Euphytica (2016) 209:171–179
DOI 10.1007/s10681-016-1655-9

Genomics of the hop pseudo-autosomal regions

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Received: 25 September 2015 / Accepted: 1 February 2016 / Published online: 23 February 2016
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Abstract Hop is one of the few dioecious plants with dimorphic sex chromosomes. Because the entire Cannabaceae family is dioecious, hop and other members of this family are thought to have a relatively older sex chromosomal system than other plant species. Hop cones are only produced in female hops with or without fertilization. This has lead to most genomic research being directed toward female plants. The work we present provides genomic resources surrounding male plants. We have produced a draft genome for the male hop line USDA 21422M using a novel genome assembly method. In addition, we identified a 1.3 Mb set of scaffolds, which appear to be the male specific region based upon specificity with male hop accessions. This set includes a smaller high confidence total length 18 Kb set of scaffolds, which are supported by over 500 individuals, including the USDA world collection of hop varieties and two mapping populations, with genotyping by sequencing. We also have identified a portion of the Teamaker × 21422M linkage map to be associated with the pseudo-autosomal region (PAR). Within the genomic scaffolds, we identified a set of genes that are sex-linked and likely located in the PAR.

Keywords Genomics · Humulus · Pseudo-autosomal region · Sex chromosomes · Sexual determining region

Introduction

Humulus lupulus L. var. lupulus (European hop) is a dioecious ($2n = 2X = 18A + XX/XY$), perennial, climbing plant that is harvested for its female flowers. Its primary use is as flavoring and bittering additive in

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Electronic supplementary material The online version of this article (doi:10.1007/s10681-016-1655-9) contains supplementary material, which is available to authorized users.

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beer. Female hops produce lupulin glands, which in-turn produce more than 1000 known essential oils (Eri et al. 2000) as well as the bittering acids responsible for beer bittering. Although males do not produce cones, they do produce lupulin glands (with much lower production) in both flowers and on leaves (Fig. 1). This suggests female versus male differences in fitness and evolutionary function of these compounds. Because of the importance of the female flower, breeding and genomic work has been almost entirely focused on females. Less than 6 % of all flowering plants are dioecious, and only a few of these are documented as heterogametic like hop (Ming et al. 2011). In the family *Cannabaceae*, *Cannabis sativa* (2n = 18 + XY), *Humulus japonicus* (2n = 14 + XY1Y2), and *Humulus lupulus* (2n = 18 + XY) all have heterogametic sex chromosomes.

Although hop typically has two sex chromosomes, there are six systems known to exist, spanning from one to three pairs of sex chromosomes with various sizes of the Y-chromosome. Differences primarily occur within *var. cordifolious* (Table 1) (Ono 1961). *Humulus lupulus* is furthermore one of the only plants to have flowers that morphologically diverge early in development (Shephard et al. 2000). These traits contribute to the hypothesis that sex chromosomes in Cannabaceae suggest a relatively well-established and presumably older system (Charlesworth 2015).

Hop is sometimes known to exhibit pseudo-monoecious flowering, however male flowers on pseudo-monoecious hop plants have never been reported to produce viable pollen. Hop is known to have an X:A ratio for sex determination, suggesting the structural genes are located on the autosomes, while the genes responsible for completing pollen development are in the sex determining region (SDR), that is the region specifically responsible for determining sex (Shephard et al. 2000).

Fig. 1 Male and female flowers. Male flowers (left) typically shed pollen prior to female flowers (right) are receptive for pollination

Thus far, there have been several cytogenetic experiments involving the sex chromosomes of Hop, *Humulus japonicus*, and Hemp (Divashuk et al. 2014), (Grabowska-Joachimiak et al. 2011), (Divashuk et al. 2011). Additionally, a SSR marker was developed for screening male hops at a young age (Jakse et al. 2008). However no work has been done to genomically unravel the control of sex determination in hop.

Traditionally, sex chromosomes have been studied in animals. More recent studies have focused upon the evolution of sex chromosomes and floral development in plant species. The primary difference between sex determination in plants and mammals is that morphological differences between sexes appear very late in the life cycle of plants. Most research on sexual differentiation and sex chromosomes has been done on *Silene,* papaya and *Rumex* (Liu et al. 2004); (Filatov 2005); (Hough et al. 2014). Unlike *Rumex*, the Y-chromosome is essential in hop for development of pollen in male plants (Shephard et al. 2000). Nevertheless, *Humulus* remains largely unstudied, even though plants in this family show evolutionary advanced stages of sex chromosomes, particularly, between stages 5 and 6 (Divashuk et al. 2011). Stage five is recognized by a small, degenerating Y-chromosome undergoing heavy recombination suppression that is enriched with repetitive elements. Stage 6 occurs with the loss of the Y-chromosome and an X:Autosome sex determination ratio (Ming et al. 2011).

The pseudo-autosomal region (PAR) is defined as the recombining region of sex chromosomes. Recombination within PAR does not follow normal segregation patterns as in autosomal chromosomes – portions of the region may be genetically linked to the sexual determining region causing recombination suppression of the alleles near the SDR. This leads to major differences in allelic frequency between sexes for loci in the PAR near the SDR boundry. This can cause an aggregation of genes with different fitness levels for both sexes. Genes with different allele frequencies in each sex may then gain tighter linkage to the SDR

Table 1 List of sex chromosome systems in *Humulus lupulus*

Name	Sex chromosomes	Description
Winge	XX/XY	2:1 X–Y size ratio
New winge	XX/XY	1.25:1 X–Y size ratio
Heteromorphic	XX/XY	Very small Y-chromosome
Sinoto	X–A–A–X/X–A–A–Y	14:12:10:7 XAAY size ratio
New sinoto	X–A–A–X/X–A–A–Y	13:11:10:3 XAAY size ratio

causing a cascade effect until the loci is ultimately subsumed by the SDR. Loci in the SDR undergo recombination suppression due to a lack of pairing during meiosis and are considered completely sex linked.

While several cytogenetic studies on hop sex chromosomes exist there are no studies on the molecular basis for sex determination. The objectives of this study were to identify the pseudo-autosomal region in hop sex chromosomes as well as identify male specific regions of the Y-chromosome along with putative genes located on these regions.

Materials and methods

Plant material, DNA extraction and library preparation

All accessions used in the study were maintained at the USDA-ARS Hop Breeding and Genomics program located outside Corvallis, OR. Rhizome cuttings were obtained from each accession and grown out under clean conditions in a glasshouse at Oregon State University (Corvallis, OR) with disease and insect infestations controlled with regular chemical applications. Young leaves of approximately 4 cm^2 were collected and placed under ice until prepared for DNA extraction in the lab. DNA extraction was performed the same day leaf tissue samples were collected. Qiagen Plant DNAeasy Kits (Qiagen Inc, USA) were used with modifications to the protocol as outlined by Henning et al. (2015). These modifications resulted in samples possessing high quality DNA samples with large fragment sizes of approximately 25 kb. Library preparation for genotyping by sequencing (GBS) was performed as reported by (Elshire et al. 2011). GBS sequencing was performed on the Illumina HiSeq 2000 platform (Illumina Inc) with 48 genotypes per lane. A total of **511** accessions were GBS-sequenced to a depth on average of 5X (Table 2).

SNP identification

All SNPs utilized in the study were identified using TASSEL 3 (Glaubitz et al. 2014) GBS pipeline and two different hop genome assemblies. SNP identification was performed twice, once against the variety ‘Shinsuwase’ assembly (Natsume et al. 2015) and another against the 21422M MSR (see below). Default settings for TASSEL GBS pipeline were used for SNP ID. This provided two sets of SNPs (male specific and autosomal) for further analysis. The resulting data sets provided initial SNP sets of 1,098,285 SNPs for Shinsuwase-based and 80,168 SNPs for 21422M MSR. Further filtration of the raw SNPs for both data sets was utilized so that only SNPs present in 80 % of all accessions were obtained: 260,318 for Shinsuwase and 23,943 for 21422M MSR.

21422M genome assembly

The genome for 21422M was assembled using a novel method called “transcriptome guided genome assembly”, which uses transcript sequences as a guide for local gene-space genome assembly. The transcripts were taken from the HopBase (http://hopbase.org/)

Table 2 List of hop accessions utilized for genotyping-by-sequencing (GBS)

Type	Males	Females
Unnamed cultivars	72	119
Named cultivars	0	138
Dwarf pop	27	64
Downy mildew pop	18	73
Total	117	394

transcriptome assembly. Reads for the assembly were acquired from the DNA Databank of Japan (DDJP) id: DRP002426. RNAseq reads corresponding to cultivar ‘Shinsuwase’ tissue types leaf, flower, immature cone, intermediate cone, mature cone, and lupulin glands. Reads were cleaned and QCed using Skewer v0.1.120 with a mean quality score required of 30 (Jiang et al. 2014). The HopBase transcriptome was assembled using SOAPdenovo-trans v1.03 with a K-value of 23 and default settings (Xie et al. 2014). Contigs smaller than 1000 were removed, as they were most likely fragmented transcripts. This resulted in a set of 37,324 contigs. Contigs were then filtered for contaminants using BLAST against the NCBI non-redundant database (NR) (Johnson et al. 2008). After removing all non-plant hits, this resulted in a remaining set of 36,808 contigs.

Our implementation of transcript guided assembly, called Cantina (Hill et al. unpublished) is available at (https://github.com/hillst/Cantina). The assembly resulted in .081 Gb out of the estimate 2.8 for *Humulus lupulus cv 21422M* with an N50 of 3654. The small size is due to the focus of assembly around the known gene space. A total of 25,185 out of 36,808 transcripts were successfully assembled. This is likely due to the low coverage of genomic sequencing, however it is still a valuable resource for investigating the male hop plant and is included in this publication.

Male specific region identification

The male specific region (MSR) is defined as regions of the male genome that do not contain alignments from any female cultivars, yet contain alignments from many or most male cultivars.

The whole genome sequencing reads from 21422M, a single lane of paired-end HiSeq 2000 with a 250 bp insert size, were assembled using velvet v1.2.10 (Zerbino and Birney 2008). A K-value of 51 was used with exp_cov set to auto and scaffolding enabled. The resulting scaffolds were filtered for contigs >200 bp.

Contigs that contained alignments from sequencing reads from female whole-genome sequencing were used to filter out regions shared by both sexes. Paired-end whole genome sequencing reads from Teamaker, 21422M, and from Shinsuwase were down-sampled to 10 × to match 21422M and decrease computation time. These reads were then aligned to the scaffolds from Teamaker, 21422M and Shinsuwase. Loci with no reads from the female libraries (Teamaker and Shinsuwase) were called male specific. This resulted in an assembly of 20202198 bp. This resulting set was used for calling SNPs as described above. These regions were then further filtered using GBS reads to identify high confidence loci and to remove loci in which GBS reads from female samples aligned. Alignments were performed using BWA v0.7.12 with default settings (Li and Durbin 2009). The difference in number of male and female samples is due to the focus of sequencing on females and the uneven distribution of males and females within a population (Table 2). Contigs containing any female GBS alignments were removed, resulting in a set of 1.3 Mb. This set is denoted our putative SDR, although it is severely limited by the fragmented MSR assembly for 21422M and has much room for improvement. Contigs containing alignments present in at least 80 % of the male accessions were called male specific with high confidence due to the large number of samples. Due to limited GBS cut sites within the MSR, these contigs resulted in a small total length of 18 Kb, these loci are the best candidates for molecular male markers.

SNPs used for identifying the linkage group from Henning et al. (2015) containing the PAR region were selected from the data set consisting of 35,922 SNPs, each SNP belonged to one of the 11 linkage groups. These were ultimately chosen for use in mixed linear models (MLM) analysis in TASSEL v5.21. Kinship and Q-matrices were not utilized for MLM as the population had a clearly defined genetic make-up consisting of a full-sib family from the mapping population between ‘Teamaker × 21422M’. The statistical threshold for marker significance of 5.85 on the −log10 scale was determined by Bonferroni correction (Dunn 1961). See Supplementary Fig. 1 for a workflow diagram of the above procedures.

Results and discussion

Male Specific Region (MSR) identification

Male specific regions are typified by large stretches of repeat DNA and retrotransposons (Zhang et al. 2008); (Oyama et al. 2010); (Divashuk et al. 2014). These regions do not undergo recombination with the X-chromosome and therefore genes located in this

region will be fixed. It is presumed that borders between MSR and PAR are regions where sexually antagonistic genes are located and are undergoing evolution (Oyama et al. 2010); (Charlesworth 2015); (Hough et al. 2014). Nonetheless, little is known about the function (if any) of the repeat DNA and retro-transposons in hop.

Our study identified a 1.3 Mb set of DNA scaffolds that appear to be unique to male hop accessions. This DNA set contains a subset totaling18 Kb in length of DNA that were validated by lack of alignment from 385 female lines present in our GBS data set as well as alignments from 80 % of the 117 males making up the GBS set. The MSR (and putative SDR) identified herein provides a set of DNA useful for both the development of male markers for selection, and the exploration of markers related to sex that are shared among males and females. It may be possible to use this region as a basis for identifying molecular mechanisms for sex determination as proposed by Zhang et al. (2008). In addition, while several publications have cited the identification of "male markers" (Polley et al. 1997; Seefelder et al. 2000; Danilova and Karlov 2006; Jakse et al. 2008; McAdam et al. 2013), most have been identified by means of segregating loci—meaning recombination with the X-chromosome. A preferable marker system would be one utilizing a male marker located on the MSR of the Y-chromosome where no recombination occurs and marker evaluation could be a simple inexpensive PCR "presence/absence" of the marker.

Divashuk et al. (2011) identified the long arms of both the X and Y-chromosomes as the PAR for hop sex chromosome. It follows that the MSR we've identified would reside upon the short-arms and potentially covers the centromere. The cytogenetic research by Divashuck et al. (2011) identified the regions showing X–Y pairing to be external to the centromere. Thus, linkage maps in *Humulus* species would not show markers from the MSR as one of the linkage groups but would only show markers present in the PAR. Linkage maps are developed through genetic marker data for loci segregating in the population. Linkage distance between loci is calculated based upon the recombination rate in the population. If X- and Y-chromosomes do not pair and undergo chiasma, no recombination will be possible. Thus, only a portion of the long arms of the X- and Y-chromosome pair and undergo chiasma. Those regions not pairing would be considered the MSR on the Y-chromosome while those regions that do pair, show recombination and are thus the PAR.

Pseudo-autosomal region (PAR) identification

The natural follow-up to identifying the MSR was to attempt to find sex-linked SNPs in the female genome assembly. The only linkage group in Henning et al. (2015) that contained sex-linked SNPs was also the clearly sex enriched linkage group, linkage group 4 (LG4) (Fig. 2). Only LG4 contained GBS markers that were significantly associated with sex. To explore this relationship further, SNPs hypothesized to be present within the PAR were identified by performing a mixed linear model (MLM) by using the TASSEL v5.21 GUI on the sexual phenotypes of all the GBS individuals previously mentioned. The SNPs were also tested against the Teamaker x 21422M linkage map (Henning et al. 2015). LG4 was statistically enriched for sex-associated SNPs and thus concluded to be the pseudo-autosomal region—presumably carrying alleles from the X and Y-chromosome (Fig. 2).

The identification of the PAR opens the door for further sex chromosome studies in *Humulus*. *Humulus lupulus* is in an advanced stage of sex chromosome evolution showing relatively small estimated PAR sizes compared to other dioecious plants with heterogametic sex chromosomes (Divashuk et al. 2011, 2014). The size of Linkage group 4 after including genomic scaffolds is only 5 Mb, however this number is much lower than expected due to the fragmented genome assembly. The nature of the genes within the PAR (Supplementary Table 1), in addition to the identification of cytogenetic markers, may pave the way for understanding the unusual distribution of sex determination in the *Humulus* genus.

Sexually antagonistic selective genes

In addition to observing an increase in pairwise diversity across the PAR, albeit missing the SDR genes, we also expect to observe genes acting in a sexually antagonistic fashion as we near the SDR boundary (Hough et al. 2014; Otto et al. 2011). To identify sexually antagonistic genes we first identified markers from the overall pool of all GBS markers that were >95 % homozygous in females and at least 50 % heterozygous in males (Fig. 3). These markers were

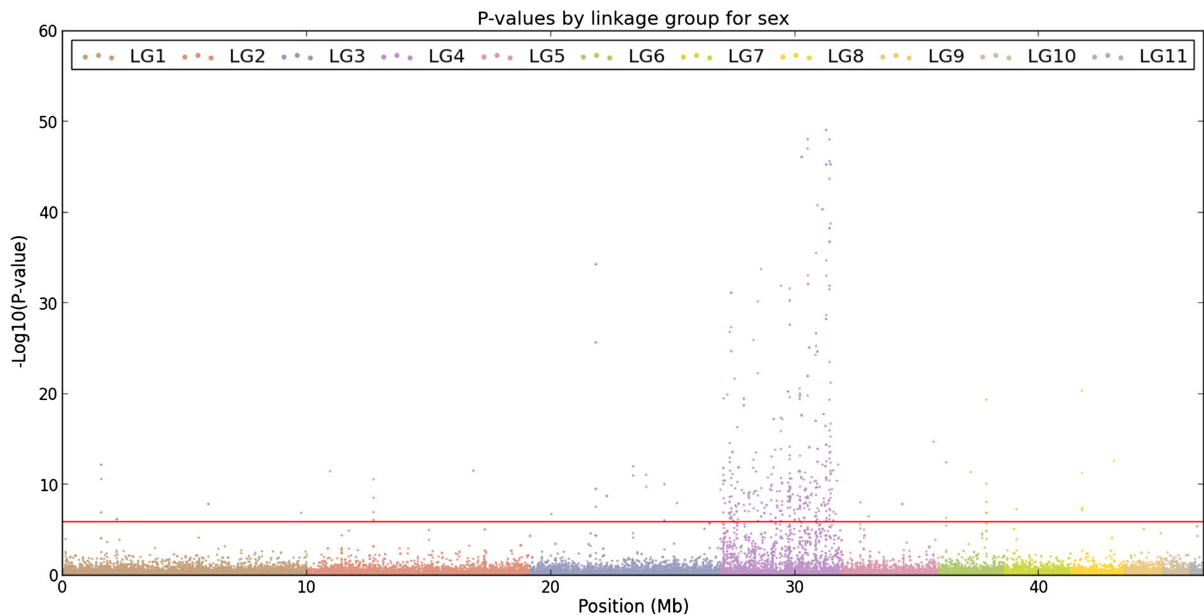

Fig. 2 Manhattan plot of mixed linear model analysis from TASSEL 5.21 showing markers with significant association with sex (females coded as "0", males coded as "1"). Linkage group 4 was saturated with highly significant markers for sex

then scanned for flanking genes to identify genes located nearby with the presumption that they act as sexually antagonistic genes (Supplementary Table 2).

Not every gene contains the ApeKi cut-sites used by GBS; many genes did not even have the possibility of being identified in the previous analysis. To explore potentially excluded genes, the PAR (LG4) was also scanned for genes. These genes were then added to our list and are putatively sex-linked, but more specifically noted as PAR genes (Supplementary Table 1). Although some of the genes identified in this step showed homology to other plant species, most of the genes had unknown function, likely due to the lack of quality annotation for hop. Specialization occurring within this region could be particularly interesting to plant breeders. If there are genes associated with any of the flavoring components, favorable alleles should be fixed on males (on the haploid X chromosome). This sort of information would allow for a nearly guaranteed inheritance of a desirable allele by selection and utilization of male parental lines possessing the desirable alleles. These regions may also be of interest for genetic engineering. If a locus is tightly linked to sex, the trait will recombine less frequently and show little change from parent to offspring. In particular, the WRKY1 transcription factor, known to be responsible for the last step of prenylation in the Xanthohumol pathway and involved in disease resistance (Majer et al. 2014), is located on LG4 (HL.SW.v1.0.G043711). Additionally a WRKY domain binding protein also exists on the PAR (HL.SW.v1.0.G020812). This further suggests female-specific specification occurring within the PAR.

One of the sex-linked genes identified within the PAR region is annotated as Acetyl-CoA carboxylase 1 (Supplementary Table 1). This gene codes for a protein that helps catalyze the first step of the humulone biosynthesis pathway. Interestingly, humulone is a compound produced predominantly in females, with trace amounts being found in male flower. This suggests that there is some specialization occurring in the PAR involving the bitter acid biosynthesis. We then used the whole genome sequence alignments described earlier to try and identify a copy number variation occurring within the genome. However, the results showed evidence of only one copy (Fig. 4). Interestingly, there was a sharp spike near the middle of the gene, showing 7 copies in females, and 14 copies in males. We then took this gene and looked for conserved protein coding domains through InterProScan5.

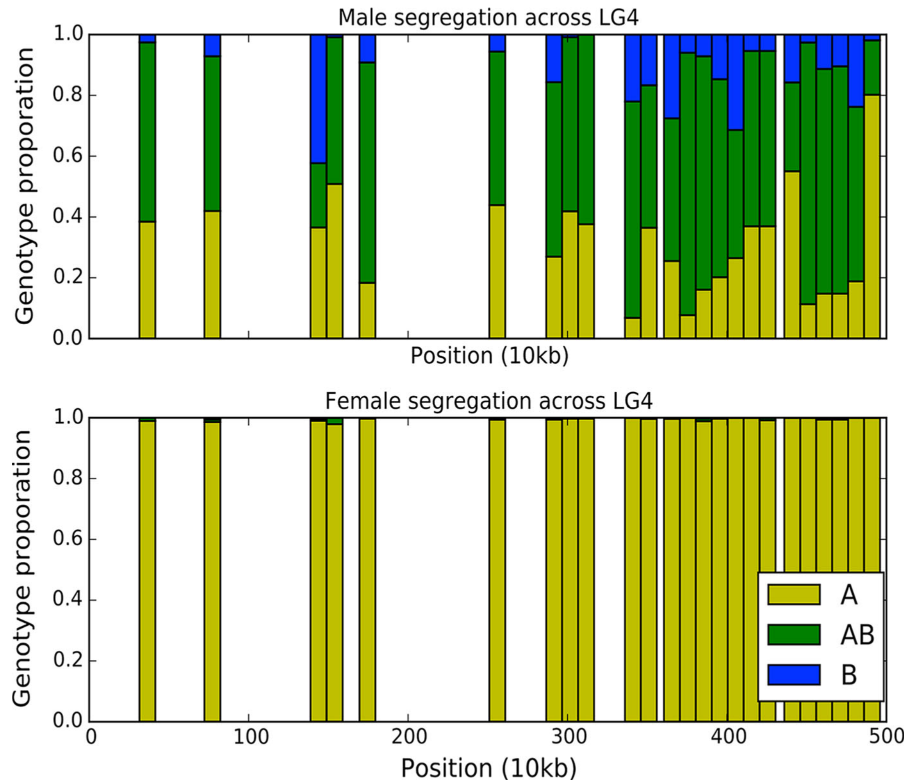

Fig. 3 Sex linked markers found on LG4 from mapping population "Teamaker x 21422M" segregating for downy mildew resistance (Henning et al. 2015) as observed across the USDA-ARS world collection of hop germplasm

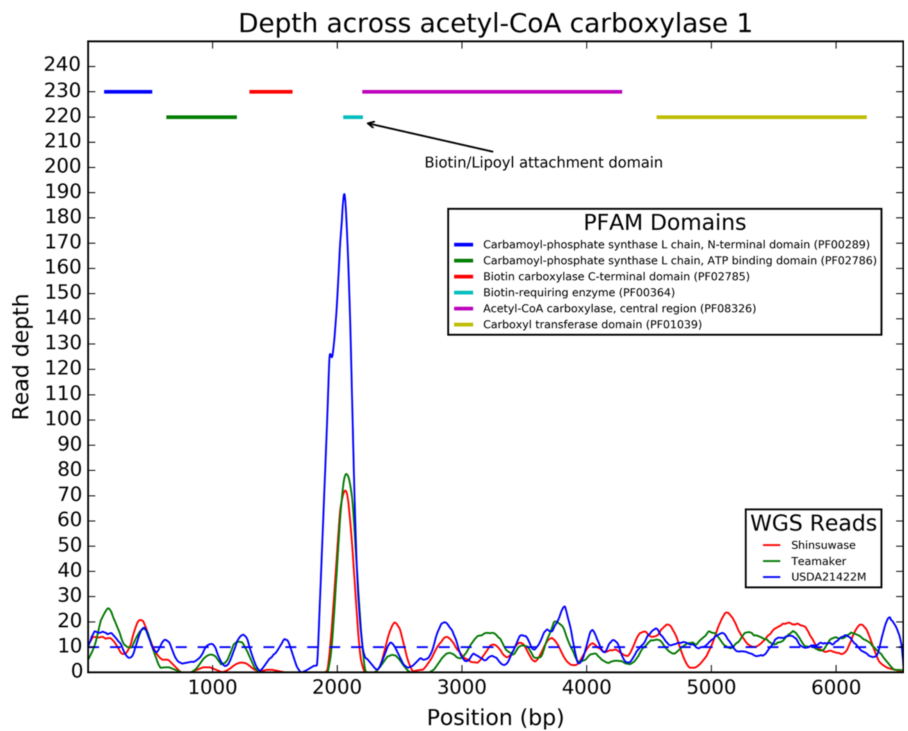

Fig. 4 Depth of read coverage across the gene space of Acetyl-CoA carboxylase 1

The peak at about 2000 bp in Fig. 4 corresponded perfectly with the biotin-lipoyl attachment domain, which is known to be critical for the function of Acetyl-CoA carboxylase 1 (Russell and Guest 1991). This suggests that the biotin-lipoyl coding domain is either extremely important for male hops, or that the region is duplicated many times on the Y-chromosome—potentially in the MSR. The latter has been observed in humans (Skaletsky et al. 2003) where there is a set of genes that are palindromic and high copy number. Although there is no direct evidence, the tight linkage of these genes with the male sex suggests it is near the MSR boundary. By further analyzing the gene family containing the biotin-lipoyl attachment domain, it may be possible to phylogenetically unravel the evolution of sex chromosomes in the Cannabaceae family.

Conclusion

The work described in this study offer a beginning for the understanding of dioecy mechanisms in *Humulus*. Unfortunately, the hop genome assembly is quite rudimentary and much of it is not assembled with only 1.8 Gb out of 2.7 Gb assembled and annotated (http://hopbase.org/). Most of the assembly resides around gene space with little or no information covering large repetitive regions that could potentially be responsible for gene regulation (Hill, unpublished data). With this in mind, it follows that the MSR for the Y-chromosome would not be included in the current hop assembly due to the theoretical presence of large regions of repetitive DNA that cannot be assembled into scaffolds using current short-read, massively-parallel sequencing. GBS data was obtained using these rudimentary genome assemblies and as such also are missing potentially a large number of SNPs and alignments that cover the whole genome. New attempts at sequencing using third generation sequencing technology are planned with the hope of covering the remaining genome and ultimately unraveling SDR and identifying sexually antagonistic genes. The results of this study, including the limited MSR and sex-linked genes, are available at http://resource-hopbase.cgrb.oregonstate.edu/HopBase/v1.0/IHS/.

References

Charlesworth B (2015) Causes of natural variation in fitness: evidence from studies of Drosophila populations. Proc Natl Acad Sci 112:1662–1669. doi:10.1073/pnas.1423275112

Danilova TV, Karlov GI (2006) Application of inter simple sequence repeat (ISSR) polymorphism for detection of sex-specific molecular markers in hop (Humulus lupulus L.). Euphytica 151:15–21

Divashuk MG, Alexandrov OS, Kroupin PY, Karlov GI (2011) Molecular cytogenetic mapping of humulus lupulus sex chromosomes. Cytogenet Genome Res 134:213–219

Divashuk MG, Alexandrov OS, Razumova OV, Kirov IV, Karlov GI (2014) Molecular cytogenetic characterization of the dioecious *Cannabis sativa* with an xy chromosome sex determination system. PLoS ONE 9:e85118. doi:10.1371/journal.pone.0085118

Dunn OJ (1961) Multiple comparisons among means. J Am Stat Assoc 56:52–64

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379

Eri S, Khoo BK, Lech J, Hartman TG (2000) 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 48:1140–1149

Filatov DA (2005) Substitution rates in a new Silene latifolia sex-linked gene. SlssX/Y Mol Biol Evol 22:402–408

Glaubitz JC, Casstevens TM, Lu F, Harriman J, Elshire RJ, Sun Q, Buckler ES (2014) TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. PLoS One 9:E90346

Grabowska-Joachimiak A, Mosiolek M, Lech A, Góralski G (2011) C-banding/DAPI and in situ hybridization reflect karyotype structure and sex chromosome differentiation in *Humulus japonicus* Siebold & Zucc. Cytogenet Genome Res 132:203–211

Henning JA, Gent DH, Twomey MC, Townsend MS, Pitra NJ, Matthews PD (2015) Precision QTL mapping of downy mildew resistance in hop (*Humulus lupulus* L.). Euphytica 202:487–498. doi:10.1007/s10681-015-1356-9

Hough J, Hollister JD, Wang W, Barrett SCH, Wright SI (2014) Genetic degeneration of old and young Y chromosomes in the flowering plant Rumex hastatulus. Proc Natl Acad Sci 111:7713–7718. doi:10.1073/pnas.1319227111

Jakse J, Stajner N, Kozjak P, Cerenak A, Javornik B (2008) Trinucleotide microsatellite repeat is tightly linked to male sex in hop (*Humulus lupulus* L.). Mol Breed 21:139–148. doi:10.1007/s11032-007-9114-x

Jiang H, Lei R, Ding S-W, Zhu S (2014) Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinform 15:182

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36:W5–W9. doi:10.1093/nar/gkn201

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760. doi:10.1093/bioinformatics/btp324

Liu Z et al (2004) A primitive Y chromosome in papaya marks incipient sex chromosome evolution. Nature 427:348–352

Majer A, Javornik B, Cerenak A, Jakse J (2014) Development of novel EST-derived resistance gene markers in hop (*Humulus lupulus* L.). Mol Breed 33:61–74

McAdam EL et al (2013) Quantitative trait loci in hop (Humulus lupulus L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry. BMC Genom 14:360

Ming R, Bendahmane A, Renner SS (2011) Sex chromosomes in land plants. Annu Rev Plant Biol 62:485–514. doi:10.1146/annurev-arplant-042110-103914

Natsume S et al (2015) The Draft Genome of Hop (Humulus lupulus), an Essence for Brewing. Plant Cell Physiol 56:428–441. doi:10.1093/pcp/pcu169

Ono T (1961) The hop native to Japan. Sasaki Printing and Publishing Company Ltd, Sendai

Otto SP et al (2011) About PAR: the distinct evolutionary dynamics of the pseudoautosomal region. Trends Genet 27:358–367

Oyama RK, Silber MV, Renner SS (2010) A specific insertion of a solo-LTR characterizes the Y-chromosome of Bryonia dioica (Cucurbitaceae). BMC research notes 3:166

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

Russell GC, Guest JR (1991) Sequence similarities within the family of dihydrolipoamide acyltransferases and discovery of a previously unidentified fungal enzyme. Biochimica et Biophysica Acta (BBA) 1076:225–232

Seefelder S, Ehrmaier H, Schweizer G, Seigner E (2000) Male and female genetic linkage map of hops, Humulus lupulus. Plant Breeding 119:249–255

Shephard HL, Parker JS, Darby P, Ainsworth CC (2000) Sexual development and sex chromosomes in hop. New Phytol 148:397–411. doi:10.1046/j.1469-8137.2000.00771.x

Skaletsky H et al (2003) The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 423:825–837

Xie Y et al (2014) SOAPdenovo-Trans: De novo transcriptome assembly with short RNA-Seq reads. Bioinformatics. doi:10.1093/bioinformatics/btu077

Zerbino DR, Birney E (2008) Velvet: algorithms for de novo short read assembly using deBruijn graphs. Genome Res 18:821–829

Zhang W, Wang X, Yu Q, Ming R, Jiang J (2008) DNA methylation and heterochromatinization in the male-specific region of the primitive Y chromosome of papaya. Genome Res 18:1938–1943