2005). DArT is a microarray-based technology that has the ability to detect all types of DNA variation: single nucleotide polymorphism (SNP), indel, copy number variation (CNV) and methylation (Kilian et al. 2005). It enables simultaneous typing of several hundred polymorphic loci in parallel, without relying on sequence data (Jaccoud et al. 2001; Kilian et al. 2005; Luikart et al. 2003; Wenzl et al. 2004; Wittenberg et al. 2005). DArT is a very high-throughput and robust system, capable of providing comprehensive genome coverage and markers of high quality, whilst also being relatively inexpensive (Jaccoud et al. 2001; Kilian et al. 2005; Luikart et al. 2003; Wenzl et al. 2004; Wittenberg et al. 2005). These factors offer significant advantages over other molecular marker technologies.

This paper evaluates the effectiveness of DArT as a high-throughput genotyping technology in hop. The robustness of DArT, in terms of the number of polymorphic markers generated from selected accessions and the quality of these markers is examined. The utility of DArT for analysis of genetic diversity is assessed in a representative of hop accessions. The results of this analysis are compared with the current understanding of hop molecular variation and phylogenetics, as a test of the accuracy and resolution of DArT. Hop is a relatively resource-poor agricultural species, dependant on limited genetic sequence information, and a comparatively small research base. We anticipate that the hop community will benefit from the combined efforts of this international consortium, and the high-throughput and cost-effective advantages of DArT.

## Materials and methods

### Plant materials

A total of 92 hop accessions were included in the DArT analysis (Table 1). These accessions were sourced from Europe, North America and Australia. The sample set comprised 32 wild and 60 cultivated accessions, both historical and currently used in commercial production. Examples of four of the five taxonomic varieties of *H. lupulus* were included: var. *lupulus*, var. *lupuloides*, var. *pubescens* and var. *neomexicanus*. Each accession was sampled from one individual plant. Replicates of six genotypes were conducted to test the consistency and robustness of the DArT marker system (Table 1). Samples were sourced in 2004 and 2005 from collections held by Wye Hops (Canterbury, Kent, England), John I. Haas Inc. (Yakima, WA, USA), USDA-ARS National Genetic Resources Programme Germplasm Resources Information Network (GRIN) (Beltsville, MD, USA), Hop Products Australia (Bushy Park, TAS, Australia; and Eurobin, VIC, Australia) and the Slovenian Institute of Hop Research and Brewing (Žalec, Slovenia). Pedigree information (where available) has been published previously (Brady et al. 1996; Jakše et al. 2010; Patzak 2001; Seefelder et al. 2000a; Šuštar-Vozlič and Javornik 1999), or was provided by Kim Hummer (NCGR Corvalis) or the authors.

### DNA extraction

DNA was extracted from dormant rhizome, bud, leaf or tissue-cultured plant samples. Extractions were performed using the common CTAB extraction protocol (Kump and Javornik 1996) with three rounds of chloroform extraction in the Chair of Genetics laboratory (Slovenia). DNA was measured by means of fluorimetry using DyNA Quant 200 (GE Healthcare). DNA quality of selected samples was verified by digesting the 1 μg of isolated DNA with four restriction enzymes *HaeIII*, *Sau3AI*, *MseI and AluI*; this DNA was run together with undigested DNA on 1.0% agarose gels to ensure that the undigested DNA formed a tight band of high molecular weight, the digested DNA formed a smear of mid- to low molecular weight, and there was no RNA contamination. DNA concentrations of samples were adjusted to 100 ng/μL. Fifteen micrograms of DNA was sent to DArT Pty Ltd according to Australian Quarantine and Inspection Service safety measurements.

### Development of DArT markers

#### *Generation of genomic representations and library construction*

Several complexity reduction methods were tested, using the rare-cutting restriction enzyme *PstI* in combination with a range of frequently cutting restriction enzymes (data not shown). The *PstI/BstNI* combination was selected as the highest performing method. For each of the 92 hop accessions, approximately 0.5 μL of DNA at a concentration of approximately 100 ng/μL was digested with

**Table 1** The identity (cultivar name or accession number) of the 92 hop accessions (including replicates) analysed in this study, along with their domestication status (cultivated or wild), taxonomic variety classification (*lupulus*, *lupuloides*, *pubescens* or *neomexicanus*), and the location from which the sample was collected (not necessarily their genetic origin)

| Name | Domestication status | Taxonomy | Geographical origin |
|---|---|---|---|
| Aurora | Cultivated | *lupulus* | Slovenia |
| Bor | Cultivated | *lupulus* | Czech Republic |
| Brewer's Gold | Cultivated | *lupulus* | UK |
| Cascade | Cultivated | *lupulus* | USA |
| Celeia[a] | Cultivated | *lupulus* | Slovenia |
| Celeia[a] | Cultivated | *lupulus* | Slovenia |
| Chang Bei 2 | Cultivated | *lupulus* | China |
| Chinook | Cultivated | *lupulus* | USA |
| Cluster[a,b] | Cultivated | *lupulus* | USA |
| Cluster[a,b] | Cultivated | *lupulus* | USA |
| Cobbs | Cultivated | *lupulus* | UK |
| Comet | Cultivated | *lupulus* | USA |
| Ellupulo | Cultivated | *lupulus* | Argentina |
| First Choice | Cultivated | *lupulus* | New Zealand |
| Fuggle | Cultivated | *lupulus* | UK |
| Galena | Cultivated | *lupulus* | USA |
| Ging Dao Do Hua[b] | Cultivated | *lupulus* | China |
| Glacier | Cultivated | *lupulus* | USA |
| Hallertauer Gold | Cultivated | *lupulus* | Germany |
| Hallertauer MTF | Cultivated | *lupulus* | Germany |
| Hallertauer Tradition | Cultivated | *lupulus* | Germany |
| Hersbrucker | Cultivated | *lupulus* | Germany |
| Huller Bitterer | Cultivated | *lupulus* | Germany |
| INT 101 | Wild | *lupulus* | Japan |
| K11 | Wild | *lupulus* | Georgia |
| K5 | Wild | *lupulus* | Georgia |
| Keyworth Midseason | Cultivated | *lupulus* | UK |
| Kirin | Cultivated | *lupulus* | Japan |
| Kitamidori | Cultivated | *lupulus* | Japan |
| Liberty | Cultivated | *lupulus* | USA |
| *lupulus* Austria | Wild | *lupulus* | Austria |
| *lupulus* Bavaria | Wild | *lupulus* | Germany |
| *lupulus* Berlin | Wild | *lupulus* | Germany |
| Magnum | Cultivated | *lupulus* | Germany |
| Merkur | Cultivated | *lupulus* | Germany |
| Millennium | Cultivated | *lupulus* | USA |
| No3-38[a] | Wild | *lupulus* | Japan |
| No3-38[a] | Wild | *lupulus* | Japan |
| Nordgard-978 | Cultivated | *lupulus* | Denmark |
| Northern Brewer | Cultivated | *lupulus* | UK |
| Nugget | Cultivated | *lupulus* | USA |
| OB21 | Cultivated | *lupulus* | UK |
| Osvald's Clone 72 | Cultivated | *lupulus* | Czech Republic |
| Pacific Gem | Cultivated | *lupulus* | New Zealand |
| Pride of Ringwood | Cultivated | *lupulus* | Australia |
| R15 | Wild | *lupulus* | Russia |
| R19 | Wild | *lupulus* | Russia |

**Table 1** continued

| Name | Domestication status | Taxonomy | Geographical origin |
|---|---|---|---|
| Saazer | Cultivated | *lupulus* | Czech Republic |
| Sereberianka[a] | Cultivated | *lupulus* | Russia |
| Sereberianka[a] | Cultivated | *lupulus* | Russia |
| Southern Brewer | Cultivated | *lupulus* | South Africa |
| Strisselspalt | Cultivated | *lupulus* | France |
| Symphony | Cultivated | *lupulus* | USA |
| Tettnanger | Cultivated | *lupulus* | Germany |
| Topaz | Cultivated | *lupulus* | Australia |
| Tutsham | Cultivated | *lupulus* | UK |
| Urozajni | Cultivated | *lupulus* | Russia |
| Warrior | Cultivated | *lupulus* | USA |
| Wild Italy | Wild | *lupulus* | Italy |
| Wye Challenger | Cultivated | *lupulus* | UK |
| Wye Target[a] | Cultivated | *lupulus* | UK |
| Wye Target[a] | Cultivated | *lupulus* | UK |
| 1000 | Wild | *Iupuloides* | USA |
| 1006 | Wild | *Iupuloides* | Canada |
| 1008 | Wild | *Iupuloides* | Canada |
| 1018 | Wild | *Iupuloides* | Canada |
| 1020 | Wild | *pubescens* | USA |
| 1355 | Wild | *neomexicanus* | USA |
| 1386 | Wild | *neomexicanus* | USA |
| 1401 | Wild | *neomexicanus* | USA |
| 1437 | Wild | *neomexicanus* | USA |
| 19058 | Cultivated | *lupulus* | USA |
| 64035 | Cultivated | *lupulus* | USA |
| 558589 | Wild | *lupulus* | USA |
| 558607 | Wild | *lupulus* | Ex-Yugoslavia |
| 558900 | Wild | *lupulus* | USA |
| 558906 | Wild | *lupulus* | USA |
| 559234 | Wild | *lupulus* | USA |
| 617471 | Wild | *pubescens* | USA |
| 1025_007 | Wild | *lupulus* | Khazakhstan |
| 14/74/209 | Cultivated | *lupulus* | UK |
| 21055 | Cultivated | *lupulus* | USA |
| 23/77/64[a] | Cultivated | *lupulus* | UK |
| 23/77/64[a] | Cultivated | *lupulus* | UK |
| 29/70/54 | Cultivated | *lupulus* | UK |
| 5/1 | Cultivated | *lupulus* | Slovenia |
| 9/2 | Cultivated | *lupulus* | Slovenia |
| A12 | Wild | *lupulus* | Russia |
| AH1-A | Wild | *lupulus* | Ex-Yugoslavia |
| AH22-I | Wild | *lupulus* | Ex-Yugoslavia |
| AH7-D | Wild | *lupulus* | Ex-Yugoslavia |
| AH9 | Wild | *lupulus* | Ex-Yugoslavia |

[a] Replication undertaken for this genotype: separate plant, clonally propagated, with the same name, collected from the same collection at the same geographical origin

[b] Accessions Cluster and Ging Dao Do Hua are the same genotype, under two cultivar names

*PstI/BstNI* restriction enzyme combination. *PstI* overhang compatible adaptors were ligated, and *PstI* fragments without *BstNI* sites were amplified using primers complementary to the adapter. The method closely followed the protocol described by Wenzl et al. (2004). Approximately 1 μL of PCR product from all accessions used in the study was mixed and used to construct a library of 6,144 clones (4,608 clones from cultivated accessions and 1,536 clones from wild accessions, generated using two independent libraries) using a pCR2.1-TOPO vector (Invitrogen), according to the manufacturer's instructions.

*Microarray preparation*

Inserts from individual clones were amplified in 384 microtiter plates using M13 primers, so that part of the polylinker region of the cloning vector was co-amplified. The amplicons were dried at 37°C, washed with 70% ethanol, and dissolved in a spotting buffer developed specifically for Erie Scientific poly-L-lysine microarray slides (Wenzl et al., in preparation). The arrays, containing inserts from the 6,144 clones, were printed in duplicate using a MicroGridII arrayer (Biorobotics, Cambridge, UK) onto poly-L-lysine-coated slides (Erie Scientific, Portsmouth, NH, USA). After printing, slides were heated to 80°C for 2 h, incubated in 95°C water bath for 2 min and dried by centrifugation.

*Preparation of sample genomic representations and hybridisation to genotyping arrays*

Genomic representations from each sample were prepared using the same method as for library construction (see above), but instead of cloning the resulting amplicons, they were precipitated with isopropanol, washed with 70% ethanol, dried and labelled with the fluorescent dyes 1 mM Cy3-dUTP or 1 mM Cy5-dUTP (Amersham). Labelled representations ('targets') were mixed with a FAM-labelled polylinker fragment of the vector that was used to clone the representation fragments (pCR2.1-TOPO). When amplifying the inserts spotted onto the DArT array the polylinker was co-amplified in two pieces at the ends of each insert so that it could be used to quantify the amount of DNA in each spot on the array. For quality control, ten accessions were genotyped twice. The labelled targets were then denatured and hybridised to the genotyping arrays overnight at 62°C.

*Slide scanning, data extraction and assessment of DArT markers*

After hybridisation, the slides were washed, following the methodology of Jaccoud et al. (2001), and scanned using a Tecan LS300 (Grödig, Salzburg, Austria) confocal laser scanner. Three images were generated from each slide. One image, produced with a 488 nm laser, was used for quality control and image processing by measuring the hybridisation intensity of the FAM-labelled reference fragments. The remaining two images were used as independent targets, one produced with 543 nm laser (Cy3-labelled targets) and one produced with a 633 nm laser (Cy5-labelled targets). The image processing and marker classification were performed using DArTsoft version 7.3 (DArT P/L, unpublished), a dedicated software package developed at DArT P/L (Yarralumla, Australia), as described previously by Wenzl et al. (2004). It should be noted that it is not necessary to obtain the DNA sequence of each marker, as the scoring of markers relies on the measure of hybridisation intensity. The program computed several quality parameters for each marker: (a) *P* value, the variance of the relative target hybridisation intensity between allelic states as a percentage of the total variance; (b) call-rate, the percentage of DNA samples with binary ('0' or '1') allele calls; and (c) reproducibility, the fraction of concordant calls for replicate assays. Markers with $P > 77\%$, call rate $> 85\%$ and 100% allele-calling consistency across the ten replicated accessions were selected as markers. Polymorphism information content (PIC), a measure of the informativeness of a genetic marker, was also calculated for each marker according to Anderson et al. (1993), using the formula:

$$\mathrm{PIC} = 1 - \sum_{i=1} nPi^2$$

where *Pi* is the population frequency of the *i*th allele and *n* is the total number of allelic states.

When using such stringent thresholds for the *P* value, high-quality markers with low frequency of minor alleles are potentially eliminated, thus reducing PIC. These quality parameters can be used to compare to other species to which the DArT marker technology has been applied and to other marker technologies applied to hop to evaluate the robustness of DArT as a high-throughput genotyping technology in hop.

*Analysis of phylogenetics and genetic diversity*

A pairwise genetic distance matrix (Nei and Li 1979) was computed on the basis of shared presence of fragments (minimising error due to non-homologous shared absences) using PAUP* version 4.0b10 (Swofford 2002). The DArTsoft-generated 0–1 scores were used as input. Markers were filtered using AFLPop, and all redundant markers were excluded. The genetic distance matrix was used to produce an unrooted Unweighted Pair Group Method with Algorithmic Mean (UPGMA) dendrogram using PAUP* version 4.0b10 (Swofford 2002). Partitioning of taxa into genetic groups was investigated by Principal Co-ordinates Analysis (PCoA). The genetic distance matrix was exported to

NTSYS-PC 2.1 (Rohlf 2000) for PCoA, which was performed using the DCENTRE, EIGEN and plotting modules. The distribution of genetic variation within the accessions included in this experiment was examined through Analysis of Molecular Variance (AMOVA) (Excoffier et al. 1992). AMOVA was calculated using Arlequin version 3.5.1.2 (Excoffier et al. 1992). Loci with >10% missing values were excluded, resulting in a distance computation based on 686 loci. Accession groupings for the AMOVA analysis were defined arbitrarily, as those identified by PCoA. Non-clustered accessions were not included in the AMOVA analysis, and nor were the following triploid samples: Celeia, Liberty, Millennium, Pacific Gem, Symphony, Topaz and Warrior. Significance of group partitioning was tested using 10,000 permutations. Pairwise genetic distances among groups (*F*-statistics) (Wright 1951), and average gene diversity over loci ($\pi_n$) (equivalent to the probability that two randomly chosen homologous nucleotides are different) (Nei 1987; Tajima 1983), were calculated using Arlequin version 3.5.1.2 (Excoffier et al. 1992), using the same conditions as for AMOVA (above). Student's *t* tests (Student 1908) were performed to determine whether differences in $\pi_n$ between groups were significant. Model-based clustering, employing a Bayesian algorithm, was applied to infer the genetic structure of the 92 hop accessions using STRUCTURE version 2.3.1 (Pritchard et al. 2000). A total of 451 loci were examined, with loci possessing >9 missing values excluded. Ten independent runs of the program were performed by setting the number of groups (*K*) from 1 to 12, each run consisted of a burn-in period of 100,000 iterations followed by 1,000,000 Monte Carlo Markov Chain (MCMC) iterations, assuming an admixture model and correlated allele frequencies. For other settings, program defaults were used and no prior information was used to define the groups. The most likely number of groups (*K*) was chosen, based on the ad hoc statistic $\Delta K$ according to Evanno et al. (2005). The data were analysed by the online version of STRUCTURE HARVESTER (Earl 2009). Any accession with a proportion of 0.993% or greater of a cluster was considered to be pure for that cluster, with the remaining 0.007% or less attributable to non-statistical variability. Graphical representation of clustering was made by CLUMPP (Jakobsson and Risenberg 2007) and DISTRUCT (Rosenberg 2004) software packages.

## Results

### Development of DArT markers in hop

A total of 6,144 DArT clones were generated from 92 hop accessions, from which 730 polymorphic markers were identified through DArTsoft analysis using highly stringent quality criteria. This resulted in 11.9% frequency of polymorphism (Table 2). Performance of the DArT markers was measured through several different parameters (Table 2). PIC values for these markers averaged 0.335. Scoring reproducibility and call rate were both close to 100%, with averages of 99.97 and 97.58%, respectively. The *P* value, which is the principal measure of marker quality, averaged 89.90%. Relaxing the marker quality thresholds slightly, by allowing up to 2% scoring inconsistency for the lower quality markers, increased the number of markers to 968 (15.8% polymorphism frequency) with only a small reduction to average marker reproducibility (99.7%) and without a decrease in average call rate (approximately 98%); however, all analysis reported in this paper were performed on the very stringent (730) set of markers.

### Analysis of phylogenetics and genetic diversity in hop

To validate the robustness of DArT in a hop system, in terms of capturing the multiplicity of sequence information available, the 730 polymorphic DArT markers were used to assess the genetic diversity of the 92 hop accessions (Table 1). Based on a pairwise genetic distance matrix (Nei and Li 1979), PCoA was undertaken (Fig. 1) and an UPGMA dendrogram was constructed (Fig. 2). In the PCoA, the first two vectors cumulatively accounted for 87% of the total variance detected, comprising 69 and 18% from the first and second vectors, respectively. Ordination of the first two vectors identified three clusters (Fig. 1). The first cluster (outlined in red) contained 13 accessions (Fig. 1), all of which were wild North American hops and included all accessions of the taxonomic varieties *lupuloides*, *pubescens* and *neomexicanus*. The second cluster (outlined in blue) contained 34 accessions (Fig. 1.), consisting of wild European hops, and cultivars of solely European genetic origin. The third cluster (outlined in green) contained 30 accessions (Fig. 1), comprising cultivars derived from hybridisation between European and North American hops. A large divergence was observed between the North American cluster and the European and hybrid clusters (Fig. 1). The European and hybrid clusters, with the hybrid cluster situated in between the North American and European clusters, were not as

**Table 2** Mean ± standard error of quality parameters for the 730 polymorphic DArT markers identified in 92 hop accessions

| | |
|---|---|
| Polymorphism (%) | 11.9 |
| PIC (polymorphism information content) | 0.335 ± 0.004 |
| *P* (%) | 89.901 ± 0.214 |
| Reproducibility (%) | 99.970 ± 0.009 |
| Call rate (%) | 97.582 ± 0.120 |

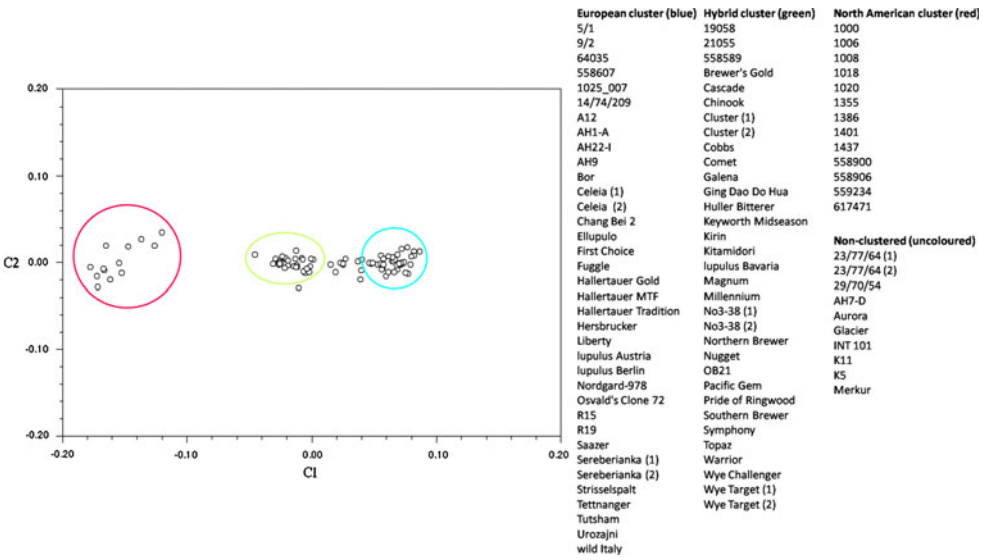



**Fig. 1** PCoA of 92 hop accessions based on 730 DArT markers, showing the ordination of the first two vectors. Principal co-ordinate 1 (*C1*) explained 69% of variation, and principal co-ordinate 2 (*C2*) explained 18% of variation. Accessions found within each cluster, as well as non-clustered, are listed

discreet (Fig. 1). Based on the available pedigree information, three accessions did not cluster as expected. Cobbs was situated in the hybrid cluster rather than the European cluster, 14/74/209 was situated in the European cluster rather than the hybrid cluster and 558589, a North American wild hop, fell within the hybrid cluster rather than the North American cluster (Fig. 1). A total of nine accessions could not be placed into any of the three clusters (Fig. 1), and according to previous pedigree information (or lack thereof) they could not form a justifiable fourth cluster. Of the nine non-clustered accessions, eight were positioned between the European and hybrid clusters; the remaining accession (INT 101), of wild Japanese origin, was situated directly below the hybrid cluster (Fig. 1), but on the third PCoA vector, accounting for 2% of total variance, this accession was separated from all other accessions (data not shown). Genotypes that were replicated in the analysis (Celeia, Cluster, No3-38, Sereberianka, Wye Target and 23/77/64) clustered consistently, as expected.

Similar patterns were observed in the UPGMA dendrogram (Fig. 2), with major disjunction occurring between North American wild hop accessions (red) and all other accessions. All North American accessions were positioned exclusively within their own cluster, while a second cluster contained both the European (blue) and hybrid (green) accessions (Fig. 2), indicative of higher genetic similarity between European and hybrid accessions than between North American and European or North American and hybrid accessions. Within the cluster containing European and hybrid accessions, all European accessions grouped together, and displayed less genetic similarity to the North American accessions than all hybrid accessions, except for Wye Target and Keyworth Midseason which grouped with the European accessions (Fig. 2). The grouping of these hybrid accessions with the European accessions indicates that these accessions had a higher genetic similarity to the European accessions than other hybrid accessions. Several hybrid accessions were found to be less genetically similar to European accessions than others, namely 21055, a cultivated hop, and 558589, included as a North American wild hop; however, these accessions still appeared to be hybrids rather than North American accessions (Fig. 2). In the case of 558589, its clustering with the hybrid accessions suggests that despite an apparent likeness to North American wild hop, its genetic composition has arisen through introgression of European genetics. Consistent with the PCoA, Cobbs again fell within the hybrid cluster, showing high genetic similarity to Nugget. This unexpected clustering suggests mislabelling of the Cobbs accession (Fig. 2). The accession 14/74/209 again fell within the European cluster. Some resolution was given to those accessions that did not cluster in the PCoA (Fig. 1). The accessions 29/70/54, Aurora and AH7-D fell within the group of European accessions (Fig. 2), indicating that they are of European genetic origin. The accessions Glacier and Merkur grouped with Wye Target, while K5, K11 and 23/77/64 fell at the periphery of this group (Fig. 2). While these accessions may be of higher genetic similarity to European accessions than other hybrid accessions, it cannot be determined whether they themselves are hybrids, genetically intermediate, or of pure European genetic origin. The accession INT 101, of wild Japanese origin, fell within the group containing the hybrid accessions next to No3-38, also of wild Japanese origin (Fig. 2), indicating that it is genetically intermediate between North American and European hops, but it is more genetically similar to European hops than to North American hops. As in the PCoA, all replications clustered consistently.

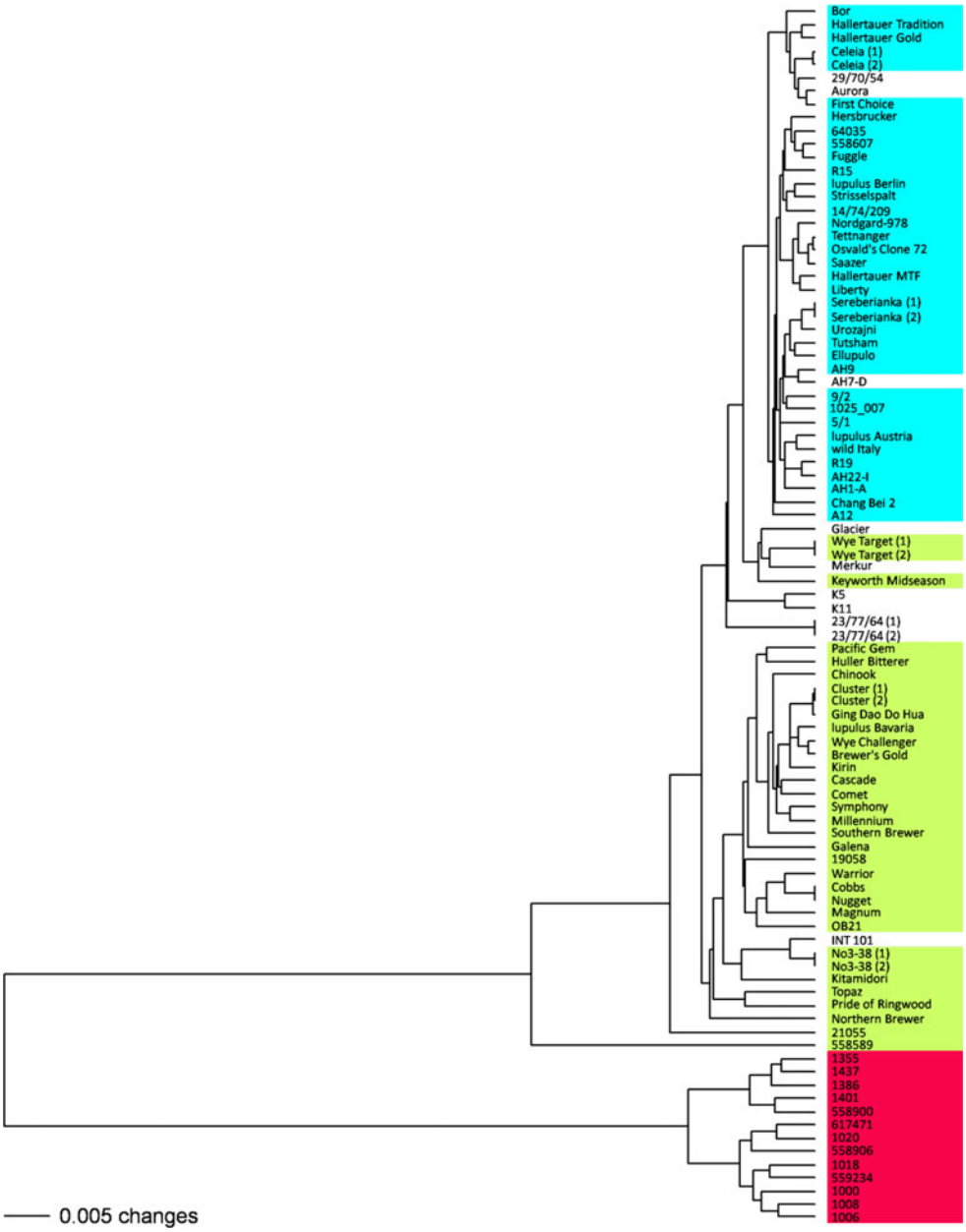



**Fig. 2** An UPGMA dendrogram (unrooted) representing the relationships between 92 hop accessions, based on 730 DArT markers

AMOVA across the three groups (North American, European and hybrid), as defined arbitrarily by PCoA clustering (Fig. 1), indicated significant partitioning of genetic variation, with 75.2% of the detected variation existing between the groups (Table 3), and the remaining 24.8% within groups ($P < 0.001$) (Table 3). Pairwise $F_{st}$ values (Table 4) further indicated that the three groups were significantly differentiated ($P < 0.001$). The relative differentiation reflected the patterns observed in the PCoA (Fig. 1) and UPGMA dendrogram (Fig. 2), with the highest level of genetic differentiation detected between North American and European accessions ($F_{st} = 0.903$) (Table 4). Less genetic differentiation was detected between North American and hybrid accessions ($F_{st} = 0.770$) (Table 4), but the hybrid accessions were genetically closest to the European accessions ($F_{st} = 0.485$) (Table 4). Genetic diversity, as inferred from the average nucleotide diversity over loci ($\pi_n$), was not significantly different among the North America and European groups ($\pi_n = 0.081 \pm 0.012$ and $\pi_n = 0.069 \pm 0.006$, respectively) (Table 4), but was significantly higher in the hybrid group ($\pi_n = 0.168 \pm 0.016$, $P < 0.001$) (Table 4). The total $\pi_n$ from all samples was 0.320 (Table 4).

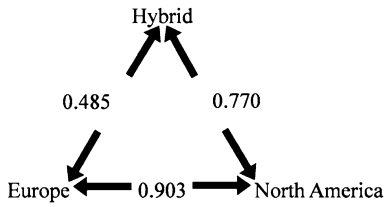
Population substructuring of the 92 hop accessions was investigated using an alternative model-based method, STRUCTURE, which assumed no predefined population structure. The results of the $\Delta K$ statistic (Evanno et al. 2005) revealed a maximum $\Delta K$ value of $K = 2$, confirming, in this group of accessions, the existence of two groups

**Table 3** AMOVA for the three groups identified in accessions of hop, as defined by PCoA, based on 730 polymorphic DArT markers

| Source of variation | *df* | Sum of squares | Variance components | Percentage of variation | Level of significance |
|---|---|---|---|---|---|
| Among groups | 2 | 5428.796 | 115.347 | 75.20 | $P < 0.001$ |
| Within groups | 71 | 2701.326 | 38.0468 | 24.80 | $P < 0.001$ |
| Total | 73 | 8130.122 | 153.393 | | |

**Table 4** Average gene diversity over loci ($\pi_n$) in the accessions of hop, and within each of the three groups defined by PCoA, based on 730 polymorphic DArT markers

| Group | *N* | $\pi_n \pm$ SE | Pairwise $F_{st}$ by group |
|---|---|---|---|
| North America | 13 | 0.081 ± 0.012 | |
| Europe | 35 | 0.069 ± 0.006 | |
| Hybrid | 28 | 0.168 ± 0.016 | |
| Total | 76 | 0.320 ± 0.010 | |




Values of $\pi_n$ were not significantly different between the North American and European groups, but were significantly higher in the hybrid group ($P < 0.001$). Pairwise $F_{st}$ values (significant, $P < 0.001$) show the degree of genetic differentiation between groups

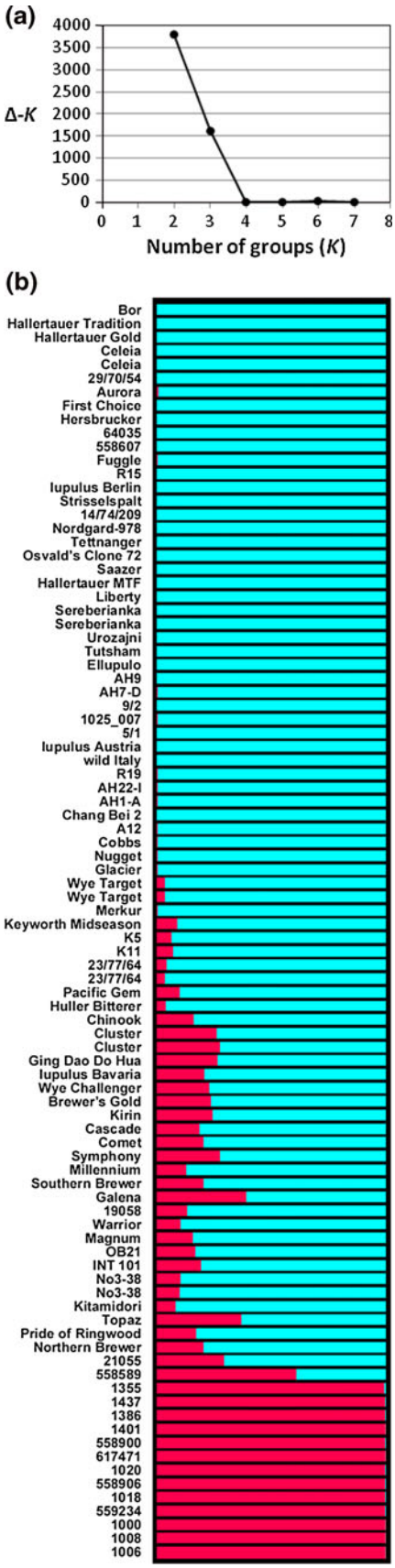
making genetic contributions (Fig. 3a). Graphical representation of membership coefficients of the 92 hop accessions is presented in Fig. 3b. The first group (red) contained all hop accessions with pure North American genetic ancestry, while the second group (blue) contained all accessions with pure European ancestry (Fig. 3b). A combination of the two colours (blue and red) reveals accessions with both North American and European genetic ancestry (i.e. hybrids) (Fig. 3b). All accessions in this hybrid group had greater than 50% European genetic ancestry (Fig. 3b), with the exception of 558589 which had greater contribution of North American genetic ancestry than European genetic ancestry (Fig. 3b), potentially due to introgression. The partitioning of groups was consistent with that revealed by PCoA (Fig. 1) and the UPGMA dendrogram (Fig. 2), with the exception of two anomalous accessions Cobbs and Nugget, possibly due to mislabelling. Consistent with the results of the UPGMA dendrogram (Fig. 2), accessions 29/70/54, Aurora and AH7-D (accessions non-clustered in PCoA, Fig. 1) were classified in the structure analysis as having only European genetic ancestry (blue only) (Fig. 3b). INT 101 (also non-clustered in PCoA, Fig. 1) had both European and North American genetic ancestry (blue and red) (Fig. 3b), indicating that it was genetically intermediate. Further resolution was given to those accessions unresolved by both PCoA (Fig. 1) and the UPGMA dendrogram (Fig. 2), with accessions Glacier and Merkur classified in the structure analysis as having pure European genetic ancestry (blue only), while accessions K5, K11 and 23-77-64 had both European and North American genetic ancestry (blue and red) (Fig. 3b). As in the PCoA and UPGMA dendrogram, all replications clustered consistently (Fig. 3b).

## Discussion

DArT has now been developed for a number of species. Studies have described the generation of hundreds and often thousands of high-quality polymorphic markers, and their utilization for a broad range of applications, including linkage mapping, marker-assisted selection for multiple phenotypic traits, genetic identity testing, guidance in the maintenance of genetic diversity, and the directed breeding of superior cultivars. We report here on the development of DArT marker technology for hop.

### Development of DArT markers in hop

A total of 730 polymorphic DArT markers were developed from 6,144 random genomic hop clones, resulting in a polymorphism rate of 11.9% (Table 2). This is comparable to other DArT studies, for example 10.4% polymorphism in barley (Wenzl et al. 2004), 9.4% in wheat (Akbari et al. 2006), 14.6% in cassava (Xia et al. 2005) and 7.0% in sugarcane (Heller-Uszynska et al. 2010). However, in hop, the polymorphic rate determined in this study is lower than the values determined using other marker systems; for example, polymorphic rates of 59.5, 43.5, 27.7 and 57.6% have been reported using AFLPs (Hartl and Seefelder




◄ **Fig. 3** Analysis of the population structure of 92 hop accessions based on 730 polymorphic DArT markers. **a** Plot of $\Delta K$ for each $K$ value (as described in Evanno et al. 2005), where $K$ is the number of groups contributing genetic information. **b** Bar plot of the proportion of each individual's genome belonging to one or other group inferred by STRUCTURE analysis. The North American group is represented in *red*, and the European group is represented in *blue*; genetic intermediates (i.e. hybrids) are observed through the combination of both colour

1998; Patzak 2001; Seefelder et al. 2000a; Townsend and Henning 2009), 57.9, 32.6 and 28.3% polymorphism has been reported using ISSR (Danilova et al. 2003; Patzak 2001), 38.6 and 42.3% polymorphism has been reported using RAPD (Patzak 2001; Šuštar-Vozlič and Javornik 1999) and 71.0% polymorphism has been reported using STS (Patzak 2001). Two main factors could account for the lower levels of polymorphism determined using DArT compared to other marker systems. One is the particular selection of hop accessions included in this study, as the efficiency of identification of polymorphic DArT markers depends on the level of genetic diversity available from the pool of accessions that is used to develop the discovery array. Alternatively, it could be due to differences in the fraction of the genome from which the respective markers are derived. SSR, ISSR, AFLP and RAPD markers are predominately derived from repetitive, non-genic fractions of the genome, whereas DArT, using a hybridisation-based platform, derives markers from low-copy genic sequences (Heller-Uszynska et al. 2010; Tinker et al. 2009). The genome usually comprises less than 10% of these sequences, and the proportion of DArT markers assayed reflects this.

The DArT markers developed for hop in this study are of high quality, as assessed by PIC, reproducibility, call rate and $P$ values (Table 2). In hop, the average PIC value of 0.34 was obtained (Table 2), and is comparable to the values of 0.38 obtained in barley (Wenzl et al. 2004), 0.31 obtained in wheat (Akbari et al. 2006) and 0.34 obtained in pigeonpea (Yang et al. 2006). However, this value is somewhat lower than the PIC values found in hop using other marker systems, for example 0.61 (Stajner et al. 2008), 0.64 (Jakše et al. 2001), 0.64 (Jakše et al. 2004) and 0.38 (Jakše et al. 2010). The average reproducibility score of 99.97% obtained (Table 2) was comparatively higher than other studies, for example 99.8% in barley (Wenzl et al. 2004), 97.71% in *Asplenium* (James et al. 2008), 99.03% in *Garovaglia* (James et al. 2008) and 99.70% in pigeonpea (Yang et al. 2006). The average call rate of 97.58% obtained (Table 2) also matched the values obtained in other studies, such as 95.0% in barley (Wenzl et al. 2004), 99.2% in wheat (Akbari et al. 2006), 92.5% in sugarcane (Heller-Uszynska et al. 2010), 91.6% in banana

(Risterucci et al. 2009) and 96.0% in pigeonpea (Yang et al. 2006). The average *P* value of 89.90% obtained (Table 2) was higher than other studies, such as 81.40% in banana (Risterucci et al. 2009) and 80.68% in sugarcane (Heller-Uszynska et al. 2010).

The results of this study show that DArT marker technology can be effectively applied to hop to detect and score hundreds of polymorphisms. Taking a maximum of 3 days to complete, the development of the hop DArT markers was rapid and efficient, relative to other marker technologies. This efficiency is a result of the fully automated nature of DArT, and its independence from DNA sequence information and gel-based procedures. DArT is also cost-effective, and much less expensive than most of the other genotyping technologies. In addition, the data quality (measured by the call rate, scoring reproducibility and *P* value) (Table 2) is comparable with other technologies, as validated in *Arabidopsis* (Wittenberg et al. 2005). Data quality is assisted by the automated nature of the array technology and the data extraction, completed automatically using dedicated software (DArT P/L, Canberra, Australia). The marker quality for hop was similar to the quality of DArT markers previously generated for other species. The percentage polymorphism and the PIC of the markers generated in this study (Table 2) were also comparable to DArT markers generated for other systems, however, these values were lower than for other marker systems developed for hop. We, therefore, propose that DArT may effectively complement the existing technologies in hop breeding and genomics, with the speed, efficiency, cost and quality of the markers, as well as the tendency towards low-copy genic sequences, compensating for the lesser polymorphism information obtained.

## Analysis of phylogenetics and genetic diversity in hop

The robustness and utility of DArT in a hop system was validated through an analysis of phylogenetics and genetic diversity. The capacity of DArT markers to resolve population differentiation and measure genetic diversity was assessed in a representative of hop accessions. The accuracy and resolution of the results were tested through comparison with the current understanding of hop molecular variation and phylogenetics.

A number of studies, utilising marker systems other than DArT, have attempted to assess the genetic diversity and understand the molecular phylogenetics of hop. These studies have utilised AFLP (Hartl and Seefelder 1998; Henning et al. 2004; Jakše et al. 2001; Patzak 2001; Seefelder et al. 2000a; Townsend and Henning 2009), RAPD (Brady et al. 1996; Patzak 2001; Šuštar-Vozlič and Javornik 1999); microsatellites (Bassil et al. 2008; Jakše et al. 2004; Murakami et al. 2006a; Patzak 2001; Patzak et al. 2010a, b; Peredo et al. 2010; Stajner et al. 2008), ISSR (Danilova et al. 2003; Patzak 2001) and STS (Brady et al. 1996; Patzak 2001; Patzak et al. 2010a, b; Patzak et al. 2007). In all studies where the material examined has included a broad coverage of accessions of European and North American genetic origin, two primary genetic groupings, Europe and North America, have been deduced, with hybrids between the two groups often detectable (Bassil et al. 2008; Henning et al. 2004; Jakše et al. 2004; Murakami et al. 2006a; Peredo et al. 2010; Seefelder et al. 2000a; Stajner et al. 2008; Šuštar-Vozlič and Javornik 1999). Some studies have resolved these groupings in greater detail, based on wild and cultivated domestication (Bassil et al. 2008; Jakše et al. 2004; Stajner et al. 2008); geographical origin (Bassil et al. 2008; Danilova et al. 2003; Henning et al. 2004; Jakše et al. 2004; Murakami 2000; Murakami et al. 2006a; Patzak et al. 2010b); breeding history (Murakami 2000; Stajner et al. 2008; Šuštar-Vozlič and Javornik 1999) or chemical content (Henning et al. 2004; Šuštar-Vozlič and Javornik 1999). Where accessions of Asian origin have been included, these accessions have additionally fallen into a separate grouping (Murakami et al. 2006a; Danilova et al. 2003).

This study separated selected hop accessions into the two genetically differentiated European and North American groupings (Figs. 1, 2, 3; Table 3). Hybrids between these two groups were clearly distinguishable (Figs. 1, 2, 3; Table 3). All results from this study indicated that the North American wild hops were widely disjunct from European hops (both wild and cultivated) (Figs. 1, 2, 3; Tables 3, 4). As expected, the hybrid accessions were genetically intermediate between the two groups, but all displayed closer genetic affinity to the European group (Figs. 1, 2, 3). This degree of similarity varied across the hybrid accessions (Figs. 1, 2, 3), and may be indicative of back-crosses to European hops after initial hybridisation with North American hops. These findings were supported by, and consistent between, the several statistical analyses of the hop DArT marker data. The PCoA (Fig. 1) clearly illustrated the wide disjunction of North American wild accessions from all other accessions, and the genetic proximity of the hybrid and European accessions, but with the hybrid accessions clustering closer to the North American accessions than the European accessions. The high percentage (69% first ordinate) of the total variance detected in the PCoA (Fig. 1) indicated that it was the major disjunction between North American and European genetic origin that was the primary factor separating all accessions. This was supported by the AMOVA (Table 3), which validated that most variation existed between groups (75.20% of the total variation), while the accessions within groups were closely related. The UPGMA dendrogram

(Fig. 2) provided an indication of the genetic relationship between all accessions, and again emphasised the clear separation of the North American and European accessions, with the hybrid accessions falling between them, but always more genetically similar to the European accessions. This finding was also supported by $F_{st}$ values (Table 3). The results of the $\Delta K$ statistic (Fig. 3a) confirmed two groups making genetic contributions, while a bar plot of the STRUCTURE modelling (Fig. 3b) established that the North American and European groups were the two sources of genetic contribution. Hybrid accessions comprised both North American and European genetics, but with greater European contribution (with the exception of accession 558589). Some possibility of an ascertainment bias exists, due to the disproportionate number of markers generated from cultivated and wild accessions. However, as the groups examined by the STRUCTURE analysis are 'North American' and 'European', rather than 'wild' and 'cultivated' this bias should have no impact on the results. In addition, wild accessions were represented approximately equally in both 'North American' (14 accessions) and 'European' (17 accessions) groups, and thus both groups should be impacted similarly by any bias. Two accessions of wild Japanese origin, INT101 and No3-38, were included in this study (Table 1). In the examination of genetic relationships, similarities were observed between INT 101, No3-38 and the hybrid accessions, indicating that they are somewhat genetically intermediate. This result is consistent with the hypothesis that the genus *Humulus* originated in China and spread to North America and Europe (Murakami et al. 2006a, b; Neve 1991); following this course of evolution, Japanese hops may be genetically intermediate between European and North American hops. However, a more comprehensive selection of Asian accessions is required to determine the genetic relationship of Asian hops to North American and European hops. Two wild accessions from the Caucasus region (K5 and K11) were also included in this study. In all analyses, these accessions fell within the hybrid cluster, at the periphery of the European cluster, indicating that they are somewhat genetically intermediate (Figs. 1, 2, 3). This result is consistent with previous studies that show wild hops from the Caucasus region to be genetically isolated from other European hops (Jakše et al. 2004; Murakami et al. 2006a; Stajner et al. 2008). A large selection of accessions of wild Caucasian origin may give rise to a distinct 'Caucasian' cluster.

The consistency of results obtained in this study across all analyses demonstrates confidence in the population differentiation determined, and in turn allows some certainty of the suitability of the DArT marker technology for assessing genetic variation and molecular phylogenetics in hop. This confidence is further increased by the consistency of these results with previous findings in hop genetic relationships obtained using other molecular markers, discussed above. While the primary genetic groupings (European, North American and hybrid) concur, several previous studies with specific selections of genotypes were able to dissect the genetic relationships between hops to greater resolution. While there are some indications of further groupings within the clustering observed in this study (for example, eight clusters could be defined in the UPGMA (Fig. 2), with the North American and European accessions each forming a cluster, and six clusters forming within the hybrid accessions) these groupings cannot be defined with conviction. This could be attributed to the genotypes included, rather than the capabilities of the DArT marker technology and its suitability for hop, as the included accessions do not have suitable distribution of numbers across the prospective groups for these groups to be definitively elucidated. This study was not designed as an analysis of hop genetic structure and diversity, but as a test of the utility, accuracy and resolution of the DArT marker technology for such an analysis. It was found that the DArT marker technology capably resolved the three groups clearly to a high statistical level ($P < 0.001$) (Table 3), and consistently with previous studies and pedigree information, indicative that with the appropriate sample set further groups would be resolved.

Genetic diversity of the hop accessions included in this study was determined through the measurement of $\pi_n$, as having a value of 0.317 (Table 4). To the best of our knowledge, this is the first time that a value for $\pi_n$ has been reported in hop. Other studies have measured gene diversity in hop, but using alternate methods, such as by comparing the number of unique alleles over specific loci in each group. For example, Jakše et al. (2004) and Peredo et al. (2010) tallied the total number of unique alleles over a number of loci groups of wild European accessions and wild North American accessions. It was reported in both studies that the number of unique alleles did not differ much between North American and European groups. This current study made similar comparisons of genetic diversity between wild North American accessions and European accessions (but both wild and cultivated), but used the more rigorous mathematical measure of $\pi_n$ (Nei and Li 1979). Gene diversity did not significantly differ between the North American and European groups (Table 4). This study also compared the gene diversity of an intermediate group (hybrids between North American and European accessions), a comparison that has not been made in previous studies. A significantly greater value of gene diversity was found in the hybrid accessions, which should be expected as these accessions capture the genetic diversity of the two phylogenetically disparate North American and European groups.

## Conclusion

This study was the first to utilise DArT marker technology in hop. An extensive number of polymorphic markers were identified, for which the quality was similar to DArT markers previously generated for other species. The newly developed DArT markers will be valuable to numerous applications in hop genetics and breeding. This study has effectively and conclusively trialled the use of the DArT markers for hop diversity analyses. We have demonstrated that the markers generated can be confidently utilised to characterise genetic diversity in hop, with the genetic relationships ascertained in this study consistent with the results of previous findings in hop genetic relationships obtained using alternate marker systems (molecular, chemical and morphological). A more systematic selection of hop accessions analysed with DArT would undoubtedly, improve the resolution of the currently accepted knowledge of hop phylogenetics.

The application of the DArT marker system to hop provides an opportunity to improve the current genetic maps of hop, such as those by Cerenak et al. (2006); Seefelder et al. (2000b); and Koie et al. (2005). DArT markers have the advantage of easy access to marker sequences (Heller-Uszynska et al. 2010; James et al. 2008; Kopecký et al. 2009), allowing the capacity to integrate diversity information with genetic and physical linkage maps. The mapping of the DArT markers will allow a much finer understanding of the structure of the hop genome and the impact of that structure on the inheritance and expression of traits in hop, hopefully assisting with the identification of markers linked with traits of interest. The hop DArT fingerprints could further assist the breeding programmes of hop through the characterisation of unknown hop accessions, the selection of superior breeding parents and the choice of individuals to introduce or retain to conserve and improve the genetics of the hop collection.

This study demonstrates that hop is well positioned to capitalise on the value of DArT genome profiling technology for a wide range of breeding applications. Currently hop breeding and genomics are constrained by limited resources, including time and funds associated with indispensible molecular technologies, and available sequence information. DArT offers a speedy, efficient and cost-effective alternative to current marker technologies, providing large numbers of high-quality polymorphic markers.

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