UNIVERSITY
OF TASMANIA

Molecular and quantitative genetic analyses of hop (*Humulus lupulus* L.)

Erin L. McAdam BBiotech (Hons)

School of Plant Science

Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

University of Tasmania, August 2013

Statements and Declarations

Declaration of originality

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text, nor does this thesis contain any material that infringes copyright.

…….. Date: ……

Erin McAdam

Statement regarding published work contained in thesis and authority of access

The publishers of the papers comprising Chapters 2 and 3 hold the copyright for that content; access to the material should be sought from the respective journals. The remaining non-published content of this thesis may be made available for loan, limited copying and communication in accordance with the *Copyright Act*, 1968.

…….. Date: ……..

Erin McAdam

Statement of co-authorship of published work

The following people and institutions contributed to the publications of work undertaken as part of this thesis:

Erin L. McAdam, School of Plant Science:
Candidate, primary author of all chapters

Andreja Cerenak, Slovenian Institute of Hop Research and Brewing:
Co-author of papers comprising Chapters 2 and 3

Andrzej Kilian, Diversity Arrays Technology Pty Ltd.:
Co-author of papers comprising Chapters 2 and 3

Anthony Koutoulis, University of Tasmania:
Co-author of papers comprising Chapters 2 and 3

Branka Javornik, University of Ljubljana:
Co-author of papers comprising Chapters 2 and 3

Cai-Hong Wang, Qingdao Agricultural University:
Co-author of paper comprising Chapter 3

Dave Andersen, The New Zealand Institute for Plant & Food Research Ltd.:
Co-author of paper comprising Chapter 3

Donna Graham, The New Zealand Institute for Plant & Food Research Ltd.:
Co-author of paper comprising Chapter 3

Emily J Buck, The New Zealand Institute for Plant & Food Research Ltd.:
Co-author of paper comprising Chapter 3

Gene Probasco, John I. Haas Inc:
Co-author of paper comprising Chapter 2

Jason Carling, Diversity Arrays Technology Pty Ltd.:
Co-author of papers comprising Chapters 2 and 3

Jernej Jakše, University of Ljubljana:
Co-author of papers comprising Chapters 2 and 3

John A. Henning, Hop Genetics and Breeding Program USDA-ARS:
Co-author of paper comprising Chapter 2

Jules S. Freeman, University of Tasmania and University of the Sunshine Coast:
Co-author of paper comprising Chapter 3

Lawrence Graham, The New Zealand Institute for Plant & Food Research Ltd.:
Co-author of paper comprising Chapter 3

Paul D. Matthews, S. S. Steiner Inc:
Co-author of paper comprising Chapter 2

Peter Darby, Wye Hops Ltd.:
Co-author of papers comprising Chapters 2 and 3

René E Vaillancourt, University of Tasmania:
Co-author of paper comprising Chapter 3

Ron Beatson, The New Zealand Institute for Plant & Food Research Ltd.:
Co-author of paper comprising Chapter 3

Simon P. Whittock, Hop Products Australia and University of Tasmania:
Co-author of papers comprising Chapters 2 and 3

Howard EL, Whittock SP, Jakše J, Carling J, Matthews PD, Probasco G, Henning JA, Darby P, Cerenak A, Javornik B, Kilian A, Koutoulis A: **High-throughput genotyping of hop (*Humulus lupulus* L.) utilising diversity arrays technology (DArT).** *Theoretical and Applied Genetics* 2011, **122:** 1265-1280.

This paper comprises the entirety of Chapter 2. Erin McAdam was the primary author (70%), and analysed all data, interpreted the results and wrote the manuscript. The co-authors contributed a cumulative total of 30% to the published work. Simon Whittock assisted with analyses and interpretation of results and revised the manuscript. Andreja Cerenak, Anthony Koutoulis, Peter Darby, John Henning, Jernej Jakše, Branka Javornik, Paul Matthews, Gene Probasco and Simon Whittock contributed intellectual input into the appropriate selection of germplasm. Andreja Cerenak, Peter Darby, John Henning, Jernej Jakše, Gene Probasco and Simon Whittock provided plant materials. Jernej Jakše also extracted the DNA for DArT marker development and assisted with both data analysis and interpretation of results. Andrzej Kilian and Jason Carling performed the DArT marker discovery analysis, developed the DArT array and assessed marker quality. Anthony Koutoulis led the international hop DArT collaboration and, was the instigator and co-ordinator of the study and revised the manuscript.

McAdam EL, Freeman JS, Whittock SP, Buck EJ, Jakše J, Cerenak A, Javornik B, Kilian A, Wang C-H, Andersen D, Vaillancourt RE, Carling J, Beatson R, Graham L, Graham D, Darby P, Koutoulis A: **Quantitative trait loci in hop (*Humulus lupulus* L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry.** *BMC Genomics* 2013, **14:** 360.

This paper comprises the entirety of Chapter 3. Erin McAdam was the primary author (75%), and performed the linkage, correlation and QTL analyses, interpreted the data and wrote the manuscript. The co-authors contributed a cumulative total of 25% to the published work. Jules Freeman assisted with linkage and QTL analyses, assisted with the data interpretation and revised the manuscript. Simon Whittock assisted with the correlation analysis and revised the manuscript. Emily Buck, with help from Cai-Hong Wang, provided DNA for DArT marker development and DArT genotyping of the New Zealand mapping population, obtained the genotypic data for the non-DArT markers used in the New Zealand mapping

population of this study and revised the manuscript. Dave Andersen contributed the chemical data for the New Zealand mapping population and revised the manuscript. Lawrence Graham and Donna Graham provided the sex phenotypic data for the New Zealand mapping population. Ron Beatson developed the New Zealand mapping population. Andreja Cerenak, Jernej Jakše and Branka Javornik provided DNA for DArT marker development. Andreja Cerenak also provided the phenotypic and chemical data for the Slovenian mapping population, while Jernej Jakše provided DNA for DArT genotyping of the Slovenian mapping population and Branka Javornik revised the manuscript. Andrzej Kilian and Jason Carling performed the DArT marker discovery analysis, developed the DArT array and genotyped the two mapping populations with the DArT markers. Peter Darby contributed to the DArT marker development. René Vaillancourt assisted with data interpretation and revised the manuscript. Anthony Koutoulis led the international hop DArT collaboration and was the instigator and co-ordinator of the study.

We the undersigned agree with the above stated proportion of work undertaken for each of the above published peer-reviewed manuscripts contributing to this thesis:

…….	…….	Date: ……..
Anthony Koutoulis	René Vaillancourt	
Primary Supervisor	Head of School	
School of Plant Science	School of Plant Science	
University of Tasmania	University of Tasmania	

Acknowledgements

This thesis would not have been possible without the valuable contributions of a number of people. Firstly, I would like to give an enormous thanks to Jules Freeman for the expert guidance in linkage and QTL analyses. Your enthusiasm and dedication to these topics has been very inspiring. I am also very appreciative of your thorough revisions and advice. Thank you to Simon Whittock for the many hours you spent assisting me with the quantitative genetics analysis and many of the other statistical procedures in this thesis, as well as sharing your extensive knowledge of hop with me. Thanks to René Vaillancourt for your general expertise and advice; and to Anthony Koutoulis for encouraging me to present my work at conferences.

I would like to acknowledge the members of the hop DArT collaboration, Andreja Cerenak, Branka Javornik, Emily Buck, Gene Probasco, Jernej Jakše, John Henning, Paul Matthews, Peter Darby and Ron Beatson for your contributions to these studies and for your supportive collaboration. I would also like to thank Hop Products Australia for providing me with materials, data, access to their hop fields and assistance with sample collection. Thanks to Matthew Hamilton and Dorothy Steane for technological assistance, Aina Price for assistance with field work and Sascha Wise for assistance in the laboratory. Thanks to Andrzej Kilian and Jason Carling from DArT Pty Ltd. for performing DArT genotyping and for technical advice. I would like to thank the organisers of the IHGC 2009 and IHGC 2011 conferences for giving me the opportunity to present work from this thesis, as well as the School of Plant Science and the University of Tasmania for providing with funding so that I could attend the conferences.

Thanks to all the other Plant Science post-grads, for sharing in the experience, the endless laughs and wildly enjoyable games of cards. I wish you all many diamond gins! I give special thanks to my Mum and Dad for your continuous support and encouragement as well as providing me with such a motivational example of the value of hard work. I always looked forward to our Sunday night phone conversations. The biggest thanks go to Scott, not only for the endless discussion of ideas, the tireless help in the field and for proofreading every last word of this thesis, but for keeping me so happy the entire time. Your passion for research and keen interest in the natural world were my biggest inspirations for this thesis. I am so grateful to you for everything.

Abstract

Beer derives bitterness, flavour and aroma from the secondary metabolites of the hop cone. Breeding programs strive to produce superior hop cultivars with higher yields and desirable brewing characteristics as well as to increase efficiency and reduce input costs. In pursuing these goals, classical breeding approaches rely on morphological and biochemical markers to assess the genetic potential of hop. These methods are, however, hampered by environmental influences and by the complex interactions between hop secondary metabolites and the brewing process. Genetic-based analyses are able to account for these environmental influences to assess quantitative variation at the genetic level. This thesis describes investigations into two of these analyses, using molecular markers for quantitative trait loci (QTL) identification and the estimation of quantitative genetic parameters. These investigations were conducted with the aim of improving our understanding of the genetic control of hop cone chemistry and important agronomic traits as well as to provide some insight as to the potential of these methods to inform hop breeding programs.

Molecular technologies are generally costly, low throughput and reliant on DNA sequence information. Diversity arrays technology (DArT) is a marker system invented specifically to overcome these barriers. This thesis examines the applicability of DArT for high-throughput, cost-effective genotyping of hop. A total of 1241 polymorphic markers were identified from 497 hop accessions. A genetic diversity analysis was conducted on representative hop accessions to validate the robustness of these markers in the hop system. Hop accessions separated into two broad, genetically distinct groups (European and North American origin), with hybrids between them clearly distinguishable. These genetic relationships concur with the current understanding of hop phylogenetics and diversity, demonstrating the accuracy and resolution of DArT markers in a hop system and their potential as an effective marker technology for this species.

The DArT markers, in conjunction with microsatellite, RAPD, STS and AFLP markers, were used to construct genetic linkage maps of two hop mapping populations; these linkage maps were then used for QTL analysis. This study focussed on identifying QTL for traits relating to three key targets in the genetic improvement of hop: expediting plant sex identification, increasing yield capacity and improving the organoleptic properties of hop cones. Sixty-three significant QTL were detected for 36 traits, including two yield traits (dry cone weight and

essential oil content) and 33 different secondary metabolite traits. A previously identified sex-linked marker (HLAGA7) was also verified in a third hop pedigree, demonstrating the utility of this marker as a routine screening tool in hop breeding programs. Many of the QTL identified were co-located, providing the first demonstration of pleiotropy/linkage influencing secondary metabolites in hop. Both pleiotropy and linkage have implications for hop breeding, as selection for specific secondary metabolites associated with such loci are likely to instigate adverse changes to other secondary metabolites, impeding the breeding for particular chemical profiles. Specific QTL influencing single secondary metabolites were also identified, demonstrating the potential for selection of particular chemical traits in isolation. The findings of this study significantly advance our understanding of the genetic control of sex, yield and secondary metabolites in hop, and provide important information on incorporating QTL for these complex traits into hop molecular selection programs.

The genetic control of hop traits was also examined through quantitative genetics analysis. Traits related to cone chemistry, yield and plant growth were assessed in a hop progeny trial, consisting of 108 families of diverse genetic backgrounds (European, North American and hybrid origins). The investigation revealed significant genetic diversity between families in emergence of shoots, vegetative morphology and all assessed cone chemical traits, but not in cone yield. Cone chemical traits were generally more heritable (0.15 to 0.29) than growth traits (0.04 to 0.20), reflecting the more intense genetic selection of hop cone chemistry and the greater environmental and agronomical influences on plant growth. Significant genetic correlations existed between cone chemistry and plant growth traits, with more vigorous plants associated with lower levels of α-acid and β-acid. This trend may reflect the underlying binary population structure of founder genotypes having either European or North American origin, or possibly the influence of selection in the Australian environment. This study also showed for the first time that sex has an effect on the phenotype of hop plants as early as emergence. It is currently held that male and female hop plants are indistinguishable until flowering, but this study found that male and female plants display differences in variation from emergence to cone maturity. This study provides valuable information on the potential genetic variation in cone chemistry and growth traits available to hop breeders, the prospective heritability of these traits and the influence that factors other than additive genetic influences have on the hop phenotype. Relationships between cone chemistry and plant growth traits present several growth measures that could be used as proxy selection indicators for particular cone chemical attributes.

This thesis provides a comprehensive investigation of two genetic-based techniques to assess quantitative genetic variation in hop traits. The findings of both molecular and quantitative genetic analyses provide important insights into the underlying genetic architecture of hop and reveal novel information on the biology of this species. The knowledge gained from both techniques demonstrates the value of their incorporation into breeding programs.

Table of Contents

CHAPTER 1

General introduction

The unique flavour, aroma and preservative properties of hop make it a highly prized commodity. Increasing demand for hops with novel organoleptic character and improved agronomic performance creates a strong impetus to develop new cultivars. Genetic research in hop is at a relatively early stage compared to many crop plants and genetic-based technologies are currently underutilised. Such technologies have the potential to provide information about the inheritance of key traits, which is fundamental to successful hop improvement. This introduction provides a summation of hop biology and its native distribution, followed by a description of the unique properties of hop that make it a valuable plant to human society and its resulting commercial applications. A brief account of the history of hop breeding is given, encapsulating the initial cultivation of the species, early selection methods that were employed to improve hop properties and the limitations that these methods have for delivering desired outcomes. The increasing application of more sophisticated molecular and quantitative genetic based technologies for hop breeding is reviewed, including a description of the techniques that have been employed and the resulting information gained; as well as reasons why the use of these techniques has not yet become widespread for hop improvement. The further potential of molecular and quantitative genetic based technologies for application to hop breeding is then discussed. Finally, a premise to this thesis is given, which focuses on utilising molecular and quantitative genetic analyses to expand the understanding of genetic variation in hop and facilitate the selective improvement of cone chemistry, yield and agronomic characteristics.

Hop biology and native distribution

There are ten genera in the family Cannabaceae; only two of these, hop (*Humulus*) and hemp (*Cannabis*), have any commercial significance. *Humulus* consists of three species: *H. japonicus*, *H. yunnanensis* and the cultivated species *H. lupulus* [1]. Five subspecies of *H. lupulus* have been described (varieties *lupulus, cordifolius, neomexicanus, pubescens* and *lupuloides*) on the basis of morphological characteristics and biogeography [2]. *H. lupulus* has a native distribution between latitudes of approximately 35° and 70° North, from Western Europe, east to Siberia and Japan and across North America, except in highlands and deserts [2-3]. *H. japonicus* is native to China, Japan, Korea and Taiwan, while *H. yunnanensis* is native to southern China [2]. Molecular evidence suggests that the genus *Humulus* evolved in

China, where all three species currently occur [4]. The three species appear to have diverged recently (within the last 6.4 million years); following the most recent glaciation, dispersal both east and west from refugia in Asia is likely to have led to the establishment of the distinct populations and subspecies in Europe and North America [4]. Although indigenous only to the Northern Hemisphere, hop plants were introduced to the Southern Hemisphere following European settlement [5]. Hops are now cultivated in many parts of the world, including Europe, North America, South Africa, Australia, New Zealand and China.

Hops are herbaceous plants with a climbing growth habit [6], and are found naturally in marshy or wet hollows in fen carr and moist alder-oak woodland habitats [7]. They are perennial, regrowing each spring from rhizomes of underground rootstock [6]. The above-ground parts of the plant die back to ground level each winter, a response triggered by shortening daylength, where they remain dormant until warmer temperatures trigger resting buds to break [6]. The emerging bines are covered with many cystolith hairs, a type of non-glandular trichome, which are used to grasp and twine [2]. In cultivation, hops are grown up strings suspended from a 5-8 m trellis. Lateral branches develop at the nodes, producing flowers at their terminal buds [8]. Hops must initiate a minimum number of nodes before flowering, which is induced by shortening daylength [8-9]. Hops are dioecious, with male and female flowers produced on separate plants, although monoecious plants (male and female flowers on the same plant) sometimes occur [10-11]. The flowers are the only morphological basis on which the sex of the plant can be determined [10]. The flowers are wind pollinated [6], after which the female inflorescence develops into a strobilus (commonly known as a cone) [10]. Many glandular trichomes (commonly called lupulin glands) form on the bracts of the cone [12-13]; it is in these glands that commercially important secondary metabolites (potentially up to 1000 different compounds) accumulate [14-15].

Commercial importance of hop

More than 1.23 billion L of beer are produced globally every year [16]. Beer is an alcoholic beverage produced by the saccharification of starch (from carbohydrate sources such as malted barley or wheat) and fermentation of the resulting sugar by brewer's yeast [17]. Hops are an essential ingredient to beer, added to the brew as both a flavouring and preservative [17]. Hops derive their preservative activity and organoleptic properties from secondary metabolites that accumulate in their cones [6, 18-20]. Hop acids are a natural preservative; their antimicrobial effects sterilise most microorganisms but do not kill brewer's yeast [6, 18-

20]. Hop acids also contribute bitterness that balances the sweetness of the malt in beer, and essential oils that add fruit, spice, resin and floral flavours and aromas [6]. Grain, yeast, water and hops have been brewed together to make beer since the Middle Ages [21]. The combination of secondary metabolites found in hop are unique to the species, such that there is no natural alternative that could replace hop as a flavouring or preservative ingredient in beer [17, 22]. All hop cones contain hop acids and essential oils, but the presence, amounts and relative proportions of specific secondary metabolites differ significantly between hop cultivars. Their unique secondary metabolite profiles give hop cultivars specific bittering, flavouring and aroma potentials [23-24]. Because of this, specific hop cultivars are capable of defining beer brands and as a result, there is considerable appetite for different flavours, aromas and ranges of bitterness. This offers significant potential for new hop cultivars to be developed.

More than 128,874 tonnes of hops are produced worldwide each year [25]. While hops are used almost exclusively in the production of beer, the use of hops outside the brewing industry is growing in importance. Hop acids are being used as naturally occurring antimicrobials for a range of purposes [26-29]. Other hop secondary metabolites have potential in the neutraceutical market; the most significant of these are 8-prenylnarigenin, a potent phytoestrogen [30], and xanthohumol, which has anti-cancer properties [31]. Consequently, there are also opportunities for the development of hop cultivars with particular secondary metabolite profiles to meet these non-brewing needs.

Traditional hop breeding

Wild hops have been gathered for a variety of purposes since antiquity, but it was not until the Middle Ages, when the preservative qualities of hops in beer was discovered, that demand became great enough to necessitate their cultivation [3]. The original hop gardens were in Europe and contained a mixture of several different genotypes, asexually propagated from cuttings of plants from the local area in possession of desirable characteristics [6, 32]. Some of the most commonly used cultivars today, including Tettnanger, Saaz, Spalt and Hallertauer Mittelfruh are propagations of these original hops. They are referred to as ‘noble hops’ and are still esteemed for their distinctive low bitterness and intense aroma [33-34]. From these earliest cultivars, selections for future generations were made in attempts to improve the yield, agronomic properties and brewing quality [6, 32]. Many successful hop varieties (e.g. Cascade) have been selected from the open-pollinated seed of desirable female plants [35].

As an understanding developed that these features were influenced by the genetic constitution of the variety rather than entirely on the country of origin, additional methods of obtaining superior varieties were pursued, such as through crossing particularly selected parents, each possessing unique, desired traits, from which seed could then be collected that would hopefully inherit the combination of traits. This seed was grown in trials, where phenotypic assessment was used to identify individuals that possessed favourable attributes.

Selection in this way resulted in the development of a number of cultivars with improved properties. The most significant of these was increased hop acid content. At the beginning of the twentieth century it was realised that it was hop acids that imparted both bitterness and preservation to beer; and that brewing with hops that had greater hop acid content resulted in beer with better keeping qualities [36]. Popular European cultivars with favourable aroma were subsequently crossed with wild North American hops, which were recognised as having higher hop acid contents (but inferior aroma), resulting in dramatic increases to hop acid yields [37]. Few pre-1900 cultivars exceeded 4% α-acid content, but by the end of the century varieties were available with greater than 18% α-acid content [38]. Traditional methods of selection also made improvements to the resin quality of hop, facilitated by advances in analytical techniques such as high-performance liquid chromatography (HPLC) which enabled a better understanding of the composition and total resins [39-41]. Improvements using traditional selection methods were also made to the storage capacity of hop acid, resistance to pests and diseases (including verticillium wilt, downy mildew, powdery mildew, black root rot and aphids), seedless cultivars and the suitability of cone morphology to modern picking machinery) [38, 42-44]. Successful cultivars similar to popular traditional cultivars have been developed in locations where the traditional cultivars were ill-adapted [38, 43].

Improving hop through traditional breeding methods, however, is difficult, with success strongly subject to chance. Traditional selection systems are unable to inform breeders about the genetic diversity of the subject plants and which individuals are sources of new genetic material and should thus be used as breeding parents [45]. Because of the confounding influence of environmental factors, traditional selection systems also provide little information on the inheritance of traits [46]. Understanding how much of the observable variation is heritable, as opposed to environmentally driven, is important to determine which traits are amenable to improvement by selection [47-48]. As traditional selection systems are

unable to separate environmental and genetic influences, it is difficult to ascertain the genetic relationships between traits [46-47]; this can lead to unintentional changes in seemingly unrelated traits, or conflicting outcomes where more than one trait is selected at a time [49]. In hop this is particularly relevant to the selection of multiple secondary metabolites to achieve desirable brewing properties. Using traditional selection methods, traits can only be assessed when expressed in the phenotype. This means that traits related to the hop cone, for example, cannot be assessed in individuals until cones are produced, and never in male plants [10, 50]. The improvement of hop using traditional crossing methods is also difficult because the cultivars are all derived from a very narrow genetic source and thus have low genetic variability between them [4, 51]. Traditional selection trials usually consist of progeny derived from only a single cross, thus limiting the genetic diversity available for selection. In addition, hops, being unable to self-pollinate, are naturally very heterozygous [51] and produce highly variable progeny rarely resembling either parent. It is for this reason that commercial hop production consists of clonally propagated plants of established cultivars, unlike many crops which are deployed from true-to-type seed [52].

Hop breeding with molecular and quantitative genetic based technologies

Traditional phenotype-based selection methods have led to the improvement of a number of key hop traits; however, these methods are most successful when selection is based on categorical, monogenic traits with simple ('Mendelian') inheritance, being those controlled by a single or few genes [47]. Most traits, however, are quantitative with complex inheritance, where the phenotype varies along a continuous gradient and is controlled by many genes with small effects [47, 53]. In the last few decades, expansions in the understanding of genetics, as well as major advances in biotechnology, statistics and computational procedures, have resulted in the development of a number of approaches to better understand the complexities of trait inheritance, as well as more efficient selection systems that can be used for the breeding of quantitative traits [47]. Two of the most significant of these developments are molecular and quantitative genetic analyses.

Molecular analysis

Molecular marker technologies are based on identifying sites of DNA heterozygosity which can be used as a tag for a particular location of the genome [53]. A number of different molecular marker technologies have been developed, which vary in terms of their reliability, the level of information they provide, the time and difficulty of technical procedure, the

quantity and quality of DNA required, their transferability and cost [54]. There are two basic types of molecular marker: those based on nucleotide differences and those based on differences in the amount of repetitive DNA [53]. Molecular markers have an extensive range of applications that can be utilised by breeding programs. Firstly, molecular markers can be used to examine genetic diversity by assessing the extent and distribution of genetic variation [55-56]. These assessments can be used to characterise breeding programs, providing information about: the genetic origin of germplasm; the relationship between breeding parents; the extent of inbreeding within the breeding population; and the allelic richness of individuals [55-56]. Genetic diversity assessments can also be used to assess the structure of base populations and identify potential sources of genetic variability that could be captured by breeding programs [57]. Secondly, molecular markers can be utilised to ensure quality control of genetic material [58]. Genotype identity can be determined unambiguously by their unique complement of molecular markers or 'molecular fingerprint' [58]. The accurate identification of individuals to be used as crossing parents is crucial in breeding programs. It is also important for the identification of new germplasm and for the quality control of clonal propagates for commercial cultivation. Thirdly, molecular markers can be used to better understand genome organisation and the genetic control of complex traits [59-60]. Molecular markers can be arranged relative to each other along chromosomes according to their recombination frequency to produce a 'linkage map' [59-60]. This genetic map can then be used to identify marker-trait associations in the form of quantitative trait loci (QTL), which are particular loci that affect the phenotypic variation of quantitative traits [59-61]. Mapping QTL increases the understanding of the genetic control of traits, by providing important information about the number, location and magnitude of effects of loci which influence phenotypic variation of traits [59-60]. Fourthly, molecular markers can be used to improve the efficiency and effectiveness of selection. Selection of target traits can be achieved indirectly by the selection of QTL through a process called marker assisted selection (MAS) [61]. In this process, traits are selected not on the basis of phenotype, but on the basis of QTL linked to that phenotype [61]. This method offers a number of advantages over traditional phenotype-based selection systems: selection is not affected by environmental factors; traits that are difficult or expensive to measure or expressed under particular environmental conditions can be effectively selected for; and traits that are expressed at later developmental stages can be selected for very early [54]. MAS is also particularly useful for the selection of two or more traits at one time, or for multiple alleles that affect a trait [54, 62]. MAS has

been successfully used in other crop species to select for traits such as disease resistance, yield, agronomic performance and quality [63-71].

Quantitative genetics

Quantitative genetics is a statistical method of analysis which is used to distinguish between and measure the proportion of phenotypic variation in individuals attributed to genetic factors and environmental influences [46, 49, 72]. Calculations are made on the basis of phenotypic data as well as the relationship between individuals (deliniated by a pedigree) [49, 72]. From the variance components, a number of informative parameters can be calculated, including the heritability of traits, the genetic relationship between different traits and the effect that environmental factors have on both the phenotype and trait relationships [46, 48-49, 72]. Breeding programs have much to gain from the consideration of these parameters, including an understanding of how much of a trait's phenotypic variation has a genetic basis and can thus be influenced by selection; which of the possible traits should be subject to selection to most effectively achieve a desired phenotype; which individuals (including males) are most genetically equipped to contribute these factors and should therefore be used as crossing parents; and which factors can be improved through changes to environmental conditions, rather than through selection [46, 48, 73]. Breeding programs that incorporate the information gained from quantitative genetic analysis are more likely to achieve their targets, and to do so more efficiently, than the breeding programs where selection is based solely on phenotypic assessment.

Current use of molecular and quantitative genetic based technologies in hop

More sophisticated breeding technologies, such as molecular and quantitative genetic analyses, are now used routinely in the breeding programs of major crops [47, 56, 61]. Compared to major crops, genetic research in hop is less advanced. As such, the development of these technologies and their utilisation in hop breeding programs has been slower, but their potential is beginning to be realised. A number of quantitative genetic analyses have been performed on a wide range of traits [50, 74-87], but many of these studies employed rather primitive statistical calculations, or were based on a limited number of families from a narrow genetic base. As such, information on the inheritance of quantitative genetic traits in hop is still very limited. A number of molecular marker technologies have been developed for hop, including RAPD, AFLP, STS, microsatellites, as well as markers based on expressed sequence tags (EST) and candidate genes [51, 88-112]. These marker technologies have been

used to investigate genetic diversity in hop, and have increased our understanding of the population structure and level of inbreeding in the species [4, 51, 89, 92, 96, 98, 104-106, 108-109, 112-113]. Marker technologies have also been utilised for the development of linkage maps and the identification of QTL [94-95, 99]. Linkage maps have been constructed for four mapping populations, but these maps are not yet saturated with markers, and not all chromosomes have been clearly identified [88, 95, 99, 114]. Most of the focus of marker-trait association has been placed on the identification of a universal sex-linked marker [91, 115-116], but QTL have also been identified for yield traits, a few hop secondary metabolites and powdery mildew susceptibility [88, 95, 99, 110, 117]. As yet, marker assisted selection in hop has been limited. The widespread use of molecular markers in hop is mainly limited by the relative cost (in time and money) of the technologies in terms of their development and subsequent utilisation, compared to the cost of traditional breeding approaches. The feasibility of the incorporation of molecular analyses into hop breeding programs is therefore dependent on the development of a large number of markers that can be utilised in a high-throughput genotyping system at a relatively low cost.

Thesis premise

This thesis reports on the use of two genetic-based techniques, molecular analysis and quantitative genetic analysis, to investigate quantitative genetic variation in important selection traits in hop. Chapter 2 reports on the investigation of a new molecular marker system, diversity arrays technology (DArT), for hop genotyping. DArT offers high-throughput and cost-effective genotyping relative to other marker systems, producing markers with high transferability and wide genome coverage [118]. The attributes of DArT as a genotyping tool for hop is evaluated; and the accuracy and resolution of the DArT marker system in hop is tested by comparing the results of a diversity analysis employing the DArT markers with previous genetic diversity studies of the species. Chapter 3 details investigations into the organisation of the hop genome and the genetic control of commercially important traits. Linkage maps are constructed for two hop mapping populations, using the DArT markers and other marker types. QTL analyses are conducted using these linkage maps, to identify marker-trait associations for traits relating to sex, yield and cone chemistry. Chapter 4 examines the genetic control of hop cone chemistry and agronomic traits using quantitative genetics. Using this analysis method, important quantitative genetic parameters are calculated, including additive genetic variation, narrow-sense heritability, genetic correlations between traits and the degree to which variation and

covariation of traits is affected by factors other than additive genetic effects. Finally, Chapter 5 provides an evaluation of the outcomes of these studies, in terms of the efficacy of both molecular analyses and quantitative genetics to improve the understanding of the genetic control of hop traits. The new insights into the underlying genetic architecture of hop are discussed, along with the impact that this information has on the future selective improvement of hop. The potential for further utilisation of DArT markers, QTL and quantitative genetics for the selection of new hop varieties is considered and the possible directions for further research into quantitative genetic variation in hop are outlined. The three experimental chapters in this thesis are composed of self-contained units, presented in the style of scientific journal articles. Each of the experimental chapters contains an introduction to the literature, outlining the potential contribution of the study to the field of research. The results presented in each chapter are followed by a discussion of the findings and conclusions.

CHAPTER 2

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

The text and results of this chapter are taken directly from the following publication:

Howard EL, Whittock SP, Jakše J, Carling J, Matthews PD, Probasco G, Henning JA, Darby P, Cerenak A, Javornik B, Kilian A, Koutoulis A: **High-throughput genotyping of hop (*Humulus lupulus* L.) utilising diversity arrays technology (DArT).** *Theoretical and Applied Genetics* 2011, **122:** 1265-1280.

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 utilize 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.

INTRODUCTION

Hop (*Humulus lupulus* L.) is a dioecious cone-bearing plant cultivated for use predominantly by the beer brewing industry [6]. 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 [22, 119-122]. Lupulin also contains other compounds with potential for the phytoceutical industry, including 8-prenylnaringenin, a potent phytoestrogen [30, 123], and xanthohumol, which exhibits possible anti-cancer properties [30-31].

H. lupulus has a native distribution throughout the Northern Hemisphere, between latitudes of approximately 35° and 70° North [6]. 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 [2]. 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 [6]. In an attempt to expand the genetic variation of the hop breeding resource, North American wild hops have been hybridised with European cultivars [36]. The incorporation of North American germplasm has imparted several favourable qualities, including pest and disease resistance, higher yielding capacity and varying bittering potentials [6, 36]. Hops native to Asia are not commonly used in breeding programs [108].

Commercial hop cultivation occurs in many parts of the world, including Europe, North America, South Africa, Australia and New Zealand. Breeding programs, 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 [51], as despite a long cultivation history, current hop cultivars are derived from a narrow genetic source [4]. 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 [4, 33, 89, 92, 104, 108, 113, 124-125]. 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 [126-128]. The use of biochemical markers, such as essential oils and flavonoids, were later employed [129-133]. 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 utilized: random amplified polymorphic DNA (RAPD) [93, 105, 112]; amplified fragment length polymorphisms (AFLP) [51, 98, 105, 109, 125, 134]; microsatellites [33, 89, 92, 97, 104-105, 108, 113, 124]; inter-simple sequence repeats (ISSR) [105, 135] and sequence tagged sites (STS) [93, 105-106, 108, 124]. 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) [89, 92, 104, 108, 112-113, 124, 134]. Some studies have been able to further resolve the European genetic group into smaller groups, corresponding to geographical origin [89, 92, 103-104, 124, 134-135], breeding history [103, 112-113] and chemical content [112, 134]. Hybrids between the European and North American genetic groups have also been distinguished, and subgroups differentiated corresponding to geographical origin and pedigree data [109, 134].

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 programs for the purpose of hop germplasm classification and selection of accessions. Use of the marker technologies is further constrained by their low through-put capacities, as a result of their dependence on gel electrophoresis or laborious and intensive DNA sequencing processes [118, 136-138].

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 [118]. It was

developed for particular application to non-model species, mainly crop species for which limited resources may be available [118, 138]. 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 [138]. It enables simultaneous typing of several hundred polymorphic loci in parallel, without relying on sequence data [118, 136-139]. 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 [118, 136-139]. 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 2.1). These accessions were sourced from Europe, North America and Australia. The sample set was comprised of 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 2.1). Samples were sourced in 2004 and 2005 from collections held by Wye Hops (Canterbury, Kent, England), John I. Haas Inc. (Yakima, Washington, USA), USDA-ARS National Genetic Resources Program Germplasm Resources Information Network (GRIN) (Beltsville, Maryland, USA),

Hop Products Australia (Bushy Park, Tasmania, Australia; and Eurobin, Victoria, Australia) and the Slovenian Institute of Hop Research and Brewing (Žalec, Slovenia). Pedigree information (where available) has been published previously [93, 105, 109, 112, 140], or was provided by Kim Hummer (NCGR Corvalis) or the authors.

Table 2.1 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

Name	Domestication status	Taxonomy	Geographical origin
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
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	*lupuloides*	USA
1006	Wild	*lupuloides*	Canada
1008	Wild	*lupuloides*	Canada
1018	Wild	*lupuloides*	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

Name	Domestication status	Taxonomy	Geographical origin
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

DNA extraction

DNA was extracted from dormant rhizome bud, leaf or tissue cultured plant samples. Extractions were performed using the common CTAB extraction protocol [141] 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 an 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 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. [137]. Approximately 1 µL of PCR product from all accessions used in the study were mixed and used to construct a library of 6144 clones (4608 clones from cultivated accessions and 1536 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 6144 clones, were printed in duplicate using a MicroGridII arrayer (Biorobotics, Cambridge, UK) onto poly-L-lysine coated slides (Erie Scientific, Portsmouth, New Hampshire, USA). After printing, slides were heated to 80°C for two hours, incubated in 95°C water bath for two 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. [118], 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. [137]. 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. [142], using the formula:

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

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 [143] was computed on the basis of shared presence of fragments (minimising error due to non-homologous shared absences) using PAUP* version 4.0b10 [144]. 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 [144]. Partitioning of taxa into genetic groups was investigated by Principle Co-oridnates Analysis (PCoA). The genetic distance matrix was exported to NTSYS-PC 2.1 [145] 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) [146]. AMOVA was calculated using Arlequin version 3.5.1.2 [146]. 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) [147], and average gene diversity over loci (π_n) (equivalent to the probability that two randomly chosen homologous nucleotides are different) [148-149], were calculated using Arlequin version 3.5.1.2 [146], using the same conditions as for AMOVA (above). Student's t-tests [150] were performed to determine whether differences in π_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 [151]. 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 MCMC (Monte Carlo Markov Chain) 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 ΔK according to Evanno et al. [152]. The data were analysed by the online version of STRUCTURE HARVESTER [153]. Any individual 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 [154] and DISTRUCT [155] 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 2.2). Performance of the DArT markers was measured through several different parameters (Table 2.2). 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

principle 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.

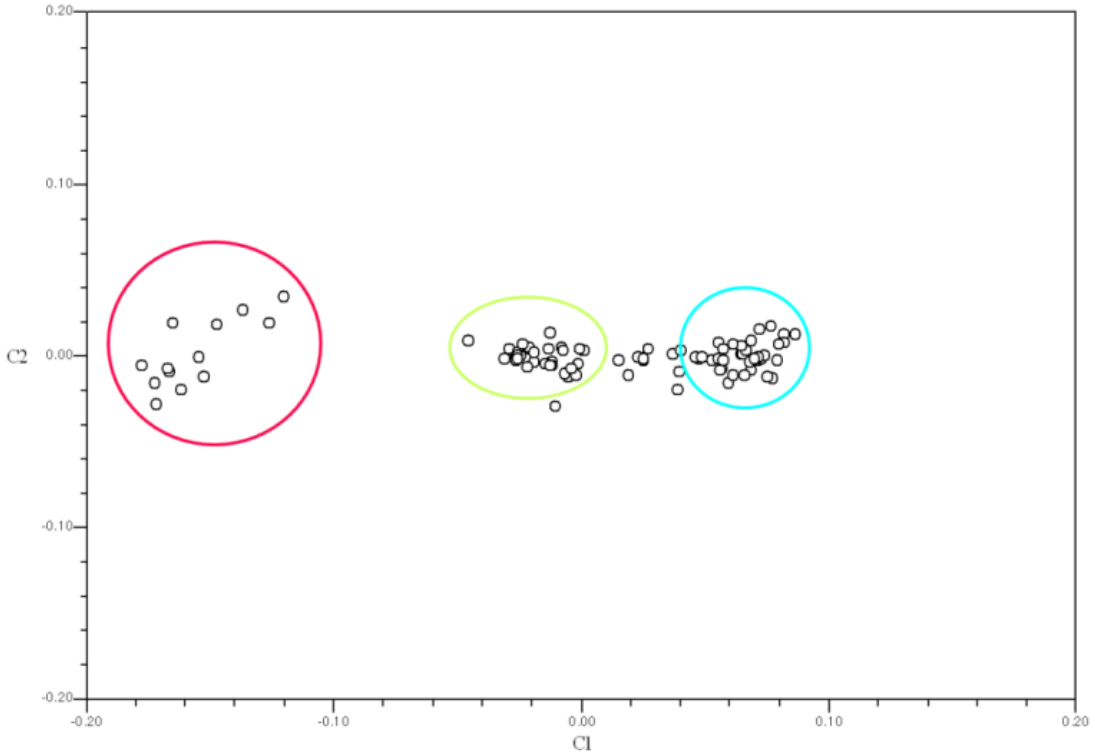
Table 2.2 Mean ± standard error of quality parameters for the 730 polymorphic markers identified in 92 hop accessions.

Quality parameter	**Mean ± SE**
% Polymorphism	11.9
PIC (polymorphism information content)	0.34 ± 0.00
P (%)	89.90 ± 0.21
Reproducibility (%)	99.97 ± 0.01
Call rate (%)	97.582 ± 0.12

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 2.1). Based on a pairwise genetic distance matrix [143], PCoA was undertaken (Figure 2.1) and an UPGMA dendrogram was constructed (Figure 2.2). 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 (Figure 2.1). The first cluster (outlined in red) contained 13 accessions (Figure 2.1), 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 (Figure 2.1), consisting of wild European hops, and cultivars of solely European genetic origin. The third cluster (outlined in green) contained 30 accessions (Figure 2.1), 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 (Figure 2.1). The European and hybrid clusters, with the hybrid cluster situated in between the North American and European clusters, were not as discreet (Figure 2.1). 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 (Figure 2.1). A total of nine accessions could not be placed into any of the three clusters (Figure 2.1), 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 (Figure 2.1), 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.



European cluster (blue)		**Hybrid cluster (green)**		**North American cluster (red)**	**Non-clustered (uncoloured)**
5/1	Hallertauer Tradition	19058	Millennium	1000	23/77/64 (1)
9/2	Hersbrucker	21055	No3-38 (1)	1006	23/77/64 (2)
64035	Liberty	558589	No3-38 (2)	1008	29/70/54
558607	lupulus Austria	Brewer's Gold	Northern Brewer	1018	AH7-D
1025_007	lupulus Berlin	Cascade	Nugget	1020	Aurora
14/74/209	Nordgard-978	Chinook	OB21	1355	Glacier
A12	Osvald's Clone 72	Cluster (1)	Pacific Gem	1386	INT 101
AH1-A	R15	Cluster (2)	Pride of Ringwood	1401	K11
AH22-I	R19	Cobbs	Southern Brewer	1437	K5
AH9	Saazer	Comet	Symphony	558900	Merkur
Bor	Sereberianka (1)	Galena	Tobaz	558906	
Celeia (1)	Sereberianka (2)	Ging Dao Do Hua	Warrior	559234	
Celeia (2)	Strisselspalt	Huller Bitterer	Wye Challenger	617471	
Chang Bei 2	Tettnanger	Keyworth Midseason	Wye Target (1)		
Ellupulo	Tutsham	Kirin	Wye Target (2)		
First Choice	Urozajni	Kitamidori			
Fuggle	wild Italy	lupulus Bavaria			
Hallertauer Gold		Magnum			

Figure 2.1 PCoA of 92 hop accessions based on 730 DArT markers, showing the ordination of the first two vectors. Principle co-ordinate 1 (C1) explained 69% of the variation and principle co-ordinate 2 (C2) explained 18% of the variation. Accessions found within each cluster, as well as non-clustered accessions, are listed.

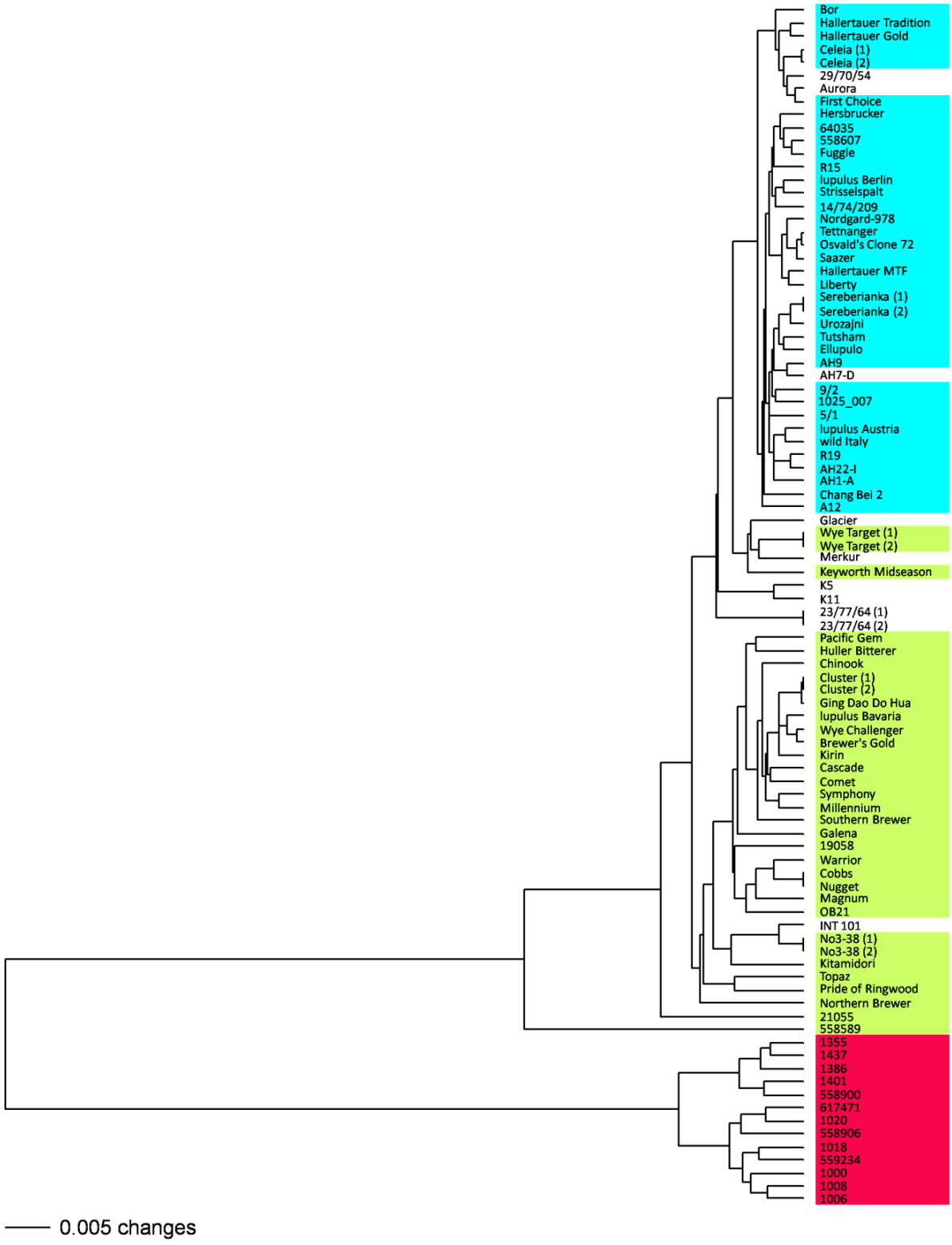


Figure 2.2 An UPGMA dendrogram (unrooted) representing the relationships between 92 hop accessions, based on 730 DArT markers.

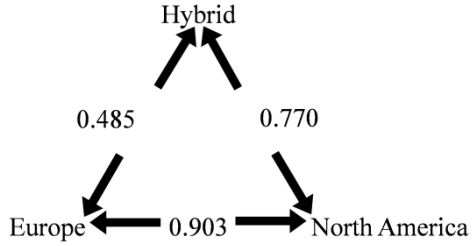
Similar patterns were observed in the UPGMA dendrogram (Figure 2.2), with major disjunction occurring between North American wild hop accessions (red) and all other accessions. All North American accessions were positioned exclusively within their own cluster, while a second cluster contained both the European (blue) and hybrid (green) accessions (Figure 2.2), 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 (Figure 2.2). 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 (Figure 2.2). 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 (Figure 2.2). 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 (Figure 2.1). The accessions 29/70/54, Aurora and AH7-D fell within the group of European accessions (Figure 2.2), 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 (Figure 2.2). 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 (Figure 2.2), 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.

AMOVA across the three groups (North American, European and hybrid), as defined arbitrarily by PCoA clustering (Figure 2.1), indicated significant partitioning of genetic variation, with 75% of the detected variation existing between the groups (Table 2.3), and the remaining 25% within groups ($P < 0.001$) (Table 2.3). Pairwise F_{st} values (Table 2.4) further indicated that the three groups were significantly differentiated ($P < 0.001$). The relative differentiation reflected the patterns observed in the PCoA (Figure 2.1) and UPGMA dendrogram (Figure 2.2), with the highest level of genetic differentiation detected between North American and European accessions ($F_{st} = 0.903$) (Table 2.4). Less genetic differentiation was detected between North American and hybrid accessions ($F_{st} = 0.770$) (Table 2.4), but the hybrid accessions were genetically closest to the European accessions ($F_{st} = 0.485$) (Table 2.4). Genetic diversity, as inferred from the average nucleotide diversity over loci (π_n), was not significantly different among the North America and European groups ($\pi_n = 0.081 \pm 0.012$ and $\pi_n = 0.070 \pm 0.006$ respectively) (Table 2.4), but was significantly higher in the hybrid group ($\pi_n = 0.168 \pm 0.016$, $P < 0.001$) (Table 2.4). The total π_n from all samples was 0.317 (Table 2.4).

Table 2.3 AMOVA for the three groups identified in accessions of hop, as defined by PCoA, based on 730 polymorphic DArT markers.

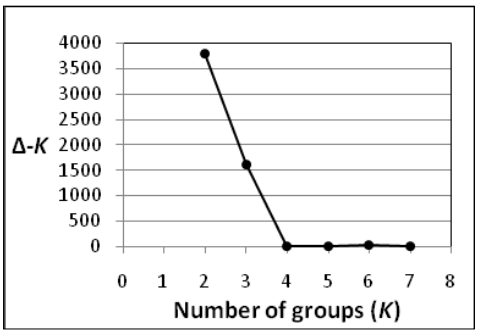
Source of variation	df	Sum of squares	Variance components	Percentage of variation	Level of significance
Among groups	2	5428.80	115.35	75.20	P < 0.001
Within groups	71	2701.33	38.05	24.80	P < 0.001
Total	73	8130.12	153.39		

Table 2.4 Average gene diversity over loci (π_n) in the accessions of hop and within each of the three groups defined by PCoA, based on 730 polymorphic DArT markers. Values of π_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.

Group	*N*	$\pi_n \pm$ SE	Pairwise F_{ST} by group
North America	13	0.08 ± 0.01	
Europe	35	0.07 ± 0.01	
Hybrid	28	0.17 ± 0.02	
Total	76	0.32 ± 0.01	

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 Δ*K* statistic (Evanno et al. 2005) revealed a maximum Δ*K* value of *K* = 2, confirming, in this group of accessions, the existence of two groups making genetic contributions (Figure 2.3a). Graphical representation of membership coefficients of the 92 hop accessions is presented in Figure 2.3b. 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 (Figure 2.3b). A combination of the two colours (blue and red) reveals accessions with both North American and European genetic ancestry (i.e. hybrids) (Figure 2.3b). All accessions in this hybrid group had greater than 50% European genetic ancestry (Figure 2.3b), with the exception of 558589 which had greater contribution of North American genetic ancestry than European genetic ancestry (Figure 2.3b), potentially due to introgression. The partitioning of groups was consistent with that revealed by PCoA (Figure 2.1) and the UPGMA dendrogram (Figure 2.2), with the exception of two anomalous accessions Cobbs and Nugget, possibly due to mislabeling. Consistent with the results of the UPGMA dendrogram (Figure 2.2), accessions 29/70/54, Aurora and AH7-D (accessions non-clustered in PCoA, Figure 2.1) were classified in the structure analysis as having only European genetic ancestry (blue only) (Figure 2.3b). INT 101 (also non-clustered in PCoA, Figure 2.1) had both European and North American genetic ancestry (blue and red) (Figure 2.3b), indicating that it was genetically intermediate. Further resolution was given to those accessions unresolved by both PCoA (Figure 2.1) and the UPGMA dendrogram (Figure 2.2), 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) (Figure 2.3b). As in the PCoA and UPGMA dendrogram, all replications clustered consistently (Figure 2.3b).

a.



b.

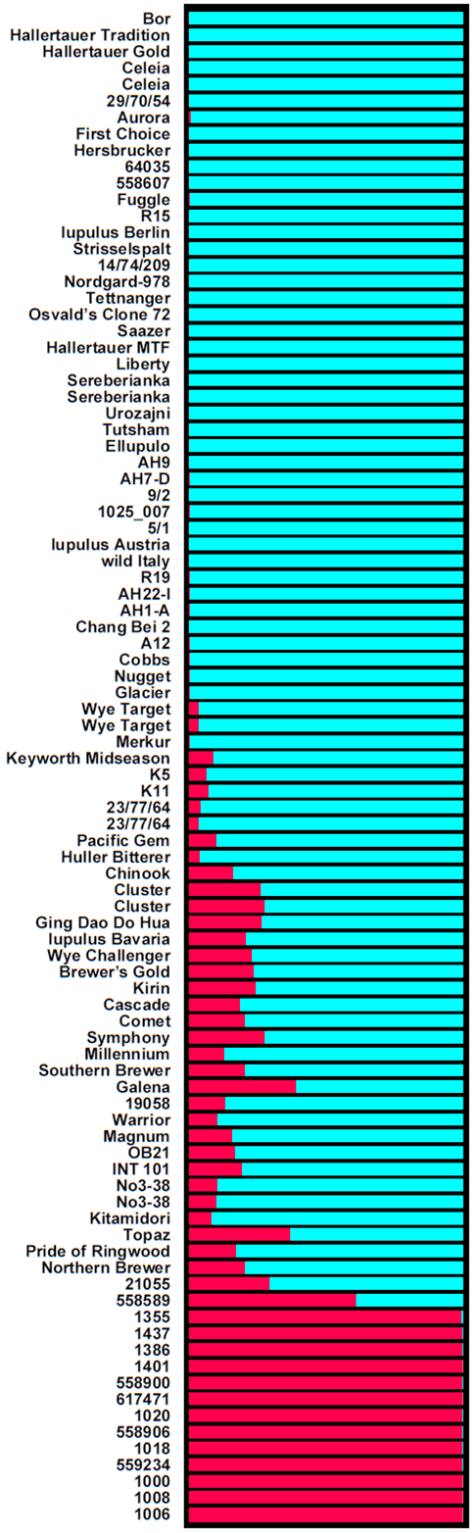


Figure 2.3 Analysis of the population structure of 92 hop accessions based on 730 polymorphic DArT markers. **a.** is a plot of ΔK for each K value [as described in 152], where K is the number of groups contributing to genetic information. **b.** is a bar plot of the proportion of each individual's genome 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 colours.

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 6144 random genomic hop clones, resulting in a polymorphism rate of 11.9% (Table 2.2). This is comparable to other DArT studies, for example 10.4% polymorphism in barley [137], 9.4% in wheat [156], 14.6% in cassava [157] and 7.0% in sugarcane [158]. However in hop, the polymorphic rate determined in this study is lower than 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 [98, 105, 109, 125], 57.9%, 32.6% and 28.3% polymorphism has been reported using ISSR [105, 135], 38.6% and 42.3% polymorphism has been reported using RAPD [105, 112] and 71.0% polymorphism has been reported using STS [105]. 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 [158-159]. 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 2.2). In hop, the average PIC value of 0.34 was obtained (Table 2.2), and is comparable to the values of 0.38 obtained in barley [137], 0.31 obtained in wheat [156] and 0.34 obtained in pigeonpea [160]. However, this value is

somewhat lower than the PIC values found in hop using other marker systems, for example 0.61 [113], 0.64 [51], 0.64 [89] and 0.38 [140]. The average reproducibility score of 99.97% obtained (Table 2.2) was comparatively higher than other studies, for example 99.8% in barley [137], 97.71% in *Asplenium* [161], 99.03% in *Garovaglia* [161] and 99.70% in pigeonpea [160]. The average call rate of 97.58% obtained (Table 2.2) also matched the values obtained in other studies, such as 95.0% in barley [137], 99.2% in wheat [156], 92.5% in sugarcane [158], 91.6% in banana [162] and 96.0% in pigeonpea [160]. The average P-value of 89.90% obtained (Table 2.2) was higher than other studies, such as 81.40% in banana [162] and 80.68% in sugarcane [158].

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 three 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 it's independence from DNA sequence information and gel-based procedure. DArT is also cost-effective, and much less expensive than most of the other genotyping technologies. Additionally, the data quality (measured by the call rate, scoring reproducibility and P-value) (Table 2.2) is comparable with other technologies, as validated in *Arabidopsis* [139]. Data quality is assisted by the automated nature of the array technology and the data extraction, completed automatically using dedicated software (Diversity Arrays Technology 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 2.2) were also comparable 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, utilizing marker systems other than DArT, have attempted to assess the genetic diversity and understand the molecular phylogenetics of hop. These studies have utilized AFLP [51, 98, 105, 109, 125, 134], RAPD [93, 105, 112]; microsatellites [33, 89, 92, 104-105, 108, 113, 124], ISSR [105, 135] and STS [33, 93, 105-106, 124]. 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 [89, 92, 104, 108-109, 112-113, 134]. Some studies have resolved these groupings in greater detail, based on wild and cultivated domestication [89, 92, 113]; geographical origin [89, 92, 103-104, 124, 134-135]; breeding history [103, 112-113] or chemical content [112, 134]. Where accessions of Asian origin have been included, these accessions have additionally fallen into a separate grouping [104, 135].

This study separated selected hop accessions into the two genetically differentiated European and North American groupings (Figures 2.1, 2.2 and 2.3; Table 2.3). Hybrids between these two groups were clearly distinguishable (Figures 2.1, 2.2 and 2.3; Table 2.3). All results from this study indicated that the North American wild hops were widely disjunct from European hops (both wild and cultivated) (Figures 2.1, 2.2 and 2.3; Tables 2.3 and 2.4). As expected, the hybrid accessions were genetically intermediate between the two groups, but all displayed closer genetic affinity to the European group (Figures 2.1, 2.2 and 2.3). This degree of similarity varied across the hybrid accessions (Figures 2.1, 2.2 and 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 (Figure 2.1) 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 (Figure 2.1) 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 2.3), which validated that most variation existed between groups (75.11% of the total variation),

while the accessions within groups were closely related. The UPGMA dendrogram (Figure 2.2) 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 2.3). The results of the ΔK statistic (Figure 2.3a) confirmed two groups making genetic contributions, while a bar plot of the STRUCTURE modeling (Figure 2.3b) 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. Additionally, 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 2.1). 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 [4, 6, 104]; 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 (Figures 2.1, 2.2 and 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 [89, 104, 113]. 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 (Figure 2.2), 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 2.3), 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 π_n, as having a value of 0.317 (Table 2.4). To the best of our knowledge, this is the first time that a value for π_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. [89] and Peredo et al. [108] 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 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 π_n [143]. Gene diversity did not significantly differ between the North American and European groups (Table 2.4). 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 utilize 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 utilized 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. [94], Seefelder et al. [114] and Koie et al. [88]. DArT markers have the advantage of easy access to marker sequences [158, 161, 163], 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 programs 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 is 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.

CHAPTER 3

Quantitative trait loci in hop (*Humulus lupulus* L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry

The text and results of this chapter are taken directly from the following publication:

McAdam EL, Freeman JS, Whittock SP, Buck EJ, Jakše J, Cerenak A, Javornik B, Kilian A, Wang C-H, Andersen D, Vaillancourt RE, Carling J, Beatson R, Graham L, Graham D, Darby P, Koutoulis A: **Quantitative trait loci in hop (*Humulus lupulus* L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry.** *BMC Genomics* 2013, **14:** 360.

ABSTRACT

Hop (*Humulus lupulus* L.) is cultivated for its cones, the secondary metabolites of which contribute bitterness, flavour and aroma to beer. Molecular breeding methods, such as marker assisted selection (MAS), have great potential for improving the efficiency of hop breeding. The success of MAS is reliant on the identification of reliable marker-trait associations. This study used quantitative trait loci (QTL) analysis to identify marker-trait associations for hop, focusing on traits related to expediting plant sex identification, increasing yield capacity and improving bittering, flavour and aroma chemistry. QTL analysis was performed on two new linkage maps incorporating transferable Diversity Arrays Technology (DArT) markers. Sixty-three QTL were identified, influencing 36 of the 50 traits examined. A putative sex-linked marker was validated in a different pedigree, confirming the potential of this marker as a screening tool in hop breeding programs. An ontogenetically stable QTL was identified for the yield trait dry cone weight; and a QTL was identified for essential oil content, which verified the genetic basis for variation in secondary metabolite accumulation in hop cones. A total of 60 QTL were identified for 33 secondary metabolite traits. Of these, 51 were pleiotropic/linked, affecting a substantial number of secondary metabolites; nine were specific to individual secondary metabolites. Pleiotropy and linkage, found for the first time to influence multiple hop secondary metabolites, have important implications for molecular selection methods. The selection of particular secondary metabolite profiles using pleiotropic/linked QTL will be challenging because of the difficulty of selecting for specific traits without adversely changing others. QTL specific to individual secondary metabolites, however, offer unequalled value to selection programs. In addition to their potential for selection, the QTL identified in this study advance our understanding of the genetic control of

traits of current economic and breeding significance in hop and demonstrate the complex genetic architecture underlying variation in these traits. The linkage information obtained in this study, based on transferable markers, can be used to facilitate the validation of QTL, crucial to the success of MAS.

INTRODUCTION

Hop is an important agronomic commodity, used mainly in the brewing industry. Rich in secondary metabolites, hop cones (female inflorescences) are an essential raw ingredient in beer, contributing the distinctive bitterness, flavour and aroma, as well as preservative activity [6, 18-20]. Traditional breeding methods have made significant progress in increasing the yield of hops and altering hop secondary metabolite profiles to improve bittering, flavour and aroma potential. Traditional breeding in hop is based on phenotypic selection of superior genotypes within segregating progenies obtained from crosses. As is the case with many perennial crops, this is a complex and lengthy process. Molecular breeding methods, such as marker assisted selection (MAS), have the potential to complement conventional phenotypic-pedigree based selection methods by providing a sophisticated, direct and precise selection system, with the capacity for higher throughput [164-167]. The successful application of MAS relies on understanding the genetic architecture underlying variation in the phenotype of traits [168]. More specifically, MAS requires the identification of molecular markers closely associated with trait variation [169]. Among other techniques, quantitative trait loci (QTL) analysis can be used to identify marker-trait associations. The genetic information acquired in QTL analysis, such as the number, location and magnitude of effects of genetic regions associated with a trait, also contributes significantly to the overall understanding of trait heritability [168].

Linkage maps are a prerequisite for QTL analysis and must be of high quality to ensure accuracy, resolution and reproducibility in the QTL identified [170]. When constructing linkage maps, transferrable markers, such as microsatellite or Diversity Arrays Technology (DArT) markers, are preferable to less transferable markers, such as amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD) markers, as they are easily employed in different mapping populations, thus facilitating direct comparison between maps and QTL verification [171]. This validation of QTL is crucial for the broad success of MAS, since QTL can be restricted to the pedigree, environment and ontogenetic stage in which they were discovered [172]. In hop, only a small number of QTL studies have been undertaken. Linkage maps have been constructed in four pseudo-testcross populations [88, 95, 99, 114] and with the exception of one [99], these are dominated by AFLP and RAPD markers, with a small proportion of transferable markers [88, 95, 114]. A few studies have identified QTL [88, 95, 99, 117] and other marker-trait associations [110, 116, 173-178];

however, only twelve traits have been examined and no validation has so far been reported. Further linkage analyses are required to gain a better understanding of loci influencing important hop traits and ultimately to enable MAS for these traits across different breeding programs. Three key areas of economic significance targeted for the genetic improvement of hop are: expedited plant sex identification, increased yield capacity and improved secondary metabolite profiles. The identification of QTL related to these targets would aid hop breeding programs in their efforts to meet the needs of the brewing industry.

Hop is a predominantly dioecious species, with male and female flowers produced on separate plants. Only female plants have economic value, producing cones containing lupulin, the secondary metabolite-packed resin, which is the commercial product. Hop has a genome size of 2.8 pg (2.7 Gbp) [179], similar to the average for all eudicots (2.8 pg) [180]. Cytogenetic studies have demonstrated that hop is diploid ($2n = 2x = 20$), with nine autosomal (A) chromosome pairs and two sex chromosomes (X and Y) [10, 175, 181-182]. Sex determination is dependent on an X/A balance, a system found in a few other plant genera, such as *Cannabis* and *Rumex* [11]. A ratio of the number of X chromosomes to the number of sets of autosomes of 1.0 gives rise to a female plant; a ratio of 0.5 gives rise to a male plant; and intermediate ratios give rise to monoecious plants (male and female flowers produced on the same plant) [10-11]. Under the X/A balance system for determination of sex phenotype, the Y chromosome is not essential to the development of the male phenotype, but is required for pollen maturation [11]; sex expression may be regulated by genes on the autosomes or may be X-linked. Definitive determination of the sex phenotype requires two seasons of growth. Sex determination at the seedling stage, using molecular markers, would drastically reduce hop breeding program costs and optimise utilisation of available land. A number of sex-linked molecular markers have been identified in hop, including RAPD markers [116, 173], inter simple sequence repeat (ISSR) markers [174], microsatellites [176] and cytogenetic markers [175]. Most markers are associated with the Y chromosome and are thus linked to maleness. However, the use of these markers has had mixed success in breeding, as the majority remain unverified across second or multiple populations. For some of these markers there is also evidence for incomplete linkage to the male sex [115]. The best described male sex-linked marker is a microsatellite, HLAGA7, being completely linked to the male sex in two Slovenian populations and on a representative sample of male hop genotypes [176]. Although HLAGA7 provides a robust sex-linked marker for use as a

screening tool in hop breeding programs, further research may detect additional polymorphic loci located on autosomes which affect sex in a broader range of genetic material.

Increasing the yield of commercial product is one of the main goals of hop breeding programs and is largely based on two methods: (i) directly increasing the content of commercially important secondary metabolites (such as hop acids, essential oils and flavonoids [183-184]) in hop cones; or (ii) indirectly increasing secondary metabolite yield, by increasing flower number and subsequently cone production. In most cultivated plant species, the inheritance of yield is complex; influenced by a multitude of integrated physiological and biochemical processes, each with their own genetic basis [185-186] and hop is no exception [79-80, 84]. Yield may also be influenced by a number of environmental factors, including water supply [9, 187-189], nutrient availability, day length [8-9], irradiance [188-189], temperature [188-189], agricultural practice [190] and infestation of pests and diseases [191-194]. The identification of QTL influencing yield and their utilisation for MAS would greatly assist breeding for increased hop yield, by eliminating confounding environmental influences as well as allowing assessments of yield potential at the seedling stage, several years before maximal cone yields, or in non-yielding male plants. An earlier study has identified putative QTL for cone yield traits, including microsatellite and AFLP markers linked to cone harvest index and dry cone weight [95]. However, given the genetic complexity of yield in other plants, there are potentially further regions of the genome associated with yield traits for which QTL could be identified.

The secondary metabolite profile of hop is diverse, consisting of three broad chemical groups: (i) hop acids (or prenylated polyketides), divisible into the subgroups α-acids and β-acids; (ii) essential oils (both terpenoid and oxygenated compounds); and (iii) polyphenols [195]. Alpha-acids impart the characteristic bitter taste to beer, while essential oils are responsible for flavour and aroma [6]. Beta-acids also contribute to beer bitterness, as well as functioning as preservative agents, possessing anti-microbial properties [6, 18-20]. The influence of polyphenols in beer brewing are not thoroughly understood, but several polyphenol compounds have been found to have potential pharmaceutical applications, particularly 8-prenylnarigenin as a phytoestrogen [30] and xanthohumol as a cancer chemopreventative agent [31]. Secondary metabolites accumulate in high concentrations in lupulin glands, which are peltate glandular trichomes found in great density on the bracteoles in hop inflorescences (cones) [12, 14]. There is evidence to show that the lupulin glands may also be involved in

the biosynthesis of the secondary metabolites [196]. In hop, differences in secondary metabolite composition are genotype-specific, with different cultivars having characteristic secondary metabolite profiles and subsequently unique bittering potentials and distinct flavour profiles [23-24]. Chemical profiles also vary with the maturation of the hop cone [197-198] and the effects of environmental stimuli. The secondary metabolite profile of kiln-dried hop cones consists of up to 30% hop acids, dominated by humulones (α-acid) and lupulones (β-acid) [195, 199]. Polyphenols and tannins comprise 3 to 6% of the hop cone weight, while essential oils are found at levels between 0.5 and 5.0 ml per 100 g [195, 199-200]. Typically, 90% of essential oils are terpenoids, dominated by myrcene, humulene, caryophyllene and farnesene [195, 199-200]. The composition of hop essential oil is diverse, with around 500 compounds currently identified and suggestions that around 1000 compounds might be present [15]. The biosynthesis of secondary metabolites is complex and not completely understood, with many of the enzymes involved yet to be identified. The three secondary metabolite chemical classes present in hop are derived from pathways of terpene metabolism, following the 2-C-methylerythritol 4-phosphate (MEP) pathway [201-202]. The biosynthesis of these hop secondary metabolites involve common precursors, including isopentenyl pyrophosphate (IPP), dimethylallyl diphosphate (DMAPP) and malonyl coenzyme A [201, 203-205]. Consequently, the synthesis of the different components may be competitive and common loci are likely to influence the concentration of each compound.

Due to the complexities of hop secondary metabolite composition and the effects of both maturation and environmental stimuli, MAS could be a useful method for breeding hops with improved brewing characteristics; allowing direct selection of hops with improved content and quality of bitter acids and essential oils in the cone. However, deployment of MAS requires a deeper understanding of the complex genetics underlying the synthesis of secondary metabolites that influence bitterness, flavour and aroma of beer. To date, QTL have been identified for a small number of important hop chemical components. In the case of hop essential oils, QTL have been identified for caryophyllene and farnesene [88]; for polyphenols, QTL have been identified for xanthohumol and desmethylxanthohumol [88, 117]; and for hop acids, QTL have been identified for α-acid, β-acid, cohumulone (as a percentage of α-acid) and colupulone (as a percentage of β-acid) [88, 95]. Five chalcone synthase genes (*vps, chs*_H1, *chs*2, *chs*3 and *chs*4) encoding enzymes directly involved in the biosynthesis of hop acids and polyphenols [201, 206-209] have been cloned; these candidate genes have been mapped in one hop population [95]. These studies have barely scratched the

surface of the hop secondary metabolite profile, warranting further analysis to identify QTL for secondary metabolites key to beer bittering, flavour and aroma.

In this study, we performed comprehensive QTL analyses, encompassing 50 traits related to three key targets in the genetic improvement of hop: expediting plant sex identification, increasing yield capacity and improving secondary metabolite composition. In order to identify QTL, male and female linkage maps were constructed from two mapping populations using a number of marker systems, including transferable DArT markers developed in this study. In one population we performed QTL analysis on two yield traits and α-acid content, with the goal of identifying environmentally and ontogenetically stable QTL. In the second population we analysed α-acid content and an additional 47 traits related to yield and secondary metabolites, the majority of which have not been previously assessed in hop QTL analyses, in order to identify QTL from single-year data. Both populations were screened for known sex-linked markers and used to search for new ones. Through the analysis of multiple traits over numerous years, this work contributes to our understanding of the genetic basis underlying phenotypic variation in hop, an essential prerequisite for future genetic improvement programs in hop.

RESULTS

Marker discovery and linkage analysis

In this study, DArT marker discovery identified 511 new polymorphic markers in hop, from 6,439 DArT clones, resulting in a frequency of polymorphism of 7.9%. A total of 834 DArT markers (511 identified in this study and 323 markers identified in a previous study [210]) were polymorphic in at least one of the two mapping populations and subsequently used for genotyping. The quality of the 834 DArT markers was assessed through several parameters. The average polymorphism information content (PIC) value was 0.36 (SE $\pm$ 0.005). Scoring reproducibility, call rate and Q values averaged at 99.8% (SE $\pm$ 0.009), 92.2% (SE $\pm$ 0.237) and 76.4 % (SE $\pm$ 0.378), respectively. The New Zealand population was genotyped with an additional 43 microsatellite markers, four RAPD markers, three sequence-tagged site (STS) markers and one marker based on a microsatellite within a candidate chalcone synthase gene (*chs*_H1). The analyses of the Slovenian population included an additional 44 microsatellite markers, 241 AFLP markers and five markers based on microsatellites within candidate

chalcone synthase genes that were genotyped in a previous study of the population (*vps*, *chs*_H1, *chs*2, *chs*3 and *chs*4) [95].

Linkage analysis of the New Zealand maternal 'Nugget' population included 337 markers (299 DArT, 34 microsatellite, 2 RAPD, 1 STS, 1 candidate gene) and resulted in a total of 286 markers (264 DArT, 20 microsatellite, 2 RAPD) placed on the map at 80 unique positions (Table 3.1; Appendix 3.1). Eleven linkage groups were formed, comprising a total map length of 231.8 cM (Table 3.1; Appendix 3.1). Linkage analysis of the New Zealand paternal Slovenian breeding line (S.B.L.) 3/3 population included 189 markers (166 DArT, 17 microsatellite, 3 RAPD, 2 STS, 1 candidate chalcone synthase gene) and resulted in a total of 157 markers (146 DArT, 8 microsatellite, 2 STS, 1 candidate gene) placed on the map at 42 unique positions (Table 3.1; Appendix 3.2). Eight linkage groups were formed, comprising a total map length of 243.0 cM (Table 3.1; Appendix 3.2). Through comparison between the maternal and paternal linkage maps, and to linkage maps of the Slovenian mapping population ('Hallertauer Magnum' x 'S.B.L. 2/1') constructed in this study, several homologous linkages were identified (Appendix 3.3). Where there were markers in common within these homologous linkage groups, the marker order was mostly conserved. There was evidence from homologous linkage groups to show that two of the linkage groups of the maternal 'Nugget' map are likely to be from the same chromosome, thus forming a total of ten linkage groups (Appendix 3.1). These ten linkage groups formed in the maternal 'Nugget' map are equal to the haploid number of chromosomes in hop (n = 10); however, only eight linkage groups were resolved in the paternal 'S.B.L. 3/3' map.

Table 3.1 Comparative features of the maternal and paternal linkage maps of the New Zealand and Slovenian mapping populations.

	New Zealand population		**Slovenian population**	
	Nugget ♀	**S.B.L. 3/3** ♂	**Hallertauer Magnum** ♀	**S.B.L. 2/1** ♂
No. markers on map	286	157	169	121
No. unique positions on map	80	42	106	63
No. linkage groups formed	10/11	8	10 / 14	10 / 11
cM of the genome covered	231.8	243.0	555.8	306.3
Average distance between markers	3.3	7.1	6.1	5.9
Largest interval between markers	36.3	36.1	40.9	32.5
No. markers with segregation distortion	136	127	68	76

Linkage analysis of the Slovenian maternal 'Hallertauer Magnum' population included 247 markers (122 DArT, 105 AFLP, 16 SSR, four candidate chalcone synthase genes) and resulted in 169 markers (100 DArT, 52 AFLP, 13 SSR, four candidate chalcone synthase genes) placed on the map at 106 unique positions (Table 3.1; Appendix 3.4). Fourteen linkage groups were formed, comprising a total map length of 555.8 cM (Table 3.1; Appendix 3.4). Linkage analysis of the Slovenian paternal S.B.L. 2/1 population included 189 markers (84 DArT, 87 AFLP, 18 SSR) and resulted in 121 markers (68 DArT, 38 AFLP, 15 SSR) placed on the map at 63 unique positions (Table 3.1; Appendix 3.5). Eleven linkage groups were formed, comprising a total map length of 306.3 cM (Table 3.1; Appendix 3.5). Through comparison between the maternal and paternal linkage maps, and to a previously reported map of the family 'Hallertauer Magnum' x 'S.B.L. 2/1' [95] (Appendix 3.3), several homologous linkage groups could be identified (Appendix 3.3). Where there were markers in common within these homologous linkage groups, the previously established marker order was mostly conserved. There was evidence from homologous linkage groups to show that several of the linkage groups within both the maternal and paternal maps were likely to be from the same chromosomes, thus forming a total of ten linkage groups in both the maternal and paternal map (Appendices 4 and 5). This is equal to the haploid chromosome number in hop.

The marker derived from the candidate chalcone synthase gene that was included in the linkage analysis of the New Zealand mapping population (*chs*_H1) was polymorphic and mapped to LG 8 of the paternal 'S.B.L. 3/3' map (Appendix 3.2). Of the five markers derived from candidate chalcone synthase genes that were included in the linkage analysis of the Slovenian population, four were polymorphic (*vps*, *chs*_H1, *chs*2 and *chs*4), and also mapped to LG 8 on the maternal 'Hallertauer Magnum' map (Appendix 3.2), following the same marker order as previously established [95].

Extensive clustering of markers was observed in the linkage maps of both the New Zealand and Slovenian mapping populations (Appendices 1 and 2). All marker types included in linkage analyses exhibited clustering within and between marker types. Before QTL analysis, superfluous markers within each cluster were eliminated to leave only one marker at each locus. In the New Zealand population, a total of 206 and 120 markers were removed from the maternal and paternal linkage maps, respectively; and a total of 63 and 58 markers were removed from the maternal and paternal linkage maps of the Slovenian population. In this

study, a significant proportion of markers demonstrated a departure from expected Mendelian segregation ratios (segregation distortion; $\alpha < 0.05$). Significant segregation distortion was found in all marker types and on all linkage maps constructed (Table 3.1). Markers with segregation distortion were frequently found close together on the linkage maps, such that the observed marker clusters consisted of markers either with or without segregation distortion. This phenomenon often resulted in entire linkage groups of exclusively distorted or non-distorted markers, or linkage groups divided into these regions (Appendices 1 and 2).

Phenotypic measurements

Sex was assessed as a binary trait; with 153 female and 25 male plants identified in the New Zealand population, giving a sex ratio of 6.1:1 (female:male). Eighty-seven female and five male plants were recognised in the Slovenian population, giving a sex ratio of 17.4:1 (female:male). All other traits assessed in this study were quantitative (Table 3.2). Of the three traits assessing hop cone yields, dry cone weight showed the smallest phenotypic variation (SD $\pm$ 0.083); followed by cone harvest index (SD $\pm$ 0.199); with green cone weight showing an eight-fold difference in variability (SD $\pm$ 0.639) compared to dry cone weight (Table 3.2). The yield of essential oil was also assessed; on average 0.64 ml (SD $\pm$ 0.08) of essential oil was obtained from 100g of dried hop cone tissue (Table 3.2).

Table 3.2 Phenotypic mean, rage and SD of secondary metabolite and yield traits quantified in the progeny of two hop mapping crosses: (i) Hallertauer Magnum x S.B.L. 2/1, grown in Slovenia; and (ii) Nugget x S.B.L. 3/3, grown in New Zealand.

Chemical group		Trait	Units	Mean	Min	Max	SD	Population	Measurement years
hop acid	α-acid	α-acid (LCV measure)	% of dry hop cone weight	8.29	2.75	15.32	2.17	Slovenia	2002-2006
		α-acid	% of dry hop cone weight	5.98	2.03	9.80	1.46	New Zealand	2009
		humulone + adhumulone	% of dry hop cone weight	4.47	1.60	7.87	1.14	New Zealand	2009
		cohumulone	% of dry hop cone weight	1.50	0.42	2.84	0.44	New Zealand	2009
		cohumulone (% of α-acid)	% of α-acid	25.25	17.53	34.71	4.18	New Zealand	2009
	β-acid	β-acid	% of dry hop cone weight	2.17	0.74	4.39	0.65	New Zealand	2009
		lupulone + adlupulone	% of dry hop cone weight	1.10	0.38	2.17	0.33	New Zealand	2009
		colupulone	% of dry hop cone weight	1.07	0.36	2.32	0.35	New Zealand	2009
		colupulone (% of β-acid)	% of β-acid	48.99	41.63	57.62	3.55	New Zealand	2009
	ratio	α-acid:β-acid	ratio of α-acid to β-acid	2.82	1.85	3.86	0.46	New Zealand	2009
essential oil	ester	geranyl acetate	% of total essential oil	0.25	0.00	0.69	0.14	New Zealand	2009
		geranyl isobutyrate	% of total essential oil	0.42	0.00	2.55	0.35	New Zealand	2009
		methyl decanoate	% of total essential oil	0.29	0.00	0.50	0.09	New Zealand	2009
		methyl dec-4-enoate	% of total essential oil	1.24	0.26	3.33	0.55	New Zealand	2009
		methyl-4-methylhex-2-enoate	% of total essential oil	0.37	0.00	1.62	0.27	New Zealand	2009
	ketone	2-undecanone	% of total essential oil	0.33	0.06	0.87	0.19	New Zealand	2009

Chemical group	Trait	Units	Mean	Min	Max	SD	Population	Measurement years
ether	humulene diepoxide a	% of total essential oil	0.50	0.00	2.37	0.39	New Zealand	2009
	humulene epoxide I	% of total essential oil	0.29	0.00	1.41	0.21	New Zealand	2009
	humulene epoxide II	% of total essential oil	0.66	0.19	2.84	0.41	New Zealand	2009
	humulene epoxide III	% of total essential oil	0.74	0.12	2.54	0.43	New Zealand	2009
monoterpene alcohol	geraniol	% of total essential oil	0.78	0.09	2.92	0.39	New Zealand	2009
	limonene-10-ol	% of total essential oil	0.29	0.00	1.94	0.25	New Zealand	2009
	linalool	% of total essential oil	0.43	0.00	1.02	0.21	New Zealand	2009
sesquiterpene alcohol	caryolan-1-ol	% of total essential oil	0.35	0.00	1.15	0.19	New Zealand	2009
	humulenol II	% of total essential oil	0.06	0.00	0.29	0.08	New Zealand	2009
	humulol	% of total essential oil	0.22	0.00	0.58	0.10	New Zealand	2009
	t-cadinol	% of total essential oil	0.14	0.00	0.40	0.13	New Zealand	2009
alkane	tetradecane	% of total essential oil	0.10	0.00	0.20	0.05	New Zealand	2009
monoterpene	β-pinene	% of total essential oil	0.26	0.00	0.69	0.16	New Zealand	2009
	camphene	% of total essential oil	0.05	0.00	0.37	0.07	New Zealand	2009
	limonene	% of total essential oil	0.68	0.00	3.42	0.48	New Zealand	2009
	myrcene	% of total essential oil	28.47	1.13	59.65	0.30	New Zealand	2009
	ρ-cymene	% of total essential oil	0.21	0.00	0.65	13.86	New Zealand	2009
	terpinene	% of total essential oil	0.47	0.00	2.72	0.09	New Zealand	2009

Chemical group		Trait	Units	Mean	Min	Max	SD	Population	Measurement years
	sesquiterpene	α-capaene	% of total essential oil	0.32	0.00	0.65	0.13	New Zealand	2009
		α-selinene	% of total essential oil	1.21	0.34	2.91	0.52	New Zealand	2009
		β-selinene	% of total essential oil	0.47	0.00	1.33	0.20	New Zealand	2009
		δ-cadinene	% of total essential oil	0.70	0.09	3.24	0.54	New Zealand	2009
		γ-cadinene	% of total essential oil	1.58	0.00	3.73	0.86	New Zealand	2009
		caryophyllene	% of total essential oil	12.37	4.64	22.80	3.90	New Zealand	2009
		caryophyllene oxide	% of total essential oil	0.21	0.00	0.58	0.12	New Zealand	2009
		farnesene	% of total essential oil	7.29	0.06	28.13	7.66	New Zealand	2009
		humulene	% of total essential oil	29.70	9.90	55.92	9.29	New Zealand	2009
		muurolene	% of total essential oil	0.92	0.29	1.74	0.79	New Zealand	2009
	ratio	humulene:caryophyllene	ratio of humulene to caryophyllene	2.50	1.36	3.55	0.66	New Zealand	2009
polyphenol	polyphenol	xanthohumol	% of dry hop cone weight	0.24	0.08	0.51	0.46	New Zealand	2009
yield	secondary metabolites	essential oil content	ml of oil per 100g of hop cone tissue	0.64	0.17	1.71	0.08	New Zealand	2009
	cones	cone harvest index	ratio of cone weight to whole plant weight	0.31	0.11	1.27	0.20	Slovenia	2002-2006
		dry cone weight	kg of dry cones per plant	0.15	0.04	0.40	0.08	Slovenia	2002-2006
		green cone weight	kg green cones per plant	1.54	0.30	3.35	0.64	New Zealand	2009

The secondary metabolite profile of hop was examined in the progeny of a New Zealand mapping cross through a total of 45 traits from all hop secondary metabolite groups (hop acids, essential oils and polyphenols). Quantitatively, the hop acid component of the secondary metabolite profile of the New Zealand mapping population was dominated by α-acid (average 6.0% of dry cone weight), the largest component of which was the humulone + adhumulone fraction (average 4.5% of dry cone weight) (Table 3.2). The essential oil component of the secondary metabolite profile was dominated by the sesquiterpenes humulene (average 29.7% of total essential oil), caryophyllene (average 12.4% of total essential oil) and farnesene (average 7.3% of total essential oil); and the monoterpene myrcene (average 28.5% of total essential oil) (Table 3.2). A single polyphenol was assessed, xanthohumol, which comprised an average of 0.2% of the dry cone weight (Table 3.2). Correlations were evident between a number of the secondary metabolites, both within and between the major structural groups (Figure 3.1). The strongest correlations were exhibited within the hop acid groups, where the six secondary metabolite traits measured (α-acid, β-acid, humulone + adhumulone, cohumulone, lupulone + adlupulone and colupulone) all shared very strong positive correlations (Pearson's $r > 0.80$) (Figure 3.1). Very strong positive correlations were also observed between several of the other secondary metabolite traits, although many of these correlations did not form cohesive patterns either within or between major chemical groups (Figure 3.1). The highest phenotypic correlations were between the two hop acids, cohumulone (% of α-acid) and colupulone (% of β-acid) ($r = 0.88$); the polyphenol and hop acid, xanthohumol and cohumulone ($r = 0.87$); the polyphenol and hop acid, xanthohumol and colupulone ($r = 0.85$); the two monoterpenes β-pinene and myrcene ($r = 0.96$); and the ketone and sesquiterpene, 2-undecanone and farnesene ($r = 0.91$) (Figure 3.1). No very strong negative correlations ($r < -0.80$) were observed between the secondary metabolites (Figure 3.1). One secondary metabolite trait, α-acid, was examined in the Slovenian population, measuring an average of 8.3% of dry cone weight. Alpha-acid was not strongly correlated with any other trait measured in the Slovenian population.

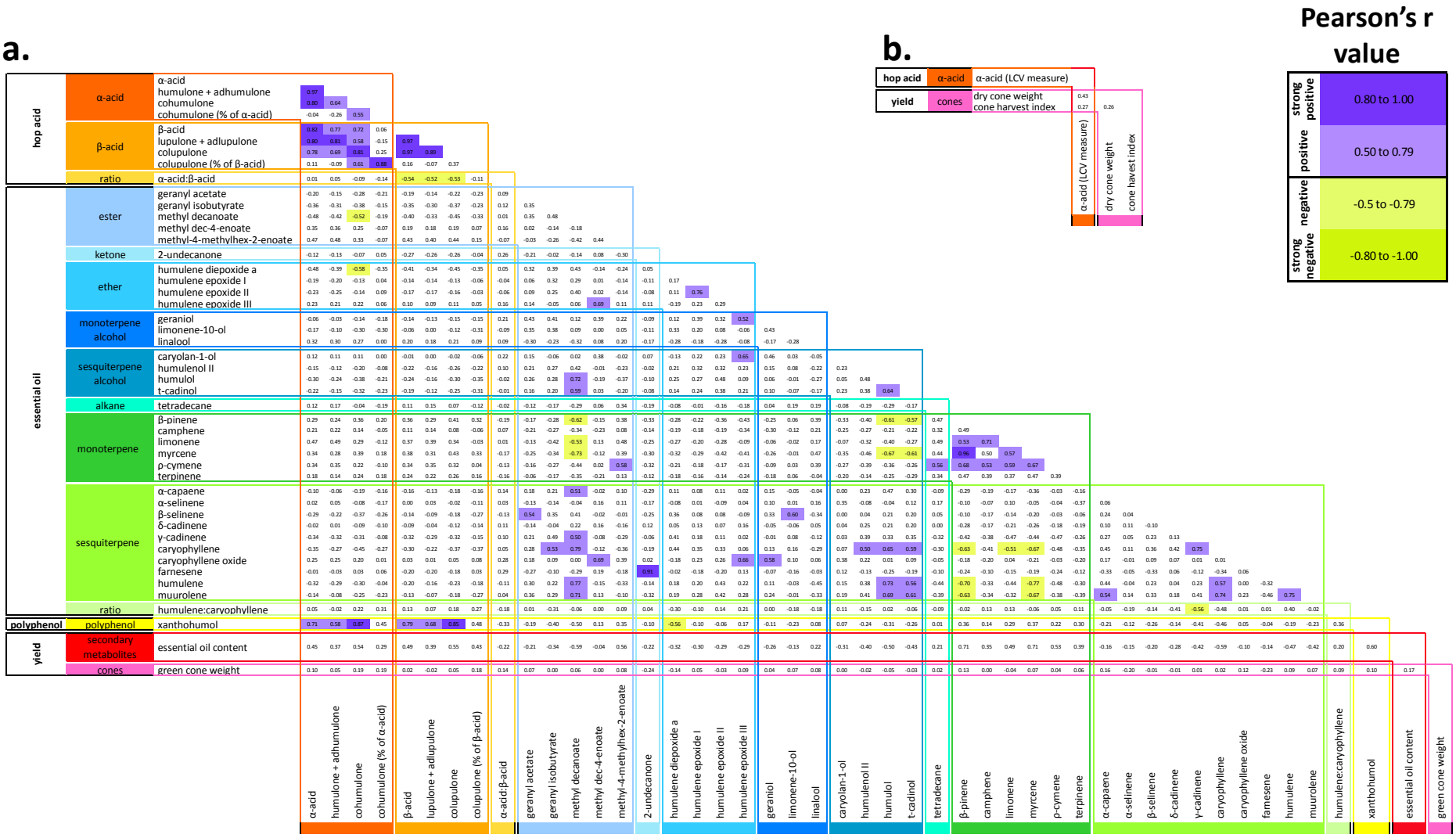


Figure 3.1 Correlation coefficients (Pearson's r) for the relationships between secondary metabolite and yield traits examined in hop, in (a) a New Zealand mapping population (n = 47) and (b) a Slovenian mapping population (n = 3).

QTL analysis

Sex trait

One sex-linked marker was detected in this study, identified for the first time in the New Zealand population and confirming its previous identification in the Slovenian population (Table 3.3; Figures 3.2 and 3.3). In both cases, the microsatellite marker HLAGA7 segregated from the male parent and showed complete linkage to the male character. While highly significant in both populations, differences in the level of significance were apparent, with a higher significance observed in the Slovenian population (LOD 1441; Table 3.3b) than in the New Zealand population (LOD 14; Table 3.3a).

Table 3.3 Quantitative trait loci identified for sex, yield and cone chemistry traits in hop, in (**a.**) a New Zealand mapping population and (**b.**) a Slovenian mapping population.

a.

Chemical group		Trait	QTL	Linkage group	Seg. [a]	Adjacent marker	Position (cM)	LOD [b]	% exp [c]	Add. [d]	Kruskal-Wallis
hop acid	α-acid	α-acid	α-acid-1	Nugget 1	F	D-hPb-718465-l-4af	0.0	2.91	8.6	0.43	P < 0.0005
			α-acid-2	Nugget 5	F	D-hPb-618369-l-1f	34.4	2.62	7.1	0.40	P < 0.0005
		humulone + adhumulone	humulone + adhumulone-1	Nugget 5	F	D-hPb-618369-l-1f	34.4	4.26	11.3	0.39	P < 0.0001
			humulone + adhumulone-2	Nugget 1	F	D-hPb-718465-l-4af	0.0	2.58	6.7	0.29	P < 0.001
		cohumulone	cohumulone-1	Nugget 2	F	D-hPb-364480-l-4c*f	1.8	8.14	21.9	-0.21	P < 0.0001
			cohumulone-2	Nugget 1	F	D-hPb-718465-l-4af	0.0	3.29	8.1	0.12	P < 0.0005
		cohumulone (% of α-acid)	cohumulone (% of α-acid)-1	Nugget 2	F	S-GT4-J12-15-lf	3.5	24.78	43	-2.78	P < 0.0001
	β-acid	β-acid	β-acid -1	Nugget 5	F	D-hPb-366221-l-1f	39.0	5.01	11.9	0.23	P < 0.0001
			β-acid -2	Nugget 2	F	D-hPb-364480-l-4c*f	1.8	5.01	11.9	-0.23	P < 0.0005
			β-acid -3	S.B.L. 3/3 3	M	S-HLGT14*-n*m	0.0	4.39	10.3	-0.28	P < 0.05
			β-acid-4	Nugget 8	F	D-hPb-362051-l-4af	5.9	2.37	5.4	0.15	P < 0.05
		lupulone + adlupulone	lupulone + adlupulone-1	Nugget 5	F	D-hPb-366221-l-1f	39.0	5.72	14.7	0.13	P < 0.0001
			lupulone + adlupulone-2	S.B.L. 3/3 3	M	S-HLGT14*-n*m	0.0	3.70	9.2	-0.13	P < 0.005
			lupulone + adlupulone-3	Nugget 8	F	D-hPb-362051-l-4af	5.9	3.17	7.8	0.09	P < 0.005
		colupulone	colupulone-1	Nugget 2	F	D-hPb-364480-l-4c*f	1.8	8.40	21.8	-0.16	P < 0.0001
			colupulone-2	Nugget 5	F	D-hPb-366221-l-1f	39.0	3.55	8.5	0.10	P < 0.0005
		colupulone (% of β-acid)	colupulone (% of β-acid)-1	Nugget 2	F	D-hPb-719407-l-4af	2.8	20.54	44.4	-2.4	P < 0.0001
	ratio	α-acid:β-acid	α-acid:β-acid-1	Nugget 8	F	D-hPb-362051-l-4af	5.9	4.44	10.5	-0.15	P < 0.0005
			α-acid:β-acid-2	Nugget 2	F	D-hPb-364957-l-4af	2.3	4.40	10.4	0.15	P < 0.0005
			α-acid:β-acid-3	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	3.63	8.5	0.13	P < 0.001
			α-acid:β-acid-4	Nugget 1	F	D-hPb-716855-l-4af	29.5	2.65	6.1	0.11	P < 0.01

Chemical group		Trait	QTL	Linkage group	Seg. [a]	Adjacent marker	Position (cM)	LOD [b]	% exp [c]	Add. [d]	Kruskal-Wallis
essential oil	ester	methyl decanoate	methyl decanoate-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	6.91	17.2	0.04	P < 0.0001
			methyl decanoate-2	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	3.70	8.7	-0.03	P < 0.0001
			methyl decanoate-3	Nugget 1	F	D-hPb-366735-l-1*f	0.0	2.45	5.6	-0.03	P < 0.001
		methyl dec-4-enoate	methyl dec-4-enoate-2	Nugget 1	F	D-hPb-366735-l-1*f	0.0	3.81	12.3	0.21	P < 0.0001
		methyl-4-methylhex-2-enoate	methyl-4-methylhex-2-enoate-2	Nugget 5	F	S-AP20_600-lf	50.4	2.36	6.1	0.07	P < 0.01
	ketone	2-undecanone	2-undecanone-1	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	25.1	53.8	0.14	P < 0.0001
			2-undecanone-2	Nugget 5	F	S-AP20_600-lf	50.4	4.62	6.8	-0.05	P < 0.01
	ether	humulene diepoxide a	humulene diepoxide a-1	Nugget 2	F	S-GA8-K15-4-lf	2.4	2.37	7.8	0.11	P < 0.05
	monoterpene alcohol	linalool	linalool-1	Nugget 5	F	D-hPb-618369-l-1f	34.4	2.68	8.8	0.06	P < 0.005
	sesquiterpene alcohol	humulol	humulol-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	3.81	11.2	0.04	P < 0.0001
			humulol-2	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	2.80	8.1	-0.03	P < 0.0005
		t-cadinol	t-cadinol-1	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	3.34	10.1	-0.03	P < 0.0005
	monoterpene	β-pinene	β-pinene-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	5.42	16.1	-0.07	P < 0.0001
		limonene	limonene-1	S.B.L. 3/3 3	M	S-HLGT14*-n*	0.0	2.33	7.7	-0.17	P < 0.001
		myrcene	myrcene-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	5.54	16	-5.61	P < 0.0001
			myrcene-2	Nugget 5	F	D-hPb-618369-l-1f	34.4	3.56	9.9	4.43	P < 0.005
		ρ-cymene	ρ-cymene-1	Nugget 5	F	D-hPb-362315-l-1f	44.5	2.84	9.3	0.04	P < 0.0005
		terpinene	terpinene-1	S.B.L. 3/3 6	M	D-hPb-619280-n-1*m	14.2	3.19	9.3	0.14	P < 0.0001
			terpinene-2	Nugget 2	F	D-hPb-719075-l-4a*f	0.0	2.73	7.9	-0.13	P < 0.0001

Chemical group		Trait	QTL	Linkage group	Seg. [a]	Adjacent marker	Position (cM)	LOD [b]	% exp [c]	Add. [d]	Kruskal-Wallis
	sesquiterpene	α-capaene	α-capaene-1	S.B.L. 3/3 3	M	D-hPb-619412-n-1m	35.1	3.52	11.4	-0.05	P < 0.0005
		α-selinene	α-selinene-1	Nugget 1	F	D-hPb-718465-l-4af	0.0	4.01	12.9	-0.19	P < 0.0001
		β-selinene	β-selinene-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	3.88	11.5	0.07	P < 0.0001
		δ-cadinene	δ-cadinene-1	Nugget 1	F	D-hPb-618333-l-1f	45.2	6.05	18.8	0.23	P < 0.0001
		γ-cadinene	γ-cadinene-1	Nugget 1	F	D-hPb-618333-l-1f	45.2	7.87	22.6	0.41	P < 0.0001
			γ-cadinene-2	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	2.67	7	-0.23	P < 0.05
		caryophyllene	caryophyllene-1	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	6.23	13.7	-1.45	P < 0.0001
			caryophyllene-2	Nugget 1	F	D-hPb-715569-l-1	28.3	5.45	12.6	1.39	P < 0.005
			caryophyllene-3	Nugget 2	F	D-hPb-364957-l-4af	2.3	5.10	11	1.32	P < 0.0001
		farnesene	farnesene-1	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	39.78	71.3	6.45	P < 0.0001
			farnesene-2	Nugget 5	F	S-AP20_600-lf	50.4	4.62	4.2	-1.62	P < 0.05
		humulene	humulene-1	Nugget 1	F	D-hPb-362665-l-1f	39.3	7.15	15.3	-3.67	P < 0.0001
			humulene-2	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	6.42	13.8	-3.45	P < 0.0001
			humulene-3	Nugget 2	F	D-hPb-719075-l-4a*f	0.0	3.61	7.3	2.55	P < 0.01
			humulene-4	Nugget 5	F	D-hPb-618369-l-1f	34.4	2.66	5.3	-2.22	P < 0.005
		muurolene	muurolene-1	S.B.L. 3/3 3	M	D-hPb-716654-n-1m	36.2	8.83	22.6	-0.15	P < 0.0001
			muurolene-2	Nugget 2	F	D-hPb-364957-l-4af	2.3	5.17	12.4	0.11	P < 0.0001
	ratio	humulene:caryophyllene	humulene:caryophyllene-1	Nugget 1	F	D-hPb-362665-l-1f	39.3	38.44	66.9	-0.54	P < 0.0001
polyphenol	polyphenol	xanthohumol	xanthohumol-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	11.5	29.5	-0.05	P < 0.0001
			xanthohumol-2	Nugget 1	F	D-hPb-715569-l-1f	28.3	2.46	5.4	-0.02	P < 0.01

Chemical group		Trait	QTL	Linkage group	Seg. [a]	Adjacent marker	Position (cM)	LOD [b]	% exp [c]	Add. [d]	Kruskal-Wallis
yield	secondary metabolites	essential oil content	essential oil content-1	Nugget 2	F	D-hPb-364957-l-4af	2.3	6.66	20.1	-0.16	P < 0.0001
sex	sex	sex	sex-1	S.B.L. 3/3 5	M	S-HLAGA7-a*m	2.1	13.84	22.6	-0.28	P < 0.0001

b.

Chemical group		Trait	QTL	Linkage group	Seg. [a]	Adjacent marker	Position (cM)	LOD [b]	% exp [c]	Add. [d]	Kruskal-Wallis
yield	cone	dry cone weight	dry cone weight-1	Hallertauer Magnum 1	F	D-hPb-716855-l-4a*f	16.0	7.49	35.0	0.05	P < 0.0001
sex	sex	sex	sex-1	S.B.L. 2/1 5	M	S-HLAGA7-e*	20.5	1441.23	81	0.50	P < 0.0001

a Seg. indicates the segregation of the QTL from either the maternal (F) or paternal (M) parent.
b LOD indicates the peak LOD score for the QTL at the genome-wide significance level.
c % exp. indicates the percentage of the phenotypic variation of the trait explained by the QTL.
d Add. indicates the estimated additive effect of the allele (i.e. (mean of the distribution of the quantitative trait associated with the female genotype – mean of the distribution of the quantitative trait associated with the male genotype)/2.

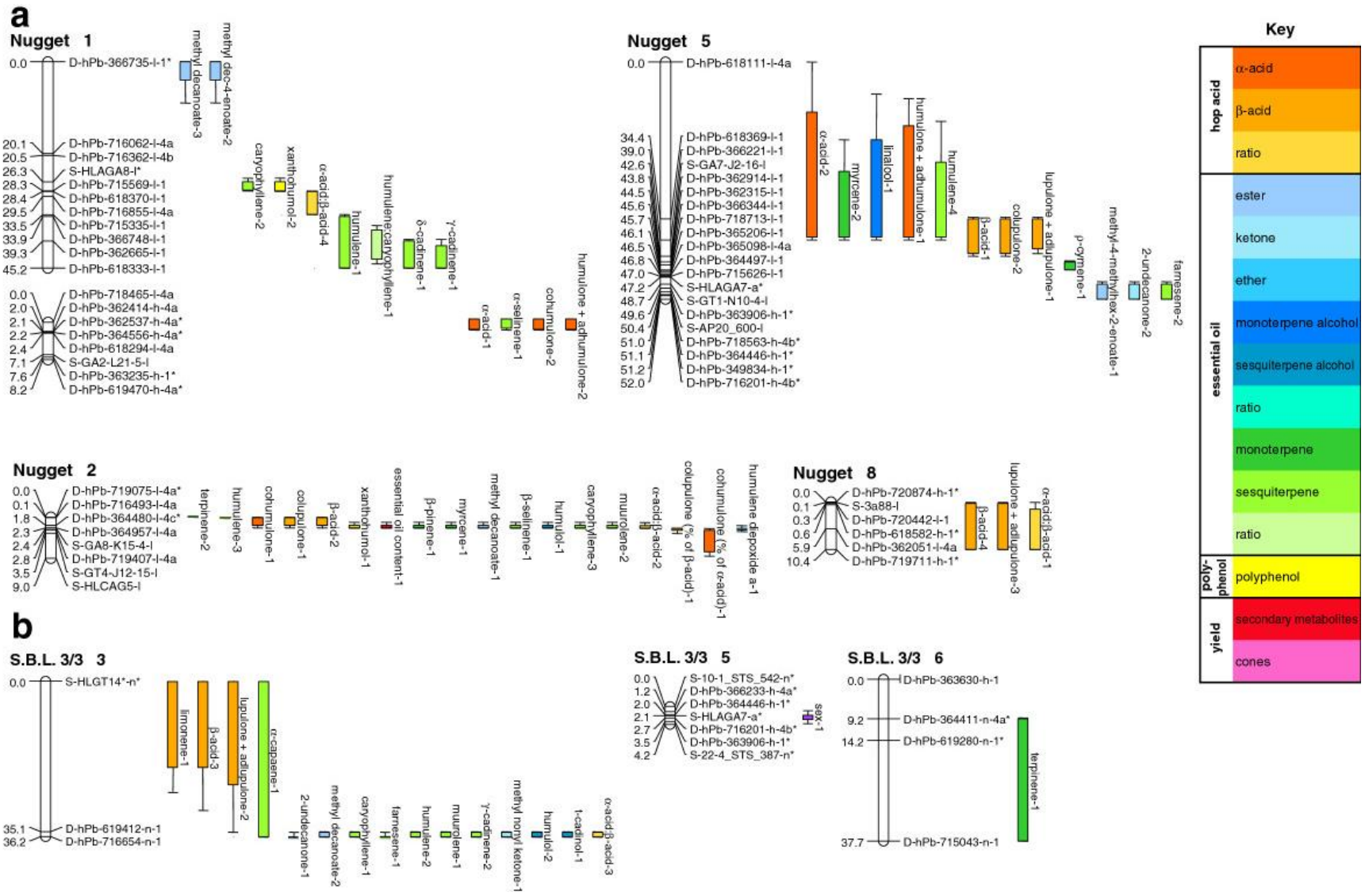


Figure 3.2 Location of QTL for sex, yield and secondary metabolite traits on (**a.**) maternal and (**b.**) paternal linkage groups of hop from the New Zealand mapping population.

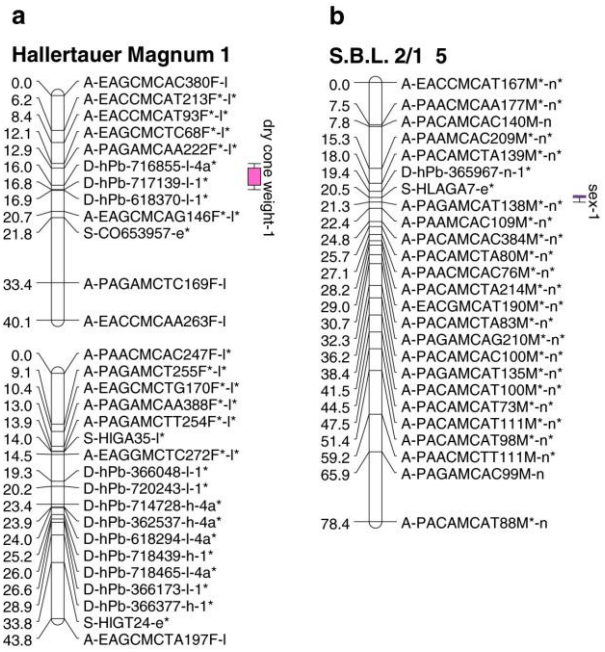


Figure 3.3 Location of QTL for sex and yield traits on (**a.**) maternal and (**b.**) paternal linkage groups of hop from the Slovenian mapping population.

Yield traits

Of the four yield traits assessed in this study, QTL were identified for two: one QTL for essential oil content (Table 3.3a; Figure 3.2a) and one QTL for dry cone weight (Table 3.3b; Figure 3.3b). Both QTL segregated from the female parent, explaining 20.1% and 35.0% of the phenotypic variation, respectively (Table 3.3). QTL were not identified for cone harvest index or green cone weight.

Secondary metabolite traits

A total of 60 putative QTL were identified for hop secondary metabolite traits, above the genome-wide significance level ($\alpha < 0.05$) (Table 3.3a). For 33 of the 45 assessed secondary metabolite traits, between one and four QTL were identified, each explaining an estimated 4.2% to 71.3% of the phenotypic variance (Table 3.3a). QTL were not identified for 12 essential oil components (camphene, caryolan-1-ol, caryophyllene oxide, geraniol, geranyl acetate, geranyl isobutyrate, humulene epoxide I, humulene epoxide II, humulene epoxide III, humulenol II, limonene-10-ol, tetradecanone). QTL were also not identified for α-acid in the Slovenian population.

The 60 putative QTL identified for hop secondary metabolite traits mapped to 13 discreet regions (defined as having QTL peaks separated by more than 5 cM) on six linkage groups (Table 3.4; Figure 3.2). Five of the QTL regions were unique to specific traits, these being

humulene (QTL region 3) on 'Nugget' linkage group (LG) 1 (39.3 cM); cadinene (both δ and γ isoforms) (QTL region 4) on 'Nugget' LG1 (45.2 cM); terpinene (QTL region 14) on 'S.B.L. 3/3' LG6 (14.2 cM); ρ-cymene (QTL region 8) on 'Nugget' LG5 (44.5 cM); and lupulone + adlupulone (QTL region 10) on 'Nugget' LG7 (5.9 cM) (Table 3.4; Figure 3.2). QTL region 10, affecting lupulone + adlupulone, was also found to affect, by extension, the traits β-acid and α-acid:β-acid (Figure 3.2b), as lupulone + adlupulone is equivalent to β-acid. QTL region 3, affecting humulene was found to affect, by extension, humulene:caryophyllene (Figure 3.2a), showing that the humulene:caryophyllene ratio is biased towards humulene. The remaining eight QTL regions affected multiple traits (Table 3.4; Figure 3.2). Two of these (QTL regions 2 and 6) were found to influence compounds from all three groups of secondary metabolites (hop acids, essential oils and polyphenols), affecting three and 18 different components, respectively (Table 3.4; Figure 3.2). The other six QTL regions influenced compounds from one or two different secondary metabolite classes: (i) QTL region 5 influenced four traits, three α-acid compounds and a sesquiterpene (relatively isolated in the biosynthetic pathway from the other sesquiterpenes included in this study); (ii) QTL region 7 influenced eight traits from the hop acid and essential oil groups; (iii) QTL region 9 influenced three traits: an ester, a ketone and a sesquiterpene (isolated in the biosynthetic pathway from the other secondary metabolites included in this study); (iv) QTL region 11 influenced three traits: two from the β-acid group and one sesquiterpene; (v) QTL region 12 influenced 11 traits from the hop acid and essential oil groups (Table 3.4; Figure 3.2); and (vi) QTL region 1 influenced two traits, both esters (Table 3.4; Figure 3.2). Each of the QTL regions identified in this study were sex-specific; 10 of the 13 QTL regions segregated from the female parent 'Nugget' (Table 3.4), a significant bias ($\chi^2_1 = 3.8$, $P < 0.05$).

Table 3.4 Discrete QTL, both specific and pleiotropic/linked, identified in hop; and the sex, yield and cone chemistry traits affected by each locus.

QTL region	Specificity	Linkage group	No. traits	Traits
QTL region 1	pleiotropic	Nugget 1	2	methyl decanoate; methyl dec-4-enoate
QTL region 2	pleiotropic	Nugget 1 & Hallertauer Magnum 1	4	α-acid:β-acid; caryophyllene; xanthohumol; dry cone weight
QTL region 3	specific	Nugget 1	2	humulene; humulene:caryophyllene
QTL region 4	specific	Nugget 1	2	δ-cadinene; γ-cadinene
QTL region 5	pleiotropic	Nugget 1	4	α-acid; humulone + adhumulone; cohumulone; α-selinene
QTL region 6	pleiotropic	Nugget 2	18	cohumulone; cohumulone (% of α-acid); β-acid; colupulone; colupulone (% of β-acid); α-acid:β-acid; methyl decanoate; humulene diepoxide a; humulol;, β-pinene; myrcene; terpinene; β-selinene; caryophyllene; humulene; muurolene; xanthohumol; essential oil content
QTL region 7	pleiotropic	Nugget 5	8	α-acid; humulone + adhumulone; β-acid; lupulone + adlupulone; colupulone; linalool; myrcene; humulene
QTL region 8	specific	Nugget 5	1	ρ-cymene
QTL region 9	pleiotropic	Nugget 5	3	methyl-4-methylhex-2-enoate; 2-undecanone; farnesene
QTL region 10	specific	Nugget 8	3	β-acid; lupulone + adlupulone; α-acid:β-acid
QTL region 11	pleiotropic	S.B.L. 3/3 3	3	β-acid; lupulone + adlupulone; limonene
QTL region 12	pleiotropic	S.B.L. 3/3 3	11	α-acid:β-acid; methyl decanoate; 2-undecanone; humulol; t-cadinol; α-capaene; γ-cadinene; caryophyllene; farnesene; humulene; muurolene
QTL region 13	specific	S.B.L. 3/3 5 & S.B.L. 2/1 5	1	sex
QTL region 14	specific	S.B.L. 3/3 6	1	terpinene

Comparisons between QTL identified in the New Zealand and Slovenian populations were made using markers found in common between linkage maps constructed from the two populations. The QTL for dry cone weight that was identified in the Slovenian population (Table 3.3; Figure 3.3a) co-located with QTL region 2, on 'Nugget' LG1 (29.5 cM) of the New Zealand population, influencing the three traits α-acid:β-acid, caryophyllene and xanthohumol (Table 3.4). Of the five markers based on candidate chalcone synthase genes (*vps*, *chs*_H1, *chs*2, *chs*3 and *chs*4) included in linkage analysis of the Slovenian population in

this study, four were mapped, in the maternal 'Hallertauer Magnum' LG8 of the Slovenian population. Although these genes encode enzymes involved in the biosynthesis of hop acids and poloyphenols, no QTL for α-acid was identified in the Slovenian population, associated with these chalcone synthase genes or on any other marker. Two QTL were, however, identified for α-acid in the New Zealand mapping population (explaining 8.6% and 7.1% of the phenotypic variation, respectively), as well as an additional 19 QTL for other hop acid traits and two QTL for xanthohumol (Table 3.3). None of the QTL identified were associated with the marker based on a candidate chalcone synthase gene (*chs*_H1) that mapped to 'S.B.L. 3/3' LG8 of the New Zealand population.

DISCUSSION

Marker discovery and linkage analysis

Although linkage maps derived from four hop mapping populations have been published [88, 95, 99, 114], a highly saturated linkage map is still to be constructed for this species. The high resolution of such a map is an essential component for the identification of accurate and reproducible QTL, particularly those with small effects. The goal of this study was to construct linkage maps for a new mapping population from New Zealand and to build upon a pre-existing linkage map from a Slovenian mapping population [95], through the addition of transferable DArT markers. The 511 new DArT markers identified in this study were found to be of a similar high quality (in terms of PIC, reproducibility and call rate) to those identified in a previous hop study [210] and in other plant species [137, 156, 158, 160, 162]. The use of these new DArT markers in linkage analysis of the Slovenian population, along with DArT, AFLP and microsatellite markers previously identified [94-95, 210], increased the number and density of markers, allowed the clear identification of ten linkage groups (corresponding to the haploid number of chromosomes in hop) and increased the transferability of the maps between mapping populations. Our study used a conservative approach; accepting only markers within designated parameters (see Methods below). This method, in combination with factors such as the amalgamation of some of the smaller groups and the exclusion of terminal markers, resulted in shorter map lengths compared with Cerenak et al. [95]. Where there were markers in common between the linkage maps of the Slovenian population constructed in this study and previous maps, marker order was mostly conserved and homologous linkage groups were identified (Appendix 3.3). Linkage group homology and marker order was also consistent between linkage maps constructed using the Slovenian

population and the New Zealand population, and between maternal and paternal linkage maps of both populations (Appendix 3.3). While ten linkage groups were identified in the maternal linkage map of the New Zealand population (Appendix 3.1), only eight were resolved in the paternal map (Appendix 3.2). The maternal linkage map also contained nearly double the number of markers of the paternal linkage map. These factors suggest that further addition of markers is required to achieve genome coverage in the paternal linkage map of the New Zealand population.

In this study, a large number of markers demonstrated significant departure from expected Mendelian ratios (Table 3.1; Appendices 1 and 2). Significant clustering of the markers was also observed (Appendices 1 and 2). These phenomena have been previously identified in other species and were attributed to biological factors, rather than experimental limitations [211-219]. Several factors indicate that this was also the case in this study. Both clustering of markers and segregation distortion (which has been identified in hop previously [95, 114]) was not limited to one marker type, but was evident in all marker types used, suggesting that they are not artefacts of genotyping error. Marker clusters were associated with regions of segregation distortion, such that they were composed of either all distorted markers or all non-distorted markers. Clustering of markers is typically symptomatic of saturation of markers on the linkage map [220], yet marker saturation in this study is unlikely, for several reasons. Intervals of greater than 10 cM exist (Table 3.1); and in one of the four linkage maps the number of linkage groups identified was fewer than the number of hop chromosomes, while in the other three linkage maps several of the hop chromosomes were split into two or more linkage groups because of insufficient linkage. These factors indicate that the linkage maps do not contain the maximal density of markers and suggest that additional markers are required if smaller sub-groups are to coalesce into a single linkage group. Also, marker saturation in clustered groups may indicate low levels of recombination in hop, for which there is no reported evidence. All of these factors suggest that marker clustering, as well as the segregation distortion of markers, shorter map length and lower marker density of the maps constructed in this study have a biological basis for which further investigation is required.

The linkage maps constructed in this study provide a valuable resource for QTL analyses in hop. The large number of QTL identified in this study (Table 3.3) provide an excellent starting point to begin to understand the complex genetic architecture underlying variation in

hop secondary metabolite composition, which is critical to the ultimate use of hop to provide bitterness, flavour and aroma in beer. Comprising a large number of transferable markers, mapped with a conservative methodology, these linkage maps will provide a basis for further comparative mapping, facilitating the identification and validation of QTL that may ultimately be applied to successful application of molecular methods in selection programs.

QTL analysis

Sex trait

In this study, the microsatellite marker HLAGA7 was linked to the sex phenotype (Table 3.3; Figures 3.2 and 3.3). The association of this marker with sex has been previously reported in two mapping populations grown in Slovenia [176], one of which was the same mapping population as used in this study. In this study the segregation of the sex-linked marker (HLAGA7) was detected for the first time in a New Zealand mapping population (Table 3.3; Figure 3.2), extending the utility of this marker. In both the New Zealand and Slovenian populations this sex-linked marker segregated from the male parent and showed complete linkage to the male sex phenotype. This is consistent with the specificity of the Y chromosome to the male sex, male plants with the heteromorphic XY configuration and female plants with homomorphic XX [10, 175, 181-182]. In both populations the significance of association of the HLAGA7 marker with sex was very high (Table 3.3); however, a greater level of significance was identified in the Slovenian population. The greater significance in the Slovenian population is likely to reflect differences such as greater map length and number of markers on the linkage group of the Slovenian population (Table 3.1; Figures 3.2b and 3.3b) as well as the smaller size of the Slovenian population, since inflation of QTL effects increases with decreasing population size [221]. Sex-linked molecular markers have been identified in hop previously [116, 173-176]; however, the HLAGA7 marker is the most definitive sex-linked marker identified in hop to date, having now been verified in multiple populations. This study has confirmed the potential for the HLAGA7 marker to be used for routine screening, allowing for the rapid identification of sex in hop breeding programs in diverse environments and populations. Further studies are required, however, to understand the influence of autosomal regions on sex differentiation, as no sex-linked markers were identified on autosomes in this study. This may be due to the existence of numerous regions, each with small effects of too low significance to be detected by this QTL analysis, or the autosomal regions may not contain polymorphism linked to sex differentiation.

Yield traits

Three traits related to hop cone yield were examined in this study. Of these, dry cone weight was the only trait for which a QTL was identified. One QTL, stable over the five year period, was detected, segregating from the female parent of the Slovenian population, explaining 35% of the phenotypic variation (Table 3.3; Figure 3.3). QTL were not identified for either green cone weight (New Zealand population) or cone harvest index (Slovenian population). The potential reasons for the failure to detect QTL differ between these two yield metrics. Quantification of green cone weight is a method used for the rapid experimental assessment of hop yield. The lack of detectable QTL influencing this trait may be due to the variable moisture content of green cones (which typically contain ~75 – 80% moisture) compared to dry cones (which contain ~8 – 10% moisture). Moisture content of harvested commercial product varies in other species [222-224] and where this is the case, most suggest that it should be corrected for. The lack of QTL for this trait suggest that caution should be applied to using green cone weight as a metric for informing yield.

Harvest index is likewise commonly used to evaluate crop yields and a number of QTL have been identified in various crop species [225-227]. Although heritability of cone harvest index has not been directly examined in hop, the heritability of another hop yield trait has been found to be high [79]. The high phenotypic variability for harvest index (Table 3.2) suggests that there should be potentially be enough power to detect QTL in this study. However, despite the high variability and potentially high heritability, no QTL, stable over the five year period, were detected for cone harvest index. This suggests that, unlike dry cone weight for which a strong QTL was detected, harvest index is influenced by multiple loci, each with small effects, which individually did not have high enough significance to be detected by this QTL analysis. Further investigations are required to elucidate the heritability and genetic basis to variation in this commercially important trait.

QTL for cone yields have been identified in a previous study in the Slovenian population [95], for both dry cone weight and cone harvest index. That QTL analysis, designed to maximally exploit QTL potential, was based on phenotypic measurements of single years (five years examined in total) and identified more than 30 QTL across the years. These QTL were found to be highly variable across the different years, probably due to seasonal variation. The present study had the goal of identifying QTL with significant effects detectable over the entire five-year experimental period. Using data averaged over five years, only a single QTL

was identified for dry cone weight that was stable across the five-year experimental period (Table 3.3; Figure 3.3). This QTL is adjacent to a marker (A-PAGAMCAA222F*l*) identified as a putative QTL for dry cone weight in a previous study of the Slovenian population [95]. This QTL, based on five-year average data, is less likely to be affected by environmental conditions or horticultural practice (reflected in annual variation) than other putative QTL identified in the previous study [95]. This QTL is an excellent candidate for MAS and warrants further investigation outside of the Slovenian growing region.

Hop essential oils are thought to be the primary contributing factors influencing the flavour and aroma of beer and as such, total essential oil content is an important yield trait. Particular essential oil profiles have historically been targeted in the genetic improvement of hop [228-229]. While it has long been understood that hop essential oil profiles have a genetic basis [230-233], the genetic control of total essential oil content has not been previously established in hop. QTL have been previously identified for only two individual essential oil components, caryophyllene and farnesene [88], but not for total essential oil content. This study is the first to report an underlying genetic basis to variation in the accumulation of essential oils in the hop cone, via the identification of a putative QTL for essential oil content (Table 3.3; Figure 3.2). The QTL identified segregated from the female parent of the New Zealand population, explaining a sizeable proportion (20.1%) of the phenotypic variation. The loci underlying variation at this QTL has great potential for hop MAS, in situations where particular levels of essential oil are the target of genetic improvement efforts. Validation outside the pedigree and experimental environment of New Zealand could provide the scope for definitive and heritable increases in yield of total essential oils.

Secondary metabolite traits

Although specific secondary metabolites constitute the commercially important hop commodity, our understanding of the genetic basis underlying their variation is in its infancy, with QTL identified for only eight traits related to the control of secondary metabolite content. In this study, we performed an extensive QTL analysis of hop secondary metabolites, investigating 45 hop secondary metabolite traits, 33 of which were found to have a significant genetic basis to their phenotypic variation. A total of 60 putative QTL were identified (Table 3.3; Figure 3.2). Between one and four QTL were identified for each of the 33 traits, varying in both their significance (LOD scores ranging between 2.3 and 39.8) and the proportion of phenotypic variance of the trait explained (average 14.9% $\pm$ 13.6 SD), suggesting that the

composition and concentration of secondary metabolites in hop is influenced by both Mendelian and quantitative inheritance. This is consistent with the genetic studies of secondary metabolites in other genera, such as *Mentha, Thymus* and *Eucalyptus* [234-235]. For example, the occurrence of a single highly significant QTL for individual compounds, such as cohumulone (expressed as percentage of α-acid) and colupulone (expressed as percentage of β-acid) (Table 3.3; Figure 3.2a), may be indicative of the influence of major loci with Mendelian inheritance; whereas a greater number of QTL of lesser significance were detected for other compounds, such as humulene, which is consistent with quantitative control (Table 3.3; Figure 3.2). No QTL were detected for 12 of the secondary metabolite traits. These secondary metabolite traits all contributed a very low percentage of the secondary metabolite profile and as such may have been subject to inaccuracies in quantification.

Two QTL were identified for α-acid content (New Zealand population) in this study, explaining 8.6% and 7.1%, respectively (Table 3.3; Figure 3.2). Although α-acid was also examined in the Slovenian population in this study, we were unable to detect these QTL for α-acid. There may be several reasons for this, including a lack of polymorphism in this population, variable loci effects in different genetic backgrounds (i.e. epistasis), instability of the QTL over varying ontogenetic stages or seasonal conditions, or confounding environmental influences. Evidence of environmental influence on the accumulation of α-acid in hop glandular trichomes has been found in a previous study of the Slovenian population [95], where QTL analysis based on phenotypic measurements of five single years identified 13 QTL for α-acid, but none of these QTL was identified in more than three years, possibly due to seasonal variation. This study re-examined QTL for α-acid in the Slovenian population, conducting QTL analysis on phenotypic data averaged over the five years, with the aim of identifying ontogenetically stable QTL. However, such a QTL was not identified. These results highlight how different ontogenetic stages and seasonal environmental conditions influence the identification of reliable and reproducible QTL and reinforce the requirement for further validation in alternate populations and different environmental conditions to improve our understanding of the genetic basis to variation in this important agronomic trait.

The QTL identified for α-acid in the New Zealand population in this study, as well as the QTL identified for other hop acids and polyphenols, may correspond to regulatory factors rather than genes encoding biosynthetic enzymes. One marker based on a candidate chalcone

synthase gene (*chs*_H1) encoding an enzyme involved in the biosynthesis of hop acids was mapped in the New Zealand population in this study, but none of the QTL identified were associated with this gene. Our findings were consistent with those of Cerenak et al. [95], who identified QTL for α-acid, but not associated with chalcone synthase genes. This observation may support the conclusions of Matoušek et al. [206, 236], that variation in regulatory factors rather than chalcone synthase genes may have a greater effect on variation in hop acids and polyphenols.

This study identified a sex bias in the inheritance of hop secondary metabolite phenotypes. A total of 13 QTL regions were identified in this study, influencing the 33 secondary metabolite traits. Each of these QTL regions were sex-specific, with 10 QTL regions associated with the female parent and three associated with the male parent (Table 3.4; Figure 3.2). This significant partiality towards inheritance from the female parent may be due to several reasons. Firstly, inheritance of the maternal phenotype may be due to dominance of the female parent at these loci; for each of the secondary metabolite traits, segregation may have occurred in the female parent while the corresponding locus in the male parent was homozygous recessive. Secondly, the bias towards maternal inheritance of secondary metabolite traits may be due to epigenetic effects, where inheritable modifications to the activation of genes have occurred to promote the natural selective advantage of a female parent with a favourable secondary metabolite profile; the maternal control over the secondary metabolite profile is maintained in the offspring, passing on the advantage [237-239]. Thirdly, the bias towards inheritance from the female parent may be due to artificial selection, where hop breeders have shown selection bias towards the commercially-relevant female plants. Artificial selection has not, however, been conducted as extensively in the male parents, which often perform as unknown pollinators (with open pollination) in the traditional crossing process [240]). Further research is required to understand the underlying genetic basis for this sex bias in inheritance of secondary metabolite variation.

Co-location of many of the putative QTL identified for secondary metabolites was a striking feature of this study. Thirteen distinct QTL regions were detected, across six linkage groups in the New Zealand population (Table 3.4). Of these 13 QTL regions, five displayed specificity for individual compounds (Table 3.4). As hop secondary metabolites are derived from pathways of terpene metabolism and involve common precursors [196, 201-205, 241], the specificity of the QTL identified for single compounds suggests that these QTL may affect

genes, transcription factors or enzymes involved in later stages of biosynthesis and modification of these compounds. Specific QTL for compounds arising from the same biosynthetic pathway have been identified amongst co-locating QTL in several other genetic analyses of secondary metabolites [234, 242]. The remaining eight QTL regions detected were found to affect multiple traits (Table 3.4). Although several strong correlations existed between many of the secondary metabolites in these seven QTL regions (Figure 3.1), in most cases there were no clear patterns amongst these correlations to coincide with the co-locating QTL or functional grouping of secondary metabolites (Appendix 3.6). Two of these QTL regions influenced compounds from all three groups of secondary metabolites (hop acids, essential oils and polyphenols), affecting three and 18 individual compounds, respectively (Table 3.4; Figure 3.2). The other four QTL regions had a less extensive influence, affecting two to eleven traits and only some of the secondary metabolite groups at one time (Table 3.4; Figure 3.2). Through comparisons between the New Zealand and Slovenian linkage maps, the QTL for dry cone weight identified in the Slovenian population was matched to one of the QTL regions on the New Zealand linkage map (QTL region 2 influencing the traits α-acid:β-acid, caryophyllene and xanthohumol; Table 3.4). Further research is required to elucidate the genetic basis of variation in these traits and the relationships between them.

There may be an underlying genetic basis for the co-location of QTL observed in this study, reflecting pleiotropic effects of single loci influencing multiple secondary metabolite compounds. Pleiotropy is consistent with the conclusion that all of the secondary metabolites of hop lupulin glands are derived from common precursors and pathways of terpene biosynthesis [196, 201-205, 241, 243]. Alternatively, the co-location of these QTL may be due to linkage between the loci associated with the secondary metabolite traits. Loci influencing secondary metabolites often exist in gene families; secondary metabolite diversity is thought to have arisen by gene duplications and consequently, the genes responsible for significant effects on variation in secondary metabolites are likely to be located very close together on the genome [244-245]. Duplication events in secondary metabolite genes, resulting in genetic linkage, have been found in a diversity of species, including *Vitis vinifera* [246], *Arabidopsis thaliana* [247], *Avena sativa* [248] and also hop [209, 249]. Therefore, the co-location of QTL identified in this study is likely to reflect the influence of both pleiotropic and linked loci, consistent with the findings of genetic studies of secondary metabolites in other taxa [234, 242, 250]. The detection of pleiotropy/linkage on the scale determined in this study would not have been possible without the simultaneous examination of an extensive

number of traits. Characterising the polymorphism and effects of pleiotropic/linked loci in diverse lineages of hop will be essential for effective application of markers linked to QTL in MAS.

The occurrence of pleiotropic or linked loci in the genetic control of secondary metabolites may have played an important ecological and evolutionary role in hop. The global hop population has been found to encompass limited levels of genetic diversity [4, 51, 124-125, 210]. Prior to artificial selection of hop, the existence of pleiotropic or linked loci may have provided an adaptive strategy, assisting in the selective adaptation of hop, as a defensive mechanism against pathogens, for example. Mutations in single genes from pleiotropic loci could affect the biosynthesis and profile of a large number of secondary metabolites, enabling a rapid diversification of secondary metabolite profiles and a broader defence response, compared to changes to single secondary metabolites by compound-specific genes. Alternatively, the occurrence of pleiotropic or linked loci may also be an artefact of selection during and since hop domestication. Artificial selection of hops for particular brewing characteristics and distinct chemical profiles may have resulted in the inheritable linkage of particular combinations of secondary metabolites. The effect of artificial selection on the genetic linkage of a number of different traits has been reported previously in a range of species [251-252].

The results obtained from these extensive QTL analyses have potentially significant implications for hop breeding. The patterns of QTL co-location observed in this study (Table 3.4) suggest that there are separate QTL regions influencing both early and late stages of secondary metabolite biosynthesis. The detection of QTL involved in the early stages of the biosynthetic pathways, either linked or with pleiotropic effects on numerous secondary metabolites, suggests that there is potential for rapid change in the levels of multiple compounds simultaneously; however, the use of these QTL in molecular hop breeding programs may have undesirable consequences. It may be difficult to select for specific secondary metabolites or combinations thereof, without causing a cascade of unpredictable changes to other secondary metabolites. Where the same QTL affects different secondary metabolites relating to opposing objectives, MAS is unlikely to succeed [185]. However, greater confidence can be placed in the specificity of the QTL identified in this study found to influence only a single trait (Table 3.4; Figure 3.2). Being compound-specific amongst a large number of secondary metabolites included in this study, these QTL may offer potential

to molecular breeding of hop, after validation in further pedigrees and a range of environmental conditions. All of the putative QTL identified present a resource to further our understanding of the genetic basis of variation in traits that influence hop quality (bitterness, flavour and aroma) in beer.

Conclusions

The QTL analyses conducted in this study revealed several important findings relating to the genetic basis of variation in three issues of relevance to hop breeding programs: expedited plant sex identification, increased yield capacity and improved secondary metabolite profiles, with important implications for the future use of molecular selection methods in hop. We verified a sex-linked marker in a third pedigree; and on the basis of its perfect association with the male sex in this and previous studies [95, 176] the HLAGA7 marker would be an effective tool for sex identification of hop plants, a key component of early stage selection in hop breeding programs. We identified an ontogenetically stable QTL for a trait associated with cone yield (dry cone weight). However, for two other metrics of cone yield (green cone weight and harvest index) currently used in routine screening of hop, no QTL were identified. The results for these traits highlight the difficulties of QTL detection for traits which may be controlled by many loci with small effects and for traits under a significant environmental influence. We identified QTL contributing towards explaining the observed phenotypic variation in secondary metabolite accumulation in hop cones through the identification of a QTL for essential oil content. We investigated a total of 45 secondary metabolite traits in this analysis and identified putative QTL affecting 33. The broad range of secondary metabolite traits included in this study provided the first demonstration of extensive pleiotropy/linkage affecting many of these compounds in hop, including many which are apparently unrelated. Pleiotropic/linked loci may present significant complications for molecular breeding, impeding the selection of specific traits without causing undesired alterations to others. In this study, we identified a number of QTL besides the pleiotropic/linked QTL that appeared to be specific to individual secondary metabolites. These QTL potentially offer a direct path to a locus influencing the phenotypic variation of specific secondary metabolites. The linkage maps constructed in this study incorporated a large number of new DArT markers. As DArT markers are transferable, these linkage maps can be employed in other mapping populations, facilitating the identification and validation of further QTL, a crucial step for the broad success of molecular breeding methods in hop. Furthermore, DArT markers can be sequenced to develop more informative co-dominant markers. This study greatly expands our

understanding of the complex genetic architecture underlying variation in hop secondary metabolite composition and yield related traits and presents a step forward in hop molecular breeding.

MATERIALS AND METHODS

Mapping populations

Two mapping populations were used in this study. Both were F_1 full-sib families. The first population (New Zealand) consisted of 178 genotypes derived from the cross 'Nugget' (female) x 'Slovenian breeding line (SBL) 3/3' (male) made in 2005. The population was placed in a randomised order, in rows spaced 2.5 m apart with 1 m between plants within each row. Plants were grown up a 5 m trellis, with 1 string per plant and 2 bines trained up each string. The mapping population was maintained by Plant & Food Research, Motueka, New Zealand. The second population (Slovenian) consisted of 89 individuals derived from the cross 'Hallertauer Magnum' (female) x 'SBL 2/1' (male) made in 1999. The population was planted in a randomised order, in rows spaced 2.4 m apart with 1.3 m between plants within each row. Plants were grown up a 6.5 m trellis, with 2 strings per plant and 3 bines trained up each string. The mapping population was maintained by the Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia. Both populations were treated with good agronomic practice, taking into consideration optimal fertilisation, irrigation and treatment against diseases and pests (based on prognosis).

Marker discovery and genotyping

DNA extraction

For the development and genotyping of DArT markers, DNA was extracted from the two mapping populations. For the Slovenian population, DNA extraction, as well as the estimation of DNA quality and concentration, was performed as described by Howard et al. [210]. For the New Zealand population, DNA was extracted as described by Buck et al. [173] and treated with RNase A (Life Technologies). DNA was quantified using the Quant-IT^{TM} Broad Range DNA Assay kit on a Qubit fluorometer (Life Technologies). DNA quality was verified by digestion with RsaI. DNA extractions and digests were run on a 1% agarose gel and stained with ethidium bromide for visualisation.

DArT marker discovery and genotyping

A first round of DArT marker discovery was conducted in a previous study, whereby 6,144 DArT clones were generated from 92 hop accessions sourced from Europe, Asia, North America and Australia [210]. From these DArT clones, 730 polymorphic markers were identified [210]. A second round of DArT marker discovery was conducted in this study to expand the array and incorporate hop material from New Zealand. DArT markers were developed and their performance evaluated, as described previously [210]. A total of 405 hop accessions were included in the analysis, sourced from New Zealand (186 individuals), Slovenia (93 individuals) and the USA (126 individuals).

A DArT microarray was constructed for the purpose of genotyping the two mapping populations used in this study (from New Zealand and Slovenia) and a third mapping population previously published (from the USA) [99]. The array was composed of markers from both the first and second rounds of markers discovery; only markers that were polymorphic within the mapping populations were included. The microarray was prepared and the two populations genotyped following the method previously described by Howard et al. [210]. DArT genotyping scoring parameters were used to assess marker quality; these parameters included *Q* value, call-rate, reproducibility and polymorphism information content (PIC), as described previously [210].

Additional markers for the New Zealand population

An additional 51 markers were used for linkage analysis of the New Zealand population in this study. This included: 43 selected microsatellite markers developed by Brady et al. [93], Jakse et al. [101], Bassil et al. [253], Hadonou et al. [97], Stajner et al. [111], Jakse et al. [90]; four RAPD based markers (Operon Technologies); three STS based markers developed by Danilova and Karlov [174]; and one intron-based DNA marker from the chalcone synthase gene *chs*_H1, produced using the CHSJ5 and CHSJ6 primers developed by Matoušek et al. 2002 [206].

Microsatellite markers were genotyped using either of two methods: independent amplification and visualisation on a CePRO 9600 TM (Combisep, Ames, IA, USA) capillary analysis system, or by undertaking amplification and high resolution melting (HRM) analysis using a Roche Light-Cycler®. Markers screened using the CePRO capillary system were initially amplified in a total volume of 15 µL containing 2 ng of DNA, 0.1 µM of each dNTPs,

1x PCR buffer (Invitrogen), 1.5 mM $MgCl_2$, 0.2 μM of each forward and reverse primer, 0.5 U Platinum *Taq*DNA polymerase (Invitrogen). Amplifications were performed in either a 9700 Geneamp Applied Biosystem or a Hybaid MBS 0.5G thermocycler. Initial denaturation at 94°C for 2 min and 30 s was followed by four cycles of 94°C for 30 s, 60°C for 1 min (reduced by 1°C per cycle), 72°C for 1 min, then followed by 30 cycles of 94°C for 30 s, 55°C for 1 min, 72°C for 1 min and a final 5 min 72°C extension. Products were desalted in 96- well microplate UNIFILTER (Whatman, Clifton, NJ, USA) using Sephadex G-75 Superfine (Amersham, Uppsala, Sweden) before analysis on the CePRO capillary system. The alternative HRM genotyping method [254] utilised a 96-well Roche Light-Cycler® 480 (Forester City, CA, USA). Amplification reactions contained 2 ng DNA, 1x Roche master mix, 2.5 mM $MgCl_2$ and 0.2 μM of each forward and reverse primer in a 10 μL total volume. These were subject to an initial denaturation step at 95°C for 5 min, followed by four cycles of 95°C for 10 s, 60°C for 30 s (reduced by 1°C per cycle) and 72°C for 15 s; and then 30 cycles of 95°C for 10 s, 55°C for 30 s and 72°C for 15 s. These reactions then underwent the HRM step of 95°C for 1 min (ramp rate 4.4°C/s) with an increase to 65°C (ramp rate 1°C/s) with 25 data acquisitions/°C for 20 min. The melting curves were then analysed using the gene scanning module of the Roche Light-Cycler® 480 collection and analysis software.

RAPD and STS markers were screened following the method outlined for RAPD markers by Buck et al. [255]. Only clear products were scored (fragment size in base pairs is indicated after primer name on linkage map). The chalcone synthase gene based marker (*chs*_H1) was genotyped using the HRM analysis on the Roche Light-Cycler® 480, as outlined above.

Additional AFLP and microsatellite markers for the Slovenian population.

An additional 241 AFLP markers and 44 microsatellite markers were used for linkage analysis of the Slovenian population in this study. Also included in this study were five markers based on microsatellites within candidate chalcone synthase genes (*vps*, *chs*_H1, *chs*2, *chs*3 and *chs*4), which encode enzymes directly involved in the biosynthesis of bitter acids [206-208]. These AFLP markers, microsatellite markers and candidate genes have been scored and mapped previously in the Slovenian population [94-95].

Linkage analysis

A highly stringent linkage analysis method was conducted, using the double pseudo-testcross strategy [256], as in other linkage analyses of hop [88, 95, 99, 114]. This was deemed an

appropriate strategy, as hop typically displays a high level of heterozygosity [6], and being dioecious, it is the best alternative to a backcross. It is also compatible with DArT markers as they are a dominant marker system [257]. Map construction was carried out using the JoinMap® 4 program [258]. All markers were re-coded by their segregation type according to the cross-pollinated coding scheme (CP) for analysis. Markers were tested for goodness of fit to their assigned Mendelian segregation ratios using the χ^2 segregation test in JoinMap® 4 [258]. Those markers with significant amounts of segregation distortion (departure from expected Mendelian segregation ratios ($\alpha \geq 0.05$)) are indicated with '*' at the end of the locus name (Appendices 1 and 2). Marker type is indicated for each locus at the beginning of the locus name as either 'D-' (DArT markers), 'A-' (AFLP markers) or 'S-' (other marker types) (Appendices 1 and 2). For each population, markers with very low polymorphism (those markers for which one allele was represented by $\leq$ 10% of the expected scores) and markers with high levels of missing data ($\geq$ 5% of the scores) were eliminated from the analysis. Individuals with high levels of missing data ($\geq$ 10% of the scores) were also eliminated from the analysis.

Separate maternal and paternal linkage maps were constructed from each of the mapping populations, based on the methods described by Keats et al. [259]. Using JoinMap® 4 [258], linkage maps were constructed by grouping significantly associated (linked) markers, statistically estimated through a logarithm (base 10) of odds (LOD) score. Establishing linkage group associations, through the selection of LOD scores is an intuitive process; the theoretical basis for the selection of LOD scores is discussed by Freeman et al. [260]. In this study, linkage groups were generally assigned with a minimum LOD threshold of 4.0, at which the contents of most groups were relatively stable. In unstable groups it was necessary to adjust the LOD threshold to achieve stability. A higher LOD was selected when a linkage group consisted of weakly linked sub-groups, which were eliminated in the process of achieving a stable marker order. The higher LOD threshold allowed the preservation of subgroups, within which there was significant association. A lower LOD was selected when a single marker dropped out of the linkage group at LOD 4.0, in order to maintain as many markers in the analysis as possible.

Within linkage groups, the optimal marker order was determined using JoinMap® 4 [258] default values of a minimum LOD threshold of 2.0, a maximum recombination threshold of 0.35, a maximum χ^2 goodness-of-fit jump threshold of 5.0 for removal of markers and a ripple

value of 1.0. The Kosambi mapping function was used to determine the distance between markers. The linkage phase of markers was determined automatically by the JoinMap® 4 program.

Linkage maps were constructed over several stages. The first stage involved the establishment of a framework map with a reliable marker order, upon which all subsequent analysis was based. This initial analysis was conducted with the highest quality markers, those segregating in a 1:1 ratio that did not show significant segregation distortion. If necessary, markers were removed from the analysis until maps were achieved within two mapping rounds, and all markers had a mean χ^2 contribution of ≤ 2.0. Markers were removed one at a time, in order of highest mean χ^2 contribution. Four subsequent stages of analysis were conducted, adding markers to the framework map in the following order of decreasing marker quality: (i) markers segregating in a 1:1 ratio with evidence of segregation distortion; (ii) markers segregating in a 3:1 ratio without evidence of segregation distortion; (iii) markers segregating in a 3:1 ratio with evidence of segregation distortion; and (iv) markers for which the genotype score of one parent was unknown and consequently estimated. At each of these stages of analysis, markers were removed as before, to achieve maps within two mapping rounds and to ensure that all markers had a mean χ^2 contribution of ≤ 2.0. Markers that contributed to the framework map were not removed and their established marker order was maintained. Markers that instigated a re-ordering of the framework markers were removed. With each subsequent round, markers added to the map in the previous round were not removed and their marker order was maintained. Iterative approaches of adding markers to a framework map, akin to the method used in this analysis, are commonly employed [261-264].

The numbering of linkage groups in all maps followed the numbering established in a previous linkage map of the Slovenian population [95]. Homology between linkage groups was inferred on the basis of shared markers. Where a linkage group was homologous with several linkage groups from the previous linkage map [95], the linkage between these groups was verified at a lower LOD threshold in JoinMap® 4; and the lowest number of the corresponding linkage groups from the previous linkage map of the Slovenian population was assigned. Linkage groups consisting entirely of the newly added markers were assigned the remaining numbers.

Significant clustering of the markers was observed in all maps constructed in this study. For the purpose of QTL analysis, clusters of markers were removed to leave only one marker at each locus (taken as the map position to one decimal place). At the completion of analysis, when all possible markers had been added to the map and the final marker order had been accepted, markers within each cluster were eliminated on the basis of high levels of missing data and then by lower *Q* values (a DArT quality measure). This resulted in between 37 and 73% of the polymorphic markers being removed from the maps.

Phenotypic measurements

Fifty traits were assessed in hop in this study, related to three issues relevant to the genetic improvement of hop: expediting plant sex identification, increasing yield capacity and improving secondary metabolite composition.

Sex trait

Sex was assessed as a binary character by field observation, as either plants bearing male flowers ("0") or plants bearing female flowers ("1"). Sex of the plants was confirmed over six seasons in the New Zealand population and for at least two years in the Slovenian population.

Yield traits

In this study, four yield traits were examined which quantify either the physical yield of cones per plant or the yield of brewing-relevant substance. Three traits assessed cone yield: (i) cone harvest index, a measure of the ratio of fresh or 'green' cone weight to the whole plant fresh weight, comparing the allocation of biomass to cone production with the allocation of biomass to vegetative growth; (ii) dry cone weight, a measure of the mass of cones per plant, after the removal of ~95% of the moisture content (leaving a moisture content of 9% by weight of the kiln-dried hop), reflecting the productive vigour of the plant; and (iii) green cone weight, also a measure of the mass of cones per plant, but of the fresh or 'green' weight, without consideration of cone moisture content. Cone harvest index and dry cone weight were assessed in the Slovenian population with the aim of identifying ontogenetically stable QTL. Phenotypic measurements were made on every plant in the trial annually over five years, between 2002 and 2006 and the arithmetic mean was calculated from these measurements to give the data used in this analysis. Dry cone weight was quantified according to the EBC 7.2 method for moisture content of hops and hop products [265], as

described by Cerenak et al. [95]; cone harvest index was also quantified as described by Cerenak et al. [95]. Green cone weight was assessed in the New Zealand population with the aim of identifying putative QTL. Phenotypic measurements were made in one year, in 2009. The fourth yield trait examined was essential oil content, a measure of the total volume of essential oil secondary metabolites. This trait was examined to determine whether variation in the accumulation of essential oil in hop glandular trichomes has a genetic basis. Essential oil content was measured in the New Zealand population, quantified by steam distillation (see below). Phenotypic measurements were made in one year, in 2009.

The relationship between the two yield traits scored in the Slovenian population (dry cone weight and cone harvest index) and the secondary metabolite trait α-acid (see below) was examined by principal components analysis using the PRINCOMP function in R version 2.11.1 [266]. The first and second vectors accounted for 30% and 10% of the variance, respectively. A correlation matrix was produced, based on Pearson's product moment correlation coefficients using the COR function (method = "PEARSON", use = "COMPLETE") in R version 2.11.1 [266] (n = 3). For the purposes of this investigation, a Pearson's r value in the range of |0.5| to |0.79| was considered a strong correlation, with |0.8| to |1.0| considered a very strong correlation [267].

Secondary metabolite traits

A total of 45 hop cone secondary metabolite traits were assessed in this study (see Appendix 3.7 for International Union of Pure and Applied Chemistry (IUPAC) names of chemical compounds), from all significant hop secondary metabolite groups (hop acids, essential oils and polyphenols). All secondary metabolite traits were assessed in the New Zealand population, with α-acid also assessed in the Slovenian population. In the New Zealand population, phenotypic measurements were made in one year, in 2009, with the aim of identifying putative QTL. In the Slovenian population, the aim was to identify environmentally and ontogenetically stable QTL and as such, phenotypic measurements were made annually over five years, between 2002 and 2006 and the data averaged. With the exception of α-acid, none of the secondary metabolite traits have been previously assessed in either the New Zealand or Slovenian populations. Hop acids comprise both α- and β-acids; a total of nine traits relating to hop acids were quantified in this study: (i) α-acid content; (ii) β-acid content; (iii) the ratio of α-acid to β-acid; (iv) the percentage of α-acid that is cohumulone (a major constituent of α-acid); (v) the percentage of β-acid that is colupulone (a

major constituent of β-acid); (vi) cohumulone content; (vii) colupulone content; (viii) humulone + adhumulone (the other major constituents of α-acid) content; and (ix) lupulone + adlupulone (the other major constituents of the β-acid) content. Essential oils comprise oxygenated compounds (esters, ketones, ethers, monoterpene alcohols and sesquiterpene alcohols) and terpenoid compounds (monoterpenes and sesquiterpenes). A total of 33 essential oil compounds were assessed in this study; these were five esters (methyl-4-methylhex-2-enoate, methyl dec-4-enoate, methyl decanoate geranyl acetate and geranyl isobutyrate), one ketone (2undecanone), four ethers (humulene diepoxide a, humulene epoxide I, II and III), three monoterpene alcohols (geraniol, linalool and limonene-10-ol), four sesquiterpene alcohols (caryolan-1-ol, humulenol II, humulol and t-cadinol), one alkane (tetradecane), six monoterpenes (β-pinene, camphene, limonene, myrcene, ρ-cymene, terpinene) and 10 sesquiterpenes (α-capaene, α-selinene, β-selinene, δ-cadinene, γ-cadinene, caryophyllene oxide, caryophyllene, farnesene, humulene, muurolene). The ratio of humulene to caryophyllene was also assessed, as it is a reliable maturity indicator [6] and is often used for varietal characterisation. One polyphenol, xanthohumol, was scored in this study.

The relationships among the 45 hop secondary metabolite traits assessed in the New Zealand population were examined using principal components analysis. The first and second vectors accounted for 55% and 26% of the total variance, respectively. A correlation matrix was produced, as described above (n = 47).

The hop acid and polyphenol components of the cone secondary metabolite profile of the New Zealand population were analysed by HPLC. Extracts were prepared in 2009, by grinding 10 g hop cone tissue with 100 mL toluene using an Omni Macro ES homogeniser (Omni International, Marietta, GA) then filtered. A volume of 3 ml of the filtrate was added to 47 ml methanol and inverted four times. The extracts were fractioned by HPLC, on a system consisting of a Shimadzu LC 6A/LC 10AS pump, a Shimadzu SIL-10AF autosampler (10 μL sample loop) and a UV/UV-Vis Shimadzu SPD 10A detector at a wavelength of 314 nm. A Kinetix reversed-phase C18 column (100 x 4.6 mm; 2.6μm particle size) was used (Phenomenex, Torrance, CA, USA), heated to 30°C with a Shimadzu CTO 10A column oven. The mobile phase used for separation was a methanol-water-phosphoric acid mixture (in a ratio of 85:17:0.25 V/V/V), at a flow rate of 1.2 ml/min, for 16 min. The sample volume injected was 10 μL. A Shimadzu LC Solution software package was used for quantification.

Standardised hop extract (ICE-3) with known content of α- and β-acids and xanthohumol were injected for identification and quantitative analysis, and their retention times and spectra compared. Five components (xanthohumol, cohumulone, humulone + adhumulone, colupulone, lupulone + adlupulone) were identified and quantified, with other traits derived by calculation from these five components (α-acid = cohumulone + (humulone + adhumulone); β-acid = colupulone + (lupulone + adlupulone); percentage of α-acid that is cohumulone = cohumulone/α-acid; percentage of β-acid that is colupulone = colupulone/β-acid; ratio of α-acid to β-acid = α-acid/β-acid).

The essential oil content of harvested cones from the New Zealand population was estimated by steam distillation, following the EBC 7.10 method for hop oil content of hops and hop products [265]; and the individual essential oil components of the cone secondary metabolite profile were analysed by GCFID on a Shimadzu GC-2010 system fitted with an AOC20i autosampler. Essential oil extracts were prepared in 2009 by steam-distillation of 100 g of ground hop cone tissue. A volume of 100 μL of essential oil was added to 1 ml of double distilled diethyl ether for GC analysis. The extracts were fractioned by GCFID, using Shimadzu GC Solution software. Each of the 33 essential oil components targeted and quantified in this analysis were expressed as the percentage of their peak area to the total area of all essential oil peaks eluted. The ratio of humulene to caryophyllene was additionally calculated.

The hop acid trait α-acid was also measured in the Slovenian population, analysed by the lead conductance value (LCV) measure, following the EBC 7.4 method for LCV of hops, powders and pellets [265], as described by Cerenak et al. [95]. Although obtained through different extraction and quantification methods, α-acid content as assessed in the Slovenian population is analogous to α-acid content as assessed in the New Zealand population, allowing direct comparison of this trait across the two separate experiments.

QTL analysis

QTL analysis was conducted using the linkage maps constructed in this study. MapQTL® 6 [268] was used for this analysis. Putative QTL were declared at the genome-wide significance level ($\alpha < 0.05$). The LOD threshold for genome-wide significance was estimated by permutation testing with 10000 iterations [269]. This method determines the LOD threshold for each phenotypic trait separately and, unlike other empirical methods,

makes no assumptions regarding probability distribution [269]. Interval mapping (IM) was conducted, using the regression algorithm and the default MapQTL® 6 parameters [268], to scan the genome for map intervals significantly associated with traits. Where map intervals exceeded the genome-wide LOD threshold, single markers with the highest LOD value were selected as cofactors for multiple QTL model (MQM) mapping. MQM mapping was performed using an iterative approach with the forward selection of cofactors until a stable set of cofactors was established.

Due to the high proportion of dominant markers (in linkage groups where markers are segregating from one parent only), MapQTL® 6 [268] was unable to reach a unique solution to the probability of the QTL genotype due to the existence of more than one solution to the set of mathematical equations, as described by Van Ooijen [268]. To overcome this problem, the two-way pseudo-testcross analysis was undertaken, whereby the marker data was separated into the two meioses (markers segregating from respective parents only) and recoded from the CP population type to the doubled haploid population type (DH), as described by Van Ooijen [268]. IM and MQM then proceeded again, as described above.

Identified QTL were confirmed with single marker non-parametric Kruskal-Wallis (KW) testing ($P < 0.05$). KW testing is a particularly robust calculation in cases where the distribution of a trait departs from normality [268]. KW testing was also used to determine whether the QTL was segregating from the male or female parent.

Male and female maps for each population were drawn using MapChart® 2.2 [270]. The QTL identified were indicated with solid bars representing 1-LOD support intervals and lines representing a 2-LOD support intervals. The 2-LOD support interval corresponds to an ~95% confidence interval [271].

CHAPTER 4

Quantitative genetic parameters for yield, plant growth and cone chemical traits in hop (*Humulus lupulus* L.)

ABSTRACT

Most of the traits targeted in the genetic improvement of hop are quantitative in nature. Improvement based on selection of quantitative traits requires a comprehensive understanding of their inheritance. This study estimated quantitative genetic parameters for 20 traits related to three key objectives for the genetic improvement of hop: cone chemistry, cone yield and agronomic characteristics. Significant heritable genetic variation was identified for α-acid and β-acid, as well as their components and relative proportions. The estimates of narrow-sense heritability for these traits (h^2 = 0.15 to 0.29) were lower than those reported in previous studies of hop, but were based on a broader suite of families (108 from European, North American and hybrid origins). Narrow-sense heritabilities are reported for hop growth traits for the first time (h^2 = 0.04 to 0.20), relating to important agronomic characteristics such as emergence, height and lateral morphology. Cone chemistry and growth traits were significantly genetically correlated, such that families with more vigorous vegetative growth were associated with lower α-acid and β-acid levels. This trend may reflect the underlying population structure of founder genotypes (European and North American origins) as well as past selection in the Australian environment. Factors besides additive genetic effects were found to influence both trait variation and correlations between traits; this has implications for attempts to achieve genetic gain in hop, particularly where selection involves multiple traits. Although male and female hop plants are thought to be indistinguishable until flowering, sex was found to influence variation in many growth traits, with male and female plants displaying differences in vegetative morphology from emergence to cone maturity. This study reveals important insights into the genetic control of quantitative hop traits, information which will be useful for the selective improvement of hop.

INTRODUCTION

In the development of new crop cultivars, breeders are confronted with choosing among many potential selection criteria. In hop (*Humulus lupulus* L.) these criteria include yield per hectare, agronomic suitability (which is based on morphological characteristics of the plant) and brewing quality (which is primarily based on the chemical characteristics of the cone). Making genetic improvements to these criteria is complex as many of the traits relevant to them are quantitative characters, likely controlled by a large number of genes, each with small effects. For these traits, it is generally impossible to determine the specific genotype (or breeding value) of an individual simply by assessing its phenotype [46]. Phenotypic assessments also provide no indication of how much variation in the trait is the result of environmental influences [46].

Quantitative genetics is the study of the effect that genetics and the environment have on phenotypic variation, and provides extensive information on the inheritance of traits [49, 72]. The basis of quantitative genetics is in statistical models, where the relative influences of genetic and environmental factors on traits are estimated from the phenotypic resemblance between relatives, usually in clonal or progeny trials [49, 72]. Quantitative genetic analysis can inform breeders as to the amount of heritable genetic variation in traits available for selection [48]. It can also provide an understanding of the genetic relationships between traits that both directly and indirectly affect the phenotype, indicating genetic correlations [49, 72]. Understanding genetic correlations can be used to identify potential proxy selection indicators where it is difficult or expensive to measure traits directly, or to avoid potentially unfavourable consequences that would arise from the selection of seemingly unrelated traits [73]. Quantitative genetic analysis can determine the degree to which environmental factors influence trait variation and the correlations between traits [46]. This knowledge is essential for the accurate prediction of genetic gains and the development of breeding strategies, as well as to inform growers how a crop can be managed more efficiently through the control of environmental factors [48]. In hop, which is dioecious [10-11], quantitative genetic analysis of progeny trials has the added benefit of providing a means of assessing the genetic potential of male plants for traits expressed only in female plants. These traits include those relating to the yield and the quality of the commercially important hop cones. The information gained from quantitative genetic analysis can simplify the hop breeding process, improving estimates of the genetic gains that can be anticipated through selection methods as well as assisting with

the choice of breeding parents and the development of clearly defined aims for hop improvement.

Hop is one of four essential ingredients of beer (the others being water, yeast and a carbohydrate source such as barley or wheat), added to provide bitterness, flavour and aroma as well as functioning as a natural preservative [17]. Female hop plants develop strobili (commonly called cones), which contain numerous glandular trichomes (lupulin glands) on their bracts [10, 12-13]. The lupulin glands contain many secondary metabolites, including resins, essential oils and tannins [6]. The resins found in hop lupulin glands have not been found in any other plant species [6]; they comprise hard resins (including xanthohumol, isoxanthohumol and flavones) and soft resins (also called hop acids), which are dominated by humulones (α-acids) and lupulones (β-acids) [195, 199]. It is the α-acids that provide the bitter taste to beer [6]. B-acids also contribute to beer bitterness, as well as providing preservative activity [6, 18-20]. The flavour and aroma of beer is derived from the hop essential oils, the composition of which is diverse (with more than 500 different compounds identified), but typically consisting of 90% terpenoids, dominated by myrcene, humulene, caryophyllene and farnesene [6, 15, 195, 199-200]. Hop cultivars differ in their secondary metabolite profiles, in terms of the presence, amount and relative proportions of these compounds. As such, different hop cultivars produce different levels of bitterness and a variety of flavours and aromas [23-24, 272]. Hop plants are perennial, wind-pollinated climbers, cultivated on strings suspended from a trellis [6]. Flowering is induced by shortening day length, after the plant has grown a minimum number of nodes [8-9]. Flowers develop at the terminal buds of lateral branches; female flowers develop into cones, which mature at the beginning of autumn [8, 10]. The vegetative parts of the plant die back each year; the underground rootstock remains dormant over winter and re-sprouts in spring [6]. Hops have a native distribution between latitudes of approximately 35° and 70° North, from Western Europe, east to Siberia and Japan and across North America, except in highlands and deserts [2-3], but many hop cultivars are of European genetic origin, or are hybrids between European and North American germplasm [6, 36].

Since the 1950s, several studies have examined the inheritance of quantitative traits in hop [50, 74-87]. Both clonal and progeny trials have been used to examine the heritability of traits relating to yield, including yield of cones (green or dry mass) per hectare and number of cones per plant [74, 77-81, 84]; cone chemistry and brewing quality, including α-acid, β-acid, their

components and their relative proportions, as well as several essential oils [74-76, 78-83, 85-87, 273]; and agronomic attributes, including hop storage index, morphology of cones, leaves, lateral and lupulin glands, vigour, flowering and cone maturity times and disease susceptibility [50, 75-78, 80-81, 84-87]. These studies have documented a wide range of heritability estimates and variable genetic relationships between traits, and have generally found that hop cone chemistry, yield and plant morphology traits have a genetic basis. Many of the earliest of these studies estimated the inheritance of traits on the basis of phenotypic observation of the transmission of traits from parent to offspring, using little statistical analysis [76, 81-82, 85-86, 273]. As such, these studies were unable to make full use of the information to separate genetic and environmental influences and therefore may be less reliable. Of those studies based on more sophisticated statistical procedures, the majority report broad-sense heritability and describe correlations on the basis of the total genetic variation [50, 74-75, 80, 84, 87]. Although the estimation of broad-sense heritability is able to discern between variation resulting from genotypic and environmental factors, it does not partition the genetic factor into additive, dominance and epistatic components [49, 72]. The additive genetic component, which is based on the average effects of alleles, is the easiest type of genetic effect to predict and use in breeding [49, 72]. As such, it is the only portion of genetic variation that is relevant to selection in current hop breeding programs [274].

Four studies have examined additive genetic variation in hop traits and have reported estimates of narrow-sense heritability (based only on additive genetic variation) [77-79, 83]. These studies have examined 13 traits: five relating to hop acids (α-acid, β-acid, α-acid:β-acid, cohumulone and colupulone), five relating to essential oils (essential oil content, myrcene, β-caryophyllene, farnesene and humulene:β-caryophyllene) one relating to polyphenols (xanthohumol), one relating to yield (yield of dry cones) and one relating to agronomic attributes (hop storage index) [77-79, 83]. While these studies provide information for selection of cone chemistry, hop storage indes and yield, the inheritance of plant growth and agronomic suitability, as well as the relationship of these factors to cone chemistry and yield, has not been examined. These four studies report heritability estimates and genetic correlations that are derived from more accurate methods of calculation, but they are based on progeny trials consisting of too few families (12-25) [77-79, 83] for the accurate estimation of quantitative genetic parameters [72, 275]. Additionally, the families examined in these four studies were derived from a narrow genetic base, using parents of primarily European genetic origin [72, 275]. As such, these results have to be treated carefully. Further heritability

estimates and genetic correlations, from quantitative genetic analyses that includes a broader range of material and larger trials and those previously conducted, would expand our current understanding of the inheritance and genetic control of traits relating to cone chemistry, yield and agronomic characteristics in hop.

QTL have been identified for a number of traits relating to hop cone chemistry and yield, including α-acid and β-acid, as well as their components and relative proportions; total essential oil content and a number of individual essential oils; the polyphenols xanthohumol and desmethylxanthohumol; yield of dry cones; cone harvest index; and powdery mildew susceptibility [88, 95, 99, 110, 117, 276]. These QTL indicate that variation in these traits has a genetic basis; but as many of these QTL have been identified in a single pedigree, environment and ontogenetic stage, a quantitative genetic analysis could offer insight into the degree of heritability of these traits in a broader range of hop material. Many of the QTL that have been identified for hop traits have been found to co-locate [276]. A quantitative genetic analysis could provide additional information about genetic correlations between traits, furthering the understanding of the genetic control of hop and providing important information for selective improvement of hop.

This study reports estimates of quantitative genetic parameters for 20 commercially important hop traits. Traits were selected on the basis of their relevance to hop breeding programs, and included α-acid and β-acid, two key brewing chemicals that impart the bitter taste and preservative activity to beer [6, 18-20], as well as their components and relative proportions. Ten plant growth traits relating to agronomic features of the hop plant were evaluated, including traits related to emergence, height, lateral morphology and cone distribution. These agronomic traits are important for the cultivation of the hop plant, as well as being possible proxy selection indicators for chemical traits, where a correlation occurs. Yield of hop cones was evaluated by the weight of green cones per plant. The quantitative genetic parameters that were assessed included additive genetic variance and narrow-sense heritability, as well as the genetic correlations between traits and the degree to which variation and correlation of traits was affected by factors other than additive genetic effects (including the environment, agricultural practice, dominance, epistasis and error). Calculations of genetic parameters were based on a progeny trial consisting of the largest number of families (108) utilised for this purpose in hop, and families were derived from a broad genetic base of genotypes from European and North American genetic origins, as well as hybrids between the two. This

study aims to increase our understanding of the inheritance of quantitative traits in hop as well as the genetic relationships between traits and the influence that elements besides additive genetic effects have on these factors. Such results would provide hop breeders with important information to assist selection and genetic gain in key traits, and would be of use in the planning of breeding programs for the development of superior hop cultivars.

MATERIALS AND METHODS

Field trial

The genetic control of hop cone chemistry, cone yield and agronomic characteristics were investigated using a field trial at Bushy Park, Tasmania (42°42′33″S 146°53′54″E). The trial consisted of open pollinated seedlings from 160 female parents that included commercial cultivars and breeding lines from Australia, Europe and the USA. The female parents were pollinated in January 2008 and the seedlots collected in March 2008. The seeds were subject to stratification in July 2008 and were germinated in September 2008. The trial, planted in December 2008, was established in a randomised incomplete block design [277], comprising five replicates of 16 incomplete blocks. Each incomplete block contained 10 families in two-plant contiguous plots, giving a total of 10 plants per family. Plants were grown in rows spaced 2.8 m apart and with 0.9 m between plants within each row (a planting density of ~3940 plants per hectare). Plants were grown up a 6 m trellis, with one string per plant and three bines trained up each string. Routine agricultural practice for hop in Australia was applied to the trial, including standard fertilisation, overhead irrigation and bine training by hand.

Families in the trial displaying evidence of monoecy (assessed by field observation) or polyploidy (assessed by pedigree record) were excluded from analysis of genetic control. Analysis proceeded on the basis of 1049 individuals from 108 families (Appendix 4.1). A pedigree of the female parents and their ancestors, to founders where possible, was constructed using records from California Fermentation Society [278], Freshops USDA named hop variety descriptions [279], Haunold et al. [280], Homer et al. [281], Jakše et al. [51], Kenny and Zimmermann [282], Miyata [283], Neve and Darby [284], Patzak [285], Reed [286], Roborgh [287], Simply Hops [288], The Germplasm Resources Information Network (GRIN) [289], Zimmermann et al. [290] and Hop Products Australia.

Trait measurements

Twenty traits were assessed in this study, including ten plant growth traits related to agronomic suitability (three associated with emergence and seven with vegetative morphology), nine traits evaluating cone chemistry and one trait assessing cone yield (Table 4.1). Plant growth traits and cone chemical traits were assessed in the trial over two cultivation seasons, while cone yield was assessed in one season (Table 4.1). Where necessary, power transformations were used to standardise the variance of traits (Table 4.1).

Table 4.1 Plant growth, yield and cone chemistry traits assessed over two seasons of the hop cultivation process. '*n* individuals' refers to the number of individuals assessed for each trait. '*n* families' refers to the number of families assessed for each trait. 'Age' refers to the age of the plants at the time that a trait was assessed in number of months after the trial was planted. 'Transformation' refers to the power transformation used to standardise the variance of each trait (x). 'Mean' refers to the backtransformed mean of all assessed individuals for each trait.

	Trait	Description	*n* individuals	*n* families	Age	Transformation	Mean
plant growth	number of shoots	Number of shoots at emergence	1049	108	11 months	$x^{0.5}$	5.51
			1049	108	24 months	None	7.42
	length of the longest shoot	Length (cm) of the longest shoot at emergence	965	108	11 months	$x^{0.5}$	51.41
			823	108	24 months	$x^{0.5}$	11.67
	number of nodes on the longest shoot	Number of nodes on the shoot of maximal length	965	108	11 months	None	7.11
			823	108	24 months	$x^{0.5}$	2.99
	height (at flower initiation)	Height of the bine from the ground to the top of the 6 m trellis (m), assessed at flower initiation	1046	108	13 months	None	3.68
			1047	108	25 months	None	5.10
	height (mid-season)	Height of the bine from the ground to the top of the 6 m trellis (m), assessed between flowering and cone maturity	976	108	14 months	None	4.45
			1047	108	26 months	None	5.68
	height (at cone maturity)	Height of the bine from the ground to the top of the 6 m trellis (m), assessed at cone maturity	1042	108	16 months	None	4.53
			1039	108	28 months	None	4.54
	lateral length	Length of a lateral at shoulder height (cm)	1012	108	16 months	$x^{0.5}$	48.22
			982	108	28 months	$x^{0.5}$	44.81
	number of nodes on lateral	Number of nodes on the same lateral measured for lateral length	1006	108	16 months	$x^{0.5}$	6.55
			1007	108	28 months	$x^{0.5}$	6.56
	internode length	Internode length on the same lateral measured for lateral length. The third internode from the main bine was measured (cm)	231	78	16 months	$x^{0.5}$	17.41
			339	96	28 months	$x^{0.5}$	26.86
	height to the cones	Height of the bine from the ground up to where the bulk of the cones began (metres)	663	108	16 months	None	1.74
			588	108	28 months	None	1.75

Trait		Description	*n* individuals	*n* families	Age	Transformation	Mean
yield	green cone weight	Fresh weight of cones per plant (g)	204	107	28 months	$x^{0.5}$	1.02
cone chemistry	cohumulone	Cohumulone (% dry weight)	397	108	16 months	$x^{0.5}$	2.71
			208	107	28 months	$x^{0.5}$	2.50
	humulone + adhumulone	Humulone + adhumulone (% dry weight)	397	108	16 months	$x^{0.5}$	6.41
			208	107	28 months	$x^{0.5}$	5.34
	colupulone	Colupulone (% dry weight)	397	108	16 months	$x^{0.5}$	2.31
			208	107	28 months	$x^{0.5}$	2.51
	lupulone + adlupulone	Lupulone + adlupulone (% dry weight)	397	108	16 months	$x^{0.5}$	2.16
			208	107	28 months	$x^{0.5}$	2.17
	α-acid	Percentage of total resin that is α-acid [cohumulone + (humulone + adhumulone)] (% dry weight)	397	108	16 months	None	9.12
			208	107	28 months	$x^{0.5}$	7.84
	β-acid	Percentage of total resin that is β-acid [colupulone + (lupulone + adlupulone)] (% dry weight)	397	108	16 months	$x^{0.5}$	4.48
			208	107	28 months	$x^{0.5}$	4.69
	cohumulone (% of α-acid)	Percentage of α-acid that is cohumulone [cohumulone / (cohumulone + humulone + adhumulone)] (% dry weight)	397	108	16 months	$x^{0.5}$	0.30
			208	107	28 months	$x^{0.5}$	0.32
	α-acid:β-acid	Ratio of α-acid to β-acid [(cohumulone + humulone +adhumulone) / (adlupulone + lupulone + adlupulone)] ratio	397	108	16 months	$x^{0.5}$	2.21
			208	107	28 months	$x^{0.74}$	1.80
	α-acid:total resin	Ratio of α-acid to total resin [(cohumulone + humulone + adhumulone) / (cohumulone + humulone + adhumulone + colupulone + lupulone + adlupulone)] ratio	397	108	16 months	$x^{0.5}$	0.67
			208	107	28 months	$x^{0.5}$	0.62

Cone samples for chemical analysis were collected at several days post commercial maturity of the majority of the trial, as hop chemistry is more stable after maturity is reached than before [197]. Hop samples were dried for eight to 12 hours at 55°C. For each plant, hop cone chemical extracts were prepared by grinding 10 g of dried hop cone tissue using a domestic coffee grinder. A quantity of 2 g of the ground tissue was then extracted with 20 ml toluene in a 30 ml glass vial with 3 x 6 mm stainless steel ball-bearings using a rotator at 75 rpm for 30 min. The samples were allowed to stand for 10 min; then diluted 1:20 with an 85% methanol solution. An 800 µL aliquot of the dilution was placed in a 1 ml HPLC vial using a two syringe (2500 µL and 250 µL) Hamilton 500 series microdiluter. Diluted samples were vortexed for 3 sec before placing into a Waters 717 autosampler. Hop acids were fractionated by HPLC, on a system consisting of a Waters 1515 pump and column heater (29°C), a Waters 717 autosampler and a Waters 2996 UV/UV-VIS photodiode array detector at wavelengths of 325 nm (α-acids) and 342 nm (β-acids). A Varian ChromSpher reversed-phase C18 column (100 x 4.6 mm; 3 µm particle size) was used, coupled with a Varian 10 x 2 mm ChromSep guard column. Column temperature was maintained at 28°C. The mobile phase used for separation comprised 86% methanol (containing 0.1 g/L dissolved tetra-sodium EDTA) and 14% 0.05 M sulphuric acid; the flow rate was 1.2 ml/min, for 8 min per sample. The sample volume injected was 10 µL. Quantification was performed using the Waters Empower software package and the International Calibration Extract (ICE-3 ASBC) for reference. ICE-3 was prepared by dissolving 1.8 g of ICE-3 in 100 ml methanol; ICE-3 samples were then prepared for injection as per the methods for other samples. Three standard vials were run with each batch of samples, with each standard vial sampled six times (three times at the start and three times at the end of each run). Four components (cohumulone, humulone + adhumulone, colupulone, and lupulone + adlupulone) were identified and quantified. The other five cone chemical traits (α-acid, β-acid, cohumulone (% of α-acid), α-acid:β-acid and α-acid:total resin) were derived by calculation from these four components (as described in Table 4.1).

Statistical procedures

ASReml [291] was used to conduct general linear mixed model analyses of the plant growth, cone yield and cone chemical data collected from the progeny trial. Residual maximum likelihood estimates of variance and covariance were obtained for each trait. The univariate model used was defined as:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z_1 c} + \mathbf{Z_2 a} + \mathbf{e}$$

where **y** is the vector of *n* observations for the dependent variable; **β** is the vector of fixed effects, which were sex (only for the plant growth traits) (as performed by Gilmour [292]) and replicate; **c** is the vector of random replicate.incomplete-block effects; **a** is the vector of random additive genetic effects; and **e** is the vector of random residuals. **X**, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are incidence matrices relating observations to factors in the model. The variance for each component was defined as:

$$\text{Var}[\mathbf{c}] = \mathbf{C} = \boldsymbol{I}\sigma_c^2$$

$$\text{Var}[\mathbf{a}] = \mathbf{G} = \boldsymbol{A}\sigma_a^2$$

$$\text{Var}[\mathbf{e}] = \mathbf{R} = \boldsymbol{I}\sigma_e^2$$

where **C**, **G** and **R** represent the random effects (family and the replicate.incomplete-block term), additive and residual covariance matrices between the observations respectively; ***A*** is the numerator relationship matrix for additive genetic effects; ***I*** is an identity matrix; and $\boldsymbol{\sigma^2}_x$ is the variance of *x*. The expected values and variances of the model were as follows:

$$\text{E}\begin{bmatrix}\mathbf{y}\\ \mathbf{c}\\ \mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{Xb}\\ \mathbf{0}\\ \mathbf{0}\\ \mathbf{0}\end{bmatrix}, \qquad \text{Var}\begin{bmatrix}\mathbf{y}\\ \mathbf{c}\\ \mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{V} & \mathbf{ZC} & \mathbf{ZG} & \mathbf{R}\\ \mathbf{CZ'} & \mathbf{C} & \mathbf{0} & \mathbf{0}\\ \mathbf{GZ'} & \mathbf{0} & \mathbf{G} & \mathbf{0}\\ \mathbf{R} & \mathbf{0} & \mathbf{0} & \mathbf{R}\end{bmatrix}$$

The phenotypic covariance matrix was:

$$\mathbf{V} = \mathbf{Z_1 C Z_1'} + \mathbf{Z_2 G Z_2'} + \mathbf{R}$$

For each trait student's *t* tests [150] were performed to determine whether additive genetic variance was significantly different from zero ($P < 0.05$). The significance of the fixed effects (replicate and sex) were also tested for each trait with F-tests ($P < 0.05$). The coefficient of additive genetic variance **($\boldsymbol{CV_A}$)** was calculated for each trait as:

$$\boldsymbol{CV_A} = \frac{\sqrt{\mathbf{a}}}{\bar{\boldsymbol{X}}}$$

where $\bar{X}$ is the phenotypic mean of the trait.

To examine the relationships between hop cone chemistry, yield and plant growth, residual maximum likelihood estimates of genetic correlation and phenotypic correlation between traits were calculated. In these bivariate analyses **y**, **c**, **a**, and **e** consist of vectors containing observations for two traits such that:

$$\mathbf{y} = (y_1', y_2'),$$

$$\mathbf{c} = (c_1', c_2'),$$

$$\mathbf{a} = (a_1', a_2'),$$

$$\mathbf{e} = (e_1', e_2'),$$

$$\mathbf{X} = \mathbf{X}_1 \oplus \mathbf{X}_2,$$

$$\mathbf{Z_1} = \mathbf{Z}_{1_1} \oplus \mathbf{Z}_{1_2},$$

$$\mathbf{Z_2} = \mathbf{Z}_{2_1} \oplus \mathbf{Z}_{2_2},$$

$$\mathbf{C} = \mathbf{I_c} \otimes \mathbf{C_o},$$

$$\mathbf{R} = \mathbf{I_N} \otimes \mathbf{R_o} \text{ and}$$

$$\mathbf{G} = \mathbf{A} \otimes \mathbf{G_o}$$

The variance-covariance matrices for the random effects (family and the replicate.incomplete-block term), additive genetic effects and residuals were represented by $\mathbf{C_o}$, $\mathbf{G_o}$ and $\mathbf{R_o}$ respectively:

$$\mathbf{C_o} = \begin{bmatrix} \sigma^2_{c_1} & \sigma_{c_{12}} \\ \sigma_{c_{12}} & \sigma^2_{c_2} \end{bmatrix}, \mathbf{G_o} = \begin{bmatrix} \sigma^2_{a_1} & \sigma_{a_{12}} \\ \sigma_{a_{12}} & \sigma^2_{e_2} \end{bmatrix} \text{ and } \mathbf{R_o} = \begin{bmatrix} \sigma^2_{e_1} & \sigma_{e_{12}} \\ \sigma_{e_{12}} & \sigma^2_{e_2} \end{bmatrix}$$

The genetic and phenotypic relationships between plant growth and cone chemistry were investigated using the emergence traits number of shoots and length of the longest shoot (both measured in the first season, 11 months after the trial was planted); the vegetative morphology traits height (at flower initiation), height (at cone maturity), height to the cones and lateral length (all measured in the second season, 28 months after the trial was planted, except for height (at flower initiation) which was measured 25 months after the trial was planted); and

cone chemical traits α-acid, β-acid, cohumulone (% of α-acid), α-acid:β-acid and α-acid:total resin (all measured in the second season, 28 months after the trial was planted). The genetic and phenotypic relationships between yield and plant growth and yield and hop chemistry were investigated using the yield trait green cone weight (measured in the second season, 28 months after the trial was planted) and the emergence, vegetative morphology and cone chemical traits listed above. The consistency of measurements of each individual trait used in these bivariate analyses was assessed by examining the genetic and phenotypic correlations between the results obtained from seasons one and two. Relationships between the different chemical traits were evaluated by investigating the genetic and phenotypic correlations between each chemical trait and every other chemical trait, with measurements from both seasons assessed. Genetic correlations between chemical components (α-acid and β-acid) and ratios between components (α-acid:β-acid and α-acid:total resin) were examined to determine whether the genetic factors influencing the amounts of chemical components also influenced the proportions of these components relative to each other and total resin content. Relationships between the different plant growth traits were also evaluated through the examination of genetic correlations. The significance of each genetic and phenotypic correlation were tested with student's *t* tests ($P < 0.05$) [150].

Narrow-sense heritability **(h^2)** was calculated for each trait. This was computed in ASReml as:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

Least squares mean for each family were computed. These were estimated for every trait from the PREDICT statement in ASReml. Where necessary the values were backtransformed; and for each trait the upper 95% and lower 95% limits were calculated.

RESULTS

Genetic variation

Significant genetic variation was found between families for all cone chemical traits assessed (Table 4.2). For some cone chemical traits (colupulone, α-acid and β-acid) genetic variance was significant in only the first growing season (Table 4.2). No significant genetic variation was found between families for cone yield (assessed in only the second growing season)

(Table 4.2); but significant genetic variation was found between families for all plant growth traits (Table 4.2). For one plant growth trait (internode length) significant genetic variance was detected in only the first growing season. For all traits, CV_A ranged from 0 to 2.67 (mean 0.12) (Table 4.2). The family least squares mean for each trait is reported in Appendix 4.2.

Table 4.2 Genetic variation and heritability of traits associated with cone chemistry, cone yield and plant growth in hop. 'Age' refers to the time that each trait was assessed after the trial was planted. 'Rep.iblock' refers to the random effect of replicate.incomplete-block. 'Additive' refers to additive genetic variance. 'Error' refers to the random effect of residuals. 'V_P' refers to the phenotypic variance. 'CV_A' refers to the coefficient of additive genetic variance. 't' refers to the t-value for Additive and 'Pr > t' refers to its significance. 'Rep' and 'Sex' refers to the fixed effects of replicate and plant sex on the trait, respectively; 'P > F' refers to their significance in each case. 'h^2' refers to the narrow-sense heritability and 'SE' refers to standard error of h^2.

			Variance components							Fixed effects				Heritability	
	Trait	Age	Rep.iblock	Additive	Error	V_P	CV_A	t	Pr > t	Rep	P > F	Sex	P > F	h^2	SE
plant growth	number of shoots	11 months	0.09	0.06	0.88	1.03	0.05	2.75	P < 0.005	8.53	P < 0.0001	885.16	P < 0.0001	0.06	0.02
		24 months	0.56	0.40	4.97	5.93	0.09	2.87	P < 0.005	3.22	P < 0.05	1556.87	P < 0.0001	0.07	0.02
	length of the longest shoot	11 months	0.32	0.33	5.86	6.52	0.01	2.31	P < 0.05	2.84	P < 0.05	1674.14	P < 0.0001	0.05	0.02
		24 months	0.09	0.37	1.84	2.31	0.05	4.28	P < 0.0001	2.39	NS	661.01	P < 0.0001	0.16	0.03
	number of nodes on the longest shoot	11 months	0.56	0.40	4.97	5.93	0.09	2.87	P < 0.005	3.22	P < 0.05	1556.87	P < 0.0001	0.07	0.02
		24 months	0.03	0.03	0.23	0.29	0.06	3.38	P < 0.0005	0.74	NS	1407.14	P < 0.0001	0.10	0.03
	height (at flower initiation)	13 months	0.05	0.09	1.32	1.45	0.08	2.73	P < 0.005	11.27	P < 0.0001	2550.49	P < 0.0001	0.06	0.02
		25 months	0.00	0.10	0.81	0.91	0.06	3.91	P < 0.0001	2.36	NS	7621.12	P < 0.0001	0.11	0.03
	height (mid-season)	14 months	0.51	0.07	1.21	1.79	0.06	2.30	P < 0.05	1.55	NS	1177.69	P < 0.0001	0.04	0.02
		26 months	0.00	0.03	0.33	0.36	0.03	3.57	P < 0.0005	1.08	NS	23768.91	P < 0.0001	0.09	0.02
	height (at cone maturity)	16 months	0.01	0.07	0.60	0.68	0.06	3.77	P < 0.0001	2.72	P < 0.05	7756.84	P < 0.0001	0.10	0.03
		28 months	0.01	0.08	0.58	0.66	0.06	4.03	P < 0.0001	2.94	P < 0.05	7709.27	P < 0.0001	0.12	0.03
	lateral length	16 months	0.10	0.38	7.17	7.65	0.01	2.28	P < 0.05	0.77	NS	3218.57	P < 0.0001	0.05	0.02
		28 months	2.58	0.21	2.96	5.74	0.01	2.50	P < 0.01	0.27	NS	511.01	P < 0.0001	0.04	0.01
	number of nodes on lateral	16 months	0.01	0.02	0.32	0.35	0.02	2.11	P < 0.05	1.51	NS	4820.12	P < 0.0001	0.04	0.02
		28 months	0.01	0.02	0.32	0.35	0.02	2.06	P < 0.05	1.50	NS	4851.78	P < 0.0001	0.04	0.02
	internode length	16 months	0.00	0.06	0.23	0.29	0.01	2.31	P < 0.05	1.51	NS	4477.69	P < 0.0001	0.20	0.08
		28 months	1.39	0.00	1.82	3.21	0.00	0.00	NS	0.06	NS	216.63	P < 0.0001	0.04	0.02

			Variance components							Fixed effects				Heritability	
	Trait	**Age**	**Rep.iblock**	**Additive**	**Error**	**V_P**	**CV_A**	**t**	**Pr > t**	**Rep**	**P > F**	**Sex**	**P > F**	**h^2**	**SE**
	height to the cones	16 months	0.01	0.03	0.33	0.38	0.10	2.54	P < 0.005	612.18	P < 0.0001	NA	NA	0.08	0.03
		28 months	0.01	0.04	0.32	0.37	0.12	2.86	P < 0.005	590.26	P < 0.0001	NA	NA	0.11	0.04
yield	green cone weight	28 months	0.01	0.00	0.11	0.12	0.02	0.33	NS	243.57	P < 0.0001	NA	NA	0.03	0.10
cone chemistry	cohumulone	16 months	0.00	0.03	0.07	0.10	0.06	4.14	P < 0.0001	1076.93	P < 0.0001	NA	NA	0.29	0.06
		28 months	NA	0.02	0.08	0.09	0.05	1.74	P < 0.05	895.61	P < 0.0001	NA	NA	0.18	0.10
	humulone + adhumulone	16 months	0.00	0.04	0.12	0.16	0.03	3.93	P < 0.0001	1714.16	P < 0.0001	NA	NA	0.26	0.06
		28 months	0.00	0.02	0.12	0.15	0.03	1.72	P < 0.05	1250.91	P < 0.0001	NA	NA	0.17	0.09
	colupulone	16 months	0.00	0.02	0.07	0.09	0.06	3.39	P < 0.0005	1105.00	P < 0.0001	NA	NA	0.21	0.06
		28 months	0.00	0.01	0.07	0.08	0.04	1.38	NS	950.66	P < 0.0001	NA	NA	0.15	0.10
	lupulone + adlupulone	16 months	0.00	0.02	0.07	0.09	0.07	3.60	P < 0.0005	1076.23	P < 0.0001	NA	NA	0.23	0.05
		28 months	0.00	0.02	0.06	0.08	0.06	1.93	P < 0.05	868.60	P < 0.0001	NA	NA	0.21	0.10
	α-acid	16 months	0.00	2.00	5.41	7.41	0.16	3.99	P < 0.0001	503.89	P < 0.0001	NA	NA	0.27	0.06
		28 months	0.01	0.02	0.11	0.14	0.02	1.45	NS	1051.50	P < 0.0001	NA	NA	0.16	0.10
	β-acid	16 months	0.00	0.03	0.12	0.15	0.04	3.34	P < 0.001	1320.40	P < 0.0001	NA	NA	0.20	0.05
		28 months	0.00	0.03	0.17	0.21	0.04	1.56	NS	1319.15	P < 0.0001	NA	NA	0.15	0.10
	cohumulone (% of α-acid)	16 months	0.00	0.00	0.00	0.00	0.11	3.90	P < 0.0001	2855.30	P < 0.0001	NA	NA	0.26	0.06
		28 months	NA	0.72	0.00	0.72	2.67	2.72	P < 0.005	3333.97	P < 0.0001	NA	NA	0.29	0.09
	α-acid:β-acid	16 months	NA	0.01	0.05	0.06	0.05	3.32	P < 0.001	1673.84	P < 0.0001	NA	NA	0.20	0.05
		28 months	0.00	0.03	0.12	0.16	0.10	2.03	P < 0.05	500.11	P < 0.0001	NA	NA	0.21	0.10
	α-acid:total resin	16 months	NA	0.00	0.00	0.00	0.04	3.52	P < 0.0005	11960.40	P < 0.0001	NA	NA	0.22	0.05
		28 months	0.00	0.00	0.00	0.00	0.05	2.05	P < 0.05	5383.75	P < 0.0001	NA	NA	0.22	0.10

The heritability of all traits assessed in the study ranged from 0.03 to 0.29 (mean 0.14) (Table 4.2). The heritability of cone chemical traits ranged from 0.15 to 0.29 (mean 0.22) and were generally higher than the heritability of growth traits, which ranged from 0.04 to 0.20 (mean 0.08) (Table 4.2). Cone yield displayed a very low heritability (h^2 = 0.03) (Table 4.2). Estimates of heritability of cone chemical traits were generally higher in the first season of growth, along with plant growth traits related to lateral branch morphology (Table 4.2). The remaining plant growth traits had higher heritability estimates in the second season (Table 4.2).

The effect of replicate was significant for all of the cone chemical traits and also for many of the plant growth traits assessed (Table 4.2). The effect of sex was highly significant ($P < 0.001$) in all traits that were assessed in both male and female plants (all plant growth traits except height to the cones) (Tables 4.2 and 4.3). For all traits related to emergence, male and female phenotypes were similar in the first season of growth (assessed in the first month of spring), but in the second season (the last month of spring), male plants had significantly greater number of shoots, greater number of nodes on the longest shoot and a longer length of the longest shoot (Table 4.3). The heights of male and female plants were also significantly different throughout the growing season, with female plants being taller than male plants (Table 4.3). In terms of lateral morphology, female plants had significantly longer lateral lengths (in season one) and greater number of nodes on laterals (both seasons), but displayed similar internode lengths to male plants (Table 4.3).

Table 4.3 Differences between male and female hop plants for growth traits. Each trait was assessed in two seasons of plant growth; 'Age' refers to the age of the plants at the time that each trait was assessed after the trial was planted. 'Female plants *n*' refers to the number of female plants assessed for each trait. 'Female plants mean ± SD' refers to the phenotypic mean and standard deviation of all female plants for each trait. 'Male plants *n*' refers to the number of male plants assessed for each trait. 'Male plants mean ± SD' refers to the phenotypic mean and standard deviation of all male plants for each trait. 'P value' refers to the significance of similarity between phenotypic variances of female and male plants.

Trait	Age	Female plants *n*	Female plants mean ± SD	Male plants *n*	Male plants mean ± SD	P value
number of shoots	11 months	671	5.51 ± 4.92	378	5.50 ± 4.33	NS
	24 months	671	6.49 ± 6.15	378	9.06 ± 6.51	P < 0.0001
length of the longest shoot	11 months	606	51.15 ± 33.79	359	51.84 ± 31.32	NS
	24 months	496	10.66 ± 10.79	327	13.21 ± 12.05	P < 0.005
number of nodes on the longest shoot	11 months	606	7.04 ± 2.44	359	7.21 ± 2.44	NS
	24 months	496	2.80 ± 1.76	327	3.28 ± 1.90	P < 0.0005
height (at flower initiation)	13 months	670	3.74 ± 1.29	376	3.58 ± 1.13	P < 0.05
	25 months	669	5.15 ± 1.00	378	5.03 ± 0.87	P < 0.05
height (mid-season)	14 months	624	4.71 ± 1.43	352	3.99 ± 1.17	P < 0.0001
	26 months	669	5.87 ± 0.46	378	5.34 ± 0.80	P < 0.0001
height (at cone maturity)	16 months	666	4.77 ± 0.79	376	4.10 ± 0.90	P < 0.0001
	28 months	663	4.79 ± 0.76	376	4.18 ± 0.70	P < 0.0001
lateral length	16 months	650	50.12 ± 26.57	362	44.80 ± 21.37	P < 0.0005
	28 months	649	44.65 ± 29.02	333	45.12 ± 20.90	NS
nodes on lateral	16 months	650	6.73 ± 3.89	356	6.01 ± 2.41	P < 0.0005
	28 months	650	6.81 ± 3.41	357	6.08 ± 2.77	P < 0.0005
internode length	16 months	152	17.53 ± 4.21	79	17.19 ± 4.88	NS
	28 months	228	28.04 ± 23.16	111	24.46 ± 18.31	NS

Genetic correlations

Trait pairwise genetic correlations were used to investigate the genetic relationships between five cone chemical traits relevant to hop breeding. α-acid and β-acid were positively genetically correlated in the first growing season, but were not correlated in the second season (Table 4.4). In both seasons, α-acid was positively genetically correlated with α-acid:β-acid and α-acid:total resin, while β-acid was negatively genetically correlated with these traits (Table 4.4). The genetic correlations between α-acid:β-acid and α-acid:total resin were strongly positive in both growing seasons (Table 4.4). In both seasons, cohumulone (% of α-acid) was positively genetically correlated with α-acid and negatively genetically correlated with β-acid; consistent with these findings, cohumulone (% of α-acid) was positively genetically correlated with α-acid:β-acid and α-acid:total resin (Table 4.4). For all of the cone

chemical traits assessed, strong positive genetic correlations were identified between assessments in the two growing seasons (Appendix 4.3a).

Table 4.4 Pairwise additive genetic (lower part of the matrix) and phenotypic (upper part of the matrix) correlations between hop cone chemical traits and also between plant growth traits in hop. The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold. **a.** refers to traits assessed in the first year of plant growth (16 months after the trial was planted); and **b.** refers to traits assessed in the second year of plant growth (28 months after the trial was planted).

a.

	α-acid		**β-acid**		**cohumulone (% of α-acid)**		**α-acid:β-acid**		**α-acid:total resin**	
α-acid			**0.44**	± 0.04	0.02	± 0.06	**0.37**	± 0.05	**0.39**	± 0.05
β-acid	**0.48**	± 0.15			0.00	± 0.06	**-0.65**	± 0.03	**-0.63**	± 0.03
cohumulone (% of α-acid)	**0.17**	± 0.18	**-0.13**	± 0.20			0.02	± 0.06	0.02	± 0.06
α-acid:β-acid	**0.52**	± 0.15	**-0.50**	± 0.15	**0.29**	± 0.20			**0.97**	± 0.00
α-acid:total resin	**0.47**	± 0.15	**-0.55**	± 0.14	**0.27**	± 0.19	**1.00**	± 0.01		

b.

	α-acid		**β-acid**		**cohumulone (% of α-acid)**		**α-acid:β-acid**		**α-acid:total resin**	
α-acid			**0.31**	± 0.06	0.09	± 0.07	**0.54**	± 0.05	**0.53**	± 0.05
β-acid	-0.08	± 0.49			-0.05	± 0.07	**-0.59**	± 0.05	**-0.20**	± 0.10
cohumulone (% of α-acid)	**0.17**	± 0.33	**-0.20**	± 0.36			0.10	± 0.07	**0.12**	± 0.07
α-acid:β-acid	**0.78**	± 0.25	**-0.60**	± 0.27	**0.19**	± 0.30			**0.95**	± 0.01
α-acid:total resin	**0.78**	± 0.26	**-1.00**	± 38.48	**0.30**	± 0.30	**0.96**	± 0.03		

Genetic relationships between hop cone chemistry and plant growth were also investigated in this study. Limited genetic correlation was observed between the emergence traits and the cone chemical traits. Number of shoots was negatively genetically correlated with β-acid and positively genetically correlated with cohumulone (% of α-acid) and α-acid:β-acid, but these correlations were only weakly significant (Table 4.5). There was a weak positive genetic correlation between length of the longest shoot and α-acid:β-acid; and a stronger positive genetic correlation between length of the longest shoot and cohumulone (% of α-acid) (Table 4.5). There was a higher degree of genetic correlation between the other plant growth traits and cone chemistry. Height, assessed at flowering and at cone maturity, was negatively genetically correlated with all chemical traits (Table 4.5). Similar results were observed for the relationships between cone chemistry and the other two traits assessing plant growth at cone maturity: height to the cones and lateral length. Height to the cones was negatively

correlated with all chemical traits, except cohumulone (% of α-acid) for which the correlation was not significantly different from zero (Table 4.5). Lateral length was negatively genetically correlated with both α-acid and β-acid, but the negative correlation was stronger with β-acid than with α-acid (Table 4.5). As a result, lateral length was positively genetically correlated with α-acid:total resin (Table 4.5). Lateral length was not significantly genetically correlated with either cohumulone (% of α-acid) or α-acid:β-acid (Table 4.5).

The genetic relationships between the different plant growth traits were assessed, with positive correlations found between most traits (Table 4.6). Exceptions to this were negative correlations between number of shoots and height to the cones, and length of the longest shoot and height at cone maturity; and no correlation between length of the longest shoot and the traits height at flowering, height to the cones and lateral length (Table 4.6). The consistency of family performance for each growth trait was also assessed across the two growing seasons in which measurements were made. For all of the plant growth traits assessed, genetic correlations between different assessments of the trait were strongly positive (Appendix 4.3b).

Table 4.5 Genetic and phenotypic correlations between cone chemical traits and plant growth traits in hop. Cone chemical traits were assessed in the second year of plant growth (28 months after the trial was planted). The plant growth traits number of shoots and length of the longest shoot were assessed in the first year of plant growth (11 months after the trial was planted) while height (at flower initiation), height (at cone maturity), height to the cones and lateral length were assessed in the second year of plant growth (height (at flower initiation) at 25 months after the trial was planted and the remaining plant growth traits at 28 months after the trial was planted). The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold.

	number of shoots		**length of the longest shoot**		**height (at flower initiation)**		**height (at cone maturity)**		**height to the cones**		**lateral length**	
	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation	Genetic correlation	Phenotypic correlation
α-acid	-0.05 ± 0.34	0.06 ± 0.07	0.03 ± 0.34	0.09 ± 0.07	**-0.56** ± 0.29	-0.09 ± 0.07	**-0.82** ± 0.26	-0.08 ± 0.07	**-0.59** ± 0.35	**-0.26** ± 0.08	**-0.49** ± 0.37	-0.03 ± 0.07
β-acid	**-0.11** ± 0.34	-0.07 ± 0.07	-0.07 ± 0.34	-0.07 ± 0.07	**-0.42** ± 0.28	**-0.20** ± 0.07	**-0.55** ± 0.25	0.04 ± 0.07	**-0.22** ± 0.35	**-0.16** ± 0.08	**-0.62** ± 0.37	**-0.14** ± 0.07
cohumulone (% of α-acid)	**0.16** ± 0.25	0.10 ± 0.07	**0.45** ± 0.24	**0.25** ± 0.06	**-0.21** ± 0.21	0.07 ± 0.07	**-0.39** ± 0.19	0.03 ± 0.07	-0.07 ± 0.24	-0.01 ± 0.08	-0.04 ± 0.26	0.03 ± 0.07
α-acid:β-acid	**0.17** ± 0.29	0.11 ± 0.07	**0.18** ± 0.29	**0.13** ± 0.07	**-0.15** ± 0.24	0.10 ± 0.07	**-0.22** ± 0.23	-0.00 ± 0.07	**-0.22** ± 0.28	-0.07 ± 0.08	0.05 ± 0.29	0.05 ± 0.07
α-acid:total resin	0.04 ± 0.29	0.10 ± 0.07	0.01 ± 0.29	**0.13** ± 0.07	**-0.12** ± 0.24	0.10 ± 0.07	**-0.13** ± 0.22	-0.01 ± 0.07	**-0.30** ± 0.30	-0.05 ± 0.08	**0.12** ± 0.27	0.09 ± 0.07

Table 4.6 Pairwise genetic (lower) and phenotypic (upper) correlations between plant growth traits in hop. The traits number of shoots and length of the longest shoot were assessed in the first year of plant growth (11 months after the trial was planted). The traits height (at flower initiation), height (at cone maturity), height to cones and lateral length were assessed in the second year of plant growth (height (at flowering) at 25 months after the trial was planted and the remaining traits at 28 months after the trial was planted). The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold.

	number of shoots	**length of the longest shoot**	**height (at flower initiation)**	**height (at cone maturity)**	**height to the cones**	**lateral length**
number of shoots		**0.57** ± 0.02	**0.14** ± 0.25	**0.22** ± 0.03	0.03 ± 0.04	**0.14** ± 0.04
length of the longest shoot	**0.87** ± 0.12		**0.28** ± 0.03	**0.23** ± 0.03	-0.02 ± 0.04	**0.12** ± 0.04
height (at flower initiation)	**0.14** ± 0.25	-0.01 ± 0.23		**0.42** ± 0.03	**0.13** ± 0.04	**0.18** ± 0.03
height (at cone maturity)	**0.18** ± 0.21	**-0.20** ± 0.24	**0.77** ± 0.10		**0.24** ± 0.04	**0.34** ± 0.03
height to the cones	**-0.22** ± 0.25	-0.01 ± 0.04	**0.62** ± 0.19	**0.66** ± 0.16		**0.21** ± 0.05
lateral length	**0.22** ± 0.26	0.04 ± 0.28	**0.56** ± 0.19	**0.99** ± 0.12	**0.16** ± 0.26	

In addition, the genetic relationships between cone yield and both the cone chemical traits and plant growth traits were assessed. Green cone weight was found to be negatively genetically correlated with α-acid, β-acid and cohumulone (% of α-acid), but positively genetically correlated with α-acid:β-acid and α-acid:total resin (Table 4.7). Green cone weight was positively genetically correlated with the emergence trait number of shoots, but was negatively genetically correlated with another emergence trait length of the longest shoot (Table 4.8). Green cone weight was negatively genetically correlated with height measured at flowering, but positively genetically correlated with height measured at cone maturity (Table 4.8). Green cone weight was negatively genetically correlated with both height to the cones and lateral length (Table 4.8).

Table 4.7 Genetic and phenotypic correlations between cone chemical traits and cone yield in hop. Traits were assessed in the second year of plant growth (28 months after the trial was planted). The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold.

	green cone weight	
	Genetic correlation	Phenotypic correlation
α-acid	**-0.93** ± 0.72	**0.22** ± 0.07
β-acid	**-0.63** ± 0.53	0.05 ± 0.07
cohumulone (% of α-acid)	**-0.44** ± 0.09	0.05 ± 0.07
α-acid:β-acid	**0.83** ± 0.21	**0.16** ± 0.07
α-acid:total resin	**0.42** ± 0.19	**0.14** ± 0.07

Table 4.8 Genetic and phenotypic correlations between plant growth traits and cone yield in hop. The traits number of shoots and length of the longest shoot were assessed in the first year of plant growth (11 months after the trial was planted). The traits height (at flower initiation), height (at cone maturity), height to the cones, lateral length and green cone weight were assessed in the second year of plant growth (height (at flowering) at 25 months after the trial was planted and the remaining traits at 28 months after the trial was planted). The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold.

	green cone weight	
	Genetic correlations	Phenotypic correlations
number of shoots	**0.62** ± 0.30	**0.24** ± 0.82
length of the longest shoot	**-0.27** ± 0.79	0.05 ± 0.07
height (at flower initiation)	**-0.95** ± 0.37	**0.20** ± 0.07
height (at cone maturity)	**-1.00** ± 0.19	**0.21** ± 0.07
height to the cones	**-0.94** ± 0.34	**-0.94** ± 0.34
lateral length	**-0.93** ± 0.25	**-0.93** ± 0.25

Phenotypic correlations

The phenotypic relationships at the family level between cone chemistry, cone yield and plant growth traits in hop were also investigated to give an indication of the influence of factors other than additive effects (including environmental and agronomic factors, as well as non-additive genetic effects and error) on these traits. Pairwise testing of the chemical traits found positive phenotypic correlations between α-acid and β-acid in both growing seasons (Table 4.4), indicating an influence of factors other than additive genetic effects in at least the second season (where no genetic correlation was identified). Other combinations of traits for which factors other than additive genetic effects were found to have an influence on phenotypic correlations included cohumulone (% of α-acid) with each of the traits β-acid, α-acid:β-acid and α-acid:total resin (first season only); in each case, no significant phenotypic correlation was identified, despite there being a positive genetic correlation (Table 4.4). Pairwise phenotypic correlations between all other cone chemical traits were similar to the genetic correlations identified earlier.

Factors other than additive genetic effects were clearly found to influence hop plant growth, evidenced by the results of pairwise tests between cone chemical traits and the plant growth traits. Either no significant phenotypic correlation was found between traits where a significant genetic correlation had been identified, or the significance of the phenotypic correlation was lower than the significance of the genetic correlation (Table 4.5). The only exception to this was a significant phenotypic correlation between length of the longest shoot and α-acid:total resin, where no significant genetic correlation was identified (Table 4.5). This trend was generally true for phenotypic correlations between cone yield and cone chemical traits and cone yield and plant growth traits, with exceptions being the relationships between green cone weight and each of the traits α-acid, height at flowering and lateral length; all of these traits were found to be strongly negatively genetically correlated but positively phenotypically correlated with green cone weight (Tables 4.7 and 4.8).

The phenotypic relationships between the different plant growth traits were generally similar to the genotypic correlations, indicating that factors besides additive genetic effects had a relatively small effect on the correlations between these traits (Table 4.6). The exceptions to this were correlations between length of the longest shoot and the traits height at flowering, height at cone maturity and lateral length, where the traits were positively phenotypically correlated with length of the longest shoot, but no genotypic correlation was identified (Table

4.6). The consistency of family performance for each growth trait was also assessed across the two growing seasons in which measurements were made. For each of the plant growth traits assessed, the phenotypic correlations between the assessments of the trait were positive across the two growing seasons (Appendix 4.3b).

DISCUSSION

Genetic variation

This study found heritable genetic variation between families in the key hop brewing substances α-acid and β-acid, as well as their components (cohumulone, humulone + adhumulone, colupulone and lupulone + adlupulone) and their relative proportions (cohumulone (% of α-acid) α-acid:β-acid and α-acid:total resin) (Table 4.2). Heritable genetic variation between families was also identified for morphological features of hop plant growth fundamental to optimal agronomic management, including emergence, height, lateral growth and distribution of cones over the hop plant (Table 4.2). Of those traits for which heritable variation was identified, the narrow-sense heritability estimates ranged from 0.04 to 0.29, with a mean of 0.15 (Table 4.2). Cone chemical traits generally had higher heritability than growth traits (Table 4.2). This may reflect the intense selection directed at hop cone chemical traits compared to growth traits. Selection of hop cone chemical traits could be due to both artificial selection by breeding or natural selection as a result of the rapid co-evolution of chemical profiles and herbivore tolerance traits [295-296]. In addition, growth traits are likely to be more susceptible to environmental/agronomic influences. The estimates of narrow-sense heritability for cone chemical traits were generally lower in this study compared to those calculated for similar traits in previous studies of hop [78-79, 83] (Appendix 4.4a). The exception to this was the value of zero for heritability of α-acid:β-acid reported by Murakami [83], compared to the estimate of 0.21 calculated in this study (Appendix 4.4a). The variability of the estimates reported illustrates the fact that heritability is a function of the genetic material upon which the calculation is based. There are several factors pertaining to the experimental design of this study that could explain the generally lower heritability estimates observed compared with the previous studies in hop. Firstly, this study utilised 108 families for estimation of heritability. Perron et al. [275] and Lynch and Walsh [72] have found that at least 75 families are generally required for accurate estimation of genetic parameters. Previous hop studies have used far fewer than 75 families [78-79, 83] (Appendix 4.4a), potentially inflating estimates. Secondly, the families used in this study were generated

from open-pollination (and open-pollination also occurred extensively within the pedigree of their ancestors), rather than controlled crosses as in the previous studies [78-79, 83]. This may have increased variability within half-sib families, decreasing heritability estimates. Thirdly, open-pollination may have reduced the accuracy of the relationship matrices, as the fathers of each family are unknown. Besides the missing parental information, the models for the calculation of heritability assume that the unknown fathers are unrelated, which is highly unlikely. These factors mean that the population size is likely to be smaller than that designated in our model, resulting in decreased heritability estimates. Inaccuracies in the relationship matrix may also have arisen due to missing information in the pedigree, where the ancestry for particular individuals (e.g. founders) is unknown. Fourthly, as suggested earlier, there is likely to be a high level of inbreeding among the parents of this study population. The models for calculation of heritability assume that the founders in our pedigree are unrelated, but this is unlikely as it is well documented that most hop cultivars descend from relatively few common ancestors that were highly prized for their brewing properties [4, 6, 51]; these cultivars have been found to have relatively limited genetic variability between them [51]. Inbreeding within the population would again result in a smaller population size than that designated in our model, possibly resulting in decreased heritability estimates.

While the heritability estimates reported in this study are possibly underestimates, the findings from this study may be more broadly applicable to hop as a species, as estimates were based on a larger number and greater diversity of families than any previous study [78-79, 83] (Appendix 4.4a). Studies which have examined the genetic diversity of hop have determined two primary genetic groupings: European and North American [89, 92, 104, 108, 112-113, 124, 134, 210]. The genotypes used in previous hop studies [74, 78-79, 83] were largely of European genetic origin and from a relatively narrow genetic base. In this study, genotypes of both European and North American origin were included, as well as hybrids between the two groups (Appendix 4.1). The accuracy of estimates of genetic parameters from this study could be improved by classifying the genotypes in the pedigree into groups to reflect the European/North American population structure; however, while the families in this study were supported by extensive pedigree information (often going back as far as founders), the records were not adequate to classify every founder or genotype into a genetic group. Accurate genetic groups could be assigned in future studies with the aid of molecular data (as in Steane et al. [297]) to improve the estimation of genetic variance.

This study is the first to report estimates of narrow-sense heritability for growth traits in hop. This assessment of the potential heritable genetic variation in growth traits provides important information for the development of new hop cultivars with improved agronomic characteristics, such as timely emergence, appropriate growth and maximal distribution of hop cones on the bine. This study also revealed an influence of sex on hop growth. Male and female hop plants have been described as being indistinguishable until they switch from the vegetative phase to the reproductive phase [10]; however this study found, for the first time, significant phenotypic differences in the growth of male and female plants as early as the emergence of shoots, in terms of the number, length of the longest shoot and number of nodes on the longest shoot (Tables 4.2 and 4.3). Male and female plants continued to display differences in morphology throughout the growing season and at maturity, for a range of plant growth traits, including height and elements of lateral morphology (Tables 4.2 and 4.3). Only a few dioecious plant species have been described as sexually dimorphic in vegetative morphology, including *Salix arctica*, *Acer negundo, Simmondsia chinensis* and *Phoradendron juniperinum* [298]. In these species, differences in photosynthetic rate and transpiration (both key traits underlying agronomic performance) between male and female plants were the cause of the observed differences in morphology [298]. An early study in hop investigated the physiological differences between male and female plants, finding differences in transpiration rate, but not in photosynthetic rate [299], however further work is required to confirm this. The sexual dimorphism in growth found in this study, suggests that there might be differences in these key physiological traits in hop, providing an opportunity to further investigate the genetic control of photosynthetic rate and water use efficiency in hop.

No significant heritable genetic variation was identified between families for yield of hop cones (green cone weight) (Table 4.2). An explanation for this might be suboptimal agricultural management of the hop plants early in the cultivation process. Hop cultivars produce uniform yields, but these yields are dependent on flowering at the optimum time, which is in turn dependent on bine control and training up the trellis at the appropriate time [8]. Flowering in hop is triggered by shortening daylength [8-9]; and different cultivars vary in their photoperiod requirements, as well as the optimum number of growing days from initial bine training to flowering and from flowering to cone maturity [8]. Yield may be significantly reduced where the bine training date is not optimal for a particular genotype on a particular site. Backdating from cone maturity to determine the optimum training date for

each individual in progeny trials, such as this one, is not feasible. This may have had a significant effect on yield, distorting the level of variation within families (Appendix 4.2d.) and resulting in no significant genetic variation between families (Table 4.2). Heritable genetic variation for yield has been reported in previous studies of hop (Appendix 4.4a) [78-79], but these trials consisted of fewer families derived from controlled crosses (reducing the genetic variability within families). As such, it is highly likely that emergence times in these trials were more uniform. Yield variance may also have been affected by inbreeding. If there was a high level of inbreeding among the parents of the study, this may reduce the variability of yield in the progeny trial [293-294].

Genetic correlations

The brewing properties of hop cultivars are defined by the chemical composition of hop cones [6, 18-20]. Of the chemical compounds that comprise hop resin, two of the most important are α-acid and β-acid. α-acids are the key source of bitterness in beer, while β-acids also contribute bitterness, but to a lesser extent [6]. The relative proportion of these compounds to each other is of high importance to the way that hops are used in brewing, with hops that have higher α-acid relative to β-acid ('high-alpha hops') used in bitter beers, and hops that have more equivalent levels of α-acid to β-acid ('aroma hops') traditionally used for (non-bitter) flavour and aroma [33, 300]. This study examined the genetic interrelationships between α-acid and β-acid and their relative proportions (α-acid:β-acid and α-acid:total resin). α-acid and β-acid were found to be positively genetically correlated in the first season of plant growth, but no relationship between them was detected in the second season (Table 4.4). The lack of correlation in the second year is a positive factor for hop breeders, as it suggests that when hop plnats reach maturity, the two compounds can be selected for independently, without changes to one compound influencing the other. α-acid was positively genetically correlated with α-acid:β-acid and α-acid:total resin while β-acid was negatively genetically correlated with these traits (Table 4.4), reflecting the trend that as levels of α-acid in hop resin increase relative to β-acid, these ratios increase, but indicating that this trend has a genetic basis. Accordingly, α-acid:β-acid was positively genetically correlated with α-acid:total resin (Table 4.4). The relationships between α-acid and β-acid reported in previous studies vary. Negative genetic correlations between the two compounds were reported by Henning et al. [77] and Henning et al. [78], but positive genetic correlations were reported by Henning et al. [79] (Appendix 4.4b). Murakami [83] examined the genetic relationships between α-acid, β-acid and α-acid:β-acid, but did not find a significant correlation between any of the traits.

Traditionally, hops with lower levels of cohumulone, one of the secondary metabolites that comprise α-acid, were considered more desirable for brewing purposes as it was thought that it contributed a harsh and unpleasant bitterness to the brew [301]. This idea probably stemmed from the fact that most 'noble hops' (traditional hops from Europe, prized for their mild bitterness and pleasant aroma) have relatively low levels of cohumulone [33-34]. More recently, the role of cohumulone has been called into question, with studies showing that quality of bitterness was not adversely affected by cohumulone [302]; and new hop varieties developed with higher levels of cohumulone that are considered not to impart a harsh bitterness. This study found significant positive genetic correlations between α-acid and cohumulone (% of α-acid) and significant negative genetic correlations between β-acid and cohumulone (% of α-acid), despite there being a positive genetic correlation between α-acid and β-acid (Table 4.4). Significant positive genetic correlations were found between cohumulone (% of α-acid) and both the traits α-acid:β-acid and α-acid:total resin (Table 4.4). These findings may reflect the history of selection for lower levels of cohumulone in 'noble'-type hops, as where α-acid:β-acid and α-acid:total resin ratios are low, the proportion of cohumulone in α-acid is also low. Previous studies have not examined the relationships between cohumulone as a percentage of α-acid and other chemical traits. Strong positive genetic correlations between seasons were found for each of the chemical traits assessed (Appendix 4.3a), indicating that families are highly consistent season to season in their chemical profiles.

While the analytical bitterness potential of hops are easily analysed, the bittering, flavour and aroma properties of hops are difficult to ascertain prior to brewing [303-304]. Attempts have been made to develop methods of selection (such as using molecular markers) that can be used to evaluate hop genotypes for particular chemical profiles prior to trial-brewing [88, 95, 117, 276]. This study examined the genetic relationships between key cone chemical traits and plant growth traits, to determine whether morphological characteristics could be used as proxy selection indicators for particular chemical attributes. A significant negative genetic relationship was found between plant vigour (characterised by a greater number of shoots at emergence, taller plants at both flower initiation and cone maturity, as well as plants with longer laterals) and α- and β-acids, where families with increased plant vigour tended to have decreased levels of α-acid and β-acid (Table 4.5). Significant positive genetic correlations were found between all of these growth traits and between measurements across seasons, indicating that vigour is maintained throughout the growing season and over years (Table 4.6;

Appendix 4.3b). These findings indicate that plant vigour could be used as an indicator of α-acid and β-acid levels in hops, with selection of families that have low vigour likely to also have higher levels of α-acid and β-acid.

The association of increased vigour with lower levels of α- and β-acids may reflect the underlying population structure of families included in this study, as well as the influence of past selection in the Australian environment. As discussed earlier, the families included in this study consisted of genotypes from both European and North American genetic groups. It has been observed that hops of European genetic origin tend to have more vigorous, leggy growth (i.e. greater heights and longer laterals) and lower levels of α-acid and β-acid when grown in the Australian environment, while hops of North American genetic origin tend to have less vigorous, more compact growth and higher levels of α-acid and β-acid when grown in the Australian environment. The genetic relationship between vigour and α- and β-acid observed in this study may be a reflection of the binary population structure of founder genotypes of European or North American genetic groups, or it may be indicative of selection for more compact growth and higher α- and β-acid levels.

Length of the longest shoot, one of the emergence traits assessed in this study, was not found to be associated with plant vigour. No genetic relationship was identified between this trait and any of the other growth traits, except for height at cone maturity, where there was a significant negative genetic correlation (Table 4.6). Length of the longest shoot was not significantly correlated with α-acid or β-acid, but did have a significant positive genetic relationship with cohumulone (% of α-acid) (Table 4.5). This relationship may again be a reflection of past selection in the Australian environment. Height to the cones was negatively genetically correlated with both α-acid and β-acid (Table 4.5), indicating that families that tend to have smaller distances between the ground and where the bulk of the hops begin on the bine also have greater levels of α-acid and β-acid in their cones. Selection of plants with a shorter height to the cones would result in concomitant increases in α-acid and β-acid. Families that displayed a greater height to the cones tended to also have reached a greater height at flowering and at cone maturity (Table 4.6).

The direct economic benefits of yield increases ensure that it is a core aim of every hop breeding program. Previous reports of the relationships between yield and the chemical traits α-acid and β-acid vary, with both positive and negative correlations between the traits

reported [77-79] (Appendix 4.4b). Yield was genetically correlated with a number of cone chemical and plant growth traits in this study (Tables 4.7 and 4.8); however, as yield was not found to be heritable in this study (Table 4.2), these genetic correlations should be treated with caution.

Phenotypic correlations

Differences between values of additive genotypic correlation and phenotypic correlation are indicative of the influence of factors other than additive genetic effects (such as the environment, agricultural practice, non-additive genetic effects and error) on the correlations between traits. In this study environmental variation was likely to have been an influential factor on the progeny trial, evidenced by replicate having significant effects on the variance of many of the traits assessed and a large proportion of the total variation attributed to replicate.incomplete block effects, particularly for plant growth traits (Table 4.2). Many of the correlations between cone chemical traits examined in this study appeared consistent at the phenotypic and additive genetic level (Table 4.4). There was evidence of influences other than additive genetic effects causing a correlation between α-acid and β-acid in one of the growth seasons, where no additive genetic correlation between the two traits had been identified (Table 4.4). There was also evidence of influences other than additive genetic effects masking the additive genetic correlations between cohumulone (% of α-acid) and all other cone chemical traits, with no phenotypic correlations but significant additive genetic correlations between the traits, identified (Table 4.4). Similarly, many of the growth traits examined in this study were significantly genetically correlated but not phenotypically correlated. The exceptions to this were relationships between length of the longest shoot and the traits height at flowering, height at cone maturity and lateral length, where factors besides additive genetic effects produced phenotypic correlations between the traits, where no additive genetic correlation had been identified (Table 4.6). The influence of factors other than additive genetic effects was prominent in the correlations between plant growth and cone chemical traits, yield and cone chemical traits, and yield and growth traits, whereby traits that were significantly genetically correlated displayed no phenotypic correlation (Tables 4.5, 4.7 and 4.8). These results have important implications for the use of these traits as proxy selection indicators for a corresponding trait in hop, as the phenotype of a trait may not be a reliable indicator of genotype, except where plants are grown under conditions where factors other than additive genetic effects no longer influence the phenotype and variation between individuals can be attributed to the additive component of the genotype alone.

Conclusions

This study presents estimates of quantitative genetic parameters for 20 hop traits related to cone chemistry, cone yield and plant growth. Calculations were based on the largest number of families and on the broadest genetic base to be assessed in this kind of study in hop. This study revealed heritable genetic variation in cone chemistry and plant growth traits in hop. In comparison to previous findings, the heritability estimates were lower for cone chemical traits, but estimates were based on a greater number of families and a more diverse genetic background, improving the accuracy of findings and offering a broader perspective on the inheritance of traits of economic importance in hop. This was the first study to report narrow-sense heritability for growth traits in hop, which were found to be generally lower than that of cone chemical traits, likely reflecting a more intense selection for cone chemistry and the greater influence of environmental factors on hop growth. Cone chemical traits were significantly genetically correlated with each other and with plant vigour, whereby increased vigour was associated with lower levels of α-acid and β-acid. This trend may reflect an underlying population structure of plants with European or North American genetic origin and past selection in the Australian environment. Factors other than additive genetic effects were found to have a significant impact on the correlations between traits, often masking genetic correlations. This study was also the first to report the effect of sex on the phenotype of hop plants, as early as emergence. Male and female plants displayed differences in morphology throughout the growing season and at maturity. The findings from this study will provide breeders with a greater understanding of the additive genetic factors which affect selection of cone chemistry, yield and agronomic characteristics in hop, aiding in the future development of improved hop cultivars.

CHAPTER 5

General conclusions and future perspectives

The main objective of hop breeding programs is the development of new cultivars with improved or novel brewing character, higher yields and better agronomic performance. An additional aim is to utilise methods that increase the likelihood of achieving these breeding aims, and to do so in less time and with reduced cost. To date, hop breeding programs have delivered a number of new cultivars with improved properties, including increased hop acid content, new and improved flavour and aroma potential, resistance to a range of pests and diseases and adaptation to new environments [37-44]. The majority of hop breeding has been conducted using traditional phenotype-based selection methods and although these methods have successfully improved some traits, their application is difficult for traits with complex inheritance and for traits strongly influenced by environmental factors [45-48]. Recently, there has been increased interest in developing more sophisticated genetic-based technologies for hop breeding, to contend with these factors. Uptake of these technologies in hop breeding programs has been limited due to the time and expense associated with their development and utilisation, relative to traditional methods. In this thesis I investigated two genetic-based technologies, molecular analysis using DArT markers and quantitative genetic analysis, as high-throughput, cost-effective sources of information about the genetic architecture underlying phenotypic trait variation of hop, to aid in the selective improvement of the species.

Development of genetic based technologies for hop breeding

Identification of molecular markers

In Chapter 2 of this thesis I evaluated the utility of DArT markers as a genotyping tool for hop. A number of different marker technologies have been used in hop, including RAPD, AFLP, STS, microsatellites, EST-based markers and candidate gene-based markers [51, 88-112], each having relative merits. However, in comparison to these techniques DArT is higher throughput and provides genome-wide coverage, at very low cost per data point [118, 136-139]. DArT markers have been particularly useful in crop species that, like hop, have relatively limited genetic resources [118, 138]. As a marker system, DArTs are dominant and so provide less segregation information compared to co-dominant markers; but unlike many other marker systems, DArT markers are transferable and easily deployed in different mapping populations [171].

More than 1200 polymorphic DArT markers were identified in this study (Chapters 2 and 3). These markers were found to be as accurate and robust as other marker systems that have been used to genotype hop, as demonstrated by the capacity of the DArT markers to resolve the genetic groups of diverse hop accessions similar to previous analyses of hop genetic diversity (Chapter 2). The capacity of DArT to rapidly generate numerous polymorphic markers at relatively low cost, as well as to capture the multiplicity of sequence information available makes it a valuable genotyping tool for hop research and breeding. The hop markers discovered in this study have numerous potential applications. Firstly, they can used for molecular fingerprinting, to confirm the identity of particular cultivars or to characterise individuals in breeding populations for which the pedigree is unknown. Secondly, the DArT markers can be used to assess hop genetic diversity, which is important to avoid inbreeding within breeding populations and to identify individuals to introduce to improve the genetic variability of breeding programs. Thirdly, the DArT markers can be incorporated into linkage maps and used to identify QTL, a type of marker-trait association, which can be utilised for marker assisted selection (MAS) of hop, as a more direct and efficient alternative to phenotypic-based selection methods.

Molecular analysis

In Chapter 3 of this thesis I utilised the DArT markers for QTL analysis, to identify marker-trait associations for some commercially important traits in hop. I constructed male and female linkage maps for two mapping populations using the DArT markers, as well as RAPD, STS, AFLP and microsatellite markers. These maps were then used to detect QTL for 50 traits related to expediting plant sex identification, increasing yield capacity and improving bittering, flavour and aroma chemistry. More than 60 QTL were identified. One of these was for dry cone weight, a trait which assesses the yield of dry cones per plant. This QTL, which explained 35% of the phenotypic variation in dry cone weight, was ontogenetically stable, being significant over a five year period. Subject to testing in multiple environments and pedigrees, this QTL can be used in breeding programs to screen for hop plants with higher cone yield potentials. QTL were identified for a second yield trait, essential oil content, as well as 33 secondary metabolites, including hop acids, essential oils and a polyphenol. With validation in different environments, pedigrees and ontogenetic stages, these QTL have the potential to be used to screen hop plants for higher yields of total essential oil, as well as higher yields of individual secondary metabolites. These QTL can also be used to select individuals that possess specific combinations or ratios of secondary metabolites, where a

particular secondary metabolite profile is identified as imparting favourable organoleptic character. While a greater understanding of the complexities of hop secondary metabolite biosynthesis and the influence of particular compounds on beer characteristics is required before secondary metabolite QTL can be fully utilised in hop MAS, the QTL I identified in this study provide a valuable resource for the future development of hop cultivars with new and improved brewing character.

In addition to QTL for yield and cone chemistry, in Chapter 3 I verified a putative sex-linked marker for hop, the microsatellite marker HLAGA7 (see Appendix 5.1 for sequence). Because hop is a dioecous species [10-11], sex determination is fundamental to breeding and cultivation, as only the female plants produce commercially relevant cones. Currently, the sex of a hop plant is distinguished by phenotypic assessment at flowering [10]. While a number of sex-linked markers have been identified in hop, these markers have not been overly successful as screening tools in breeding programs, as many are incompletely linked to the male sex, or have not been verified in more than one pedigree or environment [115-116, 173-176]. The sex-linked microsatellite, HLAGA7, now verified in three pedigrees and diverse environments as being completely linked to the male sex, can be used to distinguish male and female plants as early as the seedling stage.

The QTL identified in this study can be used to facilitate the selection of hop traits on the basis of genotype, eliminating the confounding influence of maturation and environmental variation; thus considerably increasing the chances of obtaining particular phenotypes. As hop population screens using QTL can be performed at a much earlier stage than traditional phenotypic-based screening, the QTL identified in this study may also help to reduce overhead costs and better utilise the cultivation area associated with breeding programs.

Quantitative genetic analysis

In addition to assessing the utility of DArT markers and performing QTL analysis, I investigated the potential of quantitative genetic analysis as an informative technology for the improvement of hop. Quantitative genetic analysis calculates the potential additive (selectable) genetic variation present in traits, as well as the heritability of these traits [49, 72, 274]. This information is very useful to hop breeders, since traits must be both heritable and possess variation due to additive genetic factors for selection to produce a desired phenotype [48]. Quantitative genetic analysis also calculates genetic correlations between traits and the

influence that environmental and non-additive genetic factors have on traits, which is useful for the accurate prediction of genetic gains [46, 49, 72]. Thus, quantitative genetic analysis would be a highly useful tool to assist with the development of breeding strategies for hop improvement. In addition, quantitative genetic analysis requires only a progeny trial and pedigree (as well as statistical software) [49, 72] and so is very complementary to the current phenotypic-based hop selection systems.

In Chapter 4 of this thesis I calculated quantitative genetic parameters for 20 traits related to three key selection criteria in hop: cone chemistry, cone yield and agronomic characteristics. I determined that significant heritable genetic variation occurs within hop acids (including α-acid and β-acid, as well as their derivatives and relative proportions); and within agronomic characteristics (including plant growth traits related to emergence, height and lateral morphology). Narrow-sense heritability (heritability based on the additive genetic component) was found to range between 0.15 and 0.29 for chemical traits, and 0.04 and 0.20 for agronomic traits. Cone yield (assessed as mass of green cones per plant) did not display significant genetic variation between families in this study. This information indicates that there is potential for hop improvement in terms of cone chemistry and agronomic characteristics through the selection of these hop acid and plant growth traits. Using quantitative genetic analysis, I also identified significant genetic correlations between all of the cone chemical traits, as well as significant genetic correlations between cone chemical traits and agronomic traits. Environmental and non-additive genetic factors were found to mask the effect of many of these genetic correlations and were also found to have a significant influence on the phenotype of individual traits. The information gained from this research indicates several complexities that will impact the future selection of cone chemistry and agronomic characteristics for hop improvement. This information is consequently invaluable for the development of effective breeding strategies.

Understanding the genetic architecture of hop through molecular and quantitative genetic analyses

I have shown that both molecular and quantitative genetic analyses can be directly utilised by breeding programs for hop improvement. However, the application of these techniques is also able to provide significant insight into the genetic control of traits, as well as important information on aspects of the general biology of hop. Molecular and quantitative genetic analyses can be used to better understand the genetic architecture of hop, which can feed back

into breeding programs, providing information on which breeding objectives can be achieved and how they can be realised. This may involve developing more effective breeding strategies, where each trait is weighted appropriately in terms of anticipated genetic gain, as well as directing the choice of breeding parents. An understanding of hop genetic architecture can better inform traditional phenotypic-based selection programs, as well as indicate when objectives could be achieved more effectively through molecular approaches, providing direction as to how best to use molecular markers to achieve hop improvement. Through the application of molecular and quantitative genetics to hop, I revealed a number of important insights in this thesis about the genetic control of commercially important traits and general hop biology which contribute to the overall understanding of the genetic architecture of hop.

Molecular analysis

Although linkage maps have already been constructed for four hop mapping populations [88, 95, 99, 114], in Chapter 3 of this thesis I constructed male and female linkage maps for a new hop mapping population and built upon the pre-existing linkage maps of one of the previous mapping populations through the addition of transferable DArT markers. Linkage maps were constructed using a more conservative approach than previous studies, resulting in an increased number and density of markers on the maps, as well as an increased level of accuracy, with ten linkage groups (the haploid number of hop chromosomes) clearly identified. The linkage analyses conducted in these two mapping populations add to our understanding of the organisation of the hop genome. The high levels of marker clustering and segregation distortion observed in all maps constructed in this study are indicative of as yet unidentified biological phenomena in hop. These linkage maps also provide novel sites of polymorphism that may be associated with commercially important traits and are thus a valuable resource for further investigations in the genetic control of hop.

In Chapter 3 of this thesis, the polymorphisms located on linkage maps were explored to identify potential areas of the hop genome associated with cone chemistry and yield traits. I showed for the first time that variation in total essential oil content, as well as many individual secondary metabolites has a genetic basis in hop. The results of these QTL analyses revealed a wealth of novel and important information, including the number, location and magnitude of the effect of genetic regions associated with 50 commercially important traits. Between one and four QTL were identified for 33 different secondary metabolite traits, varying in both significance and the proportion of the phenotypic variance they explained,

suggesting that the composition and concentration of secondary metabolites in hop is influenced by both Mendelian and quantitative inheritance. This is particularly significant new knowledge for hop breeders, as it indicates which traits are likely to be more amenable to selection (i.e. those traits influenced by a major locus with Mendelian inheritance). The extensive number of traits assessed in this study (the most ever assessed in a hop QTL analysis) enabled the identification of co-location between many secondary metabolites (which were mostly uncorrelated), which is indicative of pleiotropy or genetic linkage. This is the first demonstration of extensive pleiotropy/linkage affecting secondary metabolites in hop and has significant implications for hop breeders. Pleiotropy/linkage suggests that while there is potential for simultaneous and rapid changes in the levels of multiple secondary metabolites through the selection of these QTL, it may be difficult to select for specific secondary metabolites or particular profiles without causing adverse changes to others. In addition to these pleiotropic/linked loci, I identified a number of QTL in this study that appeared to be specific to individual secondary metabolites. As hop secondary metabolites are derived from common precursors, the specificity of the QTL identified for single compounds suggests that these QTL may affect genes, transcription factors or enzymes involved in later stages of biosynthesis or modification of these compounds. These QTL specific to individual secondary metabolites potentially offer hop breeders with an opportunity to directly select for these secondary metabolites.

The QTL analyses performed in Chapter 3 of this thesis also revealed a significant sex bias in inheritance of hop secondary metabolite phenotypes, where 10 of the 13 QTL regions identified were associated with the female parent. This significant partiality towards inheritance from the female parent may be due to dominance of the female parent at these loci, epigenetic effects or artificial selection. Although the nature of this sex bias is yet to be determined, the fact that it occurs has significant consequences for the choice of breeding parents for the selective improvement of hop.

Besides secondary metabolite traits, in Chapter 3 I also investigated QTL for yield traits. As mentioned earlier, I identified a significant QTL for one yield trait, dry cone weight, which explained 35% of the variation in this trait; but I did not find a QTL for two other yield traits, cone harvest index and green cone weight. Both cone harvest index and green cone weight are commonly used in hop cultivation to evaluate crop yields, but these findings indicate that these traits may have a low heritability, may be influenced by multiple loci each with small

effects, or may be confounded by environmental factors. The QTL identified for dry cone weight, however, has the potential to be used for direct selection to increase cone yield in hop.

Quantitative genetic analysis

The quantitative genetic analysis that I conducted in Chapter 4 of this study was the first quantitative genetics study (based only on additive genetic variation) that examined agronomic characteristics in hop. Besides providing estimates of additive genetic variation and narrow-sense heritability for 10 traits relating to emergence, height and lateral morphology, I identified previously undescribed differences between these agronomic traits and cone chemical traits in terms of their inheritance, as well as novel genetic relationships between these agronomic traits and hop cone chemistry. Significant genetic correlations between cone chemistry and agronomic traits were identified, such that families with more vigorous growth were associated with lower α-acid and β-acid levels and lower yields. This trend may reflect an underlying population structure of founder genotypes of European or North American genetic groups, as well as past selection and adaptation to the Australian environment. Regardless, it indicates that breeders can use low plant vigour as a proxy selection indicator of higher α-acid and β-acid levels in hop. Chemical traits were found to be more heritable than growth traits in hop, which may reflect the more intense genetic selection of hop chemical traits compared to growth traits, as a result of artificial selection in breeding or natural selection against herbivore tolerance of chemical profiles. This indicates that hop breeders can anticipate chemical traits to be more readily inherited than agronomic traits, which are likely to be influenced more by environmental factors. Male and female plants displayed significant genetic and phenotypic differences in many vegetative morphology traits. Previous studies on the sexual development of hop plants have found male and female plants to be indistinguishable until flowering [10], but I show in this study that male and female plants in the Australian environment differ in form as early as emergence and that differences in vegetative growth extend through to cone maturity.

Future perspectives

This study provides several resources which could be used to further develop genetic-based tools for hop research and breeding. The linkage maps I present in this study were constructed with a highly stringent method and incorporate a large number of transferable markers. As such, they provide a solid framework for mapping additional markers to produce a saturated consensus map for hop. In their current form, these maps can be employed in

other mapping populations, facilitating the environmental and ontogenetic validation of QTL identified in this study, as well as providing the means for further QTL analysis. There are many commercially relevant traits for which the presence of QTL could be investigated, including agronomic traits (such as those investigated in Chapter 4), physiological traits, pest and disease resistance and more secondary metabolite traits. With the addition of further markers, the linkage maps constructed in this study could also be used for further investigations of sex-linked markers located on autosomal regions of the hop genome. The linkage maps could also facilitate more precise QTL location, by methods such as association mapping or candidate gene screening, increasing their applicability for MAS. Despite early expectations that QTL could readily be used for MAS [54], successful application of QTL in many crops has mostly been with QTL that have large effects on traits and that have simple Mendelian inheritance [305]. If this is also found to be the case in hop, the markers developed in this study are still useful for genomic selection, a recently developed molecular selection technique. Genomic selection is a method based on prediction of performance, where performance is not determined by the presence of QTL, but rather the joint effects of many markers fitted as random effects in a linear model, with trait values predicted from a weighted index calculated from each marker [306]. This method has reported success for complex traits and those with low heritability, but as it focuses solely on genetic improvement, it is unable to provide the level of insight into the genetic basis of traits that QTL analysis provides [305, 307].

The findings of this study present several further avenues for research into the genetic architecture of hop. The genetic (and biological) basis for the high levels of marker clustering and segregation distortion in hop could be investigated further, to increase the understanding of genome organisation in hop. The inheritance and genetic variation of yield traits, such as green cone weight and harvest index could be further examined to deduce the reasons why these traits do not appear to be heritable in the QTL and quantitative genetic analyses, despite there being readily observable differences in these traits between cultivars. Genetic variation in α-acid could also be further investigated, as this trait was found to have heritable genetic variation in the quantitative genetic analysis, but a QTL was identified for this trait in only one of the mapping populations, suggesting that α-acid is polymorphic in only a limited range of germplasm. Further research into the sexual differentiation of hop could also be conducted, focusing in particular on the reasons for the sex bias in QTL identification as well as the underlying genetic basis of the genetic and phenotypic differences between male and female

plants identified in quantitative genetic analysis. Lastly, there is considerable scope for further quantitative genetic analysis of additional commercially relevant traits, such as essential oils, disease resistance and flowering, to provide information on the inheritance and genetic control of these traits.

Through these studies, I have improved the understanding of the genetic architecture of hop underlying variation in cone chemistry and important agronomic traits. Many of the findings can be incorporated into hop breeding programs for the development of more effective breeding strategies, or have the potential to be used directly for the selective improvement of hop. This thesis also highlights many potential areas for further research into the genetic control and biology of hop and provides a number of resources (markers, linkage maps, QTL and data) to assist with future investigations.

References

1. **Yang M-Q, van Velzen R, Bakker FT, Stattarian A, Li D-Z, Yi T-S: Molecular phylogenetics and character evolution of Cannabaceae.** ***Taxon*** **2013, 62:473-485.**
2. **Small E: A numerical and nomenclatural analysis of morpho-geographic taxa of** ***Humulus*****.** ***Syst Bot*** **1978, 3:37-76.**
3. **Delyser DY, Kasper WJ: Hopped beer: the case for cultivation.** ***Econ Bot*** **1994, 48:166-170.**
4. **Murakami A, Darby P, Javornik B, Pais MSS, Seigner E, Lutz A, Svoboda P: Molecular phylogeny of wild hops,** ***Humulus lupulus*** **L.** ***Heredity*** **2006, 97:66-74.**
5. **de Candolle A:** ***Origin of cultivated plants.*** **New York: Haffner; 1964.**
6. **Neve RA:** ***Hops.*** **London, UK: Chapman and Hall; 1991.**
7. **Wilson DG: Plant remains from the Graveny boat and the early history of** ***Humulus lupulus*** **L. in W. Europe.** ***New Phyt*** **1975, 75:627-648.**
8. **Thomas GG, Schwabe WW: Factors controlling flowering in the hop (*Humulus lupulus* L.).** ***Ann Bot*** **1969, 33:781-793.**
9. **Pavolovič V, Čerenak A, Pavolovič M, Košir IJ, Rozman Č, Bohanec M, Čeh B, Naglič B: Modelling of quality prediction for hops (*Humulus lupulus* L.) in relation to meteorological variables. In** ***BALWOIS; Ohrid, Republic of Macedonia*****. 2010**
10. **Shephard HL, Parker JS, Darby P, Ainsworth CC: Sexual development and sex chromosomes in hop.** ***New Phytol*** **2000, 148:397-411.**
11. **Parker JS, Clark MS: Dosage sex-chromosome systems in plants.** ***Plant Sci*** **1991, 80:79-92.**
12. **Oliveira MM, Pais MS: Glandular trichomes of** ***Humulus lupulus*** **var. Brewer's gold (hops): ultrastructural aspects of peltate trichomes.** ***J Submicrosc Cytol Pathol*** **1990, 22:241-248.**
13. **Menary RD, Doe PE: Some morphological and chemical changes in hops during maturation.** ***J Sci Food Agric*** **1983, 34:921-929.**
14. **Sugiyama R, Oda H, Kurosaki F: Two distinct phases of glandular trichome development in hop (*Humulus lupulus* L.).** ***Plant Biotechnol J*** **2006, 23:493-496.**
15. **Roberts MT, Dufour JP, Lewis AC: Application of comprehensive multidimensional gas chromatography combined with time-of-flight mass spectrometry (GC x GC-TOFMS) for high resolution analysis of hop essential oil.** ***J Sep Sci*** **2004, 27:473-478.**
16. **United Nations Statistics Division: Beer production. In** ***United Nations Statistical Yearbook*** **[http://unstats.un.org/unsd/syb/syb55/SYB_55.pdf]; 2011.**
17. **De Keukeleire D: Fundamentals of beer and hop chemistry.** ***Quimica Nova*** **2000, 23:108-112.**
18. **Hough JS, Howard GA, Slater CA: Bacteriostatic activities of hop resin materials.** ***J Inst Brew*** **1957, 63:331-333.**
19. **Michener HD, Snell N, Jansen EF: Antifungal activity of hop resin constituents and a new method for isolation of lupulon.** ***Arch Biochem Biophys*** **1948, 19.**
20. **Shimwell JL: On the relation between staining properties of bacteria and their reaction towards hops antiseptic.** ***J Inst Brew*** **1937, 43:111-118.**
21. **Behre KE: The history of beer additives in Europe - A review.** ***Veget Hist Archaeobot*** **1999, 8:35-48.**
22. **Verzele M: Centenary review: 100 years of hop chemistry and its relevance to brewing.** ***J Inst Brew*** **1986, 92:32-48.**
23. **Čeh B, Kač M, Košir IJ, Abram V: Relationships between xanthohumol and polyphenol content in hop leaves and hop cones with regard to water supply and cultivar.** ***Int J Mol Sci*** **2007, 8:989-1000.**
24. **Sharpe FR, Laws RJ: The essential oil of hops a review.** ***J Inst Brew*** **1981, 87:96-107.**
25. **Food and Agriculture Organisation of the United Nations: FAOSTAT. [http://faostat3.fao.org/home/index.html]; 2011.**
26. **Flythe MD: The antimicrobial effects of hops (*Humulus lupulus* L.) on ruminal hyper ammonia-producing bacteria.** ***Lett Appl Microbiol*** **2009, 48:712-717.**
27. **Larson AE, Yu RRY, Lee OA, Price S, Haas GJ, Johnson EA: Antimicrobial activity of hop extracts against** ***Listeria monocytogenes*** **in media and in food.** ***Int J Food Microbiol*** **1996, 33:195-207.**
28. **Dumas ER, Michaud AE, Bergeron C, Lafrance JL, Mortillo S, Gafner S: Deodorant effects of a supercritical hops extract: antibacterial activity against** ***Corynebacterium xerosis*** **and** ***Staphylococcus epidermidis*** **and efficacy testing of a hops/zinc ricinoleate stick in humans through the sensory evaluation of axillary deodorancy.** ***J Cosmet Dermatol*** **2009, 8:197-204.**
29. **Leite IR, Faria JR, Marquez LDS, Reis MHM, de Resende MM, Ribeiro EJ, Cardoso VL: Evaluation of hop extract as a natural antibacterial agent in contaminated fuel ethanol fermentations.** ***Fuel Process Technol*** **2013, 106:611-618.**

30. **Milligan S, Kalita J, Pocock V, Heyerick A, De Cooman L, Rong H, De Keukeleire D: Oestrogenic activity of the hop phyto-oestrogen, 8-prenylnaringenin.** ***Reproduction*** **2002, 123:235-242.**
31. **Miranda C, Stevens J, Helmrich A, Henderson M, Rodriguez R, Yang Y, Peinzer M, Barnes D, Buhler D: Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines.** ***Food Chem Toxicol*** **1999, 37:271-285.**
32. **Burgess AH:** ***Hops: botany, cultivation and utilization.*** **New York: Interscience Publishers Inc.; 1964.**
33. **Patzak J, Nesvadba V, Henychová A, Krofta K: Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses.** ***Biochem Syst Ecol*** **2010, 38:136-145.**
34. **Meilgaard M: Hop analysis, cohumulone factor and the bitterness of beer: review and critical evaluation.** ***J Inst Brew*** **1960, 66:35-50.**
35. **Haunold A: Hop production, breeding and variety development in various countries.** ***J Am Soc Brew Chem*** **1981, 39:27-34.**
36. **Moir M: Hops - a millennium review.** ***J Amer Soc Brew Chemist*** **2000, 58:131-146.**
37. **Salmon ES: The value of hop-breeding experiments.** ***J Inst Brew*** **1917, 23:60-97.**
38. **Darby P: The history of hop breeding and development.** ***Brewery History*** **2005, 121:94-112.**
39. **McMurrough I: High-performance liquid chromatography of flavonoids in barley and hops.** ***J Chromatogr A*** **1981, 218:683-693.**
40. **Buckee GK: Estimation of α-, β- and iso-α-acids in hops, hop products and beer using high performance liquid chromatography.** ***J Inst Brew*** **1985, 91:143-147.**
41. **Otter GE, Taylor L: The high performance liquid chromatrography of hop resins.** ***J Inst Brew*** **1978, 84:160-164.**
42. **Roborgh RHJ: The production of seedless varieties of hop (*Humulus lupulus*) with colchicine.** ***New Zeal J Agr Res*** **2012, 12:256-259.**
43. **Neve RA: Centenary review: hop breeding worldwide - its aims and achievements.** ***J Inst Brew*** **1986, 92:21-24.**
44. **Beatson RA: Breeding and development of hop cultivars in New Zealand. In** ***Proceedings of the Scientific Commission, International Hop Growers Convention (IHGC); Wye, England*****. 1993: 12-18.**
45. **Tester M, Langridge P: Breeding technologies to increase crop production in a changing world.** ***Science*** **2010, 327:818-822.**
46. **Allard RW, Bradshaw AD: Implications of genotype-environmental interactions in applied plant breeding.** ***Crop Sci*** **1964, 4:503-508.**
47. **Moose SP, Mumm RH: Molecular plant breeding as the foundation for 21st century crop improvement.** ***Plant Physiol*** **2008, 147:969-977.**
48. **Dudley JW, Moll RH: Interpretation and use of estimates of heritability and genetic variances in plant breeding.** ***Crop Sci*** **1969, 9:257-262.**
49. **Falconer DS, Mackay TFC:** ***Introduction to quantitative genetics.*** **Harlow, UK: Longman; 1996.**
50. **Brooks SN, Likens ST: Variability of morphological and chemical quality characters in flowers of male hops.** ***Crop Sci*** **1962, 2:189-196.**
51. **Jakše J, Kindlhofer K, Javornik B: Assessment of genetic variation and differentiation of hop genotypes by microsatellite and AFLP markers.** ***Genome*** **2001, 44:773-782.**
52. **McKey D, Elias M, Pujol B, Duputié A: The evolutionary ecology of clonally propagated domesticated plants.** ***New Phyt*** **2010, 186:318-332.**
53. **Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK: An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts.** ***Euphytica*** **2005, 142:169-196.**
54. **Collard BCY, Mackill DJ: Marker-assisted selection: an approach for precision plant breeding in the twenty-first century.** ***Philos Trans R Soc Lond B Biol Sci*** **2008, 363:557-572.**
55. **Winter P, Kahl G: Molecular marker technologies for plant improvement.** ***World J Microb Biot*** **1995, 11:438-448.**
56. **Gupta PK, Varshney RK, Sharma PC, Ramesh B: Molecular markers and their applications in wheat breeding.** ***Plant Breed*** **1999, 118:369-390.**
57. **Varshney RK, Graner A, Sorrells ME: Genomics-assisted breeding for crop improvement.** ***Trends Plant Sci*** **2005, 10:621-630.**
58. **Kumar LS: DNA markers in plant improvement: an overview.** ***Biotech Adv*** **1999, 17:143-182.**
59. **Mackay TFC: The genetic architecture of quantitative traits.** ***Annu Rev Genet*** **2001, 35:303-339.**
60. **Barton NH, Keightley PD: Understanding quantitative genetic variation.** ***Nat Rev Genet*** **2002, 3:11-21.**
61. **Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T: Genome mapping, molecular markers and marker-assisted selection in crop plants.** ***Mol Breed*** **1997, 3:87-103.**

62. **Dekkers JCM, Hospital F: The use of molecular genetics in the improvement of agricultural populations.** ***Nat Rev Genet*** **2002, 3:22-32.**
63. **Bouchez A, Hospital F, Causse M, Gallais A, Charcosset A: Marker-assisted introgression of favorable alleles at quantitative trait loci between maize elite lines.** ***Genetics*** **2002, 162:1945-1959.**
64. **Shen L, Courtois B, McNally KL, Robin S, Li Z: Evaluation of near-isogenic lines of rice introgressed with QTLs for root depth through marker-aided selection.** ***Theor Appl Genet*** **2001, 103:75-83.**
65. **Steele KA, Price AH, Shashidhar HE, Witcombe JR: Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety.** ***Theor Appl Genet*** **2006, 112:208-221.**
66. **Toojinda T, Tragoonrung S, Vanavichit A, Siangliw JL, Pa-In N, Jantaboon J, Siangliw M, Fukai S: Molecular breeding for rainfed lowland rice in the Mekong region.** ***Plant Prod Sci*** **2005, 8:330-333.**
67. **Zhong S, Toubia-Rahme H, Steffenson BJ, Smith KP: Molecular mapping and marker-assisted selection of genes for septoria speckled leaf blotch resistance in barley.** ***Phytopathology*** **2006, 96:993-999.**
68. **Yoshimura S, Yoshimura A, Iwata N, McCouch S, Abenes ML, Baraoidan MR, Mew T, Nelson RJ: Tagging and combining bacterial blight resistance genes in rice using RAPD and RFLP markers.** ***Mol Breed*** **1995, 1.**
69. **Zhou PH, Tan YF, He YQ, Xu CG, Zhang Q: Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection.** ***Theor Appl Genet*** **2003, 106:326-331.**
70. **Joseph M, Gopalakrishnan S, Sharma RK, Singh VP, Singh AK, Singh NK, Mohapatra T: Combining bacterial blight resistance and Basmati quality characteristics by phenotypic and molecular marker-assisted selection in rice.** ***Mol Breed*** **2004, 13:377-387.**
71. **Schmierer D, Kandemir N, Kudrna D, Jones B, Ullrich S, Kleinhofs A: Molecular marker-assisted selection for enhanced yield in malting barley.** ***Mol Breed*** **2004, 14:463-473.**
72. **Lynch M, Walsh B:** ***Genetics and analysis of quantitative traits.*** **Sunderland: Sinauer Associates Inc.; 1998.**
73. **Lande R, Arnold SJ: The measurement of selection on correlated characters.** ***Evolution*** **1983, 37:1210-1226.**
74. **Beatson RA, Alspach PA: The use of empirical breeding values to improve genetic progress in hops.** ***Acta Hortic (ISHS)*** **2008, 848:93-100.**
75. **Brooks SN: Effectiveness of selection within Fuggle hops (*Humulus lupulus* L.).** ***Crop Sci*** **1962, 2:5-10.**
76. **Farrar RF, Neve RA, Weston EW: Inheritance of resin characters in hops. In** ***Wye College Department of Hop Research Annual Report*****; 1955.**
77. **Henning J, Haunold A, Nickerson G: Genetic parameter estimates for five traits in male hop accesssions: a preliminary study.** ***J Am Soc Brew Chem*** **1997, 55:157-160.**
78. **Henning J, Haunold A, Nickerson G, Gampert U: Estimates of heritability and genetic correlation for five traits in female hop accessions.** ***J Am Soc Brew Chem*** **1997, 55:161-165.**
79. **Henning JA, Townsend MS: Field-based estimates of heritability and genetic correlations in hop.** ***Crop Sci*** **2005, 45:1469-1475.**
80. **Keller KR, Likens ST: Estimates of heritability in hop,** ***Humulus lupulus*** **L.** ***Agron J*** **1955, 47:518-512.**
81. **Kralj D, Haunold A: The breeding potential of native hops (*Humulus lupulus* L.) from Yugoslavia.** ***Monatsschrift Brauwiss*** **1987, 40:287-293.**
82. **Likens ST, Nickerson GB, Haunold A, Zimmermann CE: Relationship between alpha acids, beta acids and lupulin content of hops.** ***Crop Sci*** **1978, 17:380-386.**
83. **Murakami A: Inheritance of major chemical components in hops.** ***J Inst Brew*** **1999, 105:107-111.**
84. **Roberts DD, Kronstad WE, Haunold A: Genetic variability and association of maturity, yield, and quality characteristics of female hops.** ***Crop Sci*** **1980, 20:523-527.**
85. **Versluys JP: Choice of male and female parents of seedling populations of hops in relation to the alpha-acid content and the time fo ripening in the female progeny.** ***J Inst Brew*** **1969, 75:25-26.**
86. **Hautke P, Petříček D: Inheritance of biochemical characteristics in hops - report of a brief study.** ***Wallerstein Comm*** **1967, 30:135-138.**
87. **Brooks SN: Associaton of quality characters in flowers of male hops.** ***Crop Sci*** **1962, 2:192-196.**
88. **Koie K, Inaba A, Okada I, Kaneko T, Ito K: Construction of the genetic linkage map and QTL analysis on hop (*Humulus lupulus* L.).** ***Acta Hortic (ISHS)*** **2005, 668:59-66.**
89. **Jakše J, Satovic Z, Javornik B: Microsatellite variability among wild and cultivated hops (*Humulus lupulus* L.).** ***Genome*** **2004, 47:889-899.**
90. **Jakse J, Luthar Z, Javornik B: New polymorphic dinucleotide and trinucleotide microsatellite loci for hop *Humulus lupulus* L.** ***Mol Ecol Resour*** **2008, 8:769-772.**

91. Aleksandrov OS, Divashuk MG, Karlov GI: Development of a sex-specific molecular marker for Japanese hop *Humulus japonicus* Siebold & Zucc. *Russ J Genet* 2011, 47:1016-1020.

92. Bassil NV, Gilmore B, Oliphant JM, Hummer KE, Henning JA: Genic SSRs for European and North American hop (*Humulus lupulus* L.). *Genet Resour Crop Evol* 2008, 55:959-969.

93. Brady JL, Scott NS, Thomas MR: DNA typing of hops (*Humulus lupulus*) through application of RAPD and microsatellite marker sequences converted to sequence tagged sites (STS). *Euphytica* 1996, 91:277-284.

94. Cerenak A, Satovic Z, Javornik B: Genetic mapping of hop (*Humulus lupulus* L.) applied to the detection of QTLs for alpha-acid content. *Genome* 2006, 49:485-494.

95. Cerenak A, Satovic Z, Jakse J, Luthar Z, Carovic-Stanko K, Javornik B: Identification of QTLs for alpha acid content and yield in hop (*Humulus lupulus* L.). *Euphytica* 2009, 170:141-154.

96. Danilova TV, Danilov SS, Karlov GI: Analysis of molecular-genetic polymorphism in the common hop (*Humulus lupulus* L.) cultivars by use of ISSR-PCR technique. *Russ J Genet* 2003, 39:1484-1489.

97. Hadonou AM, Walden R, Darby P: Isolation and characterization of polymorphic microsatellites for assessment of genetic variation of hops (*Humulus lupulus* L.). *Mol Ecol Notes* 2004, 4:280-282.

98. Hartl L, Seefelder S: Diversity of selected hop cultivars detected by fluorescent AFLPs. *Theor Appl Genet* 1998, 96:112-116.

99. Henning JA, Townsend MS, Gent DH, Bassil N, Matthews P, Buck E, Beatson R: QTL mapping of powdery mildew susceptibility in hop (*Humulus lupulus* L.). *Euphytica* 2011, 180:411-420.

100. Jakše J, Javornik B: High throughput isolation of microsatellites in hop (*Humulus lupulus* L.). *Plant Mol Biol Rep* 2001, 19:217-226.

101. Jakse J, Bandelj D, Javornik B: Eleven new microsatellites for hop (*Humulus lupulus* L.). *Mol Ecol Notes* 2002, 2:544:546.

102. Koelling J, Coles MC, Matthews PD, Schwekendiek A: Development of new microsatellite markers (SSRs) for *Humulus lupulus*. *Mol Breed* 2012, 30:479-484.

103. Murakami A: Hop variety classification using the genetic distance based on RAPD. *J Inst Brew* 2000, 106:157-161.

104. Murakami A, Darby P, Javornik B, Pais MSS, Seigner E, Lutz A, Svoboda P: Microsatellite DNA analysis of wild hops, *Humulus lupulus* L. *Genet Resour Crop Evol* 2006, 53:1553-1562.

105. Patzak J: Comparison of RAPD, STS, ISSR and AFLP molecular methods used for assessment of genetic diversity in hop (*Humulus lupulus* L.). *Euphytica* 2001, 121:9-18.

106. Patzak J, Vrba L, Matoušek J: New STS molecular markers for assessment of genetic diversity and DNA fingerprinting in hop (*Humulus lupulus* L.). *Genome* 2007, 50:15-25.

107. Patzak J, Matoušek J: Development and evaluation of expressed sequence tag-derived microsatellite markers for hop genotyping. *Biol Plantarum* 2011, 55:761-765.

108. Peredo EL, Revilla MÁ, Reed BM, Javornik B, Cires E, Prieto JAF, Arroyo-García R: The influence of European and American wild germplasm in hop (*Humulus lupulus* L.) cultivars. *Genet Resour Crop Evol* 2010, 57:575-586.

109. Seefelder S, Ehrmaier H, Schweizer G, Seigner E: Genetic diversity and phylogenetic relationships among accessions of hop, *Humulus lupulus*, as determined by amplified fragment length polymorphism fingerprinting compared with pedigree data. *Plant Breed* 2000, 119:257-263.

110. Seefelder S, Lutz A, Seigner E: Development of molecular markers for powdery mildew resistance to support breeding for high quality hops. *Monatsschrift Brauwiss* 2006, 59:100-104.

111. Stajner N, Jakse J, Kozjak P, Javornik B: The isolation and characterisation of microsatellites in hop (*Humulus lupulus* L.). *Plant Sci* 2005, 168:213-221.

112. Šuštar-Vozlič J, Javornik B: Genetic relationships in cultivars of hop, *Humulus lupulus* L., determined by RAPD analysis. *Plant Breed* 1999, 118:175-181.

113. Stajner N, Satovic Z, Cerenak A, Javornik B: Genetic structure and differentiation in hop (*Humulus lupulus* L.) as inferred from microsatellites. *Euphytica* 2008, 161:301-311.

114. Seefelder S, Ehrmaier H, Schweizer G, Seigner E: Male and female genetic linkage map of hops, *Humulus lupulus*. *Plant Breed* 2000, 119:249-255.

115. Patzak J, Vejl P, Skupinová S, Nesvadba V: Identification of sex in F1 progenies of hop (*Humulus lupulus* L.) by molecular marker. *Plant Soil Environ* 2002, 48:318-321.

116. Polley A, Seigner E, Ganal MW: Identification of sex in hop (*Humulus lupulus*) using molecular markers. *Genome* 1997, 40:357-361.

117. Patzak J, Henychova A, Krofta K, Nesvadba V: Study of molecular markers for xanthohumol and DMX contents in hop (*Humulus lupulus* L.) by QTLs mapping analysis. *Brew Sci* 2012, 65:96-102.

118. Jaccoud D, Peng K, Feinstein D, Kilian A: Diversity arrays: a solid state technology for sequence information independent genotyping. *Nucleic Acids Res* 2001, 29:E25.

119. Goese M, Kammhuber K, Bacher A, Zenk MH, Eisenreich W: Biosynthesis of bitter acids in hops. *Eur J Biochem* 1999, 263:447-454.

120. **Tressl R, Engel KH, Kossa M, Köppler H: Characterization of tricyclic sesquiterpenes in hop (*Humulus lupulus*, var. Hersbrucker Spät). *J Agric Food Chem* 1983, 31:892-897.**
121. **Roberts JB, Stevens R: Composition and biogenesis of essential oil in hop. *J Inst Brew* 1962, 68:420-426.**
122. **Mori Y: Mechanism of humulone and lupulone synthesis in the hop cone: free amino acid in the developing cones of hops. *Bull Brew Sci* 1961, 6:19-22.**
123. **Liu J, Burdette JE, Xu H, Gu C, Van Breemen RB, Bhat KPL, Booth N, Constantinou AI, Pezzuto JM, Fong HHS, Farnsworth NR, Bolton JL: Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem* 2001, 49:2472-2479.**
124. **Patzak J, Nesvadba V, Krofta K, Henychová A, Inalovic M, Richards K: Evaluation of genetic variability of wild hops (*Humulus lupulus* L.) in Canada and Caucasus region by chemical and molecular methods. *Genome* 2010, 53:545-557.**
125. **Townsend MS, Henning JA: AFLP discrimination of native North American and cultivated hop. *Crop Sci* 2009, 49:600-607.**
126. **Davis E: Morphological complexes in hops (*Humulus lupulus* L.) with special reference to the American race. *Ann Mo Bot Gard* 1957, 44:271-294.**
127. **Small E: The relationships of hop cultivars and wild variants of *Humulus lupulus. Can J Bot* 1980, 58:676-686.**
128. **Small E: A numerical analysis of morph-geographic groups of cultivars of *Humulus lupulus* based on samples of cones. *Can J Bot* 1981, 59:311-324.**
129. **Nickerson GB, Williams PA, Haunold A: Composition of male hop oil. *J Am Soc Brew Chem* 1988, 46:14-17.**
130. **Green CP: Gas-liquid chromatography, hop oils and variety identification. *English Hops* 1986, 6:10-11.**
131. **Kralj D, Zupanec J, Vasilj D, Kralj S, Psenicnik J: Variability of essential oils of hops, *Humulus lupulus* L.. *J Inst Brew* 1991, 97:197-206.**
132. **Kammhuber K: Investigations about the contents of the lupulin gladns of hop leaves and their importance for hop breeding. *Monatsschrift Brauwiss* 1997, 50:210-213.**
133. **Stevens JF, Taylor AW, Nickerson GB, Ivancic M, Henning J, Haunold A, Deinzer ML: Prenylflavonoid variation in *Humulus lupulus:* distribution and taxonomic significance of xanthogalenol and 4'-O'methylxanthohumol. *Phytochemistry* 2000, 53:759-775.**
134. **Henning JA, Steiner JJ, Hummer KE: Genetic diversity among world hop accessions grown in the USA. *Crop Sci* 2004, 44:411-417.**
135. **Danilova TV, Danilov SS, Karlov GI: Assessment of genetic polymorphism in hop (*Humulus lupulus* L.) cultivars by ISSR-PCR analysis. *Russ J Genet* 2003, 39:1252-1257.**
136. **Luikart G, England PR, Tallmon D, Jordan S, Taberlet P: The power and promise of population genomics: from genotyping to genome typing. *Nat Rev Genet* 2003, 4:981-994.**
137. **Wenzl P, Carling J, Kudrna D, Jaccoud D, Huttner E, Kleinhofs A, Kilian A: Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proc Natl Acad Sci USA* 2004, 101:9915-9920.**
138. **Kilian A, Huttner E, Wenzl P, Jaccoud D, Carling J, Caig V, Evers M, Heller-Uszynska K, Uszynski G, Cayla C, Patarpuwadol S, Xia L, Yang S, Thomson B: The fast and the cheap: SNP and DArT-based whole genome profiling for crop improvement. In *Proceedings of the international congress in the wake of the double helix: from the green revolution to the gene revolution; Bologna, Italy*. Edited by Tuberosa R, Phillips R, Gale M. Avenue Media; 2005: 443-461.**
139. **Wittenberg AHJ, Van Der Lee T, Cayla C, Kilian A, Visser RGF, Schouten HJ: Validation of the high-throughput marker technology DArT using the model plant *Arabidopsis thaliana*. *Mol Genet Genomics* 2005, 274:30-39.**
140. **Jakše J, Stajner N, Luthar Z, Jeltsch JM, Javornik B: Development of transcript-associated microsatellite markers for diversity and linkage mapping studies in hop (*Humulus lupulus* L.). *Mol Breed* 2011, 28:227-239.**
141. **Kump B, Javornik B: Evaluation fo genetic variability among common buckwheat (*Fagopyrum exculentum* Monech) publications by RAPD markers. *Plant Sci* 1996, 114:149-158.**
142. **Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME: Optimizing parental selection for genetic-linkage maps. *Genome* 1993, 36:181-186.**
143. **Nei M, Li W-H: Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 1979, 76:5269-5273.**
144. **Swofford DL: PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts, USA: Sinauer; 2002.**
145. **Rohlf FJ: NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.1. Setauket: Exeter Software; 2000.**

146. Excoffier L, Smouse PE, Quattro JM: Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 1992, 131:479-491.

147. Wright S: The genetical structure of populations. *Ann Eugen* 1951, 15:323-354.

148. Tajima F: Evolutionary relationship of DNA sequences in finite populations. *Genetics* 1983, 105:437-460.

149. Nei M: *Molecular evolutionary genetics.* New York, USA: Columbia University Press; 1987.

150. ('Student') Gosset WS: The probable error or a mean. *Biometrika* 1908, VI:1-25.

151. Pritchard JK, Stephens M, Donnelly P: Inference of population structure using multilocus genotype data. *Genetics* 2000, 155:945-959.

152. Evanno G, Regnaut S, Goudet J: Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 2005, 14:2611-2620.

153. Earl DA: Structure Harvester v0.3 [http://users.soe.ucsc.edu/~dearl/software/struct_harvest/]; 2009.

154. Jakobsson M, Risenberg NA: CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 2007, 23:1801-1806.

155. Rosenberg NA: DISTRUCT: a program for the graphical display of population structure. *Mol Ecol Notes* 2004, 4:137-138.

156. Akbari M, Wenzl P, Caig V, Carling J, Xia L, Yang S, Uszynski G, Mohler V, Lehmensiek A, Kuchel H, Hayden MJ, Howes N, Sharp P, Vaughan P, Rathmell B, Huttner E, Kilian A: Diversity Arrays Technology (DArT) for high-throughput profiling of the hexaploid wheat genome. *Theor Appl Genet* 2006, 113:1409-1420.

157. Xia L, Peng K, Yang S, Wenzl P, Carmen De Vicente M, Fregene M, Kilian A: DArT for high-throughput genotyping of Cassava (*Manihot esculenta*) and its wild relatives. *Theor Appl Genet* 2005, 110:1092-1098.

158. Heller-Uszynska K, Uszynski G, Huttner E, Evers M, Carlig J, Caig V, Aitken K, Jackson P, Piperidis G, Cox M, Gilmour R, D'Hont A, Butterfield M, Glaszmann JC, Kilian A: Diversity Arrays Technology effectively reveals DNA polymorphism in a large and complex genome of sugarcane. *Mol Breed* 2010, 28:37-55.

159. Tinker NA, Kilian A, Wight CP, Heller-Uszynska K, Wenzl P, Rines HW, Bjørnstad Å, Howarth CJ, Jannink JL, Anderson JM, Rossnagel BG, Stuthman DD, Sorrells ME, Jackson EW, Tuvesson S, Kolb FL, Olsson O, Federizzi L, Carson ML, Ohm HW, Molnar SJ, Scoles GJ, Eckstein PE, Bonman JM, Ceplitis A, Langdon T: New DArT markers for oat provide enhanced map coverage and global germplasm characterization. *BMC Genomics* 2009, 10:39.

160. Yang S, Pang W, Ash G, Harper J, Carling J, Wenzl P, Huttner E, Zong X, Kilian A: Low level of genetic diversity in cultivated Pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theor Appl Genet* 2006, 113:585-595.

161. James KE, Schneider H, Ansell SW, Evers M, Robba L, Uszynski G, Pedersen N, Newton AE, Russell SJ, Vogel JC, Kilian A: Diversity Arrays Technology (DArT) for pan-genomic evolutionary studies of non-model organisms. *PLoS ONE* 2008, 3:e1682.

162. Risterucci AM, Hippolyte I, Perrier X, Xia L, Caig V, Evers M, Huttner E, Kilian A, Glaszmann JC: Development and assessment of diversity arrays technology for high-throughput DNA analyses in musa. *Theor Appl Genet* 2009, 119:1093-1103.

163. Kopecký D, Bartoš J, Lukaszewski AJ, Baird JH, Černoch V, Kölliker R, Rognli OA, Blois H, Caig V, Lübberstedt T, Studer B, Shaw P, Doležel J, Kilian A: Development and mapping of DArT markers within the *Festuca - Lolium* complex. *BMC Genomics* 2009, 10:473.

164. Chen J, Chang SX, Anyia AO: Gene discovery in cereals through quantitative trait loci and expression analysis in water-use efficiency measured by carbon isotope discrimination. *Plant Cell Environ* 2011, 34:2009-2023.

165. Singh D, Kumar A, Chauhan P, Kumar V, Kumar N, Singh A, Mahajan N, Sirohi P, Chand S, Ramesh B, Singh J, Kumar P, Kumar R, Yadav RB, Naresh RK: Marker assisted selection and crop management for salt tolerance: A review. *Afr J Biotechnol* 2011, 10:14694-14698.

166. Gupta PK, Kumar J, Mir RR, Kumar A: Marker assisted selection as a component of conventional plant breeding. *Plant Breed Rev* 2010, 33:145-217.

167. Kumar J, Choudhary AK, Solanki RK, Pratap A: Towards marker-assisted selection in pulses: a review. *Plant Breed* 2011, 130:297-313.

168. Churchill GA, Doerge RW: Mapping quantitative trait loci in experimental populations. In *Molecular dissection of complex traits.* Edited by Paterson AH. USA: CRC Press LLC; 1998: 31-41

169. Francia E, Tacconi G, Crosatti C, Barabaschi D, Bulgarelli D, Dall'Aglio E, Valè G: Marker assisted selection in crop plants. *Plant Cell Tiss Organ Cult* 2005, 82:317-342.

170. Kearsey MJ, Farquhar AGL: QTL analysis in plants; where are we now? *Heredity* 1997, 80:137-142

171. **Varshney RK, Graner A, Sorrells ME: Genic microsatellite markers in plants: features and applications.** ***Trends Biotechnol*** **2005, 23:48-55.**
172. **Young ND: A cautiously optimistic vision for marker-assisted breeding.** ***Mol Breed*** **1999, 5:505-510.**
173. **Buck EJ, Wiedow C, Carlisle C, Chagné D, Beatson R: Validation of new sex specific DNA markers for hops (*Humulus lupulus* L).** ***Acta Hortic (ISHS)*** **2009, 848:323-328.**
174. **Danilova TV, Karlov GI: Application of inter simple sequence repeat (ISSR) polymorphism for detection of sex-specific molecular markers in hop (*Humulus lupulus* L.).** ***Euphytica*** **2006, 151:15-21.**
175. **Divashuk MG, Alexandrov OS, Kroupin PY, Karlov GI: Molecular cytogenetic mapping of *Humulus lupulus* sex chromosomes.** ***Cytogenet Genome Res*** **2011, 134:213-219.**
176. **Jakse J, Stajner N, Kozjak P, Cerenak A, Javornik B: Trinucleotide microsatellite repeat is tightly linked to male sex in hop (*Humulus lupulus* L.).** ***Mol Breed*** **2008, 21:139-148.**
177. **Cerenak A, Kralj D, Javornik B: Compounds of essential oils as markers of hop resistance (*Humulus lupulus*) to powdery mildew (*Podosphaera macularis*).** ***Acta Agric Slov*** **2009, 93:267-273.**
178. **Seigner E, Lutz A, Radic-Miehle H, Seefelder S: Breeding and development of the hop varieties at the hop research centre Huell. In** ***Proceedings of the Scientific Commission, International Hop Growers Convention (IHGC); George, South Africa*****. 2005: 18-22.**
179. **Grabowska-Joachimiak A, Sliwinska E, Pigula M, Skomra U, Joachimiak AJ: Genome size in *Humulus lupulus* L. and *H. japonicus* Siebold & Zucc. (Cannabaceae).** ***Acta Soc Bot Pol*** **2006, 75:207-214.**
180. **Bennett MD, Leitch IJ: Angiosperm DNA C-values database (release 7.0) [http://www.kew.org/cvalues/]; 2010.**
181. **Winge Ö: On the nature of sex chromosomes in *Humulus*.** ***Hereditas*** **1929, 12::53-63.**
182. **Karlov GI, Danilova TV, Horlemann C, Weber G: Molecular cytogenetics in hop (*Humulus lupulus* L.) and identification of sex chromosomes by DAPI-banding.** ***Euphytica*** **2003, 132:185-190.**
183. **Hughes PS, Simpson WJ: Bitterness of congeners and stereoisomers of hop-derrived bitter acids found in beer.** ***J Am Soc Brew Chem*** **1996, 54:234-237.**
184. **Peacock VE, Deinzer ML: Chemistry of hop aroma in beer.** ***J Am Soc Brew Chem*** **1981, 39:136-141.**
185. **Heslop-Harrison JS, Schwarzacher T: Genetics and genomics of crop domestication. In** ***Plant biotechnology and agriculture: prospects for the 21st century.*** **Edited by Altman A, Hasegawa PM. London, UK: Academic Press; 2011: 1-16**
186. **Blum A:** ***Plant breeding for stress environments.*** **Florida, USA: CRC Press Inc.; 1988.**
187. **Svoboda P, Hniličková H, Hnilička F: Changes in yield and quality of hop depending on the irrigation.** ***Cereal Res Commun*** **2008, 36:891-894.**
188. **Srečec S, Kvaternjak I, Kaučić D, Špoljar A, Erhatić R: Influence of climatic conditions on accumulation of α-acids in hop cones.** ***Agric Conspec Sci*** **2008, 73:161-166.**
189. **Kučera J, Krofta K: Mathematical model for prediction of alpha acid contents from meteorological data for 'Saaz' aroma variety.** ***Acta Hortic (ISHS)*** **2009, 848:131-140.**
190. **Kořen J: Influence of plantation row spacing on quality and yield of hops.** ***Plant Cell Environ*** **2007, 53:276-282.**
191. **Pethybridge SJ, Wilson CR, Hay FS, Leggett GW, Sherriff LJ: Effect of viruses on agronomic and brewing characteristics of four hop (*Humulus lupulus*) cultivars in Australia.** ***Ann Appl Biol*** **2002, 140:97-105.**
192. **Weihrauch F: Evaluation of a damage threshold for two-spotted spider mites, *Tetranychus urticae* Koch (Acari: Tetranychidae), in hop culture.** ***Ann Appl Biol*** **2005, 146:501-509.**
193. **Krofta K, Nesvadba V: How hop powdery mildew influences the quality of hops and beer? In** ***Proceedings of the Scientific Commission, International Hop Growers Convention (IHGC); Žalec, Slovenia*****. 2003: 58-62.**
194. **Lorenzana A, De Hermoso Mendoza A, Seco MV: Thresholds for management of *phorodon humuli* (hemiptera: Aphididae) on hops.** ***J Entomol Sci*** **2009, 44:198-208.**
195. **Benitez JL, Forster A, De Keukeleire D, Moir M, Sharpe FR, Verhagen LC, Westwood KT: Hops and hop products. In** ***Hans Carl-Verlag; Nurenberg, Germany*****. 1997**
196. **Clark SM, Vaitheeswaran V, Ambrose SJ, Purves RW, Page JE: Transcriptome analysis of bitter acid biosynthesis and precursor pathways in hop (*Humulus lupulus*).** ***BMC Plant Biol*** **2013, 13:1.**
197. **Murphey JM, Probasco G: The development of brewing quality characteristics in hops during maturation.** ***Tech Q Master Brew Assoc Am*** **1996, 33:149-159.**
198. **De Keukeleire J, Ooms G, Heyerick A, Roldan-Ruiz I, Van Bockstaele E, De Keukeleire D: Formation and accumulation of α-acids, β-acids, desmethylxanthohumol, and xanthohumol during flowering of hops (*Humulus lupulus* L.).** ***J Agric Food Chem*** **2003, 51:4436-4441.**

199. Van Cleemput M, Cattoor K, De Bosscher K, Haegeman G, De Keukeleire D, Heyerick A: Hop (*Humulus lupulus*)-derived bitter acids as multipotent bioactive compounds. *J Nat Prod* 2009, 72:1220-1230.

200. Eri S, Khoo BK, Lech J, Hartman TG: Direct thermal desorption-gas chromatography and gas chromatography-mass spectrometry profiling of hop (*Humulus lupulus* L.) essential oils in support of varietal characterization. *J Agric Food Chem* 2000, 48:1140-1149.

201. Nagel J, Culley LK, Lu Y, Liu E, Matthews PD, Stevens JF, Page JE: EST analysis of hop glandular trichomes identifies an O-methyltransferase that catalyzes the biosynthesis of xanthohumol. *Plant Cell* 2008, 20:186-200.

202. Wang G, Tian L, Aziz N, Broun P, Dai X, He J, King A, Zhao PX, Dixon RA: Terpene biosynthesis in glandular trichomes of hop. *Plant Physiol* 2008, 148:1254-1266.

203. Zuurbier KWM, Fung S, Scheffer JJC, Verpoorte R: *In vitro* prenylation of aromatic intermediates in the biosynthesis of bitter acids in *Humulus lupulus*. *Phytochemistry* 1998, 49:2315-2322.

204. Okada Y, Yamazaki Y, Suh D, Sankawa U, Ito K: Biofunctional activities of valerophenone synthase in hop (*Humulus lupulus* L.). *J Am Soc Brew Chem* 2001, 59:163-166.

205. Wang G, Dixon RA: Heterodimeric geranyl(geranyl)diphosphate synthase from hop (*Humulus lupulus*) and the evolution of monoterpene biosynthesis. *Proc Natl Acad Sci USA* 2009, 106:9914-9919.

206. Matoušek J, Novák P, Bříza J, Patzak J, Niedermeierová H: Cloning and characterisation of chs-specific DNA and cDNA sequences from hop (*Humulus lupulus* L.). *Plant Sci* 2002, 162:1007-1018.

207. Okada Y, Ito K: Cloning and analysis of valerophenone synthase gene expressed specifically in lupulin gland of hop (*Humulus lupulus* L.). *Biosci Biotechnol Biochem* 2001, 65:150-155.

208. Paniego NB, Zuurbier KWM, Fung SY, Van Der Heijden R, Scheffer JJC, Verpoorte R: Phlorisovalerophenone synthase, a novel polyketide synthase from hop (*Humulus lupulus* L.) cones. *Eur J Biochem* 1999, 262:612-616.

209. Novák P, Matoušek J, Bříza J: Valerophenone synthase-like chalcone synthase homologues in *Humulus lupulus*. *Biol Plant* 2003, 46:375-381.

210. Howard EL, Whittock SP, Jakše J, Carling J, Matthews PD, Probasco G, Henning JA, Darby P, Cerenak A, Javornik B, Kilian A, Koutoulis A: High-throughput genotyping of hop (*Humulus lupulus* L.) utilising diversity arrays technology (DArT). *Theor Appl Genet* 2011, 122:1265-1280.

211. Xu Y, Zhu L, Xiao J, Huang N, McCouch SR: Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). *Mol Gen Genet* 1997, 253:535-545.

212. Bradshaw HD, Stettler RF: Molecular genetics of growth and development in *Populus* II. segregation distortion due to genetic load. *Theor Appl Genet* 1994, 89:551-558.

213. Myburg AA, Vogl C, Griffin AR, Sederoff RR, Whetten RW: Genetics of postzygotic isolation in *Eucalyptus*: whole-genome analysis of barriers to introgression in a wide interspecific cross of *Eucalyptus grandis* and *E. globulus*. *Genetics* 2004, 166:1405-1418.

214. Hackett CA, Broadfoot LB: Effects of genotyping errors, missing values and segregation distortion in molecular marker data on the construction of linkage maps. *Heredity* 2003, 90:33-38.

215. Milczarski P, Bolibok-Bragoszewska H, Myśków B, Stojałowski S, Heller-Uszyńska K, Góralska M, Bragoszewski P, Uszyński G, Kilian A, Rakoczy-Trojanowska M: A high density consensus map of rye (*Secale cereale* L.) based on DArT markers. *PLoS ONE* 2011, 6:12.

216. Brondani R, Williams E, Brondani C, Grattapaglia D: A microsatellite-based consensus linkage map for species of *Eucalyptus* and a novel set of 230 microsatellite markers for the genus. *BMC Plant Biol* 2006, 6:20.

217. Kammholz SJ, Campbella AW, Sutherland MW, Hollamby GJ, Martin PJ, Eastwood RF, Barclay I, Wilson RE, Brennan PS, Sheppard JA: Establishment and characterisation of wheat genetic mapping populations. *Aust J Agric Res* 2001, 52:1079-1088.

218. Mayerhofer R, Archibald C, Bowles V, Good AG: Development of molecular markers and linkage maps for the *Carthamus* species *C. tinctorius* and *C. oxyacanthus*. *Genome* 2010, 53:266-276.

219. Luo L, Xu S: Mapping viability loci using molecular markers. *Heredity* 2003, 90:459-467.

220. Menz MA, Klein RR, Mullet JE, Obert JA, Unruh NC, Klein PE: A high-density genetic map of *Sorghum bicolor* (L.) Moench based on 2926 AFLP®, RFLP and SSR markers. *Plant Mol Bio* 2002, 48:483-499.

221. Beavis WD: QTL analysis: power, precision, and accuracy. In *Molecular dissection of complex traits.* Edited by Patterson HA. Boca Raton, Florida: CRC Press; 1998: 145-162

222. Bautista RC, Siebenmorgen TJ: Individual rice kernel moisture content variability trends. *Appl Eng Agric* 2005, 21:637-643.

223. Khir R, Pan Z, Atungulu GG, Thompson JF, Shao D: Size and moisture distribution characteristics of walnuts and their components. *Food Bioprocess Tech* 2011:1-12.

224. Sala RG, Andrade FH, Westgate ME: Maize kernel moisture at physiological maturity as affected by the source-sink relationship during grain filling. *Crop Sci* 2007, 47:711-716.

225. Lanceras JC, Pantuwan GP, Jongdee B, Toojinda T: Quantitative trait loci associated with drought tolerance at reproductive stage in rice. *Plant Physiol* 2004, 135:384-399.

226. Golabadi M, Arzani A, Mirmohammadi Maibody SAM, Tabatabaei BES, Mohammadi SA: Identification of microsatellite markers linked with yield components under drought stress at terminal growth stages in durum wheat. *Euphytica* 2011, 177:207-221.

227. Rehman AU, Malhotra RS, Bett K, Tar'an B, Bueckert R, Warkentin TD: Mapping QTL associated with traits affecting grain yield in chickpea (*Cicer arietinum* L.) under terminal drought stress. *Crop Sci* 2011, 51:450-463.

228. Beatson RA, Inglis TE: Development of aroma hop cultivars in New Zealand. *J Inst Brew* 1999, 105:382-385.

229. Haunold A, Nickerson GB: Dvelopment of a hop with European aroma characteristics. *J Am Soc Brew Chem* 1987, 45:146-151.

230. Davies NW, Menary RC: Essential oils of Tasmanian grown hops. *J Inst Brew* 1982, 88:80-83.

231. Kač M, Kovačevič M: Presentation and determination of hop (*Humulus lupulus* L.) cultivars by a min-max model on composition of hop essential oil. *Monatsschrift Brauwiss* 2000, 53:180-184.

232. Kovačevič M, Kač M: Determination and verification of hop varieties by analysis of essential oils. *Food Chem* 2002, 77:489-494.

233. Likens ST, Nickerson GB: Identification of hop varieties by gas chromatographic analysis of their essential oils: constancy of oil composition under various environmental influences. *J Agric Food Chem* 1967, 15:525-530.

234. O'Reilly-Wapstra JM, Freeman JS, Davies NW, Vaillancourt RE, Fitzgerald H, Potts BM: Quantitative trait loci for foliar terpenes in a global eucalypt species. *Tree Genet Genomes* 2011, 7:485-498.

235. Theis N, Lerdau M: The evolution of function in plant secondary metabolites. *Int J Plant Sci* 2003, 164:S93-S102.

236. Matoušek J, Kocábek T, Patzak J, Füssy Z, Procházková J, Heyerick A: Combinatorial analysis of lupulin gland transcription factors from R2R3Myb, bHLH and WDR families indicates a complex regulation of chs_H1 genes essential for prenylflavonoid biosynthesis in hop (*Humulus lupulus* L.). *BMC Plant Biol* 2012, 12:27.

237. Alleman M, Doctor J: Genomic imprinting in plants: observations and evolutionary implications. *Plant Mol Bio* 2000, 43:147-161.

238. Mazzio EA, Soliman KFA: Basic concepts of epigenetics impact of environmental signals on gene expression. *Epigenetics* 2012, 7:119-130.

239. Qiao W, Fan L: Epigenetics, a mode for plants to respond to abiotic stresses. *Front Biol* 2011, 6:477-481.

240. Beránek F: Hop breeding resistance to downy mildew (*Pseudoperonospora humuli*) by artificial infections. In *Proceedings of the Scientific Commission, International Hop Growers Convention (IHGC); Žatec, Czech Republic*. 1997

241. Tsurumaru Y, Sasaki K, Miyawaki T, Uto Y, Momma T, Umemoto N, Momose M, Yazaki K: HlPT-1, a membrane-bound prenyltransferase responsible for the biosynthesis of bitter acids in hops. *Biochem Biophys Res Commun* 2012, 417:393-398.

242. Feng J, Long Y, Shi L, Shi J, Barker G, Meng J: Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. *New Phytol* 2012, 193:96-108.

243. Wagner GP, Zhang J: The pleiotropic structure of the genotype-phenotype map: the evolvability of complex organisms. *Nat Rev Genet* 2011, 12:204-213.

244. Ober D: Seeing double: gene duplication and diversification in plant secondary metabolism. *Trends Plant Sci* 2005, 10:444-449.

245. Kroymann J: Natural diversity and adaptation in plant secondary metabolism. *Curr Opin Plant Biol* 2011, 14:246-251.

246. Jaillon O, Aury JM, Noel B, Policriti A, Clepet C, Casagrande A, Choisne N, Aubourg S, Vitulo N, Jubin C, Vezzi A, Legeai F, Hugueney P, Dasilva C, Horner D, Mica E, Jublot D, Poulain J, Bruyère C, Billault A, Segurens B, Gouyvenoux M, Ugarte E, Cattonaro F, Anthouard V, Vico V, Del Fabbro C, Alaux M, Di Gaspero G, Dumas V, *et al.*: The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature* 2007, 449:463-467.

247. Kliebenstein DJ, Lambrix VM, Reichelt M, Gershenzon J, Mitchell-Olds T: Gene duplication in the diversification of secondary metabolism: tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in A*rabidopsis*. *Plant Cell* 2001, 13:681-693.

248. Qi X, Bakht S, Leggett M, Maxwell C, Melton R, Osbourn A: A gene cluster for secondary metabolism in oat: implications for the evolution of metabolic diversity in plants. *Proc Natl Acad Sci USA* 2004, 101:8233-8238.

249. Austin MB, Noel JP: The chalcone synthase superfamily of type III polyketide synthases. *Nat Prod Rep* 2003, 20:79-110.

250. Wenzl P, Li H, Carling J, Zhou M, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesná J, Cakir M, Poulsen D, Wang J, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A: A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. *BMC Genomics* 2006, 7:206.

251. Remington DL, Thornsberry JM, Matsuoka Y, Wilson LM, Whitt SR, Doebley J, Kresovich S, Goodman MM, Buckler ES: Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc Natl Acad Sci USA* 2001, 98:11479-11484.

252. Felsenstein J: The effect of linkage on directional selection. *Genetics* 1965, 52:349-363.

253. Bassil NV, Gilmore B, Oliphant JM, Hummer KE: Genbank-derived microsatellite markers in hops. *Acta Hortic (ISHS)* 2004, 668:47-51.

254. Wittwer C, Reed G, Gundry C, Vandersteen J, Pryor R: High-resolution genotyping by amplicon melting analysis using LCGreen. *Clin Chem* 2003, 49:853-860.

255. Buck E, Wiedow C, Beatson RA: Molecular marker development and use in the New Zealand hop breeding programme. In *Proceedings of the Scientific Commission, International Hop Growers Convention (IHGC); Tettnang, Germany*. 2007: 22-25.

256. Grattapaglia D, Sederoff R: Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics* 1994, 137.

257. Wenzl P, Raman H, Wang J, Zhou M, Huttner E, Kilian A: A DArT platform for quantitative bulked segregant analysis. *BMC Genomics* 2007, 8:196.

258. Van Ooijen JW: JoinMap® 4, software for the calculation of genetic linkage maps in experimental populations of diploid species. Wageningen, Netherlands: Plant Research International BV and Kayazma BV; 2006.

259. Keats BJB, Sherman SL, Morton NE, Robson EB, Buetow KH, Cartwright PE, Chakravarti A, Francke U, Green PP, Ott J: Guidelines for human linkage maps: an international system for human linkage maps (ISLM, 1990). *Ann Hum Genet* 1991, 55:1-6.

260. Freeman JS, Potts BM, Shepherd M, Vaillancourt RE: Parental and consensus linkage maps of *Eucalyptus globulus* using AFLP and microsatellite markers. *Silvae Genet* 2006, 55:202-217.

261. Ma X-F, Jensen E, Alexandrov N, Troukhan M, Zhang L, Thomas-Jones S, Farrar K, Clifton-Brown J, Donnison I, Swaller T, Flavell R: High resolution genetic mapping by genome sequencing reveals genome duplication and tetraploid genetic structure of the diploid *Miscanthus sinensis*. *PLoS ONE* 2012, 7:e33821.

262. Hudson C, Kullan A, Freeman J, Faria D, Grattapaglia D, Kilian A, Myburg A, Potts B, Vaillancourt R: High synteny and colinearity among *Eucalyptus* genomes revealed by high-density comparative genetic mapping. *Tree Genet Genomes* 2012, 8:339-352.

263. Cheema J, Dicks J: Computational approaches and software tools for genetic linkage map estimation in plants. *Brief Bioinform* 2009, 10:595-608.

264. Vision TJ, Brown DG, Shmoys DB, Durrett RT, Tanksley SD: Selective mapping: a strategy for optimizing the construction of high-density linkage maps. *Genetics* 2000, 155:407-420.

265. European Brewery Convention: Analytica EBC, section 7 - hops, methods 7.2, 7.4, and 7.10. Carl, Getränke-Fachverlag Nürnberg; 1997.

266. "R-Development-Core-Team": R version 2.11.1. Vienna, Austria: The R Foundation for Statistical Computing; 2010.

267. Cohen J: *Statistical power analysis for the behavioral sciences.* New Jersey, USA: Lawrence Erlbaum Associates Inc; 1988.

268. Van Ooijen JW: MapQTL® 6, software for the mapping of quantitative trait loci in experimental populations of diploid species. Wageningen, Netherlands: Kyazma BV; 2009.

269. Churchill GA, Doerge RW: Empirical threshold values for quantitative trait mapping. *Genetics* 1994, 138:963-971.

270. Voorrips RE: MapChart 2.2, software for the graphical presentation of linkage maps and QTLs. Wageningen, Netherlands: Plant Research International; 2006.

271. Lander ES, Botstein D: Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 1989, 121:185-199.

272. Sohn JB: *The hop aroma compendium, volumes 1 and 2.* Hans: Carl Fachverlag; 2012.

273. Nesvadba V, Černy J, Krofta K: Transfer fo the hop (*Humulus lupulus* L.) alpha-bitter acid content to progenies of F_1 and I_1 generations in selected parental components. *Plant Soil Environ* 2003, 49:269-276.

274. Hill WG: Understanding and using quantitative genetic variation. *Phil Trans R Soc B* 2010, 365:73-85.

275. Perron M, DeBlois J, Desponts M: Use of resampling to assess optimal subgroup composition for estimating genetic parameters from progeny trials. *Tree Genet Genomes* 2013, 9:129-143.

276. McAdam E, Freeman J, Whittock S, Buck E, Jakse J, Cerenak A, Javornik B, Kilian A, Wang C-H, Andersen D, Vaillancourt R, Carling J, Beatson R, Graham L, Graham D, Darby P, Koutoulis A: Quantitative trait loci in hop (*Humulus lupulus* L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry. *BMC Genomics* 2013, 14:360.

277. Patterson HD, Williams ER: A new class of resolvable incomplete block designs. *Biometrika* 1976, 63:83-92.

278. California Fermentation Society [www.calferm.org]; 2012.

279. Oregon State University High Alpha Acid Breeding Program: Freshops USDA named hop variety descriptions. [http://www.freshops.com/hops/usda-named-hop-variety-descriptions]; 2012.

280. Haunold A, Likens ST, Nickerson GB, Horner CE, Hampton RO: Registration of USDA 63015M male hop germplasm (Reg. No. GP 14). *Crop Sci* 1983, 23:600-601.

281. Homer CE, Brooks SN, Haunold A, Likens ST: Registration of hop germplasm (Reg. No. GP 2 to 4). *Crop Sci* 1974, 14:341-341.

282. Kenny ST, Zimmermann CE: Registration of 'Chinook' hop. *Crop Sci* 1986, 26:196-197.

283. Miyata J: Qualities and ancestry of prominent hop varieties. *Brewer and Distiller International* 1994, October:24-27.

284. Neve RA, Darby P: Yeoman and Zenith: two new varieties. In *Wye Report Wye College of the University of London*. Department of the Hop Research Annual Report 1983; 1984

285. Patzak J: Characterization of Czech hop (*Humulus lupulus* L.) genotypes by molecular methods. *Rost Výroba* 2002, 48:343-350.

286. Reed BM: *In vitro* storage of hop (*Humulus* L.) germplasm. *Acta Hortic (ISHS)* 2005, 668:249-256.

287. Roborgh RHJ: Classification and botanical description of imported varieties of hops in Nelson, New Zealand. *New Zeal J Bot* 1964, 2:10-18.

288. Simply Hops: www.simplyhops.co.uk]; 2012.

289. USDA ARS: Germplasm Resources Information Network (GRIN). National Germplasm Resources Laboratory, Beltsville, Maryland. [http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl]; 2012.

290. Zimmermann CE, Likens ST, Haunold A, Horner CE, Roberts DD: Registration of Comet hop (Reg. No. 3). *Crop Sci* 1975, 15:98-98.

291. Gilmour AR, Gogel BJ, Cullis BR, Thompson R: ASReml user guide release 3.0. Hemel Hempstead, HP1 1ES, UK: VSN International Ltd; 2009.

292. Gilmour AR: ASREML for testing fixed effects and estimating multiple trait variance components. *Proc Adv Anim Breed Gen* 1997, 12:386-390.

293. Charlesworth D, Willis JH: The genetics of inbreeding depression. *Nat Rev Genet* 2009, 10:783-796.

294. Duvick DN: Biotechnology in the 1930s: the development of hybrid maize. *Nat Rev Genet* 2001, 2:69-74.

295. O'Reilly-Wapstra JM, McArthur C, Potts BM: Linking plant genotype, plant defensive chemistry and mammal browsing in a Eucalyptus species. *Funct Ecol* 2004, 18:677-684.

296. Moore BD, Foley WJ: Tree use by koalas in a chemically complex landscape. *Nature* 2005, 435:488-490.

297. Steane DA, Conod N, Jones RC, Vaillancourt RE, Potts BM: A comparative analysis of population structure of a forest tree, *Eucalyptus globulus* (Myrtaceae), using microsatellite markers and quantitative traits. *Tree Genet Genomes* 2006, 2:30-38.

298. Dawson T, Geber M: Sexual dimorphism in physiology and morphology. In *Gender and sexual dimorphism in flowering plants.* New York: Springer-Verlag Berlin Heidelberg; 1999: 175-210

299. Dzhaparidze L: *Sex in plants, part II. Biochemical and physiological sex differences in dioecious plants: problem of influencing sex formation.* Jerusalem: IPST Press; 1969.

300. Krofta K: Comparison of quality parameters of Czech and foreign hop varieties. *Plant Soil Environ* 2003, 49:261-268.

301. Rigby FL: A theory on the hop flavour of beer. *Proc Am Soc Brew Chem* 1972, 46:46-50.

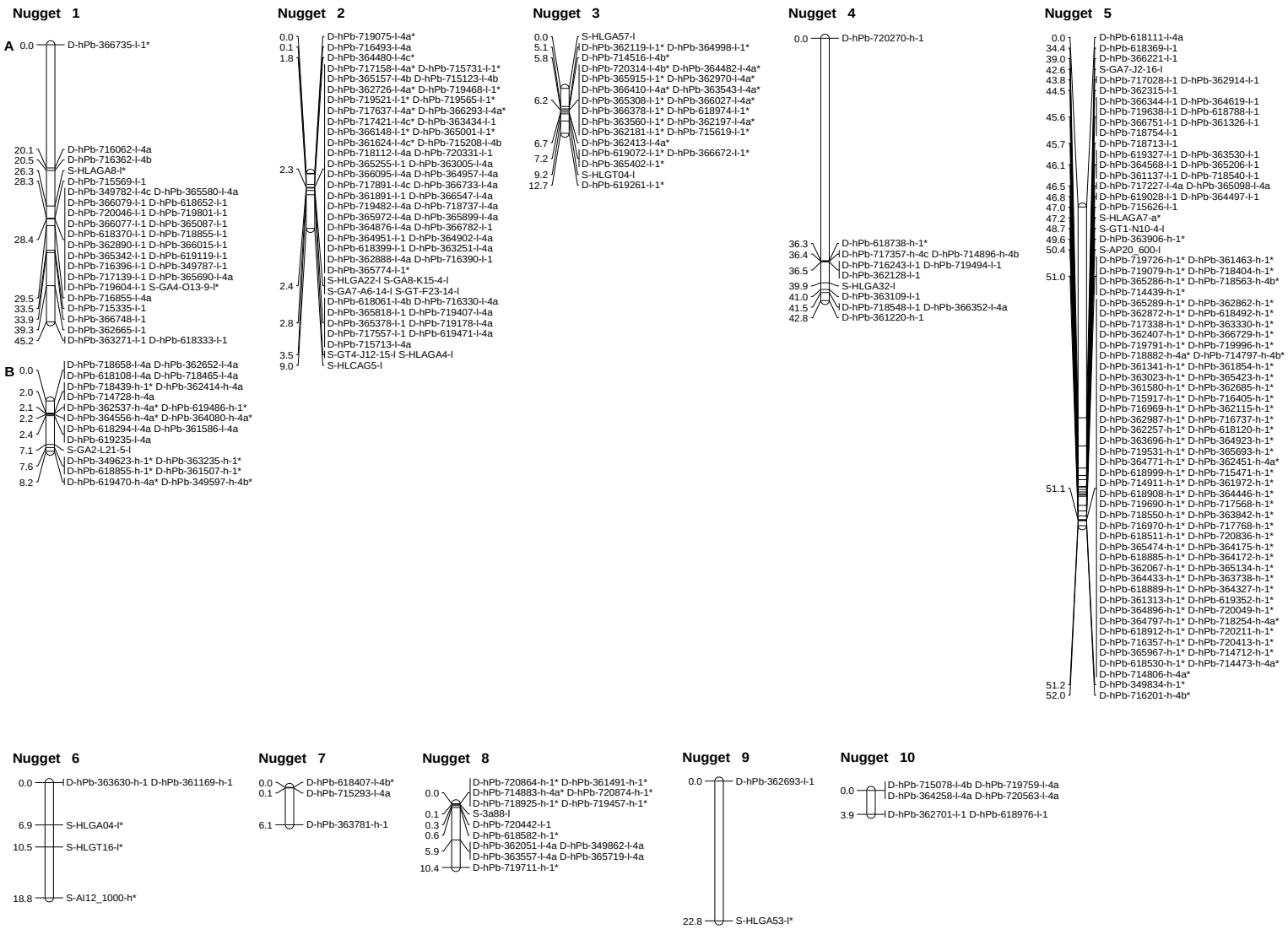
302. Wackerbauer K, Balzer U: Hop bitter compounds in beer, part II: the influence of cohumulone on beer quality. *BRAUWELT Int* 1993, 11:116-118.

303. Kollmannsberger H, Biendl M, Nitz S: Occurence of glycosidically bound flavour compounds in hops, hop products and beer. *Monatsschrift Brauwiss* 2006, 5:83-89.

304. Takoi K, Koie K, Itoga Y, Katayama Y, Shimase M, Nakayama Y, Watari J: Biotransformation of hop-derived monoterpene alcohols by lager yeast and their contribution to the flavor of hopped beer. *J Agric Food Chem* 2010, 58:5050-5058.

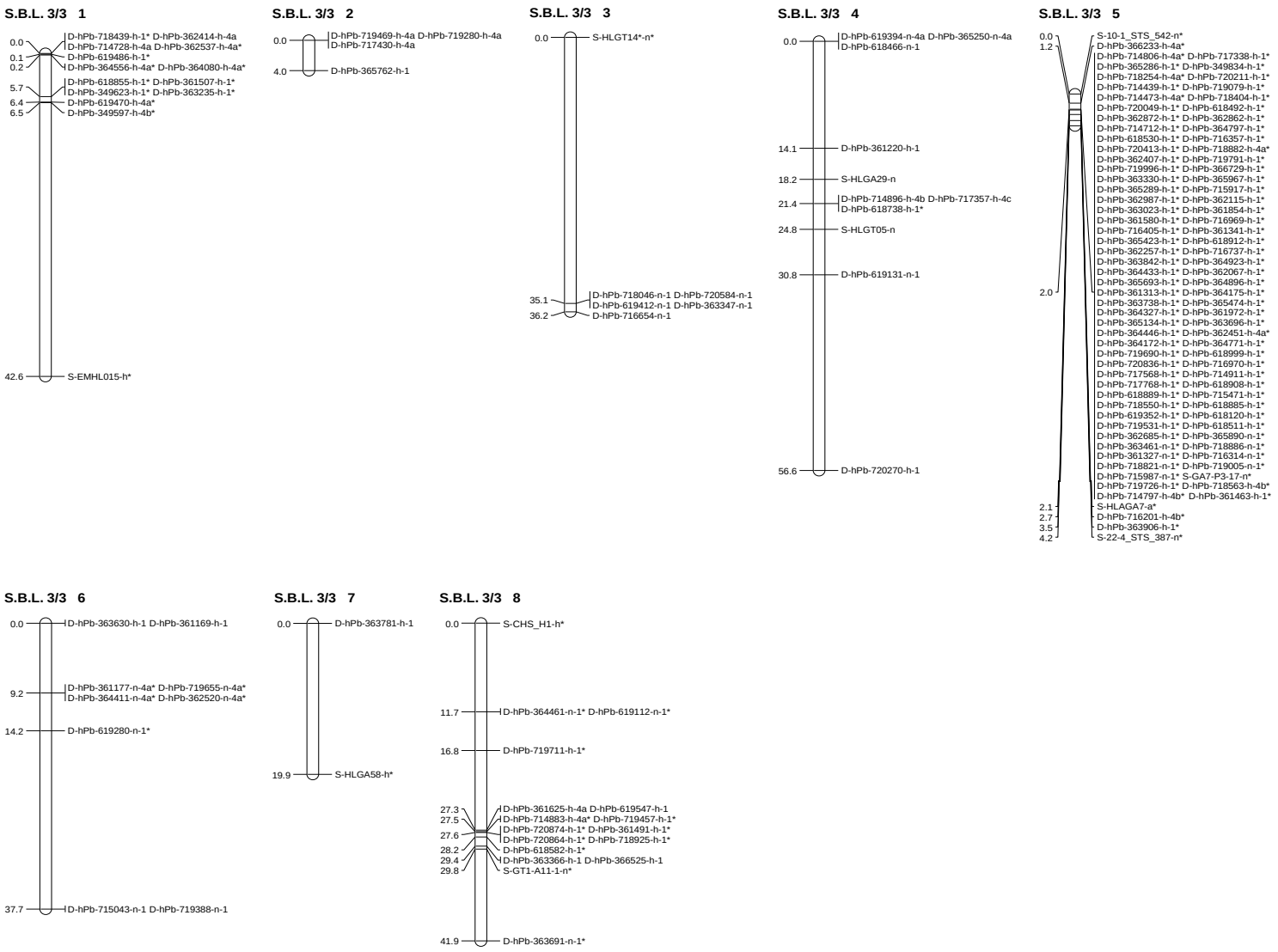
305. Heffner EL, Sorrells ME, Jannink JL: Genomic selection for crop improvement. *Crop Sci* 2009, 49:1-12.
306. Meuwissen THE, Hayes BJ, Goddara ME: Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 2001, 157:1819-1829.
307. Jannink JL, Lorenz AJ, Iwata H: Genomic selection in plant breeding: From theory to practice. *Brief Funct Genomic Proteomic* 2010, 9:166-177.

APPENDIX 3.1 Maternal linkage map of the New Zealand hop mapping population



Appendix 3.1 Linkage map constructed for the female parent 'Nugget' of the New Zealand mapping population. There is evidence from homology to the linkage map of a Slovenian mapping population also constructed in this study that the two linkage groups from the maternal 'Nugget' map can be linked to form 'Nugget' LG1. In our study, these linkage groups form one group at LOD 2. Ten linkage groups (seven major, one triplet and two doublets) were identified in the maternal 'Nugget' map.

APPENDIX 3.2 Paternal linkage map of the New Zealand hop mapping population



Appendix 3.2 Linkage map constructed for the male parent 'S.B.L. 3/3' of the New Zealand mapping population. Eight linkage groups (five major, one triplet and two doublets) were identified in the paternal 'S.B.L. 3/3' map.

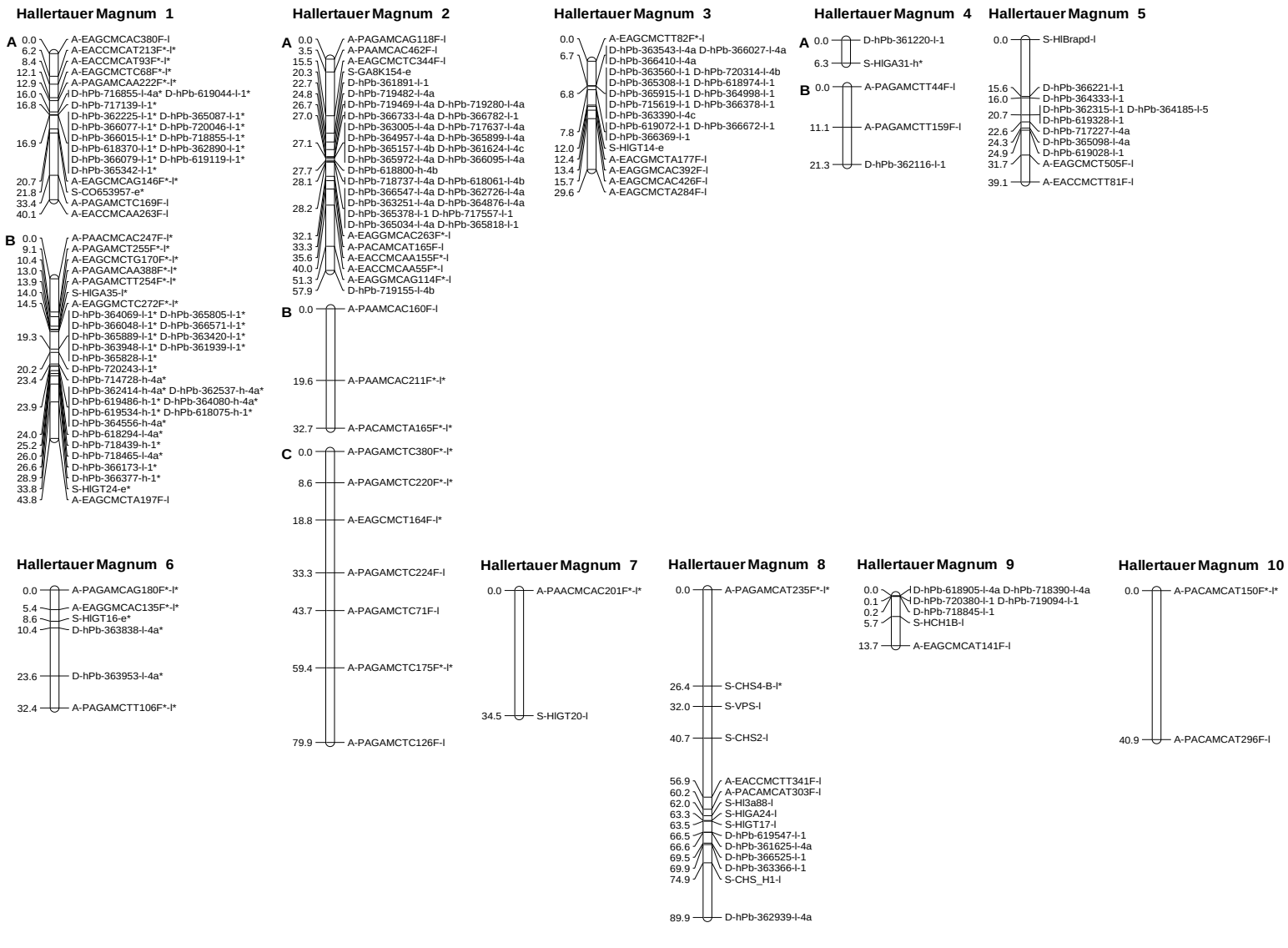
APPENDIX 3.3 Homology between linkage maps constructed in this study and between linkage maps of a previous study

Appendix 3.3 Homologous linkage groups were identified between maternal and paternal linkage maps of the New Zealand and Slovenian populations. Homologous linkage groups were also identified between linkage maps of the Slovenian population constructed in this study and linkage maps of the Slovenian population constructed in a previous study.

Linkage map of New Zealand population, this study		**Linkage map of Slovenian population, this study**		**Linkage map of Slovenian population, previous study**	
Nugget ♀	**S.B.L. 3/3 ♂**	**Hallertauer Magnum ♀**	**S.B.L. 2/1 ♂**	**Hallertauer Magnum ♀**	**S.B.L. 2/1 ♂**
1a[2]		1a[2,3,4]	1a[3,4]	1[4]	1[4]
1b[1,2]	1[1,2]	1b[2,3,4]	1b[2,3,4]	1+11[4]	1[4]
2[2]	2	2a[2,3,4]	2[3,4]	2+10+12[4]	2[4]
		2b[4]		2+9[4]	
		2c[4]		2[4]	
3[2]	3[2]	3[2,3,4]	3[2,3,4]	3[4]	3[4]
4[1,2]	4[1,2]	4a[2,4]	4[2]	4[4]	4
		4b[4]		4[4]	
5[1,2]	5[1,2]	5[2,4]	5[2,4]	5[4]	5+15+18[4]
6[1]	6[1,2]	6[3,4]	6[2,3,4]	6[4]	6[4]
7[1]	7[1]	7[4]	7[4]	7+9[4]	7[4]
8[1]	8[1]	8[4]	8[4]	8[4]	19[4]
9		9[4]	9[4]	14[4]	17[4]
10		10	10		16
				13	20

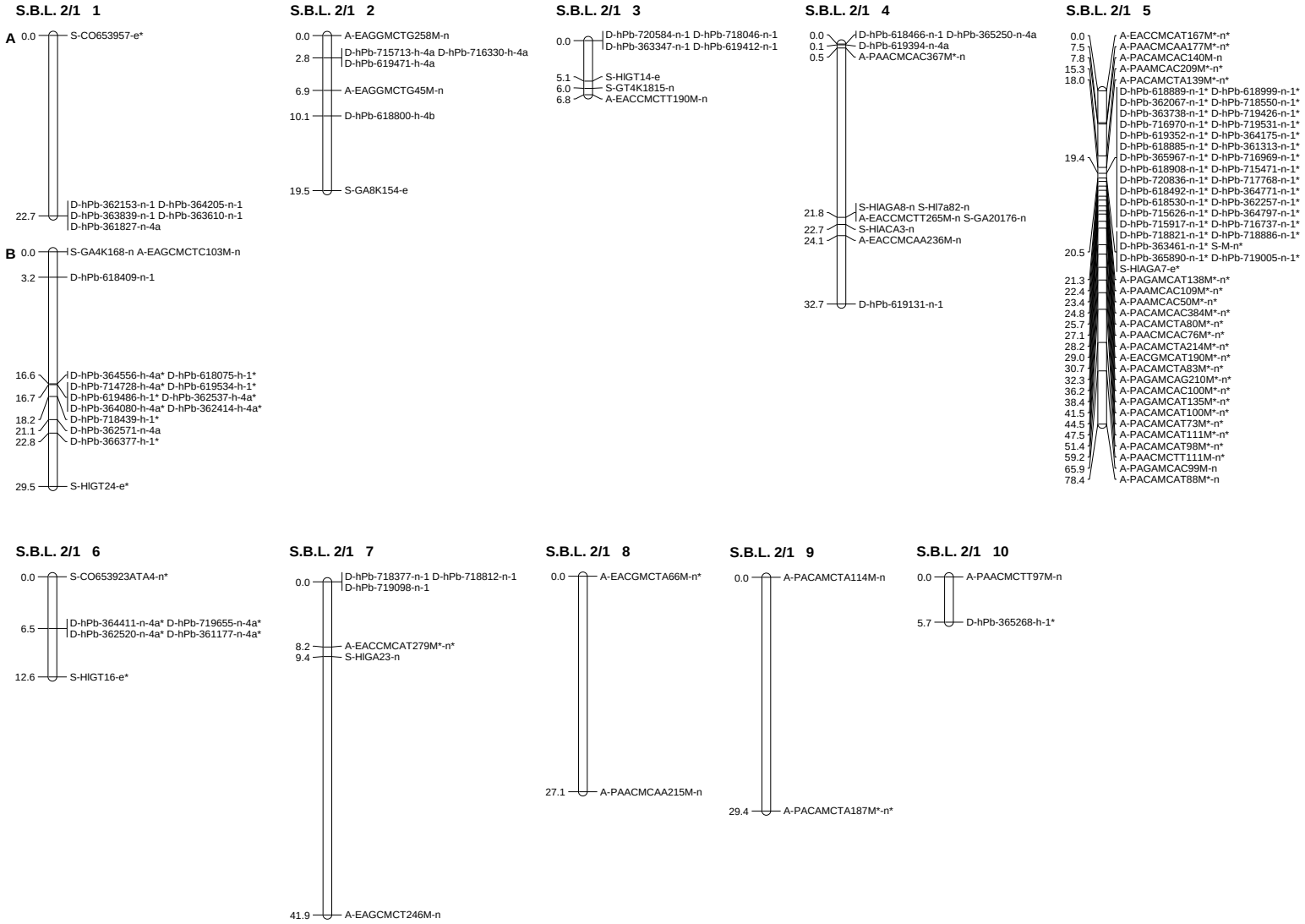
[1] = match between Nugget (this study) and S.B.L. 3/3 (this study)
[2] = match between New Zealand population (this study) and Slovenian population (this study)
[3] = match between Hallertauer Magnum (this study) and S.B.L. 2/1 (this study)
[4] = match between Slovenian population (this study) and Slovenian population (previous study)

APPENDIX 3.4 Maternal linkage map of the Slovenian hop mapping population



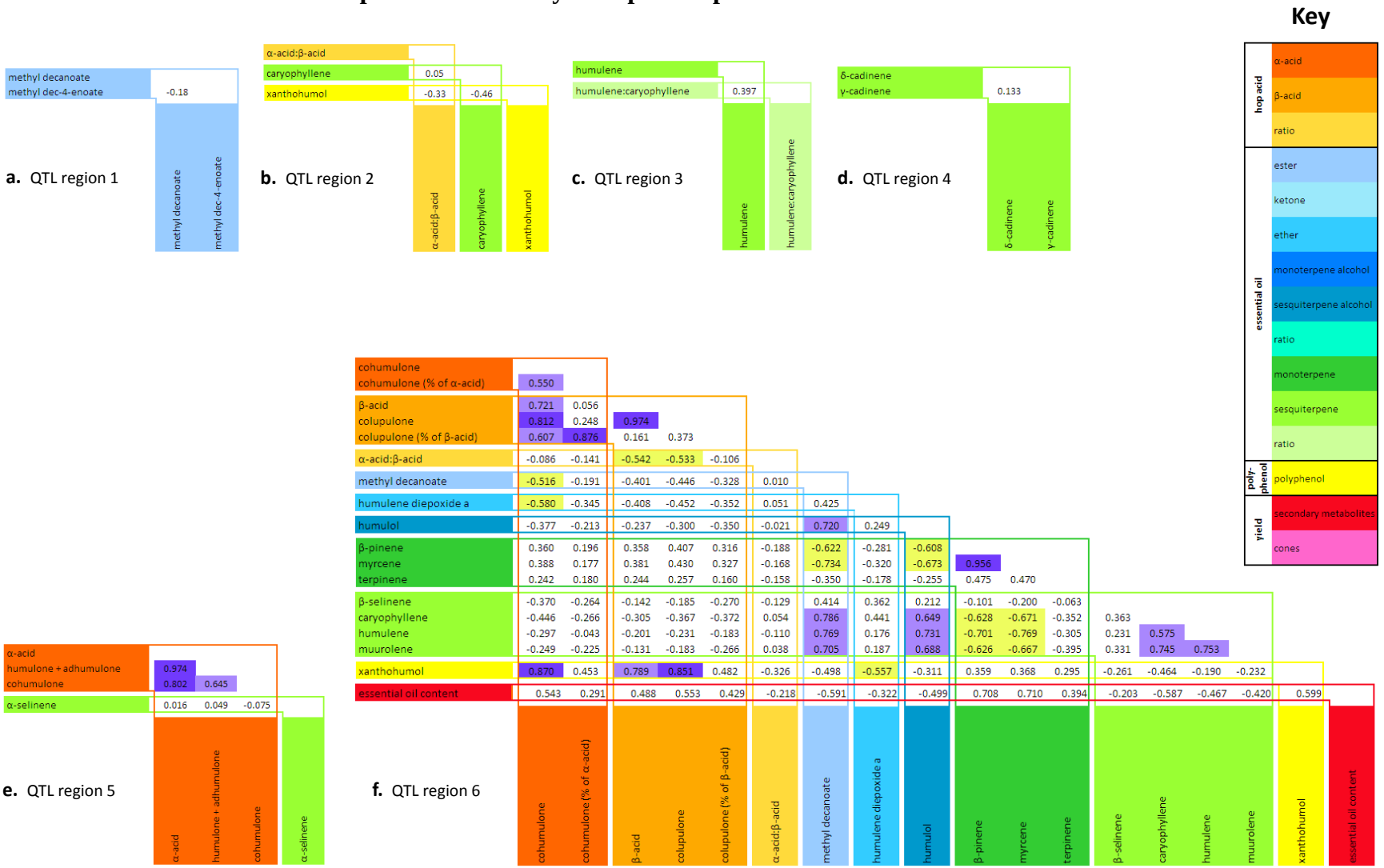
Appendix 3.4 Linkage map constructed for the female parent 'Hallertauer Magnum' of the Slovenian mapping population. There is evidence from homology to a previous linkage map of the Slovenian mapping population [95] that several of the linkage groups from the maternal linkage map can be linked. The two linkage groups that link to form the 'Hallertauer Magnum' LG1 in this study form one group at LOD 5. The three linkage groups that link to form 'Hallertauer Magnum' LG2 in this study form one group at LOD 3. Ten linkage groups (eight major and two doublets) were identified in the maternal 'Hallertauer Magnum' map.

APPENDIX 3.5 Paternal linkage map of the Slovenian hop mapping population

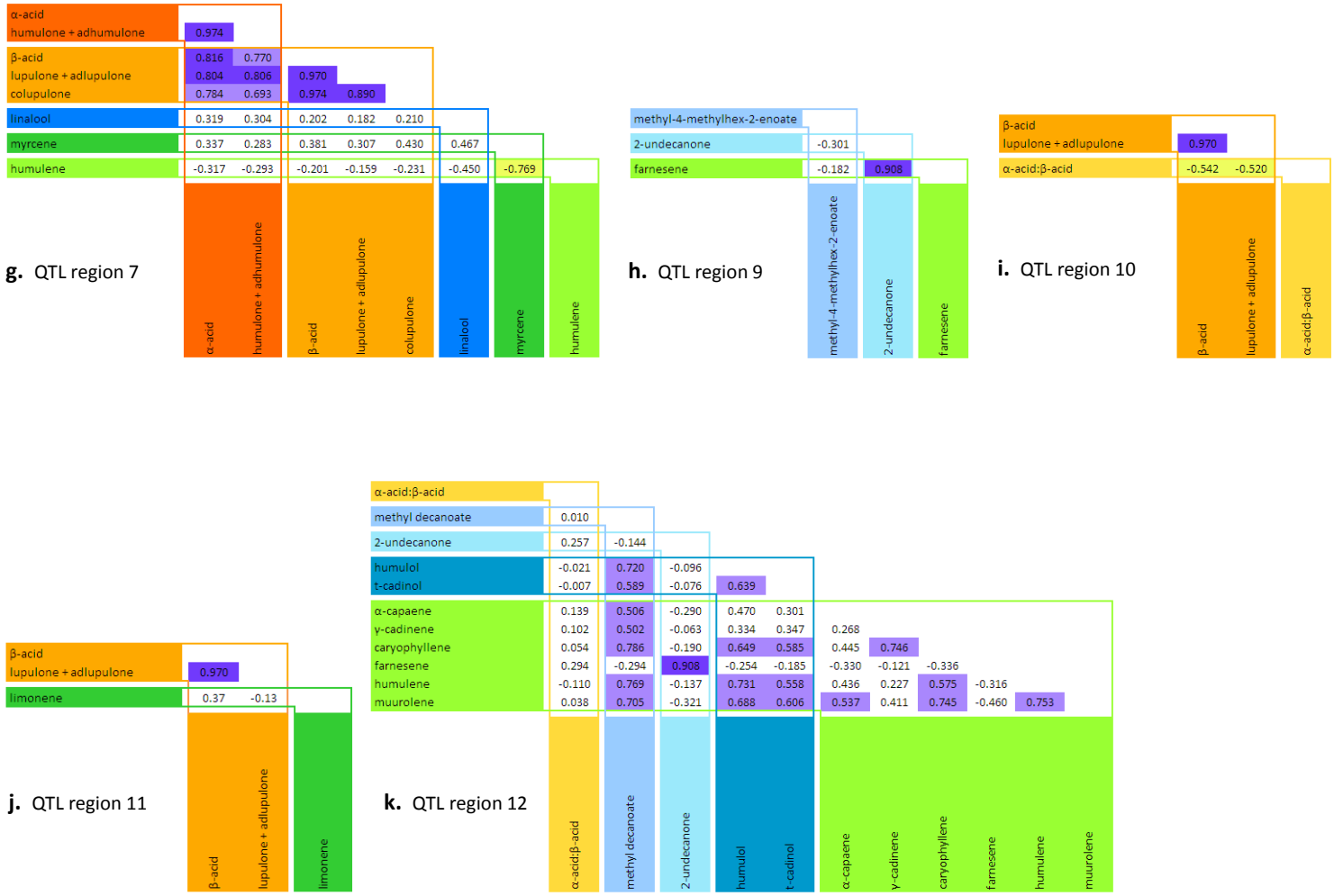


Appendix 3.5 Linkage map constructed for the male parent 'S.B.L. 2/1' of the Slovenian mapping population. There is evidence from homology to a previous linkage map of the Slovenian mapping population [95] that two linkage groups from the paternal linkage map can be linked. The two linkage groups that link to form 'S.B.L. 2/1' LG1 in this study form one group at LOD 4. Ten linkage groups (six major, one triplet and three doublets) were identified in the paternal 'S.B.L. 2/1' map.

APPENDIX 3.6 Correlations between hop traits affected by each pleiotropic/linked locus



Appendix 3.6 Correlation coefficients (Pearson's r) for the relationships between yield and secondary metabolite traits affected by pleiotropic/linked loci in hop.



Appendix 3.6 Correlation coefficients (Pearson's r) for the relationships between yield and secondary metabolite traits affected by pleiotropic/linked loci in hop. *(Continued)*

APPENDIX 3.7 IUPAC naming of secondary metabolites quantified in hop

Appendix 3.7 International Union of Pure and Applied chemistry (IUPAC) naming of 41 secondary metabolites quantified in hop, including hop acids, esters, ketones, ethers, monoterpene alcohols, sesquiterpene alcohols, monoterpenes and sesquiterpenes.

Chemical group		Trait name	IUPAC name
hop acid	α-acid	humulone	(6*R*)-3,5,6-Trihydroxy-2-(3-methylbutanoyl)-4,6-bis(3-methylbut-2-en-1-yl)cyclohexa-2,4-dien-1-one
		adhumulone	3,5,6-trihydroxy-2-(2-methylbutanoyl)-4,6-bis(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one
		cohumulone	3,5,6-trihydroxy-4,6-bis(3-methylbut-2-enyl)-2-(2-methylpropanoyl)cyclohexa-2,4-dien-1-one
	β-acid	lupulone	3,5-dihydroxy-2-(3-methylbutanoyl)-4,6,6-tris(3-methylbut-2-enyl)cyclohexa-2,4-dien-1-one
		adlupulone	3,5-Dihydroxy-2,6,6-tris(3-methyl-2-butenyl)-4-(2-methyl-1-oxobutyl)-2,4-cyclohexadien-1-one
		colupulone	3,5-dihydroxy-4,6,6-tris(3-methylbut-2-enyl)-2-(2-methylpropanoyl)cyclohexa-2,4-dien-1-one
essential oil	ester	geranyl acetate	[(2E)-3,7-dimethylocta-2,6-dienyl] acetate
		geranyl isobutyrate	(2E)-3,7-dimethylocta-2,6-dienyl] 2-methylpropanoate
		methyl decanoate	methyl decanoate
		methyl dec-4-enoate	methyl dec-4-enoate
		methyl-4-methylhex-2-enoate	(E)-4-methylhex-2-enoate
	ketone	2-undecanone	Undecan-2-one
	ether	humulene diepoxide a	6,7-diepoxy-9-[2,6,6,9-tetramethylcycloundecatriene]
		humulene epoxide I	(4E,7E,11R)-1,5,9,9-tetramethyl-12-oxabicyclo[9.1.0]dodeca-4,7-diene
		humulene epoxide II	(3E,7E,11R)-1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene
		humulene epoxide III	(3E,7E)-3,7,10,10-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene
	monoterpene alcohol	geraniol	(2E)-3,7-dimethylocta-2,6-dien-1-ol
		limonene-10-ol	2-[(1R)-4-methyl-1-cyclohex-3-enyl]prop-2-en-1-ol
		linalool	3,7-dimethylocta-1,6-dien-3-ol

Chemical group		Trait name	IUPAC name
	sesquiterpene alcohol	caryolan-1-ol	(1*S*,2*R*,5*S*,8*R*)-4,4,8-trimethyltricyclo[6.3.1.0]dodecan-1-ol
		humulenol II	(1R,4E,8E)-6,6,9-Trimethyl-2-methylene-4,8-cycloundecadien-1-ol
		humulol	humulol
		t-cadinol	(1S,4S,4aR,8aR)-1,6-dimethyl-4-propan-2-yl-3,4,4a,7,8,8a-hexahydro-2H-naphthalen-1-ol
	alkane	tetradecane	tetradecane
	monoterpene	β-pinene	7,7-dimethyl-4-methylidenebicyclo[3.1.1]heptane
		camphene	6,6-dimethyl-5-methylidenebicyclo[2.2.1]heptane
		limonene	1-methyl-4-(1-methylethenyl)-cyclohexene
		myrcene	7-methyl-3-methylideneocta-1,6-diene
		ρ-cymene	1-methyl-4-propan-2-ylbenzene
		terpinene	1-methyl-4-propan-2-ylcyclohexa-1,3-diene
	sesquiterpene	α-copaene	8-isopropyl-1,3-dimethyl tricycle(4.4.0.02,7)dec-3-ene
		α-selinene	5,8a-dimethyl-3-prop-1-en-2-yl-2,3,4,4a,7,8-hexahydro-1H-naphthalene
		β-selinene	(3S,4aR,8aS)-8a-methyl-5-methylidene-3-prop-1-en-2-yl-1,2,3,4,4a,6,7,8-octahydronaphthalene
		δ-cadinene	4,7-dimethyl-1-propan-2-yl-1,2,3,5,6,8a-hexahydronaphthalene
		γ-cadinene	7-methyl-4-methylidene-1-propan-2-yl-2,3,4a,5,6,8a-hexahydro-1H-naphthalene
		caryophyllene	(4Z)-4,11,11-trimethyl-8-methylenebicyclo(7.2.0)undec-4-ene
		caryophyllene oxide	[1R-(1R*,4R*,6R*,10S*)]-4,12,12-trimethyl-9-methylene-5-oxatricyclo[8.2.0.04,6]dodecane
		farnesene	(3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene
		humulene	2,6,6,9-tetramethylcycloundecatriene
		muurolene	(1S,4aS,8aR)-1-isopropyl-4,7-dimethyl-1,2,4a,5,6,8a-hexahydronaphthalene
poly-phenol	poly-phenol	xanthohumol	(E)-1-[2,4-dihydroxy-6-methoxy-3-(3-methylbut-2-enyl)phenyl]-3-(4-hydroxyphenyl)prop-2-en-1-one

APPENDIX 4.1 Hop accessions used as female parents for quantitative genetics progeny trial

Appendix 4.1 The identity (cultivar name or accession number) of the hop accessions used as female parents of a progeny trial utilised for an investigation of quantitative genetic variation in hop. 'Origin' refers to the country where the hop accessions were produced.

Hop accession	Origin
21055	USA
21194	USA
00-999	Australia
15-72-1	unknown
17/54/12	UK
21055 (2n)	USA
27/70/4	UK
30-74-32	Australia
71-75-1	Australia
8428-036	Australia
8451-077	Australia
89-012-017	Australia
89-012-024	Australia
89-012-052	Australia
89-022-020	Australia
89-022-033	Australia
89-103-005	Australia
89-123-001	Australia
89-124-018	Australia
89-134-022	Australia
89-135-011	Australia
89-135-016	Australia
90-014-095	Australia
91-004-013	Australia
91-008-046	Australia
91-021-020	Australia
91-031-021	Australia
94-138-006 (2n)	Australia
97-113-003	Australia
AF 3/26	South Africa
AHIL	Slovenia
AQUILA	USA
AURORA	Slovenia
B23	Australia
B24/23	Australia
BANNER	USA
BH 2/72	South Africa
BOBEK	Slovenia
BREWERS GOLD	UK
BRUNY ISLAND	Australia
BUKET	Slovenia
BULLION	UK
C10	Australia
CHINOOK	USA
CLUSTER	USA
COLUMBUS	USA
D6	Australia
E2	USA
E-85-16	Australia

Hop accession	Origin
E-85-20	Australia
EASTERN GOLD	Japan
ED-85-36	Australia
EG-85-17	Australia
EI-85-33	Australia
ELSASSER	France
EN-85-32	Australia
EP-86-4	Australia
EROICA	USA
EX FRF	Australia
F-84-19	Australia
FK21	Japan
G-85-9	Australia
GALENA	USA
GOLDING	UK
HALLERTAU	Germany
HERSBRUCKER	Germany
HERSBRUCKER G	Germany
JAPANESE ROGUE	Japan
K56	USA
K-85-9	Australia
L1	USA
L1 EX FRF	USA
LA-85-70	Australia
LB-85-2	Australia
ME-85-33	Australia
NORTHERN BREWER	UK
NUGGET	USA
OM26	UK
PERLE	Germany
S-5524	Australia
SAAZ 36	Czech Republic
SHINSHUWASE	Japan
SMOOTHCONE	New Zealand
SOUTHERN BREWER	South Africa
STYRIAN	Australia
SWISS TETTNANG	Germany
TILLICUM	USA
U-85-10	Australia
UNKNOWN 09	Australia
V-85-9	Australia
VOJVODINA	Slovenia
W-86-26	Australia
WYE CHALLENGER	UK
WYE NORTHDOWN	UK
WYE TARGET	UK
YEOMAN	UK
ZENITH	UK

Each accession was used as a female parent once, except for accessions 21194, 21055(2n), NUGGET, CLUSTER and SWISS TETTNANG which were used twice; and individuals 21055 and 94-138-006 (2n) which were used four times.

APPENDIX 4.2 Least squares means by family for hop cone chemistry, yield and plant growth traits

Appendix 4.2 Least squares means (lower 95% limit, upper 95% limit) for each family included in the quantitative genetic analysis, for traits relating to plant growth, yield and cone chemistry in hop. '*' indicates traits for which the means have been backtransformed**. a.** refers to the traits number of shoots, length of the longest shoot and number of nodes on the longest shoot, relating to the emergence stage of plant growth. **b.** refers to the trait height, assessed at three different time points of the growing season (flower initiation, mid-season and cone maturity). **c.** refers to the traits lateral length, number of nodes on lateral and internode length, relating to the cone maturity stage of plant growth. **d.** refers to the trait height to the cones, relating to the cone maturity stage of plant growth; and green cone weight, relating to cone yield. **e.** refers to the cone chemical traits cohumulone, humulone + adhumulone, colupulone and lupulone + adlupulone. **f.** refers to the cone chemical traits α-acid and β-acid. **g.** refers to the cone chemical traits cohumulone (% of α-acid), α-acid:β-acid and α-acid:total resin. All traits were assessed in two seasons of plant growth, except for green cone weight, which was assessed in only the second season.

a.

Family	number of shoots 11 months *		number of shoots 24 months		length of the longest shoot 11 months *		length of the longest shoot 24 months *		number of nodes on the longest shoot 11 months		number of nodes on the longest shoot 24 months *	
2008003	3.71	(2.98 , 4.51)	7.46	(6.04 , 8.89)	42.60	(36.53 , 49.13)	8.10	(5.97 , 10.56)	6.80	(6.30 , 7.30)	2.69	(2.28 , 3.13)
2008190	4.44	(3.64 , 5.31)	8.17	(6.75 , 9.59)	44.93	(38.69 , 51.65)	8.94	(6.77 , 11.41)	7.26	(6.75 , 7.76)	2.68	(2.29 , 3.11)
2008209	4.46	(3.65 , 5.36)	6.75	(5.29 , 8.22)	47.64	(41.10 , 54.67)	10.18	(7.56 , 13.18)	6.93	(6.42 , 7.44)	2.80	(2.36 , 3.28)
2008151	4.12	(3.35 , 4.96)	5.67	(4.25 , 7.09)	32.71	(27.51 , 38.36)	8.40	(6.15 , 11.01)	6.01	(5.51 , 6.50)	2.77	(2.34 , 3.23)
2008039	4.88	(4.04 , 5.80)	8.92	(7.50 , 10.34)	47.77	(41.43 , 54.56)	9.04	(6.86 , 11.52)	7.62	(7.13 , 8.11)	2.72	(2.32 , 3.15)
2008152	3.76	(3.03 , 4.57)	5.72	(4.30 , 7.15)	48.41	(41.91 , 55.37)	8.51	(6.23 , 11.14)	7.28	(6.78 , 7.79)	2.82	(2.39 , 3.29)
2008001	4.87	(4.00 , 5.82)	8.19	(6.68 , 9.70)	49.10	(42.45 , 56.23)	7.54	(5.56 , 9.81)	7.93	(7.42 , 8.45)	2.62	(2.24 , 3.05)
2008073	3.36	(2.67 , 4.13)	5.68	(4.26 , 7.10)	40.51	(34.39 , 47.14)	5.38	(3.61 , 7.51)	6.63	(6.10 , 7.15)	2.34	(1.95 , 2.76)
2008168	4.55	(3.74 , 5.43)	10.96	(9.54 , 12.38)	48.27	(41.90 , 55.09)	18.45	(15.38 , 21.80)	7.24	(6.75 , 7.73)	3.40	(2.96 , 3.86)
2008161	4.32	(3.54 , 5.19)	7.86	(6.44 , 9.29)	45.66	(39.26 , 52.55)	7.39	(5.44 , 9.65)	6.69	(6.18 , 7.21)	2.92	(2.51 , 3.36)
2008290	3.57	(2.83 , 4.39)	5.52	(4.01 , 7.03)	41.10	(34.81 , 47.92)	6.48	(4.33 , 9.05)	7.10	(6.57 , 7.64)	2.40	(1.98 , 2.87)
2008208	3.72	(2.99 , 4.52)	6.35	(4.93 , 7.78)	38.16	(32.43 , 44.37)	10.07	(7.47 , 13.05)	6.66	(6.16 , 7.16)	2.74	(2.30 , 3.22)
2008251	4.30	(3.52 , 5.17)	9.02	(7.60 , 10.44)	40.93	(34.99 , 47.35)	7.25	(5.38 , 9.41)	7.16	(6.66 , 7.66)	2.65	(2.27 , 3.06)
2008052	5.83	(4.90 , 6.85)	10.93	(9.46 , 12.39)	54.08	(47.21 , 61.42)	13.46	(10.86 , 16.34)	7.34	(6.84 , 7.84)	3.08	(2.67 , 3.52)
2008037	4.34	(3.54 , 5.22)	4.28	(2.82 , 5.74)	37.99	(32.18 , 44.29)	5.59	(3.70 , 7.86)	6.95	(6.44 , 7.46)	2.10	(1.72 , 2.52)
2008154	4.42	(3.62 , 5.29)	9.19	(7.76 , 10.61)	45.27	(39.10 , 51.89)	10.47	(8.19 , 13.03)	7.13	(6.64 , 7.63)	2.93	(2.53 , 3.37)
2008136	5.90	(4.98 , 6.90)	10.80	(9.37 , 12.22)	53.42	(46.70 , 60.60)	14.87	(12.21 , 17.79)	7.79	(7.30 , 8.28)	3.61	(3.17 , 4.08)
2008096	4.52	(3.72 , 5.40)	9.69	(8.26 , 11.11)	44.13	(38.05 , 50.66)	15.55	(12.54 , 18.89)	7.40	(6.91 , 7.90)	3.31	(2.86 , 3.80)
2008071	3.87	(3.12 , 4.69)	6.18	(4.75 , 7.60)	38.52	(32.65 , 44.87)	5.66	(3.91 , 7.74)	6.58	(6.07 , 7.09)	2.05	(1.70 , 2.44)
2008124	5.20	(4.34 , 6.15)	10.72	(9.30 , 12.14)	51.69	(45.09 , 58.74)	13.43	(10.91 , 16.21)	7.79	(7.29 , 8.28)	2.90	(2.51 , 3.32)
2008145	5.67	(4.77 , 6.66)	7.86	(6.44 , 9.29)	45.99	(39.76 , 52.66)	8.54	(6.49 , 10.87)	7.35	(6.86 , 7.84)	2.98	(2.58 , 3.42)
2008084	5.14	(4.28 , 6.08)	7.05	(5.62 , 8.47)	45.31	(39.14 , 51.92)	6.13	(4.15 , 8.50)	7.09	(6.60 , 7.59)	2.14	(1.75 , 2.56)
2008083	5.14	(4.28 , 6.07)	8.63	(7.21 , 10.05)	48.29	(41.92 , 55.12)	11.26	(8.81 , 14.01)	7.66	(7.17 , 8.15)	3.14	(2.71 , 3.59)
2008056	4.17	(3.40 , 5.02)	6.11	(4.69 , 7.53)	41.81	(35.80 , 48.28)	8.12	(5.80 , 10.82)	6.55	(6.05 , 7.05)	2.97	(2.51 , 3.46)

Family	number of shoots 11 months *		number of shoots 24 months		length of the longest shoot 11 months *		length of the longest shoot 24 months *		number of nodes on the longest shoot 11 months		number of nodes on the longest shoot 24 months *	
2008261	3.80	(3.07 , 4.62)	5.62	(4.20 , 7.04)	43.02	(36.71 , 49.84)	6.08	(4.26 , 8.23)	7.19	(6.66 , 7.71)	2.21	(1.84 , 2.60)
2008157	4.91	(4.07 , 5.82)	8.78	(7.36 , 10.20)	49.14	(42.72 , 56.02)	6.25	(4.57 , 8.18)	7.10	(6.61 , 7.59)	2.64	(2.27 , 3.04)
2008006	4.92	(4.08 , 5.83)	10.98	(9.56 , 12.40)	53.38	(46.67 , 60.54)	15.78	(13.04 , 18.79)	7.58	(7.08 , 8.07)	3.78	(3.33 , 4.25)
2008240	5.37	(4.49 , 6.33)	8.13	(6.71 , 9.55)	50.69	(44.16 , 57.68)	10.33	(8.07 , 12.87)	7.29	(6.80 , 7.78)	2.75	(2.36 , 3.17)
2008103	4.57	(3.76 , 5.46)	8.56	(7.14 , 9.98)	45.20	(38.94 , 51.94)	12.20	(9.55 , 15.18)	7.24	(6.73 , 7.74)	3.27	(2.82 , 3.75)
2008148	4.99	(4.14 , 5.91)	8.13	(6.70 , 9.55)	47.20	(40.89 , 53.96)	7.79	(5.84 , 10.02)	7.32	(6.83 , 7.81)	2.49	(2.12 , 2.89)
2008236	4.83	(4.00 , 5.74)	7.35	(5.93 , 8.78)	51.08	(44.51 , 58.09)	8.14	(6.00 , 10.60)	7.39	(6.89 , 7.88)	2.55	(2.16 , 2.98)
2008259	5.84	(4.92 , 6.84)	11.30	(9.88 , 12.72)	56.34	(49.33 , 63.82)	15.04	(12.28 , 18.08)	7.70	(7.20 , 8.20)	3.45	(3.02 , 3.92)
2008191	4.79	(3.94 , 5.72)	7.95	(6.48 , 9.41)	44.62	(38.39 , 51.32)	6.48	(4.58 , 8.70)	6.78	(6.27 , 7.28)	2.28	(1.90 , 2.68)
2008242	5.10	(4.24 , 6.04)	7.27	(5.85 , 8.70)	49.69	(43.10 , 56.74)	8.28	(6.26 , 10.57)	7.42	(6.91 , 7.92)	2.41	(2.05 , 2.81)
2008248	4.29	(3.50 , 5.17)	7.84	(6.37 , 9.30)	45.79	(39.38 , 52.69)	9.20	(7.00 , 11.70)	6.84	(6.32 , 7.35)	2.95	(2.54 , 3.40)
2008010	4.16	(3.39 , 5.01)	11.34	(9.91 , 12.76)	46.16	(39.82 , 52.96)	14.77	(12.12 , 17.68)	7.16	(6.66 , 7.66)	3.39	(2.97 , 3.85)
2008263	4.55	(3.74 , 5.44)	8.57	(7.15 , 9.99)	40.04	(34.16 , 46.39)	10.80	(8.40 , 13.49)	6.75	(6.25 , 7.26)	3.03	(2.61 , 3.48)
2008153	4.78	(3.95 , 5.69)	7.47	(6.05 , 8.90)	48.11	(41.52 , 55.18)	9.39	(7.23 , 11.83)	7.22	(6.70 , 7.73)	2.82	(2.42 , 3.24)
2008119	4.67	(3.85 , 5.57)	6.55	(5.12 , 7.97)	42.45	(36.39 , 48.99)	10.54	(7.88 , 13.59)	7.19	(6.69 , 7.70)	2.75	(2.31 , 3.22)
2008206	3.94	(3.19 , 4.77)	8.04	(6.62 , 9.47)	41.64	(35.63 , 48.11)	7.79	(5.84 , 10.03)	6.96	(6.46 , 7.47)	2.58	(2.20 , 2.98)
2008139	3.83	(3.09 , 4.64)	5.22	(3.80 , 6.65)	43.25	(36.90 , 50.11)	5.68	(3.86 , 7.85)	6.78	(6.25 , 7.31)	2.27	(1.89 , 2.69)
2008106	4.85	(4.02 , 5.76)	11.24	(9.82 , 12.66)	52.61	(45.83 , 59.84)	17.39	(14.41 , 20.64)	7.79	(7.29 , 8.30)	3.85	(3.39 , 4.35)
2008122	4.44	(3.63 , 5.33)	8.34	(6.88 , 9.80)	50.86	(44.20 , 57.97)	12.51	(9.92 , 15.40)	7.24	(6.74 , 7.74)	3.30	(2.86 , 3.77)
2008155	5.01	(4.16 , 5.93)	7.46	(6.04 , 8.88)	46.80	(40.53 , 53.53)	9.16	(6.89 , 11.75)	7.17	(6.68 , 7.66)	2.69	(2.28 , 3.12)
2008088	4.62	(3.81 , 5.51)	10.42	(9.00 , 11.84)	45.09	(38.94 , 51.70)	10.03	(7.80 , 12.53)	7.48	(6.98 , 7.97)	3.08	(2.67 , 3.52)
2008111	5.24	(4.37 , 6.19)	7.65	(6.23 , 9.07)	41.61	(35.72 , 47.96)	9.73	(7.45 , 12.31)	7.03	(6.54 , 7.53)	2.83	(2.42 , 3.27)
2008202	4.49	(3.68 , 5.39)	5.88	(4.42 , 7.34)	50.82	(44.16 , 57.94)	7.75	(5.60 , 10.26)	7.74	(7.24 , 8.24)	2.63	(2.22 , 3.08)
2008013	4.22	(3.45 , 5.08)	6.44	(5.02 , 7.86)	42.30	(36.36 , 48.70)	8.60	(6.32 , 11.23)	6.50	(6.01 , 6.99)	2.55	(2.14 , 2.99)
2008279	4.88	(4.04 , 5.79)	8.09	(6.67 , 9.51)	47.95	(41.39 , 55.00)	9.27	(7.06 , 11.78)	7.48	(6.97 , 7.99)	2.76	(2.36 , 3.19)
2008007	4.01	(3.25 , 4.84)	6.76	(5.34 , 8.18)	45.43	(39.15 , 52.17)	8.96	(6.72 , 11.53)	7.08	(6.58 , 7.58)	2.61	(2.21 , 3.04)
2008090	6.13	(5.19 , 7.15)	10.14	(8.72 , 11.56)	49.15	(42.71 , 56.04)	13.97	(11.32 , 16.91)	7.93	(7.44 , 8.42)	3.29	(2.86 , 3.74)
2008055	4.33	(3.54 , 5.19)	7.63	(6.21 , 9.05)	46.15	(39.92 , 52.84)	7.90	(5.87 , 10.24)	6.77	(6.28 , 7.27)	2.38	(2.01 , 2.79)
2008271	3.74	(3.02 , 4.55)	6.58	(5.16 , 8.00)	47.14	(40.63 , 54.14)	9.61	(7.28 , 12.26)	7.26	(6.75 , 7.78)	2.96	(2.54 , 3.42)
2008035	5.00	(4.15 , 5.92)	9.04	(7.62 , 10.46)	47.53	(41.11 , 54.42)	9.06	(6.95 , 11.45)	7.56	(7.06 , 8.06)	2.97	(2.56 , 3.40)
2008212	3.79	(3.06 , 4.61)	8.19	(6.77 , 9.61)	38.59	(32.82 , 44.83)	8.78	(6.64 , 11.22)	6.76	(6.26 , 7.27)	2.57	(2.19 , 2.99)
2008149	5.90	(4.98 , 6.90)	7.34	(5.92 , 8.76)	48.86	(42.45 , 55.72)	8.92	(6.68 , 11.48)	7.82	(7.32 , 8.31)	2.78	(2.37 , 3.22)
2008170	5.50	(4.61 , 6.47)	8.08	(6.66 , 9.50)	49.04	(42.62 , 55.92)	9.30	(7.23 , 11.64)	7.47	(6.98 , 7.97)	2.62	(2.25 , 3.02)
2008247	3.88	(3.14 , 4.70)	6.32	(4.90 , 7.74)	45.65	(39.35 , 52.41)	7.14	(5.22 , 9.36)	7.07	(6.56 , 7.57)	2.38	(2.01 , 2.78)
2008110	4.12	(3.34 , 4.97)	6.60	(5.13 , 8.06)	42.46	(36.30 , 49.11)	6.87	(4.99 , 9.04)	6.83	(6.32 , 7.34)	2.03	(1.69 , 2.41)

Family	number of shoots 11 months *		number of shoots 24 months		length of the longest shoot 11 months *		length of the longest shoot 24 months *		number of nodes on the longest shoot 11 months		number of nodes on the longest shoot 24 months *	
2008043	3.34	(2.65 , 4.10)	6.51	(5.09 , 7.94)	41.99	(35.85 , 48.62)	6.72	(4.79 , 8.97)	6.76	(6.25 , 7.28)	2.43	(2.04 , 2.85)
2008188	4.11	(3.35 , 4.95)	6.76	(5.34 , 8.18)	48.08	(41.62 , 55.01)	15.98	(12.80 , 19.52)	7.01	(6.51 , 7.51)	2.94	(2.50 , 3.41)
2008080	4.75	(3.89 , 5.69)	6.80	(5.29 , 8.31)	43.43	(37.08 , 50.27)	6.81	(4.88 , 9.07)	6.75	(6.23 , 7.27)	2.50	(2.11 , 2.93)
2008239	3.46	(2.77 , 4.24)	6.76	(5.34 , 8.18)	48.38	(41.79 , 55.46)	6.31	(4.57 , 8.32)	7.11	(6.59 , 7.62)	2.36	(2.00 , 2.74)
2008075	4.62	(3.81 , 5.51)	9.55	(8.12 , 10.97)	46.55	(40.29 , 53.27)	13.03	(10.55 , 15.78)	7.43	(6.94 , 7.92)	2.66	(2.28 , 3.06)
2008114	3.93	(3.15 , 4.79)	6.42	(4.91 , 7.93)	35.58	(29.95 , 41.69)	7.09	(5.03 , 9.50)	6.29	(5.77 , 6.80)	2.31	(1.93 , 2.74)
2008166	5.26	(4.39 , 6.21)	10.07	(8.65 , 11.49)	41.17	(35.31 , 47.49)	10.74	(8.35 , 13.43)	6.80	(6.31 , 7.29)	3.21	(2.77 , 3.67)
2008252	5.02	(4.17 , 5.95)	8.62	(7.19 , 10.04)	49.60	(43.14 , 56.52)	10.00	(7.77 , 12.51)	7.55	(7.06 , 8.04)	2.62	(2.24 , 3.03)
2008150	4.40	(3.61 , 5.27)	5.55	(4.12 , 6.97)	39.32	(33.49 , 45.61)	6.30	(4.44 , 8.48)	6.90	(6.40 , 7.41)	2.51	(2.12 , 2.94)
2008009	4.27	(3.49 , 5.13)	7.22	(5.80 , 8.65)	43.89	(37.72 , 50.53)	8.99	(6.74 , 11.56)	6.98	(6.47 , 7.48)	2.51	(2.11 , 2.93)
2008081	3.61	(2.87 , 4.43)	8.61	(7.10 , 10.12)	44.69	(38.24 , 51.65)	10.59	(8.13 , 13.38)	6.98	(6.46 , 7.51)	3.10	(2.66 , 3.57)
2008041	4.79	(3.96 , 5.70)	8.78	(7.36 , 10.21)	46.32	(40.07 , 53.02)	9.09	(6.90 , 11.58)	7.67	(7.18 , 8.16)	2.87	(2.46 , 3.31)
2008061	5.40	(4.50 , 6.38)	8.98	(7.52 , 10.44)	47.55	(41.12 , 54.45)	9.51	(7.27 , 12.05)	7.13	(6.63 , 7.63)	3.01	(2.59 , 3.46)
2008087	3.70	(2.98 , 4.50)	8.11	(6.69 , 9.53)	44.84	(38.61 , 51.54)	9.21	(7.01 , 11.71)	7.18	(6.68 , 7.69)	3.01	(2.59 , 3.46)
2008147	4.53	(3.72 , 5.41)	9.22	(7.79 , 10.64)	44.49	(38.28 , 51.18)	12.65	(10.20 , 15.36)	7.28	(6.78 , 7.78)	3.09	(2.69 , 3.53)
2008258	4.50	(3.70 , 5.38)	9.32	(7.90 , 10.74)	49.60	(42.92 , 56.76)	9.10	(6.91 , 11.59)	7.53	(7.02 , 8.04)	2.81	(2.41 , 3.25)
2008130	4.20	(3.43 , 5.05)	6.45	(5.03 , 7.87)	41.98	(35.84 , 48.61)	9.17	(6.81 , 11.88)	6.98	(6.46 , 7.49)	2.85	(2.42 , 3.31)
2008100	4.17	(3.38 , 5.03)	6.64	(5.18 , 8.10)	40.29	(34.40 , 46.66)	12.89	(10.26 , 15.82)	6.54	(6.04 , 7.05)	2.91	(2.50 , 3.36)
2008132	5.03	(4.18 , 5.96)	10.03	(8.61 , 11.45)	48.35	(41.98 , 55.17)	13.24	(10.66 , 16.09)	7.62	(7.13 , 8.11)	3.14	(2.72 , 3.58)
2008256	5.73	(4.82 , 6.72)	11.04	(9.62 , 12.46)	48.50	(42.12 , 55.33)	17.33	(14.46 , 20.47)	7.66	(7.17 , 8.15)	3.21	(2.80 , 3.65)
2008093	4.12	(3.36 , 4.97)	6.04	(4.62 , 7.46)	40.20	(34.31 , 46.56)	5.21	(3.53 , 7.20)	6.59	(6.09 , 7.09)	2.01	(1.66 , 2.40)
2008108	5.75	(4.84 , 6.74)	11.37	(9.95 , 12.79)	57.26	(50.30 , 64.67)	13.63	(11.09 , 16.43)	7.77	(7.28 , 8.26)	3.30	(2.89 , 3.75)
2008179	4.11	(3.34 , 4.95)	6.67	(5.25 , 8.09)	42.08	(36.04 , 48.58)	5.68	(3.98 , 7.68)	7.18	(6.68 , 7.68)	2.02	(1.68 , 2.39)
2008192	4.37	(3.57 , 5.25)	6.20	(4.74 , 7.67)	40.00	(34.13 , 46.35)	11.66	(8.73 , 15.02)	6.59	(6.09 , 7.10)	3.18	(2.70 , 3.71)
2008089	3.69	(2.97 , 4.50)	6.91	(5.49 , 8.34)	37.48	(31.80 , 43.62)	9.00	(6.74 , 11.58)	6.43	(5.93 , 6.94)	2.57	(2.17 , 3.00)
2008064	4.40	(3.61 , 5.27)	8.29	(6.87 , 9.71)	45.41	(39.24 , 52.03)	12.03	(9.58 , 14.76)	7.04	(6.55 , 7.53)	2.87	(2.47 , 3.29)
2008269	4.82	(3.97 , 5.74)	7.86	(6.40 , 9.33)	46.21	(39.89 , 53.01)	6.68	(4.89 , 8.75)	7.48	(6.97 , 7.98)	2.59	(2.22 , 3.00)
2008059	4.12	(3.36 , 4.97)	5.25	(3.83 , 6.67)	39.97	(34.19 , 46.19)	6.14	(4.31 , 8.31)	6.73	(6.24 , 7.22)	2.21	(1.85 , 2.62)
2008047	4.98	(4.14 , 5.91)	5.98	(4.56 , 7.40)	42.84	(36.76 , 49.39)	3.88	(2.55 , 5.49)	6.98	(6.47 , 7.48)	1.86	(1.54 , 2.21)
2008194	4.07	(3.31 , 4.91)	7.54	(6.12 , 8.97)	44.31	(38.22 , 50.86)	7.94	(5.91 , 10.27)	7.09	(6.60 , 7.59)	2.49	(2.11 , 2.90)
2008232	3.87	(3.13 , 4.69)	7.20	(5.78 , 8.62)	39.21	(33.40 , 45.50)	8.82	(6.68 , 11.27)	6.41	(5.90 , 6.91)	2.41	(2.03 , 2.81)
2008173	4.14	(3.37 , 4.98)	8.57	(7.15 , 10.00)	38.79	(33.10 , 44.94)	7.67	(5.68 , 9.96)	6.36	(5.87 , 6.85)	2.59	(2.20 , 3.01)
2008120	3.78	(3.03 , 4.60)	6.54	(5.08 , 8.01)	43.80	(37.41 , 50.69)	7.62	(5.70 , 9.83)	6.98	(6.46 , 7.51)	2.50	(2.13 , 2.90)
2008262	4.53	(3.71 , 5.43)	6.88	(5.42 , 8.35)	39.70	(33.85 , 46.01)	7.02	(5.11 , 9.23)	6.70	(6.20 , 7.20)	2.36	(1.98 , 2.76)
2008187	4.56	(3.75 , 5.45)	8.55	(7.13 , 9.97)	45.77	(39.56 , 52.42)	10.89	(8.63 , 13.41)	6.79	(6.30 , 7.29)	2.95	(2.56 , 3.37)

Family	number of shoots 11 months *		number of shoots 24 months		length of the longest shoot 11 months *		length of the longest shoot 24 months *		number of nodes on the longest shoot 11 months		number of nodes on the longest shoot 24 months *	
2008051	3.87	(3.12 , 4.71)	7.16	(5.69 , 8.62)	42.20	(35.94 , 48.95)	12.41	(9.64 , 15.53)	6.82	(6.30 , 7.35)	3.16	(2.71 , 3.65)
2008198	3.94	(3.15 , 4.82)	7.05	(5.48 , 8.61)	45.98	(39.18 , 53.32)	9.41	(6.91 , 12.29)	7.32	(6.77 , 7.87)	2.76	(2.32 , 3.24)
2008054	4.84	(4.01 , 5.75)	7.12	(5.70 , 8.54)	47.29	(40.77 , 54.31)	7.25	(5.31 , 9.48)	7.58	(7.07 , 8.10)	2.57	(2.19 , 2.99)
2008244	4.96	(4.11 , 5.88)	5.50	(4.07 , 6.92)	43.53	(37.40 , 50.14)	7.96	(5.77 , 10.49)	7.11	(6.60 , 7.61)	2.36	(1.97 , 2.78)
2008177	3.40	(2.71 , 4.17)	5.09	(3.67 , 6.51)	42.07	(35.94 , 48.69)	5.78	(3.77 , 8.22)	6.71	(6.19 , 7.22)	2.27	(1.86 , 2.72)
2008117	4.58	(3.77 , 5.47)	6.71	(5.28 , 8.13)	48.15	(41.77 , 54.98)	10.53	(7.86 , 13.58)	6.87	(6.38 , 7.36)	2.98	(2.52 , 3.48)
2008016	5.09	(4.23 , 6.02)	6.54	(5.12 , 7.96)	45.67	(39.38 , 52.44)	13.02	(10.03 , 16.39)	7.00	(6.50 , 7.50)	3.12	(2.65 , 3.63)
2008267	4.20	(3.42 , 5.07)	7.86	(6.40 , 9.33)	47.33	(40.81 , 54.34)	8.79	(6.72 , 11.15)	7.19	(6.67 , 7.70)	2.65	(2.27 , 3.06)
2008040	4.00	(3.25 , 4.83)	7.69	(6.27 , 9.11)	47.76	(41.42 , 54.55)	10.43	(8.16 , 12.98)	6.98	(6.49 , 7.48)	2.84	(2.44 , 3.26)
2008218	4.12	(3.35 , 4.96)	6.94	(5.51 , 8.36)	45.24	(38.85 , 52.11)	7.43	(5.12 , 10.17)	7.26	(6.74 , 7.77)	2.44	(2.01 , 2.90)
2008070	4.49	(3.69 , 5.36)	5.70	(4.28 , 7.12)	42.74	(36.76 , 49.17)	10.24	(7.73 , 13.10)	6.81	(6.32 , 7.30)	2.54	(2.13 , 2.98)
2008102	3.02	(2.36 , 3.76)	5.83	(4.36 , 7.30)	39.53	(33.24 , 46.36)	5.37	(3.59 , 7.50)	6.70	(6.15 , 7.25)	2.11	(1.74 , 2.51)
2008078	6.22	(5.27 , 7.24)	10.85	(9.43 , 12.27)	45.74	(39.55 , 52.39)	10.67	(8.44 , 13.16)	7.32	(6.82 , 7.81)	2.79	(2.41 , 3.20)
2008074	4.06	(3.29 , 4.92)	8.54	(7.07 , 10.01)	39.36	(33.32 , 45.91)	10.93	(8.43 , 13.76)	6.42	(5.89 , 6.94)	2.89	(2.46 , 3.34)

b.

Family	height (at flower initiation) 13 months		height (at flower initiation) 25 months		height (mid-season) 14 months		height (mid-season) 26 months		height (at cone maturity) 16 months		height (at cone maturity) 28 months	
2008003	3.53	(3.30 , 3.77)	4.87	(4.66 , 5.09)	4.21	(3.97 , 4.44)	5.36	(5.23 , 5.49)	4.32	(4.14 , 4.50)	4.31	(4.13 , 4.50)
2008190	3.78	(3.55 , 4.02)	5.00	(4.79 , 5.21)	4.42	(4.18 , 4.66)	5.67	(5.54 , 5.80)	4.55	(4.36 , 4.73)	4.56	(4.37 , 4.74)
2008209	3.63	(3.40 , 3.87)	4.81	(4.59 , 5.02)	4.35	(4.09 , 4.61)	5.48	(5.34 , 5.61)	4.42	(4.24 , 4.61)	4.43	(4.24 , 4.62)
2008151	3.45	(3.21 , 3.68)	5.03	(4.81 , 5.24)	4.25	(4.01 , 4.49)	5.75	(5.62 , 5.88)	4.38	(4.20 , 4.57)	4.55	(4.36 , 4.74)
2008039	3.90	(3.66 , 4.13)	5.10	(4.89 , 5.32)	4.55	(4.31 , 4.78)	5.58	(5.45 , 5.71)	4.62	(4.44 , 4.81)	4.64	(4.45 , 4.82)
2008152	3.69	(3.45 , 3.92)	5.13	(4.92 , 5.35)	4.45	(4.20 , 4.70)	5.67	(5.54 , 5.80)	4.65	(4.46 , 4.83)	4.66	(4.48 , 4.85)
2008001	3.80	(3.56 , 4.05)	5.16	(4.94 , 5.39)	4.57	(4.32 , 4.81)	5.69	(5.55 , 5.83)	4.56	(4.36 , 4.75)	4.56	(4.37 , 4.76)
2008073	3.64	(3.40 , 3.87)	4.80	(4.59 , 5.02)	4.37	(4.13 , 4.61)	5.68	(5.55 , 5.81)	4.47	(4.29 , 4.65)	4.47	(4.29 , 4.66)
2008168	3.58	(3.35 , 3.82)	4.97	(4.75 , 5.18)	4.34	(4.10 , 4.58)	5.49	(5.36 , 5.62)	4.34	(4.16 , 4.53)	4.34	(4.15 , 4.53)
2008161	3.77	(3.54 , 4.01)	5.40	(5.19 , 5.61)	4.42	(4.18 , 4.65)	5.70	(5.57 , 5.84)	4.51	(4.32 , 4.69)	4.51	(4.33 , 4.70)
2008290	3.64	(3.39 , 3.88)	4.82	(4.59 , 5.05)	4.26	(4.02 , 4.50)	5.47	(5.33 , 5.61)	4.36	(4.17 , 4.56)	4.36	(4.17 , 4.56)
2008208	3.62	(3.38 , 3.86)	5.02	(4.80 , 5.23)	4.29	(4.05 , 4.53)	5.70	(5.57 , 5.83)	4.32	(4.14 , 4.50)	4.31	(4.13 , 4.50)
2008251	3.53	(3.30 , 3.77)	4.97	(4.76 , 5.19)	4.16	(3.92 , 4.40)	5.55	(5.42 , 5.68)	4.26	(4.08 , 4.44)	4.25	(4.07 , 4.43)
2008052	4.04	(3.80 , 4.28)	5.27	(5.05 , 5.48)	4.54	(4.30 , 4.78)	5.77	(5.64 , 5.91)	4.56	(4.37 , 4.75)	4.57	(4.38 , 4.76)

Family	height (at flower initiation) 13 months		height (at flower initiation) 25 months		height (mid-season) 14 months		height (mid-season) 26 months		height (at cone maturity) 16 months		height (at cone maturity) 28 months	
2008037	3.80	(3.56 , 4.04)	4.84	(4.62 , 5.05)	4.48	(4.24 , 4.73)	5.76	(5.62 , 5.89)	4.69	(4.50 , 4.88)	4.71	(4.52 , 4.90)
2008154	3.55	(3.31 , 3.78)	4.88	(4.66 , 5.09)	4.10	(3.86 , 4.34)	5.50	(5.37 , 5.63)	4.13	(3.95 , 4.31)	4.11	(3.93 , 4.30)
2008136	3.52	(3.29 , 3.76)	4.90	(4.69 , 5.11)	4.17	(3.93 , 4.41)	5.44	(5.31 , 5.57)	4.35	(4.16 , 4.53)	4.34	(4.15 , 4.52)
2008096	3.65	(3.41 , 3.88)	4.94	(4.73 , 5.15)	4.43	(4.19 , 4.67)	5.45	(5.32 , 5.58)	4.27	(4.09 , 4.45)	4.26	(4.08 , 4.45)
2008071	3.84	(3.61 , 4.08)	4.76	(4.54 , 4.97)	4.56	(4.32 , 4.81)	5.69	(5.56 , 5.83)	4.59	(4.41 , 4.77)	4.60	(4.42 , 4.78)
2008124	3.76	(3.53 , 4.00)	5.40	(5.19 , 5.61)	4.46	(4.22 , 4.70)	5.76	(5.63 , 5.89)	4.55	(4.37 , 4.74)	4.56	(4.38 , 4.75)
2008145	3.63	(3.39 , 3.86)	5.13	(4.92 , 5.34)	4.26	(4.02 , 4.51)	5.59	(5.46 , 5.72)	4.59	(4.41 , 4.77)	4.60	(4.42 , 4.79)
2008084	3.82	(3.59 , 4.06)	4.88	(4.67 , 5.09)	4.57	(4.34 , 4.81)	5.66	(5.52 , 5.79)	4.77	(4.58 , 4.95)	4.79	(4.60 , 4.97)
2008083	3.62	(3.39 , 3.86)	5.32	(5.11 , 5.54)	4.45	(4.21 , 4.68)	5.67	(5.54 , 5.80)	4.38	(4.20 , 4.57)	4.38	(4.20 , 4.56)
2008056	3.73	(3.49 , 3.96)	5.24	(5.03 , 5.46)	4.46	(4.23 , 4.70)	5.85	(5.72 , 5.98)	4.68	(4.50 , 4.87)	4.70	(4.52 , 4.89)
2008261	3.84	(3.61 , 4.08)	5.09	(4.88 , 5.31)	4.56	(4.32 , 4.79)	5.65	(5.52 , 5.78)	4.73	(4.55 , 4.92)	4.76	(4.57 , 4.94)
2008157	4.07	(3.84 , 4.31)	5.54	(5.32 , 5.75)	4.54	(4.30 , 4.78)	5.71	(5.58 , 5.84)	4.61	(4.42 , 4.79)	4.62	(4.44 , 4.80)
2008006	3.60	(3.37 , 3.84)	4.92	(4.71 , 5.14)	4.30	(4.06 , 4.55)	5.62	(5.49 , 5.75)	4.14	(3.96 , 4.33)	4.13	(3.94 , 4.31)
2008240	3.84	(3.60 , 4.07)	5.28	(5.07 , 5.50)	4.36	(4.13 , 4.60)	5.62	(5.49 , 5.75)	4.47	(4.29 , 4.65)	4.47	(4.29 , 4.66)
2008103	3.16	(2.92 , 3.39)	4.86	(4.65 , 5.07)	3.88	(3.65 , 4.12)	5.42	(5.29 , 5.55)	3.92	(3.73 , 4.10)	3.88	(3.70 , 4.06)
2008148	3.56	(3.33 , 3.80)	5.05	(4.83 , 5.26)	4.23	(3.98 , 4.48)	5.69	(5.56 , 5.83)	4.38	(4.20 , 4.57)	4.38	(4.19 , 4.56)
2008236	4.00	(3.77 , 4.24)	5.47	(5.26 , 5.68)	4.65	(4.41 , 4.89)	5.65	(5.52 , 5.79)	4.73	(4.54 , 4.91)	4.74	(4.56 , 4.93)
2008259	3.68	(3.45 , 3.92)	5.02	(4.81 , 5.23)	4.43	(4.20 , 4.67)	5.49	(5.36 , 5.62)	4.36	(4.18 , 4.54)	4.36	(4.17 , 4.54)
2008191	3.57	(3.33 , 3.81)	5.13	(4.91 , 5.35)	4.33	(4.09 , 4.57)	5.69	(5.56 , 5.83)	4.43	(4.24 , 4.62)	4.42	(4.24 , 4.61)
2008242	3.79	(3.55 , 4.03)	5.41	(5.19 , 5.62)	4.43	(4.18 , 4.69)	5.65	(5.51 , 5.78)	4.46	(4.28 , 4.65)	4.47	(4.28 , 4.65)
2008248	3.67	(3.43 , 3.91)	5.19	(4.97 , 5.41)	4.44	(4.20 , 4.69)	5.75	(5.62 , 5.89)	4.61	(4.42 , 4.80)	4.62	(4.43 , 4.81)
2008010	3.35	(3.12 , 3.59)	5.00	(4.79 , 5.21)	4.19	(3.96 , 4.43)	5.65	(5.52 , 5.78)	4.44	(4.26 , 4.62)	4.43	(4.25 , 4.62)
2008263	3.49	(3.25 , 3.72)	4.65	(4.43 , 4.86)	4.32	(4.09 , 4.56)	5.50	(5.37 , 5.63)	4.13	(3.94 , 4.31)	4.10	(3.92 , 4.29)
2008153	3.72	(3.48 , 3.95)	5.20	(4.99 , 5.41)	4.50	(4.26 , 4.74)	5.65	(5.52 , 5.79)	4.48	(4.30 , 4.67)	4.49	(4.30 , 4.67)
2008119	3.59	(3.35 , 3.82)	4.90	(4.69 , 5.12)	4.35	(4.11 , 4.60)	5.55	(5.41 , 5.68)	4.52	(4.34 , 4.70)	4.73	(4.54 , 4.92)
2008206	3.54	(3.30 , 3.77)	5.39	(5.18 , 5.60)	4.34	(4.10 , 4.57)	5.70	(5.57 , 5.83)	4.26	(4.08 , 4.44)	4.24	(4.06 , 4.43)
2008139	3.55	(3.31 , 3.78)	5.24	(5.03 , 5.45)	4.45	(4.21 , 4.70)	5.66	(5.53 , 5.79)	4.44	(4.26 , 4.62)	4.43	(4.25 , 4.62)
2008106	3.52	(3.28 , 3.75)	5.12	(4.90 , 5.33)	4.45	(4.21 , 4.68)	5.53	(5.40 , 5.66)	4.44	(4.25 , 4.62)	4.44	(4.25 , 4.62)
2008122	3.45	(3.21 , 3.69)	5.18	(4.97 , 5.40)	4.20	(3.96 , 4.44)	5.44	(5.31 , 5.58)	4.22	(4.03 , 4.41)	4.21	(4.02 , 4.40)
2008155	3.74	(3.50 , 3.97)	5.13	(4.92 , 5.34)	4.33	(4.10 , 4.57)	5.61	(5.48 , 5.75)	4.43	(4.25 , 4.62)	4.43	(4.25 , 4.62)
2008088	3.47	(3.24 , 3.71)	4.92	(4.71 , 5.13)	4.22	(3.98 , 4.45)	5.49	(5.36 , 5.63)	4.26	(4.08 , 4.45)	4.25	(4.07 , 4.44)
2008111	3.38	(3.15 , 3.62)	4.61	(4.40 , 4.83)	4.28	(4.04 , 4.53)	5.08	(4.95 , 5.21)	4.19	(4.00 , 4.37)	4.17	(3.99 , 4.36)
2008202	3.88	(3.64 , 4.12)	5.14	(4.92 , 5.36)	4.55	(4.31 , 4.79)	5.53	(5.40 , 5.67)	4.53	(4.35 , 4.72)	4.54	(4.35 , 4.73)
2008013	3.76	(3.53 , 4.00)	5.37	(5.16 , 5.58)	4.53	(4.29 , 4.76)	5.66	(5.53 , 5.79)	4.79	(4.61 , 4.97)	4.82	(4.64 , 5.00)
2008279	3.88	(3.64 , 4.11)	5.23	(5.02 , 5.45)	4.59	(4.36 , 4.83)	5.68	(5.55 , 5.81)	4.68	(4.50 , 4.86)	4.70	(4.52 , 4.89)

Family	height (at flower initiation) 13 months		height (at flower initiation) 25 months		height (mid-season) 14 months		height (mid-season) 26 months		height (at cone maturity) 16 months		height (at cone maturity) 28 months	
2008007	3.75	(3.51 , 3.99)	4.85	(4.64 , 5.06)	4.19	(3.96 , 4.43)	5.48	(5.35 , 5.62)	4.26	(4.07 , 4.44)	4.25	(4.06 , 4.44)
2008090	3.36	(3.13 , 3.60)	4.90	(4.69 , 5.11)	4.12	(3.88 , 4.36)	5.56	(5.43 , 5.69)	4.10	(3.92 , 4.28)	4.08	(3.89 , 4.26)
2008055	3.85	(3.62 , 4.09)	5.38	(5.17 , 5.60)	4.57	(4.34 , 4.81)	5.72	(5.59 , 5.86)	4.62	(4.44 , 4.81)	4.63	(4.45 , 4.82)
2008271	3.60	(3.36 , 3.83)	5.14	(4.93 , 5.35)	4.37	(4.13 , 4.61)	5.53	(5.40 , 5.66)	4.46	(4.27 , 4.64)	4.46	(4.27 , 4.64)
2008035	3.65	(3.41 , 3.88)	5.30	(5.08 , 5.51)	4.35	(4.11 , 4.58)	5.73	(5.60 , 5.86)	4.44	(4.25 , 4.62)	4.60	(4.41 , 4.79)
2008212	3.62	(3.39 , 3.86)	5.03	(4.82 , 5.25)	4.25	(4.01 , 4.48)	5.60	(5.47 , 5.73)	4.41	(4.22 , 4.59)	4.40	(4.22 , 4.59)
2008149	3.67	(3.44 , 3.91)	5.12	(4.90 , 5.33)	4.33	(4.09 , 4.57)	5.65	(5.52 , 5.78)	4.54	(4.35 , 4.72)	4.54	(4.36 , 4.73)
2008170	3.87	(3.63 , 4.10)	5.42	(5.21 , 5.63)	4.54	(4.30 , 4.77)	5.73	(5.60 , 5.86)	4.65	(4.47 , 4.84)	4.67	(4.48 , 4.85)
2008247	3.64	(3.40 , 3.87)	4.86	(4.65 , 5.08)	4.25	(4.01 , 4.49)	5.62	(5.49 , 5.75)	4.25	(4.07 , 4.43)	4.23	(4.05 , 4.41)
2008110	3.64	(3.40 , 3.88)	5.00	(4.78 , 5.22)	4.33	(4.09 , 4.57)	5.54	(5.41 , 5.68)	4.35	(4.15 , 4.54)	4.35	(4.15 , 4.54)
2008043	3.65	(3.42 , 3.89)	5.14	(4.93 , 5.36)	4.47	(4.24 , 4.71)	5.70	(5.57 , 5.83)	4.55	(4.36 , 4.73)	4.55	(4.37 , 4.74)
2008188	3.76	(3.53 , 4.00)	5.40	(5.19 , 5.61)	4.39	(4.16 , 4.63)	5.60	(5.47 , 5.74)	4.55	(4.36 , 4.73)	4.56	(4.37 , 4.74)
2008080	3.77	(3.52 , 4.01)	5.01	(4.78 , 5.23)	4.47	(4.22 , 4.71)	5.69	(5.56 , 5.83)	4.49	(4.29 , 4.69)	4.50	(4.30 , 4.70)
2008239	3.50	(3.27 , 3.74)	5.19	(4.97 , 5.40)	4.16	(3.92 , 4.40)	5.77	(5.64 , 5.90)	4.40	(4.22 , 4.59)	4.40	(4.22 , 4.59)
2008075	3.59	(3.36 , 3.83)	5.27	(5.06 , 5.48)	4.29	(4.05 , 4.52)	5.55	(5.42 , 5.68)	4.45	(4.26 , 4.63)	4.45	(4.26 , 4.63)
2008114	3.67	(3.42 , 3.91)	5.54	(5.31 , 5.76)	4.43	(4.19 , 4.67)	5.61	(5.47 , 5.75)	4.62	(4.43 , 4.82)	4.64	(4.44 , 4.83)
2008166	3.69	(3.45 , 3.92)	5.09	(4.88 , 5.30)	4.44	(4.20 , 4.68)	5.73	(5.60 , 5.86)	4.65	(4.47 , 4.83)	4.67	(4.48 , 4.85)
2008252	3.80	(3.56 , 4.03)	5.36	(5.15 , 5.57)	4.35	(4.11 , 4.59)	5.65	(5.52 , 5.78)	4.54	(4.35 , 4.73)	4.55	(4.36 , 4.74)
2008150	3.77	(3.53 , 4.00)	5.25	(5.04 , 5.46)	4.35	(4.11 , 4.59)	5.76	(5.63 , 5.89)	4.40	(4.22 , 4.58)	4.40	(4.22 , 4.58)
2008009	3.76	(3.53 , 4.00)	5.09	(4.88 , 5.31)	4.32	(4.09 , 4.56)	5.52	(5.39 , 5.66)	4.41	(4.23 , 4.60)	4.41	(4.23 , 4.60)
2008081	3.37	(3.13 , 3.62)	4.73	(4.51 , 4.96)	4.27	(4.01 , 4.53)	5.47	(5.34 , 5.61)	4.18	(3.98 , 4.37)	4.16	(3.96 , 4.35)
2008041	3.64	(3.40 , 3.88)	4.93	(4.72 , 5.14)	4.31	(4.07 , 4.55)	5.57	(5.44 , 5.70)	4.49	(4.31 , 4.67)	4.49	(4.31 , 4.68)
2008061	4.14	(3.90 , 4.38)	5.43	(5.21 , 5.65)	4.64	(4.40 , 4.88)	5.75	(5.62 , 5.89)	4.90	(4.71 , 5.09)	4.93	(4.74 , 5.12)
2008087	3.35	(3.12 , 3.59)	4.66	(4.45 , 4.88)	4.07	(3.84 , 4.31)	5.33	(5.20 , 5.46)	3.85	(3.66 , 4.03)	3.81	(3.63 , 4.00)
2008147	3.58	(3.35 , 3.82)	4.95	(4.74 , 5.17)	4.49	(4.25 , 4.72)	5.59	(5.46 , 5.73)	4.42	(4.24 , 4.60)	4.42	(4.24 , 4.60)
2008258	3.55	(3.31 , 3.78)	4.81	(4.60 , 5.02)	4.25	(4.01 , 4.49)	5.60	(5.47 , 5.73)	4.46	(4.27 , 4.64)	4.46	(4.27 , 4.64)
2008130	3.79	(3.55 , 4.03)	5.07	(4.86 , 5.28)	4.41	(4.18 , 4.65)	5.54	(5.41 , 5.67)	4.38	(4.20 , 4.56)	4.38	(4.19 , 4.56)
2008100	3.46	(3.22 , 3.70)	4.87	(4.65 , 5.09)	4.24	(3.99 , 4.49)	5.27	(5.14 , 5.41)	4.25	(4.06 , 4.44)	4.24	(4.05 , 4.43)
2008132	3.53	(3.30 , 3.77)	4.76	(4.55 , 4.97)	4.32	(4.09 , 4.56)	5.43	(5.30 , 5.56)	4.39	(4.21 , 4.57)	4.39	(4.21 , 4.58)
2008256	3.45	(3.21 , 3.68)	4.69	(4.47 , 4.90)	4.26	(4.02 , 4.49)	5.27	(5.14 , 5.40)	4.24	(4.06 , 4.43)	4.23	(4.05 , 4.42)
2008093	3.60	(3.36 , 3.83)	5.42	(5.20 , 5.63)	4.31	(4.07 , 4.55)	5.68	(5.54 , 5.81)	4.40	(4.22 , 4.58)	4.40	(4.22 , 4.58)
2008108	4.04	(3.81 , 4.28)	5.17	(4.96 , 5.38)	4.60	(4.36 , 4.83)	5.70	(5.57 , 5.83)	4.74	(4.56 , 4.93)	4.77	(4.59 , 4.95)
2008179	3.83	(3.59 , 4.06)	5.27	(5.06 , 5.49)	4.48	(4.25 , 4.72)	5.73	(5.59 , 5.86)	4.57	(4.39 , 4.76)	4.58	(4.40 , 4.77)
2008192	3.77	(3.53 , 4.01)	5.27	(5.05 , 5.49)	4.41	(4.17 , 4.65)	5.60	(5.46 , 5.73)	4.45	(4.27 , 4.64)	4.45	(4.26 , 4.64)
2008089	3.34	(3.10 , 3.57)	4.79	(4.57 , 5.00)	4.06	(3.83 , 4.30)	5.54	(5.41 , 5.67)	4.13	(3.95 , 4.31)	4.12	(3.93 , 4.30)

Family	height (at flower initiation) 13 months		height (at flower initiation) 25 months		height (mid-season) 14 months		height (mid-season) 26 months		height (at cone maturity) 16 months		height (at cone maturity) 28 months	
2008064	3.82	(3.58 , 4.05)	5.43	(5.21 , 5.64)	4.39	(4.15 , 4.62)	5.55	(5.42 , 5.68)	4.56	(4.37 , 4.74)	4.57	(4.38 , 4.75)
2008269	3.78	(3.54 , 4.02)	5.18	(4.96 , 5.39)	4.46	(4.22 , 4.70)	5.72	(5.58 , 5.85)	4.60	(4.41 , 4.79)	4.62	(4.43 , 4.81)
2008059	3.76	(3.52 , 3.99)	5.01	(4.79 , 5.22)	4.48	(4.25 , 4.72)	5.62	(5.49 , 5.76)	4.62	(4.44 , 4.80)	4.63	(4.45 , 4.82)
2008047	4.04	(3.80 , 4.27)	5.32	(5.10 , 5.53)	4.61	(4.36 , 4.85)	5.80	(5.67 , 5.94)	4.68	(4.50 , 4.87)	4.70	(4.52 , 4.89)
2008194	3.61	(3.38 , 3.85)	5.02	(4.81 , 5.23)	4.18	(3.93 , 4.42)	5.62	(5.49 , 5.75)	4.40	(4.22 , 4.58)	4.40	(4.22 , 4.59)
2008232	3.67	(3.44 , 3.91)	5.14	(4.93 , 5.36)	4.43	(4.19 , 4.68)	5.68	(5.54 , 5.81)	4.44	(4.26 , 4.62)	4.44	(4.26 , 4.62)
2008173	3.93	(3.69 , 4.16)	5.45	(5.24 , 5.66)	4.39	(4.15 , 4.64)	5.66	(5.53 , 5.79)	4.62	(4.44 , 4.80)	4.64	(4.45 , 4.82)
2008120	3.53	(3.29 , 3.77)	5.24	(5.02 , 5.46)	4.15	(3.91 , 4.39)	5.59	(5.46 , 5.73)	4.32	(4.13 , 4.51)	4.32	(4.13 , 4.51)
2008262	3.57	(3.33 , 3.81)	4.71	(4.50 , 4.93)	4.31	(4.07 , 4.55)	5.53	(5.40 , 5.67)	4.53	(4.34 , 4.72)	4.54	(4.35 , 4.73)
2008187	3.67	(3.44 , 3.91)	4.98	(4.77 , 5.20)	4.29	(4.04 , 4.53)	5.50	(5.37 , 5.63)	4.16	(3.98 , 4.34)	4.14	(3.95 , 4.32)
2008051	3.36	(3.13 , 3.60)	4.85	(4.64 , 5.07)	4.07	(3.83 , 4.31)	5.62	(5.49 , 5.76)	4.22	(4.03 , 4.41)	4.21	(4.02 , 4.40)
2008198	3.41	(3.16 , 3.66)	4.61	(4.38 , 4.85)	4.00	(3.76 , 4.25)	5.20	(5.06 , 5.34)	3.98	(3.78 , 4.18)	3.94	(3.74 , 4.14)
2008054	3.74	(3.51 , 3.98)	5.06	(4.84 , 5.28)	4.19	(3.95 , 4.43)	5.61	(5.48 , 5.74)	4.18	(3.99 , 4.36)	4.16	(3.98 , 4.34)
2008244	4.01	(3.77 , 4.24)	5.45	(5.24 , 5.66)	4.55	(4.31 , 4.79)	5.78	(5.65 , 5.91)	4.63	(4.44 , 4.81)	4.64	(4.46 , 4.82)
2008177	3.94	(3.70 , 4.18)	5.37	(5.16 , 5.58)	4.50	(4.24 , 4.75)	5.80	(5.67 , 5.94)	4.58	(4.40 , 4.76)	4.59	(4.41 , 4.77)
2008117	3.73	(3.49 , 3.97)	5.13	(4.92 , 5.34)	4.26	(4.02 , 4.51)	5.52	(5.38 , 5.65)	4.48	(4.30 , 4.67)	4.49	(4.30 , 4.67)
2008016	3.94	(3.71 , 4.18)	5.39	(5.18 , 5.60)	4.64	(4.40 , 4.89)	5.65	(5.52 , 5.78)	4.67	(4.49 , 4.86)	4.69	(4.51 , 4.87)
2008267	3.80	(3.56 , 4.04)	5.49	(5.27 , 5.70)	4.57	(4.32 , 4.81)	5.70	(5.56 , 5.83)	4.59	(4.40 , 4.78)	4.60	(4.41 , 4.79)
2008040	3.41	(3.18 , 3.65)	4.93	(4.72 , 5.15)	4.07	(3.83 , 4.31)	5.48	(5.35 , 5.61)	4.27	(4.08 , 4.46)	4.25	(4.07 , 4.44)
2008218	3.63	(3.40 , 3.87)	5.16	(4.95 , 5.38)	4.37	(4.14 , 4.61)	5.73	(5.60 , 5.86)	4.37	(4.19 , 4.55)	4.36	(4.18 , 4.55)
2008070	3.70	(3.46 , 3.93)	5.04	(4.83 , 5.26)	4.39	(4.15 , 4.64)	5.72	(5.59 , 5.85)	4.54	(4.35 , 4.72)	4.55	(4.36 , 4.73)
2008102	3.36	(3.12 , 3.60)	4.75	(4.53 , 4.98)	4.13	(3.88 , 4.38)	5.51	(5.37 , 5.65)	4.05	(3.86 , 4.24)	4.03	(3.84 , 4.21)
2008078	3.52	(3.28 , 3.75)	4.69	(4.47 , 4.90)	4.29	(4.05 , 4.52)	5.56	(5.43 , 5.69)	4.32	(4.14 , 4.51)	4.32	(4.14 , 4.50)
2008074	3.52	(3.28 , 3.76)	5.25	(5.03 , 5.47)	4.20	(3.95 , 4.45)	5.72	(5.58 , 5.85)	4.45	(4.27 , 4.64)	4.46	(4.27 , 4.64)

c.

Family	lateral length 16 months *		lateral length 28 months *		number of nodes on lateral 16 months *		number of nodes on lateral 28 months *		internode length 16 months *		internode length 28 months *	
2008003	43.18	(38.64 , 47.98)	38.57	(33.70 , 43.77)	6.43	(5.91 , 6.98)	6.42	(5.90 , 6.96)	15.41	(14.08 , 16.79)	22.56	(20.40 , 24.83)
2008190	42.65	(38.14 , 47.42)	35.04	(30.41 , 40.00)	5.55	(5.07 , 6.06)	5.57	(5.09 , 6.07)	17.60	(16.19 , 19.07)	22.56	(20.40 , 24.83)
2008209	41.69	(36.97 , 46.68)	35.65	(30.85 , 40.79)	5.94	(5.41 , 6.49)	5.94	(5.42 , 6.49)	15.67	(14.24 , 17.17)	22.56	(20.40 , 24.83)
2008151	47.61	(42.83 , 52.64)	39.59	(34.59 , 44.91)	5.89	(5.39 , 6.42)	5.90	(5.41 , 6.42)	18.41	(16.74 , 20.16)	22.56	(20.40 , 24.83)
2008039	45.53	(40.86 , 50.45)	47.60	(42.17 , 53.36)	6.39	(5.86 , 6.94)	6.39	(5.86 , 6.93)	17.30	(15.90 , 18.77)	22.56	(20.40 , 24.83)
2008152	47.20	(42.43 , 52.21)	42.21	(37.11 , 47.64)	5.98	(5.48 , 6.51)	5.99	(5.49 , 6.51)	17.88	(16.45 , 19.37)	22.56	(20.40 , 24.83)
2008001	39.52	(35.02 , 44.29)	33.27	(28.65 , 38.23)	5.41	(4.91 , 5.93)	5.43	(4.94 , 5.94)	18.48	(16.94 , 20.10)	22.56	(20.40 , 24.83)
2008073	46.44	(41.73 , 51.41)	40.86	(35.85 , 46.20)	6.73	(6.20 , 7.29)	6.72	(6.19 , 7.27)	19.24	(17.53 , 21.03)	22.56	(20.40 , 24.83)
2008168	42.44	(37.94 , 47.20)	37.71	(32.76 , 43.01)	6.16	(5.65 , 6.70)	6.17	(5.66 , 6.69)	17.96	(16.69 , 19.29)	22.56	(20.40 , 24.83)
2008161	44.27	(39.67 , 49.13)	38.26	(33.42 , 43.42)	6.06	(5.55 , 6.59)	6.07	(5.56 , 6.59)	17.95	(16.51 , 19.45)	22.56	(20.40 , 24.83)
2008290	41.10	(36.51 , 45.96)	34.88	(30.14 , 39.98)	5.83	(5.31 , 6.37)	5.84	(5.33 , 6.37)	16.69	(15.10 , 18.36)	22.56	(20.40 , 24.83)
2008208	44.52	(39.91 , 49.40)	37.67	(32.87 , 42.80)	5.82	(5.32 , 6.34)	5.83	(5.34 , 6.34)	16.47	(15.10 , 17.90)	22.56	(20.40 , 24.83)
2008251	39.91	(35.47 , 44.62)	32.89	(28.35 , 37.75)	5.76	(5.26 , 6.29)	5.78	(5.28 , 6.30)	17.61	(15.83 , 19.49)	22.56	(20.40 , 24.83)
2008052	45.01	(40.29 , 50.00)	37.74	(32.78 , 43.05)	6.06	(5.55 , 6.60)	6.07	(5.56 , 6.60)	20.78	(19.24 , 22.37)	22.56	(20.40 , 24.83)
2008037	45.77	(41.00 , 50.79)	39.61	(34.60 , 44.96)	6.13	(5.61 , 6.67)	6.13	(5.62 , 6.67)	18.45	(16.78 , 20.21)	22.56	(20.40 , 24.83)
2008154	44.73	(40.10 , 49.61)	39.11	(34.21 , 44.34)	5.96	(5.46 , 6.49)	5.97	(5.47 , 6.49)	15.81	(14.27 , 17.44)	22.56	(20.40 , 24.83)
2008136	45.17	(40.43 , 50.17)	36.52	(31.66 , 41.73)	5.83	(5.32 , 6.36)	5.84	(5.34 , 6.36)	13.89	(12.70 , 15.12)	22.56	(20.40 , 24.83)
2008096	42.06	(37.58 , 46.80)	37.32	(32.55 , 42.41)	5.88	(5.38 , 6.40)	5.89	(5.39 , 6.40)	14.93	(13.63 , 16.29)	22.56	(20.40 , 24.83)
2008071	45.61	(40.92 , 50.55)	37.05	(32.21 , 42.23)	5.77	(5.28 , 6.29)	5.78	(5.29 , 6.30)	19.49	(17.76 , 21.29)	22.56	(20.40 , 24.83)
2008124	50.90	(45.96 , 56.09)	43.91	(38.70 , 49.44)	6.32	(5.80 , 6.86)	6.32	(5.80 , 6.85)	19.71	(17.98 , 21.53)	22.56	(20.40 , 24.83)
2008145	44.37	(39.75 , 49.24)	38.45	(33.59 , 43.65)	5.97	(5.46 , 6.49)	5.97	(5.47 , 6.50)	18.63	(17.16 , 20.16)	22.56	(20.40 , 24.83)
2008084	43.41	(38.85 , 48.21)	37.45	(32.49 , 42.77)	6.15	(5.63 , 6.68)	6.15	(5.64 , 6.68)	16.01	(14.65 , 17.43)	22.56	(20.40 , 24.83)
2008083	44.62	(40.00 , 49.49)	38.13	(33.31 , 43.28)	6.33	(5.81 , 6.87)	6.33	(5.81 , 6.86)	16.20	(14.84 , 17.61)	22.56	(20.40 , 24.83)
2008056	48.87	(44.04 , 53.96)	40.93	(35.93 , 46.26)	7.11	(6.56 , 7.68)	7.09	(6.54 , 7.65)	19.30	(17.82 , 20.84)	22.56	(20.40 , 24.83)
2008261	47.59	(42.81 , 52.61)	40.62	(35.63 , 45.93)	6.04	(5.53 , 6.57)	6.04	(5.54 , 6.56)	17.60	(16.34 , 18.91)	22.56	(20.40 , 24.83)
2008157	49.31	(44.45 , 54.43)	43.31	(38.17 , 48.77)	6.12	(5.61 , 6.65)	6.12	(5.62 , 6.65)	16.96	(15.35 , 18.64)	22.56	(20.40 , 24.83)
2008006	37.71	(33.31 , 42.37)	31.99	(27.46 , 36.87)	5.53	(5.03 , 6.05)	5.55	(5.05 , 6.06)	17.21	(15.59 , 18.90)	22.56	(20.40 , 24.83)
2008240	47.24	(42.48 , 52.25)	41.20	(36.17 , 46.56)	6.92	(6.37 , 7.48)	6.90	(6.36 , 7.46)	14.92	(13.42 , 16.50)	22.56	(20.40 , 24.83)
2008103	36.81	(32.46 , 41.42)	32.13	(27.56 , 37.04)	5.61	(5.11 , 6.14)	5.63	(5.13 , 6.15)	15.37	(14.05 , 16.75)	22.56	(20.40 , 24.83)
2008148	41.28	(36.83 , 45.98)	36.04	(31.34 , 41.06)	5.80	(5.30 , 6.32)	5.81	(5.32 , 6.33)	15.37	(13.84 , 16.98)	22.56	(20.40 , 24.83)
2008236	49.00	(44.15 , 54.10)	40.65	(35.64 , 45.99)	6.59	(6.06 , 7.14)	6.58	(6.06 , 7.13)	17.49	(16.07 , 18.96)	22.56	(20.40 , 24.83)
2008259	43.98	(39.32 , 48.90)	39.81	(34.80 , 45.16)	5.89	(5.38 , 6.42)	5.90	(5.40 , 6.42)	16.73	(15.50 , 18.01)	22.56	(20.40 , 24.83)
2008191	45.48	(40.72 , 50.50)	41.44	(36.32 , 46.91)	5.97	(5.46 , 6.51)	5.98	(5.47 , 6.51)	16.63	(15.39 , 17.92)	22.56	(20.40 , 24.83)

Family	lateral length 16 months *		lateral length 28 months *		number of nodes on lateral 16 months *		number of nodes on lateral 28 months *		internode length 16 months *		internode length 28 months *	
2008242	43.02	(38.39 , 47.92)	35.83	(31.08 , 40.92)	6.20	(5.68 , 6.75)	6.20	(5.69 , 6.74)	16.16	(14.59 , 17.81)	22.56	(20.40 , 24.83)
2008248	44.44	(39.74 , 49.39)	38.16	(33.25 , 43.42)	6.18	(5.66 , 6.73)	6.18	(5.67 , 6.72)	16.83	(15.45 , 18.27)	22.56	(20.40 , 24.83)
2008010	45.81	(41.13 , 50.75)	39.65	(34.72 , 44.90)	6.39	(5.87 , 6.94)	6.39	(5.87 , 6.93)	16.48	(14.90 , 18.15)	22.56	(20.40 , 24.83)
2008263	45.54	(40.87 , 50.46)	40.06	(35.10 , 45.35)	6.41	(5.89 , 6.96)	6.41	(5.89 , 6.95)	18.09	(16.66 , 19.59)	22.56	(20.40 , 24.83)
2008153	43.95	(39.36 , 48.80)	37.29	(32.51 , 42.41)	6.12	(5.61 , 6.65)	6.12	(5.61 , 6.65)	16.41	(15.04 , 17.85)	22.56	(20.40 , 24.83)
2008119	44.96	(40.32 , 49.86)	38.80	(33.85 , 44.09)	6.01	(5.50 , 6.54)	6.01	(5.51 , 6.54)	16.10	(14.74 , 17.52)	22.56	(20.40 , 24.83)
2008206	41.40	(36.95 , 46.11)	37.55	(32.75 , 42.68)	6.06	(5.55 , 6.59)	6.06	(5.56 , 6.59)	17.09	(15.48 , 18.79)	22.56	(20.40 , 24.83)
2008139	43.74	(39.07 , 48.66)	37.24	(32.40 , 42.42)	6.02	(5.51 , 6.56)	6.03	(5.52 , 6.56)	17.90	(16.25 , 19.63)	22.56	(20.40 , 24.83)
2008106	43.96	(39.37 , 48.80)	37.68	(32.89 , 42.81)	5.98	(5.48 , 6.51)	5.99	(5.49 , 6.51)	18.06	(16.63 , 19.55)	22.56	(20.40 , 24.83)
2008122	40.53	(35.97 , 45.36)	34.81	(30.07 , 39.90)	6.13	(5.60 , 6.68)	6.13	(5.61 , 6.68)	15.52	(13.99 , 17.13)	22.56	(20.40 , 24.83)
2008155	44.54	(39.92 , 49.41)	39.05	(34.16 , 44.26)	6.19	(5.67 , 6.72)	6.19	(5.68 , 6.72)	16.49	(15.12 , 17.92)	22.56	(20.40 , 24.83)
2008088	41.00	(36.49 , 45.77)	35.14	(30.38 , 40.25)	5.74	(5.23 , 6.26)	5.75	(5.25 , 6.27)	15.41	(13.74 , 17.18)	22.56	(20.40 , 24.83)
2008111	41.25	(36.82 , 45.94)	36.27	(31.42 , 41.47)	5.83	(5.34 , 6.35)	5.84	(5.35 , 6.36)	15.98	(14.64 , 17.38)	22.56	(20.40 , 24.83)
2008202	46.69	(41.88 , 51.77)	40.23	(35.19 , 45.60)	6.50	(5.96 , 7.05)	6.49	(5.96 , 7.04)	16.67	(15.09 , 18.34)	22.56	(20.40 , 24.83)
2008013	49.25	(44.39 , 54.36)	43.68	(38.51 , 49.18)	6.34	(5.82 , 6.88)	6.33	(5.82 , 6.87)	18.37	(17.08 , 19.71)	22.56	(20.40 , 24.83)
2008279	44.75	(40.13 , 49.63)	39.27	(34.37 , 44.50)	5.98	(5.47 , 6.50)	5.98	(5.48 , 6.50)	19.10	(17.40 , 20.88)	22.56	(20.40 , 24.83)
2008007	52.02	(46.83 , 57.48)	44.75	(39.34 , 50.50)	6.48	(5.94 , 7.04)	6.47	(5.93 , 7.03)	17.00	(15.40 , 18.68)	22.56	(20.40 , 24.83)
2008090	42.79	(38.18 , 47.67)	36.77	(31.96 , 41.92)	6.27	(5.75 , 6.82)	6.27	(5.75 , 6.82)	16.66	(15.08 , 18.33)	22.56	(20.40 , 24.83)
2008055	47.09	(42.33 , 52.10)	38.91	(33.96 , 44.19)	6.57	(6.04 , 7.13)	6.56	(6.04 , 7.11)	16.66	(15.07 , 18.32)	22.56	(20.40 , 24.83)
2008271	45.48	(40.81 , 50.41)	38.13	(33.23 , 43.36)	6.27	(5.75 , 6.81)	6.27	(5.76 , 6.80)	17.90	(16.25 , 19.64)	22.56	(20.40 , 24.83)
2008035	44.68	(39.97 , 49.64)	40.60	(35.54 , 46.00)	5.89	(5.38 , 6.42)	5.89	(5.39 , 6.42)	16.90	(15.51 , 18.35)	22.56	(20.40 , 24.83)
2008212	45.09	(40.45 , 49.99)	38.92	(34.04 , 44.13)	6.35	(5.83 , 6.89)	6.35	(5.83 , 6.88)	17.56	(15.93 , 19.28)	22.56	(20.40 , 24.83)
2008149	42.83	(38.31 , 47.61)	36.11	(31.41 , 41.13)	6.06	(5.55 , 6.59)	6.06	(5.56 , 6.58)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008170	48.87	(44.03 , 53.97)	41.92	(36.85 , 47.32)	6.27	(5.75 , 6.81)	6.27	(5.75 , 6.80)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008247	39.06	(34.74 , 43.63)	32.53	(28.02 , 37.38)	5.63	(5.14 , 6.14)	5.64	(5.16 , 6.15)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008110	37.49	(33.11 , 42.15)	32.22	(27.66 , 37.12)	5.57	(5.06 , 6.10)	5.67	(5.16 , 6.19)	15.16	(13.64 , 16.75)	22.56	(20.40 , 24.83)
2008043	45.44	(40.68 , 50.46)	39.85	(34.84 , 45.20)	6.17	(5.65 , 6.72)	6.17	(5.66 , 6.71)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008188	43.19	(38.64 , 47.98)	36.28	(31.52 , 41.38)	6.55	(6.02 , 7.10)	6.54	(6.02 , 7.09)	17.37	(15.75 , 19.08)	22.56	(20.40 , 24.83)
2008080	45.10	(40.29 , 50.18)	38.14	(33.19 , 43.44)	6.12	(5.59 , 6.67)	6.12	(5.60 , 6.66)	17.42	(15.65 , 19.29)	22.56	(20.40 , 24.83)
2008239	43.64	(38.90 , 48.65)	38.58	(33.59 , 43.91)	6.21	(5.67 , 6.76)	6.21	(5.68 , 6.75)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008075	45.03	(40.38 , 49.93)	39.53	(34.60 , 44.78)	6.20	(5.69 , 6.74)	6.20	(5.69 , 6.74)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008114	41.47	(36.85 , 46.36)	35.90	(31.08 , 41.06)	5.95	(5.43 , 6.49)	5.95	(5.44 , 6.49)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008166	45.54	(40.87 , 50.46)	38.58	(33.71 , 43.78)	6.37	(5.85 , 6.92)	6.37	(5.86 , 6.91)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008252	45.87	(41.18 , 50.82)	39.46	(34.54 , 44.70)	6.19	(5.67 , 6.72)	6.19	(5.68 , 6.72)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008150	45.34	(40.68 , 50.26)	39.25	(34.35 , 44.48)	6.18	(5.66 , 6.71)	6.18	(5.67 , 6.71)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)

Family	lateral length 16 months *		lateral length 28 months *		number of nodes on lateral 16 months *		number of nodes on lateral 28 months *		internode length 16 months *		internode length 28 months *	
2008009	41.42	(36.96 , 46.12)	35.53	(30.88 , 40.51)	5.95	(5.45 , 6.48)	5.96	(5.46 , 6.48)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008081	44.80	(40.00 , 49.87)	39.04	(33.93 , 44.51)	6.15	(5.62 , 6.70)	6.15	(5.63 , 6.69)	17.09	(15.48 , 18.79)	22.56	(20.40 , 24.83)
2008041	42.81	(38.29 , 47.60)	36.95	(32.17 , 42.05)	6.00	(5.49 , 6.53)	6.01	(5.51 , 6.53)	17.32	(15.70 , 19.02)	22.56	(20.40 , 24.83)
2008061	50.19	(45.20 , 55.45)	43.53	(38.29 , 49.11)	6.68	(6.14 , 7.24)	6.66	(6.13 , 7.22)	17.69	(16.26 , 19.18)	22.56	(20.40 , 24.83)
2008087	36.18	(31.80 , 40.84)	31.63	(26.96 , 36.68)	5.29	(4.80 , 5.81)	5.31	(4.82 , 5.83)	15.44	(13.91 , 17.05)	22.56	(20.40 , 24.83)
2008147	48.46	(43.54 , 53.64)	40.04	(34.94 , 45.50)	6.49	(5.95 , 7.04)	6.48	(5.95 , 7.03)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008258	43.12	(38.58 , 47.92)	33.62	(29.10 , 38.47)	6.10	(5.59 , 6.64)	6.11	(5.60 , 6.63)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008130	40.86	(36.44 , 45.53)	33.78	(29.23 , 38.65)	5.80	(5.30 , 6.32)	5.81	(5.32 , 6.33)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008100	38.27	(33.92 , 42.87)	33.85	(29.25 , 38.79)	5.48	(4.99 , 6.00)	5.50	(5.01 , 6.01)	17.64	(16.01 , 19.35)	22.56	(20.40 , 24.83)
2008132	44.66	(40.04 , 49.53)	36.65	(31.78 , 41.86)	6.35	(5.83 , 6.89)	6.35	(5.83 , 6.88)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008256	37.40	(33.19 , 41.87)	33.95	(29.34 , 38.89)	5.39	(4.91 , 5.89)	5.40	(4.93 , 5.90)	17.86	(16.22 , 19.59)	22.56	(20.40 , 24.83)
2008093	39.23	(34.91 , 43.81)	33.21	(28.72 , 38.03)	5.93	(5.43 , 6.46)	5.94	(5.44 , 6.46)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008108	51.32	(46.36 , 56.54)	40.98	(35.96 , 46.33)	6.41	(5.89 , 6.95)	6.40	(5.89 , 6.94)	15.88	(14.33 , 17.51)	22.56	(20.40 , 24.83)
2008179	46.36	(41.64 , 51.33)	40.81	(35.81 , 46.14)	6.87	(6.33 , 7.43)	6.85	(6.32 , 7.41)	18.54	(16.86 , 20.29)	22.56	(20.40 , 24.83)
2008192	43.89	(39.23 , 48.82)	38.56	(33.65 , 43.81)	5.84	(5.33 , 6.37)	5.85	(5.35 , 6.37)	17.29	(15.52 , 19.16)	22.56	(20.40 , 24.83)
2008089	38.04	(33.71 , 42.64)	32.29	(27.80 , 37.12)	5.78	(5.27 , 6.30)	5.79	(5.29 , 6.31)	17.11	(15.71 , 18.57)	22.56	(20.40 , 24.83)
2008064	44.82	(40.11 , 49.79)	38.75	(33.82 , 44.02)	6.45	(5.92 , 7.00)	6.44	(5.92 , 6.99)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008269	44.80	(40.09 , 49.77)	39.54	(34.38 , 45.05)	6.23	(5.70 , 6.79)	6.23	(5.71 , 6.78)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008059	49.83	(44.94 , 54.97)	43.34	(38.17 , 48.84)	6.05	(5.54 , 6.58)	6.05	(5.55 , 6.57)	16.63	(15.04 , 18.30)	22.56	(20.40 , 24.83)
2008047	44.58	(39.88 , 49.54)	36.46	(31.52 , 41.76)	5.78	(5.27 , 6.30)	5.79	(5.29 , 6.31)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008194	41.09	(36.66 , 45.77)	34.55	(29.89 , 39.56)	5.90	(5.39 , 6.42)	5.90	(5.41 , 6.42)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008232	39.94	(35.58 , 44.56)	34.31	(29.60 , 39.37)	6.50	(5.97 , 7.05)	6.50	(5.97 , 7.04)	17.09	(15.47 , 18.78)	22.56	(20.40 , 24.83)
2008173	45.47	(40.80 , 50.39)	39.90	(34.95 , 45.17)	6.21	(5.69 , 6.76)	6.21	(5.69 , 6.75)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008120	38.12	(33.70 , 42.82)	31.61	(27.10 , 36.46)	5.46	(4.96 , 5.98)	5.48	(4.98 , 5.99)	16.60	(15.13 , 18.14)	22.56	(20.40 , 24.83)
2008262	49.57	(44.61 , 54.79)	45.03	(39.69 , 50.72)	6.47	(5.94 , 7.03)	6.46	(5.94 , 7.01)	18.49	(17.04 , 20.00)	22.56	(20.40 , 24.83)
2008187	39.11	(34.71 , 43.77)	35.79	(31.05 , 40.87)	5.72	(5.22 , 6.24)	5.73	(5.23 , 6.25)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008051	41.25	(36.73 , 46.02)	36.02	(31.26 , 41.10)	6.02	(5.49 , 6.56)	6.02	(5.50 , 6.56)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008198	38.81	(34.17 , 43.75)	32.91	(28.16 , 38.03)	5.57	(5.05 , 6.12)	5.59	(5.07 , 6.12)	16.79	(15.05 , 18.63)	22.56	(20.40 , 24.83)
2008054	39.64	(35.29 , 44.25)	35.98	(31.29 , 40.99)	5.70	(5.21 , 6.22)	5.71	(5.23 , 6.22)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008244	43.15	(38.61 , 47.94)	37.33	(32.55 , 42.45)	6.56	(6.03 , 7.11)	6.56	(6.03 , 7.10)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008177	48.94	(44.01 , 54.13)	41.28	(36.12 , 46.80)	6.37	(5.84 , 6.92)	6.37	(5.85 , 6.91)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008117	42.08	(37.59 , 46.83)	33.10	(28.59 , 37.94)	5.88	(5.38 , 6.40)	5.88	(5.39 , 6.40)	17.98	(16.32 , 19.71)	22.56	(20.40 , 24.83)
2008016	47.87	(42.99 , 53.01)	44.12	(38.84 , 49.74)	6.42	(5.89 , 6.98)	6.42	(5.89 , 6.97)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008267	45.94	(41.17 , 50.98)	41.40	(36.29 , 46.84)	6.23	(5.71 , 6.78)	6.23	(5.71 , 6.77)	19.65	(17.92 , 21.45)	22.56	(20.40 , 24.83)
2008040	44.73	(39.93 , 49.81)	38.96	(33.94 , 44.34)	5.83	(5.31 , 6.36)	5.84	(5.33 , 6.37)	17.14	(15.53 , 18.83)	22.56	(20.40 , 24.83)

Family	lateral length 16 months *		lateral length 28 months *		number of nodes on lateral 16 months *		number of nodes on lateral 28 months *		internode length 16 months *		internode length 28 months *	
2008218	44.14	(39.54 , 48.99)	33.74	(29.20 , 38.61)	6.23	(5.71 , 6.77)	6.23	(5.72 , 6.76)	17.51	(15.88 , 19.22)	22.56	(20.40 , 24.83)
2008070	43.65	(39.09 , 48.47)	38.39	(33.49 , 43.64)	6.37	(5.85 , 6.91)	6.36	(5.85 , 6.90)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008102	38.90	(34.35 , 43.74)	33.03	(28.35 , 38.08)	6.16	(5.62 , 6.72)	6.16	(5.63 , 6.72)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)
2008078	42.14	(37.66 , 46.87)	36.55	(31.83 , 41.59)	6.03	(5.52 , 6.55)	6.03	(5.53 , 6.55)	16.00	(14.80 , 17.24)	22.56	(20.40 , 24.83)
2008074	43.86	(39.19 , 48.79)	35.62	(30.88 , 40.70)	6.06	(5.54 , 6.61)	6.07	(5.55 , 6.61)	17.12	(15.15 , 19.21)	22.56	(20.40 , 24.83)

d.

Family	height to the cones 16 months		height to the cones 28 months		green cone weight 28 months *	
2008003	1.68	(1.54 , 1.83)	1.68	(1.52 , 1.84)	0.94	(0.82 , 1.08)
2008190	1.80	(1.66 , 1.94)	1.82	(1.67 , 1.97)	0.91	(0.78 , 1.04)
2008209	1.85	(1.70 , 1.99)	1.87	(1.71 , 2.04)	0.90	(0.78 , 1.03)
2008151	1.76	(1.61 , 1.91)	1.71	(1.54 , 1.88)	0.89	(0.77 , 1.03)
2008039	1.88	(1.73 , 2.02)	1.91	(1.75 , 2.06)	0.93	(0.80 , 1.07)
2008152	1.86	(1.72 , 1.99)	1.89	(1.75 , 2.04)	0.86	(0.74 , 0.99)
2008001	1.86	(1.71 , 2.00)	1.89	(1.72 , 2.05)	0.93	(0.81 , 1.07)
2008073	1.90	(1.75 , 2.05)	1.95	(1.79 , 2.12)	0.92	(0.79 , 1.06)
2008168	1.62	(1.47 , 1.76)	1.59	(1.43 , 1.74)	0.93	(0.80 , 1.06)
2008161	1.78	(1.64 , 1.92)	1.77	(1.61 , 1.94)	0.93	(0.81 , 1.06)
2008290	1.72	(1.58 , 1.87)	1.73	(1.57 , 1.89)	0.85	(0.73 , 0.98)
2008208	1.61	(1.47 , 1.75)	1.59	(1.44 , 1.74)	0.95	(0.82 , 1.09)
2008251	1.84	(1.69 , 1.98)	1.86	(1.71 , 2.02)	0.88	(0.76 , 1.01)
2008052	1.83	(1.68 , 1.98)	1.86	(1.69 , 2.02)	0.87	(0.74 , 1.00)
2008037	1.77	(1.61 , 1.92)	1.79	(1.62 , 1.96)	0.92	(0.80 , 1.06)
2008154	1.61	(1.47 , 1.75)	1.63	(1.47 , 1.79)	0.94	(0.82 , 1.08)
2008136	1.63	(1.49 , 1.77)	1.63	(1.47 , 1.79)	0.84	(0.72 , 0.97)
2008096	1.76	(1.62 , 1.91)	1.78	(1.62 , 1.93)	0.94	(0.81 , 1.08)
2008071	1.85	(1.72 , 1.99)	1.88	(1.74 , 2.03)	0.93	(0.81 , 1.07)
2008124	1.83	(1.69 , 1.98)	1.86	(1.70 , 2.03)	0.91	(0.78 , 1.04)
2008145	1.60	(1.47 , 1.74)	1.57	(1.43 , 1.72)	0.93	(0.81 , 1.07)
2008084	1.85	(1.71 , 2.00)	1.91	(1.74 , 2.08)	0.91	(0.78 , 1.05)
2008083	1.79	(1.64 , 1.93)	1.96	(1.79 , 2.13)	0.88	(0.75 , 1.01)

Family	height to the cones 16 months		height to the cones 28 months		green cone weight 28 months *	
2008056	1.84	(1.68 , 2.00)	1.89	(1.71 , 2.06)	0.92	(0.79 , 1.06)
2008261	1.80	(1.65 , 1.95)	1.83	(1.67 , 1.99)	0.91	(0.78 , 1.04)
2008157	1.75	(1.60 , 1.89)	1.76	(1.60 , 1.92)	0.91	(0.78 , 1.04)
2008006	1.79	(1.64 , 1.93)	1.81	(1.63 , 1.98)	0.85	(0.73 , 0.98)
2008240	1.62	(1.47 , 1.76)	1.60	(1.44 , 1.75)	0.87	(0.74 , 1.00)
2008103	1.67	(1.53 , 1.80)	1.66	(1.50 , 1.81)	0.94	(0.81 , 1.07)
2008148	1.79	(1.65 , 1.92)	1.82	(1.68 , 1.96)	0.93	(0.81 , 1.06)
2008236	1.96	(1.82 , 2.10)	1.88	(1.72 , 2.04)	0.88	(0.76 , 1.01)
2008259	1.65	(1.49 , 1.81)	1.63	(1.45 , 1.80)	0.94	(0.81 , 1.08)
2008191	1.91	(1.77 , 2.05)	1.98	(1.83 , 2.13)	0.85	(0.73 , 0.98)
2008242	1.81	(1.68 , 1.94)	1.85	(1.70 , 1.99)	0.92	(0.80 , 1.05)
2008248	1.87	(1.72 , 2.01)	1.90	(1.75 , 2.06)	0.88	(0.76 , 1.01)
2008010	1.63	(1.49 , 1.77)	1.62	(1.47 , 1.77)	0.86	(0.74 , 0.99)
2008263	1.61	(1.47 , 1.75)	1.58	(1.43 , 1.73)	0.91	(0.79 , 1.05)
2008153	1.48	(1.34 , 1.61)	1.44	(1.30 , 1.58)	0.92	(0.80 , 1.05)
2008119	1.68	(1.54 , 1.81)	1.59	(1.44 , 1.74)	0.91	(0.79 , 1.05)
2008206	1.69	(1.56 , 1.82)	1.66	(1.51 , 1.81)	0.90	(0.77 , 1.03)
2008139	1.85	(1.72 , 1.98)	1.87	(1.73 , 2.01)	0.88	(0.75 , 1.01)
2008106	1.65	(1.51 , 1.80)	1.64	(1.49 , 1.80)	0.88	(0.76 , 1.01)
2008122	1.80	(1.64 , 1.95)	1.81	(1.64 , 1.99)	0.89	(0.76 , 1.02)
2008155	1.70	(1.56 , 1.85)	1.69	(1.54 , 1.85)	0.87	(0.75 , 1.00)
2008088	1.52	(1.38 , 1.66)	1.53	(1.37 , 1.69)	0.91	(0.78 , 1.04)
2008111	1.74	(1.57 , 1.90)	1.74	(1.55 , 1.92)	0.87	(0.75 , 1.00)

Family	height to the cones 16 months		height to the cones 28 months		green cone weight 28 months *	
2008202	1.69	(1.54 , 1.83)	1.69	(1.54 , 1.85)	0.89	(0.77 , 1.03)
2008013	1.85	(1.70 , 2.00)	1.87	(1.71 , 2.03)	0.87	(0.75 , 1.00)
2008279	1.76	(1.60 , 1.92)	1.77	(1.59 , 1.94)	0.91	(0.79 , 1.05)
2008007	1.61	(1.46 , 1.75)	1.59	(1.43 , 1.74)	0.89	(0.77 , 1.02)
2008090	1.69	(1.55 , 1.83)	1.76	(1.60 , 1.92)	0.94	(0.81 , 1.07)
2008055	1.65	(1.51 , 1.78)	1.83	(1.67 , 2.00)	0.92	(0.80 , 1.06)
2008271	1.77	(1.63 , 1.91)	1.77	(1.62 , 1.93)	0.92	(0.80 , 1.06)
2008035	1.88	(1.74 , 2.03)	1.92	(1.76 , 2.08)	0.94	(0.81 , 1.07)
2008212	1.74	(1.59 , 1.88)	1.73	(1.57 , 1.89)	0.94	(0.81 , 1.07)
2008149	1.70	(1.56 , 1.85)	1.70	(1.54 , 1.86)	0.88	(0.76 , 1.01)
2008170	1.75	(1.60 , 1.89)	1.77	(1.62 , 1.93)	0.98	(0.85 , 1.12)
2008247	1.66	(1.51 , 1.80)	1.65	(1.49 , 1.81)	1.00	(0.87 , 1.14)
2008110	1.82	(1.66 , 1.97)	1.84	(1.67 , 2.01)	0.90	(0.78 , 1.04)
2008043	1.77	(1.64 , 1.90)	1.79	(1.65 , 1.93)	0.91	(0.79 , 1.05)
2008188	1.77	(1.62 , 1.92)	1.79	(1.62 , 1.95)	0.89	(0.76 , 1.02)
2008080	1.76	(1.60 , 1.92)	1.77	(1.59 , 1.94)	0.91	(0.79 , 1.05)
2008239	1.80	(1.66 , 1.94)	1.82	(1.67 , 1.97)	0.89	(0.77 , 1.03)
2008075	1.63	(1.49 , 1.76)	1.61	(1.45 , 1.76)	0.84	(0.72 , 0.97)
2008114	1.81	(1.67 , 1.96)	1.83	(1.67 , 1.98)	0.88	(0.75 , 1.01)
2008166	1.74	(1.59 , 1.88)	1.76	(1.59 , 1.92)	0.91	(0.78 , 1.04)
2008252	1.85	(1.72 , 1.99)	1.88	(1.74 , 2.03)	0.89	(0.77 , 1.02)
2008150	1.57	(1.43 , 1.71)	1.54	(1.39 , 1.69)	0.93	(0.80 , 1.06)
2008009	1.80	(1.67 , 1.94)	1.82	(1.67 , 1.96)	0.88	(0.76 , 1.01)
2008081	1.80	(1.64 , 1.96)	1.78	(1.58 , 1.97)	0.87	(0.74 , 1.00)
2008041	1.67	(1.53 , 1.81)	1.67	(1.53 , 1.82)	0.89	(0.77 , 1.03)
2008061	1.61	(1.46 , 1.76)	1.58	(1.42 , 1.74)	0.95	(0.82 , 1.09)
2008087	1.61	(1.45 , 1.77)	1.58	(1.40 , 1.76)	0.89	(0.76 , 1.02)
2008147	1.63	(1.49 , 1.77)	1.66	(1.51 , 1.81)	0.92	(0.79 , 1.05)
2008258	1.58	(1.44 , 1.72)	1.63	(1.46 , 1.81)	0.89	(0.77 , 1.03)
2008130	1.87	(1.73 , 2.01)	1.93	(1.76 , 2.09)	0.90	(0.78 , 1.03)
2008100	1.68	(1.53 , 1.83)	1.68	(1.51 , 1.84)	0.92	(0.79 , 1.06)
2008132	1.66	(1.50 , 1.82)	1.65	(1.47 , 1.82)	0.90	(0.78 , 1.04)

Family	height to the cones 16 months		height to the cones 28 months		green cone weight 28 months *	
2008256	1.79	(1.63 , 1.94)	1.78	(1.61 , 1.96)	0.93	(0.80 , 1.07)
2008093	1.86	(1.72 , 2.00)	1.89	(1.74 , 2.05)	0.88	(0.76 , 1.01)
2008108	1.73	(1.58 , 1.87)	1.70	(1.53 , 1.86)	0.91	(0.79 , 1.05)
2008179	1.97	(1.83 , 2.10)	2.00	(1.84 , 2.15)	0.88	(0.75 , 1.01)
2008192	1.61	(1.46 , 1.75)	1.58	(1.43 , 1.74)	0.90	(0.77 , 1.03)
2008089	1.57	(1.43 , 1.71)	1.53	(1.38 , 1.68)	0.88	(0.76 , 1.01)
2008064	1.69	(1.54 , 1.83)	1.68	(1.52 , 1.84)	0.89	(0.77 , 1.03)
2008269	1.73	(1.56 , 1.89)	1.74	(1.56 , 1.92)	0.90	(0.77 , 1.04)
2008059	1.76	(1.61 , 1.90)	1.76	(1.60 , 1.91)	0.86	(0.74 , 0.99)
2008047	1.78	(1.63 , 1.94)	1.79	(1.62 , 1.96)	0.89	(0.77 , 1.02)
2008194	1.77	(1.63 , 1.92)	1.78	(1.61 , 1.94)	0.88	(0.76 , 1.02)
2008232	1.76	(1.62 , 1.91)	1.78	(1.62 , 1.93)	0.89	(0.77 , 1.02)
2008173	2.09	(1.96 , 2.23)	2.25	(2.09 , 2.40)	0.88	(0.76 , 1.01)
2008120	1.80	(1.65 , 1.94)	1.81	(1.65 , 1.96)	0.89	(0.77 , 1.02)
2008262	1.66	(1.50 , 1.81)	1.65	(1.48 , 1.82)	0.89	(0.76 , 1.02)
2008187	1.72	(1.57 , 1.86)	1.65	(1.49 , 1.81)	0.90	(0.78 , 1.04)
2008051	1.67	(1.52 , 1.82)	1.66	(1.50 , 1.82)	0.90	(0.77 , 1.04)
2008198	1.58	(1.44 , 1.73)	1.55	(1.40 , 1.71)	0.97	(0.84 , 1.11)
2008054	1.78	(1.64 , 1.91)	1.75	(1.60 , 1.91)	0.89	(0.77 , 1.02)
2008244	1.71	(1.56 , 1.85)	1.71	(1.55 , 1.86)	0.88	(0.75 , 1.01)
2008177	1.86	(1.71 , 2.01)	1.80	(1.64 , 1.97)	0.86	(0.74 , 0.99)
2008117	1.76	(1.63 , 1.89)	1.94	(1.78 , 2.10)	0.90	(0.78 , 1.04)
2008016	1.86	(1.72 , 2.00)	1.89	(1.74 , 2.04)	0.88	(0.76 , 1.02)
2008267	1.72	(1.58 , 1.87)	1.72	(1.56 , 1.87)	0.90	(0.78 , 1.04)
2008040	1.82	(1.67 , 1.96)	1.85	(1.68 , 2.01)	0.86	(0.73 , 0.99)
2008218	1.58	(1.44 , 1.72)	1.72	(1.55 , 1.89)	0.93	(0.80 , 1.06)
2008070	1.68	(1.52 , 1.83)	1.69	(1.52 , 1.87)	0.91	(0.79 , 1.05)
2008102	1.79	(1.66 , 1.92)	1.74	(1.59 , 1.89)	0.88	(0.76 , 1.01)
2008078	1.77	(1.62 , 1.93)	1.78	(1.62 , 1.95)	0.96	(0.83 , 1.09)
2008074	1.66	(1.52 , 1.80)	1.55	(1.38 , 1.73)	0.96	(0.84 , 1.10)

e.

Family	cohumulone 16 months *		cohumulone 28 months *		humulone + adhumulone 16 months *		humulone + adhumulone 28 months *		colupulone 16 months *		colupulone 28 months *		lupulone + adlupulone 16 months *		lupulone + adlupulone 28 months *	
2008003	2.95	(2.57 , 3.36)	2.56	(2.22 , 2.91)	6.59	(5.86 , 7.35)	5.22	(4.62 , 5.85)	2.35	(2.04 , 2.69)	2.58	(2.27 , 2.90)	2.03	(1.75 , 2.33)	2.12	(1.83 , 2.44)
2008190	2.03	(1.74 , 2.34)	2.08	(1.78 , 2.40)	5.30	(4.70 , 5.94)	4.80	(4.23 , 5.41)	1.94	(1.68 , 2.22)	2.29	(2.00 , 2.59)	1.87	(1.61 , 2.14)	2.12	(1.83 , 2.44)
2008209	3.09	(2.66 , 3.55)	2.56	(2.22 , 2.92)	7.24	(6.40 , 8.12)	5.39	(4.78 , 6.03)	2.16	(1.84 , 2.51)	2.49	(2.19 , 2.81)	2.04	(1.73 , 2.38)	2.11	(1.81 , 2.42)
2008151	2.91	(2.50 , 3.36)	2.41	(2.09 , 2.76)	7.06	(6.23 , 7.93)	5.65	(5.03 , 6.31)	2.40	(2.06 , 2.77)	2.51	(2.21 , 2.83)	2.24	(1.92 , 2.59)	2.38	(2.07 , 2.71)
2008039	2.79	(2.38 , 3.22)	2.45	(2.09 , 2.83)	5.59	(4.86 , 6.37)	5.36	(4.71 , 6.06)	1.95	(1.64 , 2.28)	2.42	(2.11 , 2.76)	1.60	(1.33 , 1.90)	2.07	(1.76 , 2.42)
2008152	2.76	(2.45 , 3.10)	2.09	(1.79 , 2.42)	7.30	(6.64 , 7.99)	4.73	(4.17 , 5.33)	2.56	(2.27 , 2.86)	2.47	(2.17 , 2.78)	2.28	(2.01 , 2.56)	2.11	(1.82 , 2.43)
2008001	2.73	(2.40 , 3.09)	2.54	(2.21 , 2.90)	6.26	(5.60 , 6.95)	5.73	(5.11 , 6.39)	2.13	(1.85 , 2.42)	2.39	(2.10 , 2.70)	1.88	(1.63 , 2.16)	2.07	(1.78 , 2.38)
2008073	2.03	(1.72 , 2.37)	2.29	(1.94 , 2.66)	4.86	(4.24 , 5.52)	4.86	(4.24 , 5.52)	1.97	(1.69 , 2.28)	2.36	(2.05 , 2.69)	1.79	(1.52 , 2.07)	1.91	(1.61 , 2.24)
2008168	2.58	(2.19 , 3.00)	2.11	(1.81 , 2.44)	6.84	(6.03 , 7.71)	4.72	(4.16 , 5.32)	2.59	(2.24 , 2.97)	2.47	(2.17 , 2.78)	2.59	(2.24 , 2.96)	2.15	(1.85 , 2.46)
2008161	2.63	(2.28 , 3.02)	2.23	(1.93 , 2.54)	7.03	(6.28 , 7.82)	5.24	(4.69 , 5.83)	2.11	(1.82 , 2.43)	2.51	(2.23 , 2.81)	2.09	(1.80 , 2.40)	2.34	(2.06 , 2.65)
2008290	2.59	(2.26 , 2.94)	2.38	(2.06 , 2.73)	7.01	(6.32 , 7.74)	5.46	(4.85 , 6.10)	2.00	(1.73 , 2.29)	2.27	(1.99 , 2.58)	2.01	(1.75 , 2.30)	2.01	(1.72 , 2.31)
2008208	2.63	(2.23 , 3.05)	2.22	(1.91 , 2.56)	6.79	(5.98 , 7.66)	5.27	(4.67 , 5.90)	2.10	(1.78 , 2.44)	2.37	(2.08 , 2.68)	2.06	(1.74 , 2.39)	2.20	(1.90 , 2.52)
2008251	2.46	(2.08 , 2.87)	2.17	(1.86 , 2.51)	6.31	(5.53 , 7.13)	5.05	(4.46 , 5.67)	2.31	(1.98 , 2.67)	2.36	(2.07 , 2.67)	2.34	(2.01 , 2.70)	2.21	(1.91 , 2.53)
2008052	2.19	(1.91 , 2.49)	2.32	(2.00 , 2.66)	5.43	(4.86 , 6.03)	4.81	(4.24 , 5.42)	1.80	(1.56 , 2.06)	2.16	(1.89 , 2.46)	1.70	(1.47 , 1.95)	1.74	(1.47 , 2.02)
2008037	1.87	(1.57 , 2.20)	2.12	(1.82 , 2.45)	5.36	(4.70 , 6.05)	5.15	(4.56 , 5.78)	1.76	(1.50 , 2.05)	2.16	(1.88 , 2.46)	1.82	(1.55 , 2.11)	1.98	(1.70 , 2.29)
2008154	3.53	(3.11 , 3.97)	3.13	(2.75 , 3.52)	6.60	(5.87 , 7.37)	5.51	(4.90 , 6.16)	2.60	(2.27 , 2.95)	2.71	(2.40 , 3.05)	1.99	(1.71 , 2.29)	1.99	(1.70 , 2.29)
2008136	2.84	(2.54 , 3.16)	2.47	(2.16 , 2.80)	7.27	(6.65 , 7.92)	5.39	(4.83 , 5.99)	2.31	(2.05 , 2.58)	2.45	(2.17 , 2.75)	2.36	(2.10 , 2.63)	2.15	(1.87 , 2.44)
2008096	2.27	(2.00 , 2.56)	2.31	(1.99 , 2.65)	5.11	(4.59 , 5.66)	4.89	(4.32 , 5.51)	1.97	(1.74 , 2.23)	2.27	(1.99 , 2.58)	1.84	(1.61 , 2.08)	1.90	(1.63 , 2.20)
2008071	2.80	(2.48 , 3.14)	2.27	(1.98 , 2.59)	6.78	(6.14 , 7.45)	5.01	(4.47 , 5.58)	2.13	(1.87 , 2.41)	2.23	(1.97 , 2.52)	2.14	(1.88 , 2.41)	1.98	(1.72 , 2.26)
2008124	2.64	(2.25 , 3.07)	2.35	(2.00 , 2.73)	6.52	(5.73 , 7.36)	5.01	(4.38 , 5.68)	2.27	(1.94 , 2.62)	2.33	(2.02 , 2.66)	2.21	(1.89 , 2.56)	1.95	(1.64 , 2.29)
2008145	3.17	(2.87 , 3.49)	2.57	(2.26 , 2.91)	7.43	(6.83 , 8.05)	5.68	(5.10 , 6.29)	2.56	(2.30 , 2.83)	2.50	(2.22 , 2.80)	2.39	(2.14 , 2.64)	2.17	(1.90 , 2.47)
2008084	2.20	(1.92 , 2.50)	2.47	(2.11 , 2.85)	5.93	(5.33 , 6.56)	5.39	(4.74 , 6.09)	2.17	(1.91 , 2.45)	2.59	(2.26 , 2.93)	2.21	(1.95 , 2.49)	2.32	(1.98 , 2.68)
2008083	2.72	(2.36 , 3.11)	2.61	(2.27 , 2.98)	7.09	(6.34 , 7.89)	5.86	(5.23 , 6.52)	2.25	(1.95 , 2.57)	2.34	(2.05 , 2.65)	2.30	(2.00 , 2.63)	2.03	(1.74 , 2.33)
2008056	2.55	(2.19 , 2.93)	2.30	(1.95 , 2.67)	6.02	(5.32 , 6.75)	5.06	(4.43 , 5.74)	2.49	(2.17 , 2.83)	2.54	(2.22 , 2.89)	2.29	(1.99 , 2.62)	2.33	(1.99 , 2.70)
2008261	2.93	(2.58 , 3.31)	2.49	(2.16 , 2.84)	6.62	(5.94 , 7.33)	5.29	(4.69 , 5.92)	2.32	(2.03 , 2.63)	2.40	(2.11 , 2.71)	2.10	(1.83 , 2.39)	2.03	(1.74 , 2.34)
2008157	2.61	(2.22 , 3.03)	2.21	(1.89 , 2.54)	6.24	(5.46 , 7.06)	4.73	(4.17 , 5.33)	2.16	(1.84 , 2.51)	2.27	(1.99 , 2.58)	2.07	(1.75 , 2.40)	1.90	(1.62 , 2.20)
2008006	2.29	(1.96 , 2.65)	2.74	(2.39 , 3.11)	6.06	(5.36 , 6.79)	5.63	(5.01 , 6.28)	2.08	(1.79 , 2.40)	2.47	(2.18 , 2.79)	2.38	(2.07 , 2.71)	2.08	(1.79 , 2.40)
2008240	2.65	(2.34 , 2.97)	2.72	(2.38 , 3.09)	5.88	(5.28 , 6.50)	5.83	(5.20 , 6.50)	2.57	(2.28 , 2.87)	2.46	(2.16 , 2.77)	2.08	(1.83 , 2.35)	2.13	(1.83 , 2.44)
2008103	2.29	(1.93 , 2.69)	2.61	(2.29 , 2.95)	6.35	(5.57 , 7.18)	5.69	(5.11 , 6.30)	2.01	(1.70 , 2.35)	2.57	(2.29 , 2.88)	2.14	(1.82 , 2.48)	2.35	(2.07 , 2.66)
2008148	2.65	(2.34 , 2.98)	2.54	(2.22 , 2.87)	6.18	(5.57 , 6.82)	5.18	(4.63 , 5.77)	2.28	(2.01 , 2.57)	2.49	(2.21 , 2.79)	2.08	(1.82 , 2.35)	2.05	(1.78 , 2.33)
2008236	2.90	(2.60 , 3.22)	2.38	(2.05 , 2.72)	5.04	(4.52 , 5.58)	4.49	(3.94 , 5.08)	2.20	(1.95 , 2.46)	2.36	(2.07 , 2.67)	1.51	(1.31 , 1.73)	1.72	(1.46 , 2.00)
2008259	2.48	(2.10 , 2.89)	2.39	(2.04 , 2.77)	6.02	(5.26 , 6.83)	5.22	(4.58 , 5.90)	2.11	(1.80 , 2.46)	2.34	(2.03 , 2.67)	1.95	(1.65 , 2.28)	1.99	(1.68 , 2.33)
2008191	3.05	(2.70 , 3.43)	2.58	(2.27 , 2.92)	4.85	(4.27 , 5.46)	4.37	(3.86 , 4.91)	2.36	(2.07 , 2.67)	2.51	(2.23 , 2.81)	1.59	(1.36 , 1.85)	1.70	(1.46 , 1.96)

Family	cohumulone 16 months *		cohumulone 28 months *		humulone + adhumulone 16 months *		humulone + adhumulone 28 months *		colupulone 16 months *		colupulone 28 months *		lupulone + adlupulone 16 months *		lupulone + adlupulone 28 months *	
2008242	2.35	(2.04 , 2.68)	1.99	(1.71 , 2.28)	5.13	(4.54 , 5.75)	4.55	(4.03 , 5.10)	2.61	(2.30 , 2.93)	2.34	(2.07 , 2.63)	2.24	(1.96 , 2.53)	2.11	(1.84 , 2.40)
2008248	1.63	(1.37 , 1.91)	2.04	(1.74 , 2.36)	4.58	(4.02 , 5.17)	4.94	(4.37 , 5.56)	1.76	(1.51 , 2.03)	2.18	(1.90 , 2.48)	1.90	(1.64 , 2.17)	2.08	(1.79 , 2.39)
2008010	2.58	(2.25 , 2.92)	2.54	(2.21 , 2.90)	6.32	(5.66 , 7.01)	5.13	(4.54 , 5.75)	1.91	(1.65 , 2.19)	2.37	(2.08 , 2.68)	1.90	(1.64 , 2.17)	1.99	(1.71 , 2.29)
2008263	2.58	(2.25 , 2.92)	2.54	(2.21 , 2.90)	6.62	(5.95 , 7.33)	5.36	(4.76 , 6.00)	2.26	(1.98 , 2.57)	2.50	(2.21 , 2.82)	2.23	(1.95 , 2.53)	2.18	(1.88 , 2.50)
2008153	2.70	(2.40 , 3.01)	2.39	(2.09 , 2.71)	6.76	(6.16 , 7.38)	5.26	(4.71 , 5.85)	2.08	(1.84 , 2.34)	2.37	(2.09 , 2.66)	2.04	(1.80 , 2.29)	2.08	(1.81 , 2.37)
2008119	2.44	(2.10 , 2.81)	2.31	(1.99 , 2.65)	5.77	(5.10 , 6.49)	4.94	(4.36 , 5.55)	1.96	(1.68 , 2.26)	2.28	(2.00 , 2.59)	1.85	(1.58 , 2.14)	1.92	(1.64 , 2.22)
2008206	3.02	(2.66 , 3.39)	2.63	(2.31 , 2.97)	5.87	(5.23 , 6.54)	5.31	(4.75 , 5.90)	2.31	(2.02 , 2.62)	2.55	(2.26 , 2.85)	1.85	(1.60 , 2.12)	2.05	(1.79 , 2.34)
2008139	1.82	(1.57 , 2.10)	2.02	(1.73 , 2.35)	4.70	(4.17 , 5.25)	4.55	(4.00 , 5.14)	2.39	(2.11 , 2.68)	2.31	(2.03 , 2.62)	2.41	(2.14 , 2.70)	2.11	(1.82 , 2.42)
2008106	2.42	(2.11 , 2.76)	2.33	(2.01 , 2.68)	5.77	(5.15 , 6.44)	4.98	(4.40 , 5.59)	1.90	(1.64 , 2.17)	2.51	(2.21 , 2.82)	1.84	(1.59 , 2.11)	2.27	(1.97 , 2.60)
2008122	2.38	(2.00 , 2.78)	2.55	(2.21 , 2.91)	5.40	(4.68 , 6.17)	5.49	(4.88 , 6.13)	2.35	(2.01 , 2.71)	2.61	(2.30 , 2.93)	2.15	(1.84 , 2.50)	2.26	(1.95 , 2.58)
2008155	2.95	(2.60 , 3.32)	2.60	(2.26 , 2.96)	5.18	(4.59 , 5.81)	5.37	(4.77 , 6.01)	2.31	(2.03 , 2.62)	2.61	(2.30 , 2.93)	1.60	(1.37 , 1.85)	2.16	(1.86 , 2.47)
2008088	2.70	(2.36 , 3.05)	2.81	(2.46 , 3.19)	6.66	(5.99 , 7.37)	5.94	(5.30 , 6.61)	2.31	(2.02 , 2.62)	2.65	(2.34 , 2.97)	2.17	(1.90 , 2.46)	2.38	(2.07 , 2.71)
2008111	2.31	(1.94 , 2.71)	2.34	(1.99 , 2.72)	6.18	(5.41 , 7.01)	5.27	(4.63 , 5.96)	2.18	(1.86 , 2.53)	2.52	(2.20 , 2.87)	2.18	(1.86 , 2.53)	2.28	(1.95 , 2.64)
2008202	3.18	(2.86 , 3.52)	2.64	(2.30 , 3.01)	6.28	(5.70 , 6.89)	5.17	(4.57 , 5.79)	2.19	(1.94 , 2.46)	2.51	(2.21 , 2.83)	1.81	(1.59 , 2.05)	1.92	(1.64 , 2.22)
2008013	2.03	(1.75 , 2.34)	2.13	(1.83 , 2.46)	5.93	(5.30 , 6.60)	4.98	(4.40 , 5.59)	1.96	(1.69 , 2.24)	2.24	(1.96 , 2.54)	2.14	(1.87 , 2.43)	2.06	(1.77 , 2.37)
2008279	3.38	(2.93 , 3.86)	2.43	(2.07 , 2.81)	7.00	(6.18 , 7.87)	4.92	(4.29 , 5.58)	2.57	(2.22 , 2.95)	2.37	(2.06 , 2.71)	2.15	(1.83 , 2.49)	1.89	(1.59 , 2.22)
2008007	2.97	(2.59 , 3.37)	2.62	(2.28 , 2.99)	6.57	(5.84 , 7.34)	5.43	(4.83 , 6.07)	2.10	(1.81 , 2.42)	2.28	(1.99 , 2.58)	1.88	(1.61 , 2.18)	1.90	(1.62 , 2.20)
2008090	3.15	(2.82 , 3.51)	2.75	(2.42 , 3.09)	6.73	(6.10 , 7.40)	5.74	(5.15 , 6.35)	2.18	(1.91 , 2.46)	2.39	(2.12 , 2.69)	1.99	(1.75 , 2.26)	2.04	(1.78 , 2.33)
2008055	2.40	(2.11 , 2.71)	2.22	(1.91 , 2.56)	6.27	(5.66 , 6.92)	4.95	(4.37 , 5.56)	2.44	(2.16 , 2.74)	2.48	(2.19 , 2.80)	2.54	(2.26 , 2.84)	2.22	(1.92 , 2.55)
2008271	2.67	(2.36 , 3.00)	2.49	(2.16 , 2.85)	6.33	(5.71 , 6.97)	5.16	(4.57 , 5.79)	2.04	(1.79 , 2.31)	2.28	(1.99 , 2.58)	1.80	(1.57 , 2.05)	1.85	(1.58 , 2.15)
2008035	2.32	(2.01 , 2.65)	2.38	(2.05 , 2.73)	6.01	(5.37 , 6.69)	5.33	(4.73 , 5.97)	2.05	(1.78 , 2.35)	2.41	(2.12 , 2.73)	2.09	(1.82 , 2.38)	2.13	(1.84 , 2.45)
2008212	2.38	(2.04 , 2.75)	2.56	(2.20 , 2.96)	5.75	(5.07 , 6.47)	5.40	(4.74 , 6.09)	2.63	(2.30 , 2.98)	2.63	(2.30 , 2.99)	2.43	(2.12 , 2.77)	2.25	(1.92 , 2.61)
2008149	3.93	(3.53 , 4.36)	2.41	(2.08 , 2.76)	6.87	(6.18 , 7.59)	4.98	(4.40 , 5.60)	2.86	(2.54 , 3.20)	2.44	(2.14 , 2.75)	2.19	(1.92 , 2.49)	2.04	(1.75 , 2.35)
2008170	2.30	(2.01 , 2.61)	2.45	(2.12 , 2.80)	6.31	(5.69 , 6.95)	5.38	(4.78 , 6.02)	1.94	(1.69 , 2.21)	2.19	(1.91 , 2.49)	2.03	(1.78 , 2.29)	1.86	(1.59 , 2.16)
2008247	2.31	(1.95 , 2.71)	2.73	(2.38 , 3.10)	6.11	(5.35 , 6.93)	5.46	(4.85 , 6.10)	1.93	(1.63 , 2.26)	2.40	(2.10 , 2.71)	1.89	(1.59 , 2.21)	1.90	(1.62 , 2.20)
2008110	2.84	(2.43 , 3.28)	2.41	(2.05 , 2.79)	6.17	(5.40 , 6.99)	5.03	(4.40 , 5.70)	2.30	(1.96 , 2.65)	2.51	(2.19 , 2.86)	2.00	(1.70 , 2.34)	2.09	(1.77 , 2.43)
2008043	1.85	(1.62 , 2.09)	2.16	(1.87 , 2.46)	5.03	(4.54 , 5.54)	4.61	(4.09 , 5.16)	2.01	(1.78 , 2.26)	2.14	(1.88 , 2.42)	2.01	(1.79 , 2.25)	1.78	(1.53 , 2.05)
2008188	2.57	(2.19 , 3.00)	2.43	(2.07 , 2.81)	5.93	(5.18 , 6.74)	4.86	(4.24 , 5.52)	2.54	(2.19 , 2.92)	2.60	(2.28 , 2.95)	2.18	(1.86 , 2.53)	2.07	(1.75 , 2.41)
2008080	2.21	(1.85 , 2.60)	2.28	(1.93 , 2.65)	5.94	(5.19 , 6.74)	5.01	(4.38 , 5.68)	2.18	(1.86 , 2.53)	2.29	(1.98 , 2.62)	2.34	(2.01 , 2.70)	1.97	(1.66 , 2.31)
2008239	2.50	(2.12 , 2.91)	2.49	(2.16 , 2.85)	5.52	(4.79 , 6.30)	5.11	(4.53 , 5.74)	2.50	(2.15 , 2.87)	2.78	(2.46 , 3.11)	2.11	(1.80 , 2.45)	2.29	(1.98 , 2.62)
2008075	2.77	(2.43 , 3.13)	2.27	(1.97 , 2.58)	7.58	(6.86 , 8.34)	5.12	(4.57 , 5.70)	2.34	(2.05 , 2.65)	2.45	(2.17 , 2.74)	2.49	(2.20 , 2.81)	2.28	(2.00 , 2.58)
2008114	2.12	(1.79 , 2.46)	2.01	(1.72 , 2.34)	6.40	(5.69 , 7.16)	4.82	(4.25 , 5.42)	2.04	(1.75 , 2.35)	2.42	(2.12 , 2.73)	2.36	(2.05 , 2.69)	2.46	(2.15 , 2.80)
2008166	3.09	(2.70 , 3.50)	2.27	(1.95 , 2.61)	5.35	(4.70 , 6.04)	4.67	(4.11 , 5.27)	2.18	(1.88 , 2.50)	2.18	(1.90 , 2.48)	1.60	(1.35 , 1.87)	1.70	(1.44 , 1.98)
2008252	2.51	(2.16 , 2.88)	2.17	(1.86 , 2.50)	6.37	(5.65 , 7.12)	5.22	(4.62 , 5.85)	2.09	(1.80 , 2.40)	2.31	(2.02 , 2.61)	2.07	(1.78 , 2.38)	2.20	(1.90 , 2.52)
2008150	2.97	(2.68 , 3.28)	2.45	(2.09 , 2.83)	6.54	(5.98 , 7.12)	5.08	(4.45 , 5.76)	2.01	(1.78 , 2.25)	2.40	(2.09 , 2.74)	1.69	(1.48 , 1.91)	2.00	(1.69 , 2.34)

Family	cohumulone 16 months *		cohumulone 28 months *		humulone + adhumulone 16 months *		humulone + adhumulone 28 months *		colupulone 16 months *		colupulone 28 months *		lupulone + adlupulone 16 months *		lupulone + adlupulone 28 months *	
2008009	2.61	(2.28 , 2.96)	2.39	(2.08 , 2.71)	7.51	(6.79 , 8.26)	5.81	(5.22 , 6.43)	1.83	(1.57 , 2.10)	2.11	(1.85 , 2.39)	1.97	(1.71 , 2.25)	1.90	(1.65 , 2.18)
2008081	3.21	(2.77 , 3.68)	2.72	(2.34 , 3.12)	8.22	(7.33 , 9.16)	6.19	(5.49 , 6.93)	2.58	(2.22 , 2.96)	2.59	(2.26 , 2.93)	2.54	(2.19 , 2.91)	2.43	(2.08 , 2.80)
2008041	3.54	(3.19 , 3.92)	2.41	(2.11 , 2.74)	6.61	(5.98 , 7.27)	5.66	(5.08 , 6.27)	2.45	(2.17 , 2.74)	2.29	(2.02 , 2.58)	1.73	(1.50 , 1.97)	1.98	(1.72 , 2.26)
2008061	2.22	(1.94 , 2.53)	2.36	(2.01 , 2.74)	5.81	(5.22 , 6.43)	5.20	(4.56 , 5.89)	2.01	(1.76 , 2.28)	2.37	(2.06 , 2.71)	2.00	(1.75 , 2.27)	2.01	(1.70 , 2.35)
2008087	2.73	(2.33 , 3.16)	2.68	(2.31 , 3.08)	6.41	(5.62 , 7.24)	5.27	(4.62 , 5.95)	2.15	(1.83 , 2.50)	2.64	(2.31 , 2.99)	2.02	(1.71 , 2.35)	2.22	(1.89 , 2.57)
2008147	3.31	(3.00 , 3.64)	2.74	(2.41 , 3.09)	7.30	(6.71 , 7.92)	5.95	(5.36 , 6.58)	2.18	(1.94 , 2.43)	2.61	(2.32 , 2.92)	1.94	(1.72 , 2.17)	2.25	(1.97 , 2.55)
2008258	2.82	(2.51 , 3.13)	2.43	(2.10 , 2.78)	7.89	(7.25 , 8.57)	5.49	(4.88 , 6.14)	3.12	(2.82 , 3.44)	3.17	(2.84 , 3.53)	3.35	(3.05 , 3.67)	3.09	(2.73 , 3.47)
2008130	2.62	(2.29 , 2.97)	2.74	(2.39 , 3.11)	6.45	(5.79 , 7.15)	5.71	(5.08 , 6.36)	1.97	(1.71 , 2.25)	2.38	(2.08 , 2.69)	1.92	(1.67 , 2.20)	1.98	(1.70 , 2.29)
2008100	2.61	(2.28 , 2.96)	2.53	(2.17 , 2.92)	7.12	(6.42 , 7.86)	5.58	(4.91 , 6.28)	2.14	(1.87 , 2.44)	2.71	(2.38 , 3.07)	2.28	(2.00 , 2.58)	2.51	(2.16 , 2.88)
2008132	2.99	(2.60 , 3.40)	2.27	(1.92 , 2.64)	7.07	(6.32 , 7.87)	4.93	(4.31 , 5.59)	2.47	(2.15 , 2.81)	2.45	(2.13 , 2.79)	2.28	(1.98 , 2.60)	2.12	(1.80 , 2.47)
2008256	2.89	(2.51 , 3.30)	2.14	(1.81 , 2.50)	6.66	(5.93 , 7.43)	4.64	(4.04 , 5.29)	3.31	(2.94 , 3.70)	3.01	(2.66 , 3.39)	2.91	(2.56 , 3.27)	2.77	(2.40 , 3.16)
2008093	2.24	(1.97 , 2.53)	2.17	(1.88 , 2.48)	5.45	(4.92 , 6.02)	4.70	(4.18 , 5.26)	1.75	(1.52 , 1.99)	2.18	(1.92 , 2.46)	1.65	(1.44 , 1.88)	1.90	(1.65 , 2.18)
2008108	2.26	(1.99 , 2.55)	2.14	(1.83 , 2.47)	5.54	(5.00 , 6.11)	4.71	(4.15 , 5.31)	2.28	(2.02 , 2.55)	2.36	(2.07 , 2.67)	2.31	(2.06 , 2.58)	2.17	(1.87 , 2.48)
2008179	2.13	(1.85 , 2.42)	2.29	(1.97 , 2.63)	6.57	(5.94 , 7.23)	5.52	(4.91 , 6.17)	2.23	(1.96 , 2.51)	2.47	(2.17 , 2.78)	2.52	(2.24 , 2.82)	2.36	(2.05 , 2.70)
2008192	3.13	(2.74 , 3.55)	2.20	(1.87 , 2.57)	5.82	(5.14 , 6.54)	4.77	(4.16 , 5.42)	3.38	(3.00 , 3.77)	2.48	(2.16 , 2.82)	2.43	(2.12 , 2.76)	2.09	(1.77 , 2.43)
2008089	2.90	(2.53 , 3.30)	2.46	(2.13 , 2.81)	6.97	(6.22 , 7.76)	5.27	(4.67 , 5.90)	2.25	(1.94 , 2.57)	2.35	(2.06 , 2.66)	2.17	(1.88 , 2.49)	2.04	(1.75 , 2.35)
2008064	2.66	(2.27 , 3.09)	2.35	(2.00 , 2.72)	6.52	(5.73 , 7.37)	5.28	(4.64 , 5.97)	2.02	(1.71 , 2.36)	2.34	(2.03 , 2.67)	1.89	(1.60 , 2.22)	2.08	(1.76 , 2.42)
2008269	2.81	(2.40 , 3.25)	2.40	(2.01 , 2.82)	5.30	(4.59 , 6.07)	5.18	(4.48 , 5.93)	2.42	(2.08 , 2.79)	2.43	(2.09 , 2.80)	1.91	(1.61 , 2.24)	2.10	(1.74 , 2.49)
2008059	2.73	(2.32 , 3.17)	2.38	(2.05 , 2.72)	6.47	(5.68 , 7.31)	4.98	(4.40 , 5.59)	2.30	(1.96 , 2.66)	2.61	(2.30 , 2.93)	2.12	(1.81 , 2.47)	2.25	(1.94 , 2.57)
2008047	2.78	(2.41 , 3.18)	2.46	(2.13 , 2.81)	5.90	(5.21 , 6.63)	4.81	(4.24 , 5.41)	2.21	(1.90 , 2.53)	2.27	(1.99 , 2.57)	1.82	(1.55 , 2.11)	1.70	(1.43 , 1.98)
2008194	3.27	(2.87 , 3.70)	2.27	(1.95 , 2.61)	7.70	(6.91 , 8.52)	5.20	(4.60 , 5.82)	2.75	(2.42 , 3.11)	2.47	(2.17 , 2.79)	2.55	(2.23 , 2.89)	2.25	(1.95 , 2.57)
2008232	2.06	(1.74 , 2.40)	2.03	(1.73 , 2.35)	5.58	(4.91 , 6.28)	4.64	(4.08 , 5.23)	2.26	(1.96 , 2.59)	2.61	(2.30 , 2.93)	2.36	(2.05 , 2.69)	2.45	(2.14 , 2.79)
2008173	2.17	(1.81 , 2.56)	2.33	(2.01 , 2.68)	6.28	(5.50 , 7.11)	5.12	(4.53 , 5.75)	2.20	(1.87 , 2.55)	2.39	(2.10 , 2.70)	2.45	(2.11 , 2.82)	2.14	(1.84 , 2.45)
2008120	2.29	(1.95 , 2.65)	2.47	(2.14 , 2.83)	6.36	(5.65 , 7.12)	5.69	(5.07 , 6.35)	2.45	(2.13 , 2.79)	2.72	(2.40 , 3.05)	2.20	(1.91 , 2.52)	2.38	(2.07 , 2.72)
2008262	2.67	(2.31 , 3.05)	2.31	(1.96 , 2.68)	6.42	(5.70 , 7.18)	5.10	(4.46 , 5.77)	2.17	(1.87 , 2.49)	2.40	(2.09 , 2.74)	1.96	(1.68 , 2.26)	2.11	(1.79 , 2.46)
2008187	2.16	(1.84 , 2.51)	2.11	(1.80 , 2.43)	5.52	(4.86 , 6.23)	4.80	(4.23 , 5.40)	1.76	(1.49 , 2.05)	2.16	(1.88 , 2.46)	1.81	(1.54 , 2.10)	1.89	(1.61 , 2.19)
2008051	3.11	(2.72 , 3.53)	2.54	(2.18 , 2.93)	6.54	(5.82 , 7.30)	5.27	(4.62 , 5.95)	2.75	(2.42 , 3.11)	2.58	(2.25 , 2.93)	2.25	(1.95 , 2.57)	2.09	(1.77 , 2.44)
2008198	2.36	(2.03 , 2.73)	2.23	(1.92 , 2.57)	5.38	(4.73 , 6.08)	4.74	(4.17 , 5.34)	2.04	(1.75 , 2.35)	2.19	(1.91 , 2.49)	1.74	(1.48 , 2.03)	1.78	(1.51 , 2.07)
2008054	1.89	(1.65 , 2.15)	2.20	(1.91 , 2.51)	4.87	(4.36 , 5.41)	4.68	(4.15 , 5.23)	1.60	(1.39 , 1.83)	2.36	(2.09 , 2.65)	1.57	(1.36 , 1.79)	2.01	(1.75 , 2.30)
2008244	2.64	(2.33 , 2.97)	2.49	(2.16 , 2.85)	6.81	(6.18 , 7.48)	5.38	(4.78 , 6.02)	2.26	(1.99 , 2.55)	2.66	(2.35 , 2.99)	2.05	(1.80 , 2.32)	2.22	(1.92 , 2.54)
2008177	2.66	(2.26 , 3.09)	2.13	(1.82 , 2.46)	6.41	(5.62 , 7.24)	4.98	(4.40 , 5.60)	2.20	(1.88 , 2.56)	2.45	(2.15 , 2.76)	2.08	(1.76 , 2.42)	2.41	(2.10 , 2.75)
2008117	3.07	(2.71 , 3.45)	2.70	(2.38 , 3.04)	5.84	(5.21 , 6.51)	5.01	(4.46 , 5.58)	2.26	(1.98 , 2.57)	2.52	(2.24 , 2.82)	1.68	(1.44 , 1.94)	1.84	(1.59 , 2.11)
2008016	2.14	(1.88 , 2.42)	2.59	(2.25 , 2.95)	5.79	(5.24 , 6.38)	5.61	(4.99 , 6.26)	2.29	(2.03 , 2.56)	2.60	(2.29 , 2.92)	2.46	(2.20 , 2.73)	2.29	(1.99 , 2.62)
2008267	2.41	(2.07 , 2.78)	2.53	(2.19 , 2.89)	6.83	(6.09 , 7.61)	5.29	(4.69 , 5.92)	2.06	(1.77 , 2.37)	2.32	(2.03 , 2.63)	2.13	(1.84 , 2.44)	1.91	(1.63 , 2.21)
2008040	3.20	(2.76 , 3.67)	2.41	(2.06 , 2.79)	7.26	(6.42 , 8.15)	5.02	(4.39 , 5.69)	2.38	(2.04 , 2.75)	2.28	(1.97 , 2.61)	2.14	(1.82 , 2.48)	1.87	(1.57 , 2.20)

Family	cohumulone		cohumulone		humulone + adhumulone		humulone + adhumulone		colupulone		colupulone		lupulone + adlupulone		lupulone + adlupulone	
	16 months *		28 months *		16 months *		28 months *		16 months *		28 months *		16 months *		28 months *	
2008218	2.95	(2.62 , 3.29)	2.70	(2.36 , 3.07)	7.94	(7.25 , 8.66)	5.94	(5.30 , 6.61)	2.22	(1.96 , 2.51)	2.39	(2.10 , 2.70)	2.23	(1.97 , 2.51)	2.06	(1.77 , 2.37)
2008070	2.64	(2.28 , 3.02)	1.94	(1.62 , 2.28)	6.38	(5.67 , 7.14)	5.11	(4.47 , 5.78)	2.53	(2.20 , 2.87)	2.03	(1.74 , 2.34)	2.50	(2.18 , 2.84)	2.05	(1.74 , 2.39)
2008102	3.29	(2.85 , 3.76)	2.47	(2.14 , 2.83)	7.20	(6.36 , 8.08)	5.23	(4.64 , 5.86)	2.50	(2.15 , 2.88)	2.52	(2.22 , 2.84)	2.29	(1.96 , 2.64)	2.19	(1.89 , 2.51)
2008078	2.93	(2.51 , 3.38)	2.43	(2.07 , 2.81)	7.58	(6.72 , 8.48)	4.97	(4.34 , 5.64)	2.41	(2.07 , 2.78)	2.57	(2.24 , 2.91)	2.38	(2.04 , 2.74)	2.07	(1.75 , 2.41)
2008074	2.52	(2.22 , 2.84)	2.59	(2.27 , 2.92)	6.47	(5.85 , 7.13)	5.87	(5.28 , 6.49)	1.94	(1.69 , 2.20)	2.27	(2.00 , 2.55)	2.04	(1.79 , 2.31)	2.10	(1.83 , 2.39)

f.

Family	α-acid		α-acid		β-acid		β-acid	
	16 months		28 months *		16 months *		28 months *	
2008003	9.74	(8.76 , 10.72)	7.78	(6.94 , 8.66)	4.40	(3.86 , 4.98)	4.71	(4.17 , 5.29)
2008190	7.50	(6.60 , 8.41)	6.97	(6.18 , 7.82)	3.85	(3.37 , 4.36)	4.42	(3.89 , 4.98)
2008209	10.58	(9.50 , 11.66)	7.95	(7.10 , 8.85)	4.22	(3.65 , 4.84)	4.62	(4.08 , 5.19)
2008151	10.24	(9.16 , 11.32)	8.06	(7.21 , 8.97)	4.65	(4.04 , 5.29)	4.86	(4.31 , 5.44)
2008039	8.58	(7.50 , 9.66)	7.82	(6.92 , 8.78)	3.62	(3.09 , 4.19)	4.52	(3.95 , 5.13)
2008152	10.29	(9.45 , 11.13)	6.92	(6.13 , 7.76)	4.84	(4.33 , 5.37)	4.59	(4.05 , 5.16)
2008001	9.19	(8.29 , 10.09)	8.24	(7.38 , 9.15)	4.05	(3.57 , 4.57)	4.48	(3.95 , 5.04)
2008073	7.17	(6.19 , 8.15)	7.22	(6.35 , 8.14)	3.80	(3.30 , 4.34)	4.33	(3.77 , 4.92)
2008168	9.70	(8.62 , 10.78)	6.92	(6.13 , 7.76)	5.15	(4.51 , 5.83)	4.62	(4.08 , 5.19)
2008161	9.82	(8.85 , 10.80)	7.51	(6.74 , 8.33)	4.22	(3.69 , 4.79)	4.82	(4.30 , 5.36)
2008290	9.75	(8.85 , 10.66)	7.86	(7.01 , 8.75)	4.04	(3.55 , 4.56)	4.31	(3.79 , 4.86)
2008208	9.60	(8.52 , 10.68)	7.54	(6.72 , 8.41)	4.18	(3.61 , 4.80)	4.56	(4.03 , 5.13)
2008251	8.92	(7.84 , 10.00)	7.28	(6.47 , 8.14)	4.65	(4.05 , 5.30)	4.56	(4.03 , 5.13)
2008052	7.85	(7.00 , 8.69)	7.19	(6.39 , 8.04)	3.55	(3.12 , 4.01)	3.98	(3.48 , 4.51)
2008037	7.56	(6.58 , 8.54)	7.35	(6.53 , 8.21)	3.64	(3.14 , 4.16)	4.18	(3.67 , 4.72)
2008154	10.38	(9.40 , 11.36)	8.58	(7.70 , 9.51)	4.64	(4.08 , 5.23)	4.76	(4.21 , 5.34)
2008136	10.32	(9.53 , 11.11)	7.87	(7.08 , 8.70)	4.67	(4.20 , 5.17)	4.61	(4.10 , 5.14)
2008096	7.55	(6.76 , 8.35)	7.26	(6.45 , 8.12)	3.85	(3.42 , 4.30)	4.23	(3.71 , 4.77)
2008071	9.74	(8.89 , 10.58)	7.33	(6.56 , 8.13)	4.29	(3.81 , 4.79)	4.24	(3.76 , 4.75)
2008124	9.32	(8.24 , 10.40)	7.41	(6.53 , 8.34)	4.48	(3.89 , 5.12)	4.33	(3.77 , 4.93)
2008145	10.75	(10.00 , 11.50)	8.22	(7.41 , 9.08)	4.95	(4.49 , 5.43)	4.67	(4.17 , 5.21)
2008084	8.26	(7.42 , 9.10)	7.87	(6.96 , 8.83)	4.39	(3.91 , 4.90)	4.88	(4.28 , 5.51)
2008083	10.02	(9.04 , 11.00)	8.42	(7.55 , 9.34)	4.56	(4.01 , 5.15)	4.39	(3.87 , 4.95)

Family	α-acid		α-acid		β-acid		β-acid	
	16 months		28 months *		16 months *		28 months *	
2008056	8.73	(7.75 , 9.71)	7.42	(6.54 , 8.35)	4.79	(4.22 , 5.40)	4.84	(4.25 , 5.47)
2008261	9.65	(8.75 , 10.56)	7.78	(6.94 , 8.67)	4.44	(3.93 , 4.99)	4.46	(3.93 , 5.02)
2008157	9.01	(7.93 , 10.09)	7.02	(6.22 , 7.86)	4.25	(3.67 , 4.87)	4.22	(3.71 , 4.77)
2008006	8.56	(7.58 , 9.54)	8.32	(7.46 , 9.24)	4.48	(3.93 , 5.06)	4.58	(4.05 , 5.15)
2008240	8.82	(7.98 , 9.66)	8.50	(7.63 , 9.43)	4.69	(4.20 , 5.21)	4.60	(4.06 , 5.17)
2008103	8.85	(7.77 , 9.93)	8.28	(7.47 , 9.14)	4.18	(3.61 , 4.79)	4.91	(4.39 , 5.46)
2008148	9.18	(8.33 , 10.02)	7.73	(6.95 , 8.56)	4.39	(3.91 , 4.90)	4.57	(4.07 , 5.10)
2008236	8.20	(7.41 , 8.99)	6.99	(6.20 , 7.83)	3.79	(3.36 , 4.23)	4.18	(3.67 , 4.73)
2008259	8.68	(7.60 , 9.76)	7.64	(6.75 , 8.59)	4.10	(3.53 , 4.70)	4.37	(3.81 , 4.97)
2008191	8.20	(7.29 , 9.10)	7.06	(6.31 , 7.85)	4.02	(3.54 , 4.54)	4.32	(3.84 , 4.84)
2008242	7.67	(6.76 , 8.57)	6.64	(5.92 , 7.41)	4.87	(4.33 , 5.43)	4.47	(3.97 , 4.99)
2008248	6.57	(5.66 , 7.47)	7.08	(6.28 , 7.92)	3.71	(3.24 , 4.20)	4.27	(3.76 , 4.82)
2008010	9.10	(8.19 , 10.00)	7.69	(6.86 , 8.57)	3.85	(3.38 , 4.36)	4.40	(3.88 , 4.96)
2008263	9.36	(8.45 , 10.26)	7.90	(7.05 , 8.79)	4.50	(3.99 , 5.05)	4.69	(4.15 , 5.26)
2008153	9.62	(8.82 , 10.41)	7.68	(6.89 , 8.50)	4.14	(3.70 , 4.61)	4.47	(3.97 , 4.99)
2008119	8.44	(7.46 , 9.42)	7.30	(6.49 , 8.16)	3.85	(3.34 , 4.39)	4.24	(3.73 , 4.79)
2008206	9.08	(8.17 , 9.98)	7.94	(7.14 , 8.78)	4.20	(3.70 , 4.73)	4.62	(4.11 , 5.15)
2008139	6.74	(5.89 , 7.58)	6.69	(5.92 , 7.52)	4.79	(4.29 , 5.33)	4.43	(3.91 , 4.99)
2008106	8.32	(7.42 , 9.23)	7.37	(6.55 , 8.23)	3.77	(3.30 , 4.27)	4.76	(4.22 , 5.34)
2008122	7.96	(6.88 , 9.04)	8.02	(7.17 , 8.92)	4.51	(3.92 , 5.15)	4.85	(4.30 , 5.44)
2008155	8.41	(7.50 , 9.31)	7.96	(7.12 , 8.86)	3.98	(3.50 , 4.49)	4.77	(4.22 , 5.35)
2008088	9.55	(8.65 , 10.45)	8.68	(7.79 , 9.61)	4.49	(3.98 , 5.04)	4.98	(4.43 , 5.58)
2008111	8.70	(7.62 , 9.78)	7.65	(6.75 , 8.59)	4.38	(3.79 , 5.01)	4.78	(4.19 , 5.41)

Family	α-acid 16 months		α-acid 28 months *		β-acid 16 months *		β-acid 28 months *	
2008202	9.68	(8.89 , 10.47)	7.82	(6.98 , 8.71)	4.06	(3.62 , 4.52)	4.49	(3.96 , 5.05)
2008013	8.22	(7.32 , 9.12)	7.19	(6.38 , 8.04)	4.13	(3.64 , 4.65)	4.32	(3.80 , 4.87)
2008279	10.65	(9.57 , 11.73)	7.40	(6.52 , 8.33)	4.73	(4.12 , 5.38)	4.33	(3.77 , 4.92)
2008007	9.69	(8.71 , 10.66)	8.04	(7.18 , 8.94)	4.02	(3.50 , 4.57)	4.22	(3.71 , 4.77)
2008090	10.23	(9.39 , 11.07)	8.44	(7.62 , 9.30)	4.20	(3.73 , 4.70)	4.45	(3.96 , 4.97)
2008055	8.85	(8.01 , 9.69)	7.24	(6.43 , 8.09)	4.98	(4.46 , 5.52)	4.70	(4.15 , 5.27)
2008271	9.19	(8.35 , 10.03)	7.69	(6.86 , 8.57)	3.88	(3.43 , 4.36)	4.20	(3.68 , 4.74)
2008035	8.71	(7.81 , 9.61)	7.73	(6.90 , 8.62)	4.17	(3.68 , 4.70)	4.55	(4.02 , 5.12)
2008212	8.29	(7.31 , 9.27)	7.95	(7.04 , 8.92)	5.04	(4.46 , 5.66)	4.87	(4.28 , 5.51)
2008149	11.00	(10.10 , 11.90)	7.44	(6.62 , 8.30)	5.07	(4.52 , 5.65)	4.50	(3.97 , 5.06)
2008170	8.74	(7.90 , 9.58)	7.84	(7.00 , 8.73)	3.99	(3.54 , 4.48)	4.11	(3.60 , 4.64)
2008247	8.61	(7.53 , 9.69)	8.16	(7.30 , 9.07)	3.86	(3.31 , 4.45)	4.35	(3.83 , 4.91)
2008110	9.17	(8.09 , 10.25)	7.48	(6.60 , 8.42)	4.33	(3.75 , 4.95)	4.63	(4.05 , 5.24)
2008043	7.10	(6.35 , 7.85)	6.85	(6.11 , 7.63)	4.05	(3.64 , 4.49)	3.99	(3.52 , 4.48)
2008188	8.68	(7.60 , 9.76)	7.36	(6.48 , 8.29)	4.73	(4.12 , 5.38)	4.72	(4.14 , 5.34)
2008080	8.33	(7.25 , 9.41)	7.35	(6.47 , 8.28)	4.53	(3.94 , 5.17)	4.30	(3.75 , 4.90)
2008239	8.25	(7.17 , 9.33)	7.64	(6.81 , 8.52)	4.62	(4.02 , 5.27)	5.06	(4.50 , 5.66)
2008075	10.53	(9.63 , 11.44)	7.43	(6.66 , 8.24)	4.82	(4.29 , 5.38)	4.71	(4.20 , 5.25)
2008114	8.74	(7.76 , 9.73)	6.94	(6.15 , 7.77)	4.42	(3.87 , 5.00)	4.83	(4.28 , 5.41)
2008166	8.57	(7.59 , 9.55)	7.02	(6.23 , 7.87)	3.84	(3.34 , 4.39)	3.98	(3.48 , 4.51)
2008252	9.11	(8.14 , 10.09)	7.44	(6.62 , 8.31)	4.18	(3.66 , 4.75)	4.50	(3.97 , 5.07)
2008150	9.65	(8.90 , 10.40)	7.56	(6.68 , 8.51)	3.73	(3.33 , 4.16)	4.44	(3.87 , 5.04)
2008009	10.31	(9.40 , 11.21)	8.18	(7.38 , 9.04)	3.84	(3.37 , 4.35)	4.06	(3.59 , 4.56)
2008081	11.79	(10.71 , 12.87)	8.81	(7.85 , 9.82)	5.08	(4.45 , 5.76)	4.96	(4.36 , 5.60)
2008041	10.41	(9.57 , 11.25)	8.08	(7.28 , 8.93)	4.22	(3.75 , 4.72)	4.31	(3.82 , 4.82)
2008061	8.23	(7.39 , 9.07)	7.60	(6.71 , 8.55)	4.04	(3.58 , 4.53)	4.42	(3.86 , 5.02)
2008087	9.36	(8.28 , 10.44)	7.95	(7.04 , 8.91)	4.20	(3.63 , 4.82)	4.85	(4.26 , 5.48)
2008147	10.76	(10.01 , 11.51)	8.63	(7.80 , 9.50)	4.14	(3.71 , 4.58)	4.85	(4.33 , 5.40)
2008258	11.04	(10.25 , 11.84)	7.92	(7.08 , 8.82)	6.40	(5.85 , 6.98)	6.09	(5.47 , 6.74)
2008130	9.30	(8.39 , 10.20)	8.40	(7.52 , 9.32)	3.93	(3.45 , 4.43)	4.40	(3.88 , 4.96)
2008100	10.00	(9.09 , 10.90)	8.08	(7.17 , 9.06)	4.43	(3.92 , 4.98)	5.15	(4.54 , 5.80)
2008132	10.25	(9.27 , 11.23)	7.27	(6.40 , 8.19)	4.74	(4.17 , 5.34)	4.58	(4.00 , 5.19)

Family	α-acid 16 months		α-acid 28 months *		β-acid 16 months *		β-acid 28 months *	
2008256	9.77	(8.79 , 10.75)	6.89	(6.04 , 7.79)	6.16	(5.51 , 6.84)	5.65	(5.01 , 6.33)
2008093	7.92	(7.13 , 8.71)	6.96	(6.21 , 7.74)	3.44	(3.04 , 3.87)	4.13	(3.65 , 4.63)
2008108	8.14	(7.35 , 8.93)	6.94	(6.15 , 7.78)	4.60	(4.13 , 5.09)	4.53	(4.00 , 5.09)
2008179	8.88	(8.03 , 9.72)	7.84	(7.00 , 8.73)	4.75	(4.25 , 5.27)	4.79	(4.25 , 5.37)
2008192	9.10	(8.12 , 10.08)	7.06	(6.21 , 7.97)	5.81	(5.18 , 6.47)	4.59	(4.01 , 5.20)
2008089	10.09	(9.11 , 11.07)	7.74	(6.90 , 8.62)	4.43	(3.88 , 5.01)	4.41	(3.88 , 4.96)
2008064	9.40	(8.32 , 10.48)	7.66	(6.77 , 8.61)	3.96	(3.41 , 4.56)	4.44	(3.87 , 5.04)
2008269	8.55	(7.47 , 9.63)	7.61	(6.65 , 8.65)	4.38	(3.80 , 5.01)	4.54	(3.92 , 5.21)
2008059	9.38	(8.29 , 10.46)	7.40	(6.58 , 8.27)	4.44	(3.85 , 5.07)	4.85	(4.30 , 5.43)
2008047	8.81	(7.82 , 9.79)	7.33	(6.51 , 8.19)	4.07	(3.54 , 4.63)	4.06	(3.56 , 4.60)
2008194	11.39	(10.41 , 12.36)	7.51	(6.68 , 8.38)	5.29	(4.69 , 5.92)	4.71	(4.16 , 5.28)
2008232	7.86	(6.88 , 8.83)	6.77	(5.99 , 7.60)	4.62	(4.06 , 5.21)	5.01	(4.45 , 5.60)
2008173	8.67	(7.58 , 9.75)	7.50	(6.68 , 8.38)	4.65	(4.04 , 5.30)	4.54	(4.00 , 5.10)
2008120	8.80	(7.82 , 9.78)	8.15	(7.29 , 9.06)	4.66	(4.09 , 5.26)	5.07	(4.50 , 5.66)
2008262	9.42	(8.44 , 10.40)	7.45	(6.57 , 8.39)	4.16	(3.63 , 4.72)	4.53	(3.96 , 5.14)
2008187	8.12	(7.14 , 9.10)	6.99	(6.19 , 7.83)	3.61	(3.12 , 4.14)	4.10	(3.59 , 4.63)
2008051	9.94	(8.96 , 10.92)	7.81	(6.91 , 8.77)	5.01	(4.43 , 5.63)	4.70	(4.12 , 5.32)
2008198	7.98	(7.00 , 8.95)	7.06	(6.26 , 7.90)	3.84	(3.33 , 4.38)	4.05	(3.55 , 4.59)
2008054	7.04	(6.25 , 7.84)	6.95	(6.21 , 7.74)	3.22	(2.83 , 3.63)	4.40	(3.91 , 4.92)
2008244	9.62	(8.77 , 10.46)	7.88	(7.03 , 8.77)	4.33	(3.86 , 4.84)	4.88	(4.33 , 5.47)
2008177	9.23	(8.15 , 10.32)	7.19	(6.38 , 8.04)	4.31	(3.73 , 4.94)	4.82	(4.27 , 5.40)
2008117	9.15	(8.25 , 10.06)	7.75	(6.96 , 8.57)	4.01	(3.53 , 4.53)	4.43	(3.94 , 4.96)
2008016	8.14	(7.35 , 8.94)	8.17	(7.31 , 9.08)	4.74	(4.27 , 5.24)	4.87	(4.32 , 5.46)
2008267	9.47	(8.49 , 10.45)	7.82	(6.98 , 8.71)	4.20	(3.67 , 4.77)	4.28	(3.76 , 4.82)
2008040	10.73	(9.65 , 11.81)	7.48	(6.60 , 8.42)	4.54	(3.94 , 5.18)	4.22	(3.67 , 4.81)
2008218	11.13	(10.29 , 11.97)	8.58	(7.69 , 9.51)	4.46	(3.98 , 4.97)	4.46	(3.93 , 5.02)
2008070	9.32	(8.34 , 10.30)	7.20	(6.34 , 8.12)	5.07	(4.48 , 5.69)	4.14	(3.60 , 4.73)
2008102	10.77	(9.69 , 11.85)	7.72	(6.88 , 8.60)	4.80	(4.19 , 5.46)	4.72	(4.18 , 5.29)
2008078	10.84	(9.76 , 11.92)	7.45	(6.57 , 8.39)	4.78	(4.17 , 5.43)	4.67	(4.09 , 5.29)
2008074	9.17	(8.33 , 10.01)	8.41	(7.59 , 9.27)	4.01	(3.55 , 4.49)	4.39	(3.89 , 4.91)

g.

Family	cohumulone (% of α-acid) 16 months *		cohumulone (% of α-acid) 28 months *		α-acid:β-acid 16 months *		α-acid:β-acid 28 months *		α-acid:total resin 16 months *		α-acid:total resin 28 months *	
2008003	0.31	(0.28 , 0.33)	0.33	(0.31 , 0.36)	2.29	(2.03 , 2.55)	1.78	(1.55 , 2.01)	0.68	(0.65 , 0.71)	0.62	(0.58 , 0.66)
2008190	0.28	(0.25 , 0.30)	0.29	(0.27 , 0.32)	2.04	(1.81 , 2.27)	1.61	(1.39 , 1.84)	0.66	(0.63 , 0.69)	0.60	(0.56 , 0.63)
2008209	0.30	(0.27 , 0.32)	0.32	(0.30 , 0.35)	2.43	(2.15 , 2.73)	1.80	(1.57 , 2.03)	0.70	(0.66 , 0.73)	0.63	(0.59 , 0.67)
2008151	0.29	(0.26 , 0.32)	0.29	(0.27 , 0.32)	2.16	(1.90 , 2.44)	1.73	(1.50 , 1.96)	0.67	(0.64 , 0.71)	0.62	(0.58 , 0.66)
2008039	0.33	(0.30 , 0.36)	0.31	(0.28 , 0.34)	2.47	(2.18 , 2.77)	1.83	(1.58 , 2.09)	0.70	(0.67 , 0.73)	0.63	(0.59 , 0.67)
2008152	0.27	(0.25 , 0.29)	0.30	(0.28 , 0.33)	2.11	(1.90 , 2.34)	1.54	(1.32 , 1.77)	0.67	(0.64 , 0.69)	0.59	(0.55 , 0.62)
2008001	0.30	(0.28 , 0.33)	0.30	(0.28 , 0.33)	2.29	(2.05 , 2.54)	1.97	(1.73 , 2.21)	0.69	(0.66 , 0.71)	0.65	(0.61 , 0.69)
2008073	0.30	(0.27 , 0.32)	0.32	(0.30 , 0.35)	1.95	(1.72 , 2.20)	1.74	(1.49 , 1.99)	0.65	(0.62 , 0.68)	0.62	(0.58 , 0.66)
2008168	0.28	(0.25 , 0.30)	0.31	(0.28 , 0.33)	1.95	(1.70 , 2.21)	1.59	(1.37 , 1.82)	0.64	(0.61 , 0.67)	0.58	(0.54 , 0.61)
2008161	0.27	(0.25 , 0.30)	0.29	(0.27 , 0.31)	2.32	(2.06 , 2.59)	1.63	(1.42 , 1.84)	0.69	(0.66 , 0.72)	0.59	(0.56 , 0.62)
2008290	0.27	(0.25 , 0.29)	0.30	(0.27 , 0.32)	2.45	(2.20 , 2.71)	1.93	(1.70 , 2.17)	0.70	(0.67 , 0.73)	0.64	(0.61 , 0.68)
2008208	0.28	(0.25 , 0.30)	0.28	(0.26 , 0.31)	2.34	(2.06 , 2.63)	1.71	(1.48 , 1.94)	0.69	(0.66 , 0.72)	0.61	(0.58 , 0.65)
2008251	0.28	(0.26 , 0.31)	0.29	(0.27 , 0.32)	1.97	(1.72 , 2.24)	1.63	(1.41 , 1.86)	0.65	(0.62 , 0.68)	0.61	(0.57 , 0.64)
2008052	0.29	(0.27 , 0.31)	0.33	(0.30 , 0.35)	2.32	(2.09 , 2.56)	1.95	(1.72 , 2.19)	0.69	(0.66 , 0.71)	0.65	(0.61 , 0.68)
2008037	0.26	(0.24 , 0.28)	0.28	(0.25 , 0.30)	2.13	(1.89 , 2.39)	1.85	(1.62 , 2.09)	0.67	(0.64 , 0.70)	0.64	(0.60 , 0.68)
2008154	0.33	(0.31 , 0.36)	0.37	(0.35 , 0.40)	2.24	(1.99 , 2.51)	1.89	(1.66 , 2.13)	0.68	(0.65 , 0.71)	0.64	(0.60 , 0.68)
2008136	0.28	(0.26 , 0.30)	0.31	(0.29 , 0.34)	2.21	(2.00 , 2.43)	1.77	(1.56 , 1.98)	0.68	(0.66 , 0.71)	0.63	(0.59 , 0.66)
2008096	0.31	(0.29 , 0.33)	0.32	(0.30 , 0.35)	1.99	(1.80 , 2.20)	1.83	(1.60 , 2.07)	0.66	(0.63 , 0.68)	0.63	(0.60 , 0.67)
2008071	0.29	(0.27 , 0.31)	0.31	(0.29 , 0.33)	2.31	(2.09 , 2.55)	1.81	(1.60 , 2.03)	0.69	(0.66 , 0.71)	0.63	(0.60 , 0.67)
2008124	0.29	(0.26 , 0.31)	0.32	(0.29 , 0.35)	2.14	(1.88 , 2.42)	1.80	(1.55 , 2.06)	0.67	(0.64 , 0.70)	0.63	(0.59 , 0.67)
2008145	0.30	(0.28 , 0.32)	0.31	(0.29 , 0.33)	2.15	(1.95 , 2.36)	1.83	(1.62 , 2.05)	0.67	(0.65 , 0.70)	0.64	(0.60 , 0.67)
2008084	0.27	(0.25 , 0.29)	0.31	(0.28 , 0.34)	1.99	(1.78 , 2.20)	1.69	(1.44 , 1.94)	0.65	(0.62 , 0.67)	0.61	(0.57 , 0.65)
2008083	0.28	(0.25 , 0.30)	0.31	(0.28 , 0.33)	2.18	(1.93 , 2.44)	2.08	(1.84 , 2.33)	0.68	(0.65 , 0.71)	0.66	(0.62 , 0.70)
2008056	0.29	(0.26 , 0.31)	0.31	(0.28 , 0.33)	1.89	(1.66 , 2.13)	1.59	(1.35 , 1.85)	0.64	(0.61 , 0.67)	0.59	(0.55 , 0.63)
2008261	0.31	(0.29 , 0.33)	0.32	(0.30 , 0.35)	2.19	(1.95 , 2.43)	1.82	(1.59 , 2.05)	0.68	(0.65 , 0.71)	0.63	(0.60 , 0.67)
2008157	0.29	(0.27 , 0.32)	0.32	(0.30 , 0.35)	2.18	(1.92 , 2.47)	1.72	(1.49 , 1.95)	0.67	(0.64 , 0.71)	0.62	(0.59 , 0.66)
2008006	0.27	(0.25 , 0.30)	0.33	(0.31 , 0.36)	1.93	(1.69 , 2.17)	1.96	(1.73 , 2.21)	0.65	(0.62 , 0.68)	0.64	(0.61 , 0.68)
2008240	0.32	(0.30 , 0.34)	0.32	(0.29 , 0.34)	1.93	(1.73 , 2.15)	1.98	(1.74 , 2.22)	0.64	(0.61 , 0.66)	0.65	(0.61 , 0.69)
2008103	0.26	(0.24 , 0.29)	0.30	(0.28 , 0.33)	2.17	(1.90 , 2.45)	1.73	(1.52 , 1.95)	0.67	(0.64 , 0.71)	0.62	(0.59 , 0.66)
2008148	0.31	(0.28 , 0.33)	0.33	(0.31 , 0.36)	2.13	(1.91 , 2.36)	1.80	(1.59 , 2.02)	0.66	(0.63 , 0.68)	0.61	(0.58 , 0.65)
2008236	0.36	(0.34 , 0.38)	0.36	(0.34 , 0.39)	2.20	(2.00 , 2.42)	1.70	(1.48 , 1.94)	0.68	(0.66 , 0.70)	0.61	(0.58 , 0.65)
2008259	0.29	(0.26 , 0.32)	0.31	(0.28 , 0.34)	2.16	(1.90 , 2.44)	1.86	(1.61 , 2.12)	0.67	(0.64 , 0.71)	0.64	(0.59 , 0.68)
2008191	0.38	(0.35 , 0.41)	0.39	(0.37 , 0.42)	2.09	(1.86 , 2.33)	1.69	(1.48 , 1.90)	0.67	(0.64 , 0.69)	0.62	(0.58 , 0.65)

Family	cohumulone (% of α-acid) 16 months *		cohumulone (% of α-acid) 28 months *		α-acid:β-acid 16 months *		α-acid:β-acid 28 months *		α-acid:total resin 16 months *		α-acid:total resin 28 months *	
2008242	0.31	(0.29 , 0.34)	0.30	(0.28 , 0.32)	1.69	(1.49 , 1.90)	1.51	(1.31 , 1.72)	0.61	(0.58 , 0.64)	0.58	(0.55 , 0.61)
2008248	0.26	(0.24 , 0.28)	0.27	(0.25 , 0.30)	1.88	(1.66 , 2.10)	1.76	(1.53 , 2.00)	0.63	(0.61 , 0.66)	0.61	(0.57 , 0.64)
2008010	0.28	(0.26 , 0.30)	0.34	(0.31 , 0.36)	2.43	(2.18 , 2.68)	1.87	(1.64 , 2.11)	0.70	(0.67 , 0.73)	0.64	(0.60 , 0.67)
2008263	0.28	(0.25 , 0.30)	0.32	(0.30 , 0.35)	2.11	(1.88 , 2.35)	1.76	(1.53 , 1.99)	0.67	(0.64 , 0.70)	0.63	(0.59 , 0.66)
2008153	0.28	(0.26 , 0.30)	0.31	(0.29 , 0.33)	2.34	(2.13 , 2.57)	1.79	(1.58 , 2.01)	0.69	(0.67 , 0.72)	0.63	(0.60 , 0.67)
2008119	0.30	(0.27 , 0.32)	0.32	(0.29 , 0.34)	2.29	(2.03 , 2.55)	1.79	(1.56 , 2.03)	0.69	(0.66 , 0.72)	0.63	(0.59 , 0.67)
2008206	0.34	(0.31 , 0.36)	0.33	(0.31 , 0.36)	2.22	(1.99 , 2.47)	1.79	(1.58 , 2.01)	0.68	(0.65 , 0.71)	0.63	(0.60 , 0.66)
2008139	0.28	(0.26 , 0.30)	0.30	(0.28 , 0.33)	1.53	(1.35 , 1.72)	1.53	(1.31 , 1.76)	0.57	(0.55 , 0.60)	0.58	(0.55 , 0.62)
2008106	0.29	(0.27 , 0.31)	0.32	(0.29 , 0.34)	2.26	(2.02 , 2.51)	1.59	(1.37 , 1.82)	0.69	(0.66 , 0.71)	0.59	(0.56 , 0.63)
2008122	0.31	(0.28 , 0.34)	0.32	(0.29 , 0.34)	1.85	(1.60 , 2.11)	1.76	(1.54 , 2.00)	0.63	(0.60 , 0.66)	0.61	(0.58 , 0.65)
2008155	0.36	(0.33 , 0.38)	0.33	(0.30 , 0.35)	2.20	(1.96 , 2.44)	1.74	(1.52 , 1.98)	0.68	(0.65 , 0.70)	0.62	(0.58 , 0.66)
2008088	0.29	(0.26 , 0.31)	0.32	(0.30 , 0.35)	2.13	(1.90 , 2.37)	1.81	(1.58 , 2.05)	0.67	(0.64 , 0.70)	0.63	(0.59 , 0.67)
2008111	0.27	(0.25 , 0.30)	0.30	(0.27 , 0.33)	2.02	(1.77 , 2.30)	1.66	(1.42 , 1.92)	0.66	(0.63 , 0.69)	0.61	(0.57 , 0.65)
2008202	0.33	(0.31 , 0.35)	0.35	(0.32 , 0.37)	2.38	(2.17 , 2.61)	1.82	(1.59 , 2.05)	0.70	(0.67 , 0.72)	0.63	(0.59 , 0.67)
2008013	0.25	(0.23 , 0.28)	0.29	(0.26 , 0.31)	2.11	(1.89 , 2.35)	1.75	(1.52 , 1.99)	0.66	(0.63 , 0.69)	0.61	(0.57 , 0.65)
2008279	0.31	(0.29 , 0.34)	0.34	(0.31 , 0.37)	2.20	(1.93 , 2.48)	1.79	(1.54 , 2.05)	0.68	(0.65 , 0.71)	0.63	(0.59 , 0.67)
2008007	0.31	(0.29 , 0.34)	0.33	(0.30 , 0.35)	2.46	(2.20 , 2.74)	2.10	(1.86 , 2.35)	0.70	(0.67 , 0.73)	0.66	(0.62 , 0.70)
2008090	0.32	(0.30 , 0.34)	0.33	(0.30 , 0.35)	2.39	(2.16 , 2.63)	2.02	(1.80 , 2.24)	0.70	(0.67 , 0.72)	0.66	(0.62 , 0.69)
2008055	0.27	(0.25 , 0.29)	0.30	(0.28 , 0.33)	1.82	(1.63 , 2.03)	1.59	(1.37 , 1.82)	0.63	(0.60 , 0.65)	0.59	(0.55 , 0.62)
2008271	0.30	(0.28 , 0.32)	0.33	(0.30 , 0.35)	2.41	(2.18 , 2.65)	1.98	(1.75 , 2.23)	0.69	(0.67 , 0.72)	0.65	(0.61 , 0.69)
2008035	0.28	(0.26 , 0.30)	0.31	(0.28 , 0.33)	2.07	(1.85 , 2.31)	1.78	(1.55 , 2.02)	0.66	(0.63 , 0.69)	0.63	(0.59 , 0.66)
2008212	0.29	(0.27 , 0.32)	0.32	(0.29 , 0.35)	1.73	(1.51 , 1.97)	1.70	(1.46 , 1.96)	0.61	(0.58 , 0.64)	0.61	(0.57 , 0.65)
2008149	0.36	(0.33 , 0.38)	0.33	(0.31 , 0.36)	2.15	(1.92 , 2.39)	1.71	(1.48 , 1.94)	0.67	(0.65 , 0.70)	0.61	(0.58 , 0.65)
2008170	0.27	(0.25 , 0.29)	0.31	(0.28 , 0.33)	2.29	(2.06 , 2.52)	2.09	(1.85 , 2.33)	0.68	(0.66 , 0.71)	0.66	(0.62 , 0.70)
2008247	0.27	(0.25 , 0.30)	0.34	(0.31 , 0.37)	2.28	(2.01 , 2.57)	2.03	(1.79 , 2.27)	0.69	(0.65 , 0.72)	0.65	(0.62 , 0.69)
2008110	0.31	(0.28 , 0.34)	0.33	(0.30 , 0.36)	2.18	(1.91 , 2.46)	1.68	(1.44 , 1.94)	0.67	(0.64 , 0.71)	0.61	(0.57 , 0.65)
2008043	0.27	(0.25 , 0.29)	0.32	(0.30 , 0.34)	1.84	(1.66 , 2.04)	1.79	(1.58 , 2.01)	0.63	(0.61 , 0.66)	0.63	(0.60 , 0.67)
2008188	0.30	(0.27 , 0.33)	0.35	(0.32 , 0.38)	1.89	(1.65 , 2.16)	1.62	(1.38 , 1.88)	0.64	(0.61 , 0.67)	0.60	(0.56 , 0.64)
2008080	0.27	(0.25 , 0.30)	0.31	(0.28 , 0.34)	1.96	(1.71 , 2.23)	1.80	(1.55 , 2.06)	0.64	(0.61 , 0.67)	0.63	(0.59 , 0.67)
2008239	0.31	(0.29 , 0.34)	0.34	(0.31 , 0.36)	1.86	(1.62 , 2.13)	1.56	(1.34 , 1.78)	0.63	(0.60 , 0.66)	0.58	(0.55 , 0.62)
2008075	0.27	(0.25 , 0.29)	0.30	(0.28 , 0.33)	2.15	(1.92 , 2.39)	1.62	(1.42 , 1.84)	0.68	(0.65 , 0.70)	0.60	(0.57 , 0.64)
2008114	0.25	(0.23 , 0.27)	0.28	(0.26 , 0.31)	2.08	(1.84 , 2.33)	1.47	(1.25 , 1.69)	0.66	(0.63 , 0.69)	0.57	(0.53 , 0.60)
2008166	0.36	(0.33 , 0.39)	0.33	(0.31 , 0.36)	2.33	(2.07 , 2.60)	1.93	(1.70 , 2.17)	0.69	(0.66 , 0.72)	0.64	(0.60 , 0.68)
2008252	0.28	(0.26 , 0.30)	0.28	(0.26 , 0.31)	2.27	(2.02 , 2.53)	1.75	(1.52 , 1.99)	0.67	(0.64 , 0.70)	0.61	(0.58 , 0.65)
2008150	0.31	(0.29 , 0.33)	0.33	(0.30 , 0.36)	2.59	(2.38 , 2.82)	1.79	(1.54 , 2.05)	0.72	(0.69 , 0.74)	0.63	(0.59 , 0.67)

Family	cohumulone (% of α-acid) 16 months *		cohumulone (% of α-acid) 28 months *		α-acid:β-acid 16 months *		α-acid:β-acid 28 months *		α-acid:total resin 16 months *		α-acid:total resin 28 months *	
2008009	0.26	(0.24 , 0.28)	0.28	(0.26 , 0.30)	2.76	(2.50 , 3.03)	2.26	(2.03 , 2.49)	0.72	(0.69 , 0.75)	0.67	(0.64 , 0.71)
2008081	0.28	(0.25 , 0.31)	0.30	(0.27 , 0.33)	2.26	(1.99 , 2.54)	1.88	(1.62 , 2.14)	0.68	(0.65 , 0.71)	0.64	(0.60 , 0.68)
2008041	0.34	(0.31 , 0.36)	0.29	(0.26 , 0.31)	2.48	(2.24 , 2.72)	2.01	(1.79 , 2.23)	0.70	(0.68 , 0.73)	0.66	(0.62 , 0.69)
2008061	0.28	(0.26 , 0.30)	0.31	(0.28 , 0.34)	2.10	(1.89 , 2.33)	1.82	(1.57 , 2.08)	0.67	(0.64 , 0.70)	0.63	(0.59 , 0.67)
2008087	0.30	(0.27 , 0.33)	0.34	(0.32 , 0.37)	2.24	(1.97 , 2.53)	1.71	(1.46 , 1.96)	0.68	(0.65 , 0.72)	0.61	(0.57 , 0.66)
2008147	0.31	(0.29 , 0.33)	0.32	(0.29 , 0.34)	2.61	(2.39 , 2.84)	1.87	(1.66 , 2.09)	0.71	(0.69 , 0.74)	0.64	(0.60 , 0.67)
2008258	0.26	(0.24 , 0.28)	0.30	(0.28 , 0.32)	1.74	(1.56 , 1.94)	1.41	(1.20 , 1.64)	0.61	(0.59 , 0.64)	0.55	(0.51 , 0.58)
2008130	0.29	(0.26 , 0.31)	0.32	(0.30 , 0.35)	2.39	(2.15 , 2.65)	2.10	(1.86 , 2.34)	0.70	(0.67 , 0.72)	0.66	(0.62 , 0.70)
2008100	0.27	(0.24 , 0.29)	0.31	(0.28 , 0.34)	2.26	(2.03 , 2.51)	1.65	(1.40 , 1.91)	0.68	(0.66 , 0.71)	0.60	(0.57 , 0.65)
2008132	0.30	(0.27 , 0.32)	0.31	(0.28 , 0.34)	2.13	(1.89 , 2.39)	1.63	(1.39 , 1.89)	0.67	(0.64 , 0.70)	0.60	(0.56 , 0.64)
2008256	0.30	(0.28 , 0.33)	0.32	(0.29 , 0.35)	1.67	(1.45 , 1.90)	1.37	(1.14 , 1.62)	0.60	(0.57 , 0.63)	0.51	(0.47 , 0.54)
2008093	0.30	(0.28 , 0.32)	0.32	(0.30 , 0.34)	2.40	(2.18 , 2.62)	1.80	(1.59 , 2.02)	0.70	(0.67 , 0.72)	0.63	(0.59 , 0.66)
2008108	0.29	(0.27 , 0.31)	0.31	(0.29 , 0.33)	1.83	(1.64 , 2.02)	1.59	(1.37 , 1.82)	0.61	(0.59 , 0.64)	0.59	(0.55 , 0.62)
2008179	0.24	(0.22 , 0.26)	0.28	(0.26 , 0.30)	1.95	(1.74 , 2.17)	1.72	(1.49 , 1.95)	0.65	(0.62 , 0.67)	0.61	(0.58 , 0.65)
2008192	0.34	(0.31 , 0.37)	0.32	(0.29 , 0.34)	1.67	(1.46 , 1.90)	1.58	(1.34 , 1.83)	0.60	(0.57 , 0.63)	0.58	(0.54 , 0.62)
2008089	0.29	(0.27 , 0.31)	0.32	(0.29 , 0.34)	2.25	(2.00 , 2.52)	1.81	(1.58 , 2.04)	0.68	(0.66 , 0.71)	0.63	(0.60 , 0.67)
2008064	0.28	(0.26 , 0.31)	0.30	(0.27 , 0.33)	2.42	(2.14 , 2.72)	1.81	(1.56 , 2.07)	0.70	(0.66 , 0.73)	0.63	(0.59 , 0.67)
2008269	0.34	(0.31 , 0.36)	0.31	(0.28 , 0.35)	1.94	(1.69 , 2.21)	1.77	(1.49 , 2.06)	0.64	(0.61 , 0.67)	0.62	(0.57 , 0.67)
2008059	0.30	(0.27 , 0.32)	0.33	(0.30 , 0.35)	2.14	(1.88 , 2.42)	1.57	(1.35 , 1.80)	0.67	(0.64 , 0.70)	0.59	(0.55 , 0.63)
2008047	0.32	(0.30 , 0.35)	0.35	(0.33 , 0.38)	2.21	(1.96 , 2.48)	1.92	(1.69 , 2.16)	0.68	(0.65 , 0.71)	0.64	(0.60 , 0.68)
2008194	0.29	(0.27 , 0.32)	0.30	(0.27 , 0.32)	2.05	(1.81 , 2.31)	1.64	(1.42 , 1.87)	0.66	(0.63 , 0.69)	0.61	(0.57 , 0.64)
2008232	0.27	(0.25 , 0.29)	0.30	(0.27 , 0.32)	1.79	(1.57 , 2.03)	1.38	(1.17 , 1.60)	0.62	(0.59 , 0.65)	0.54	(0.51 , 0.58)
2008173	0.26	(0.23 , 0.28)	0.31	(0.29 , 0.34)	1.91	(1.66 , 2.18)	1.73	(1.50 , 1.96)	0.64	(0.61 , 0.68)	0.62	(0.58 , 0.65)
2008120	0.26	(0.24 , 0.29)	0.30	(0.27 , 0.32)	1.96	(1.72 , 2.21)	1.67	(1.45 , 1.91)	0.65	(0.62 , 0.68)	0.61	(0.57 , 0.65)
2008262	0.29	(0.27 , 0.32)	0.31	(0.28 , 0.33)	2.28	(2.03 , 2.55)	1.71	(1.46 , 1.97)	0.69	(0.66 , 0.72)	0.61	(0.57 , 0.65)
2008187	0.28	(0.26 , 0.30)	0.30	(0.27 , 0.32)	2.23	(1.98 , 2.50)	1.78	(1.55 , 2.01)	0.68	(0.65 , 0.71)	0.63	(0.59 , 0.66)
2008051	0.32	(0.29 , 0.34)	0.33	(0.30 , 0.36)	1.98	(1.74 , 2.23)	1.74	(1.49 , 2.00)	0.65	(0.62 , 0.68)	0.62	(0.58 , 0.66)
2008198	0.30	(0.28 , 0.33)	0.32	(0.29 , 0.34)	2.13	(1.89 , 2.39)	1.85	(1.62 , 2.09)	0.67	(0.64 , 0.70)	0.64	(0.60 , 0.67)
2008054	0.28	(0.26 , 0.30)	0.31	(0.29 , 0.34)	2.22	(2.01 , 2.44)	1.62	(1.41 , 1.83)	0.68	(0.66 , 0.71)	0.59	(0.56 , 0.62)
2008244	0.28	(0.26 , 0.30)	0.31	(0.29 , 0.34)	2.27	(2.05 , 2.50)	1.71	(1.49 , 1.95)	0.68	(0.66 , 0.71)	0.61	(0.57 , 0.65)
2008177	0.29	(0.26 , 0.32)	0.29	(0.26 , 0.31)	2.16	(1.90 , 2.44)	1.53	(1.31 , 1.75)	0.67	(0.64 , 0.71)	0.58	(0.55 , 0.62)
2008117	0.34	(0.31 , 0.36)	0.36	(0.34 , 0.39)	2.28	(2.04 , 2.53)	1.79	(1.58 , 2.01)	0.69	(0.66 , 0.71)	0.63	(0.60 , 0.67)
2008016	0.27	(0.25 , 0.29)	0.31	(0.29 , 0.34)	1.75	(1.57 , 1.95)	1.74	(1.52 , 1.98)	0.62	(0.60 , 0.64)	0.62	(0.58 , 0.66)
2008267	0.26	(0.24 , 0.28)	0.32	(0.30 , 0.35)	2.22	(1.97 , 2.49)	1.92	(1.69 , 2.16)	0.68	(0.65 , 0.71)	0.64	(0.61 , 0.68)
2008040	0.30	(0.27 , 0.33)	0.33	(0.30 , 0.36)	2.35	(2.08 , 2.65)	1.92	(1.67 , 2.19)	0.69	(0.66 , 0.72)	0.64	(0.60 , 0.68)

Family	cohumulone (% of α-acid)		cohumulone (% of α-acid)		α-acid:β-acid		α-acid:β-acid		α-acid:total resin		α-acid:total resin	
	16 months *		28 months *		16 months *		28 months *		16 months *		28 months *	
2008218	0.27	(0.25 , 0.29)	0.31	(0.29 , 0.33)	2.42	(2.19 , 2.66)	2.07	(1.83 , 2.31)	0.70	(0.67 , 0.73)	0.66	(0.62 , 0.70)
2008070	0.28	(0.26 , 0.31)	0.24	(0.21 , 0.26)	1.93	(1.70 , 2.18)	1.87	(1.61 , 2.13)	0.64	(0.61 , 0.67)	0.64	(0.59 , 0.68)
2008102	0.30	(0.28 , 0.33)	0.32	(0.30 , 0.35)	2.19	(1.93 , 2.48)	1.72	(1.49 , 1.95)	0.68	(0.65 , 0.71)	0.62	(0.58 , 0.66)
2008078	0.28	(0.25 , 0.31)	0.34	(0.31 , 0.37)	2.20	(1.93 , 2.48)	1.66	(1.41 , 1.91)	0.68	(0.64 , 0.71)	0.61	(0.57 , 0.65)
2008074	0.28	(0.26 , 0.30)	0.30	(0.28 , 0.32)	2.30	(2.08 , 2.54)	2.07	(1.85 , 2.30)	0.69	(0.66 , 0.72)	0.66	(0.63 , 0.70)

APPENDIX 4.3 Genetic and phenotypic correlations between growing seasons in which the quantitative genetic analysis was conducted

Appendix 4.3 Genetic and phenotypic correlations between the two growing seasons in which hop cone chemical traits and plant growth traits were assessed. The standard error of each correlation is given. Correlations statistically different from zero ($P < 0.05$) are shown in bold. **a.** refers to cone chemical traits, which were all measured at 16 months (season 1) and 28 months (season 2) after the trial was planted. **b.** refers to plant growth traits. The traits number of shoots and length of longest shoot were assessed 11 months (season 1) and 24 months (season 2) after the trial was planted. The trait height (at flower initiation) was assessed at 13 months (season 1) and 25 months (season 2) after the trial was planted. The traits height (at cone maturity), height to the cones and lateral length were assessed at 16 months (season 1) and 28 months (season 2) after the trial was planted.

a.

	Genetic correlations	Phenotypic correlations
α-acid	**1.00** ± 0.24	**0.61** ± 0.07
β-acid	**0.99** ± 0.09	**0.85** ± 0.02
cohumulone (% of α-acid)	**0.99** ± 0.10	**0.86** ± 0.02
α-acid:β-acid	**0.97** ± 0.12	**0.81** ± 0.03
α-acid:total resin	**0.95** ± 0.09	**0.86** ± 0.02

b.

	Genetic correlations	Phenotypic correlations
number of shoots	**0.80** ± 0.12	**0.42** ± 0.03
length of the longest shoot	**0.78** ± 0.15	**0.40** ± 0.03
height (at flower initiation)	**0.94** ± 0.12	**0.45** ± 0.03
height (at cone maturity)	**1.00** ± 0.01	**1.00** ± 0.01
height to the cones	**1.00** ± 0.28	**1.00** ± 0.02
lateral length	**1.00** ± 0.03	**0.69** ± 0.03

APPENDIX 4.4 Quantitative genetic parameters calculated for hop in this study compared to results of previous studies

Appendix 4.4 Comparisons between the results of this study and results of previous studies for estimates of quantitative genetic parameters of hop cone chemical traits and yield. **a.** refers to the number of families and estimates of narrow-sense heritability calculated for cone chemical traits and yield. The values reported from this study are averages of data from two seasons for cone chemical traits and one season for yield. **b.** refers to additive genetic correlations between the traits α-acid, β-acid and cone yield from previous studies of hop, compared to the values determined in this study. Correlations statistically different from zero ($P < 0.05$) are shown in bold.

a.

Study	*n* families	cohumulone	colupulone	α-acid	β-acid	α-acid:β-acid	yield
This study	107-108	0.23	0.18	0.22	0.18	0.21	0.00
Henning et al. (2005)	25	0.87	0.89	0.76	0.57	-	0.71
Murakami (1999)	12	-	-	0.50	0.75	0.00	-
Henning et al. (1997a)	14	-	-	0.88	0.35	-	0.20

b.

Study	α-acid x β-acid	α-acid x yield	β-acid x yield
This study – season 1	**0.48**	**-**	**-**
This study – season 2	-0.08	**-0.93**	**-0.63**
Henning et al. (2005)	**0.71**	0.28	**0.73**
Murakami (1999)	-	-	-
Henning et al. (1997a)	**-0.42**	**-0.66**	**0.84**
Henning et al. (1997b)	**-0.74**	-0.12	0.04

APPENDIX 5.1 Genetic sequence for the sex-linked microsatellite marker HLAGA7

Appendix 5.1 The genetic sequence of the sex-linked microsatellite marker HLAGA7, verified in three pedigrees and diverse environments as being completely linked to the male sex.

>ENA|EF175945|EF175945.1 *Humulus lupulus* microsatellite HLAGA7 sequence.

TCCAAGTCTCTCAATTAGGAATTCTACATCCTCTTCTTCATTTACATTCTTCATTTCCAA
TTCTTTCAATTTGGGCAAAGGTGAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAAGAA
GAAGAAGAAGAAGAAGAATAAGAAGAAGAAGAAGAAGAGGATGCTTTTGATGTTGATGAT
CCTACATAATTCCTCATCATTACTGCTTGT

Jakse J., Stajner N., Kozjak P., Cerenak A., Javornik B. Submitted (10-DEC-2006) to the International Nucleotide Sequence Database Collaboration (INSDC). Agronomy Department, University of Ljubljana, Biotechnical faculty, Jamnikarjeva 101, Ljubljana SI-1000, Slovenia.