



HAL
open science

Plant genera *Cannabis* and *Humulus* share the same pair of well-differentiated sex chromosomes

Djivan Prentout, Natasa Stajner, Andreja Cerenak, Theo Tricou, Celine Brochier-Armanet, Jernej Jakse, Jos Käfer, Gabriel Marais

► To cite this version:

Djivan Prentout, Natasa Stajner, Andreja Cerenak, Theo Tricou, Celine Brochier-Armanet, et al.. Plant genera *Cannabis* and *Humulus* share the same pair of well-differentiated sex chromosomes. *New Phytologist*, 2021, 231 (4), pp.1599-1611. 10.1111/nph.17456 . hal-03254304

HAL Id: hal-03254304

<https://univ-lyon1.hal.science/hal-03254304v1>

Submitted on 8 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Plant genera *Cannabis* and *Humulus* share the same pair of well-differentiated sex chromosomes

D Prentout¹, N Stajner², A Cerenak³, T Tricou¹, C Brochier-Armanet¹, J Jakse², J Käfer^{1*}, GAB Marais^{1,4*}

1. Université de Lyon, Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR 5558, F-69622 Villeurbanne, France

2. Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia

3. Slovenian Institute of Hop Research and Brewing, Cesta Zalskega Tabora 2, SI-3310 Zalec, Slovenia

4. Current address: LEAF- Linking Landscape, Environment, Agriculture and Food, Instituto Superior de Agronomia, Universidade de Lisboa, Portugal

* These authors contributed equally to this work

Author for correspondence:

Djivan Prentout

djivan.prentout@univ-lyon1.fr

Word count

Total Main text: 7,316 (citations included) – 5 Figures (to be published in colour) – 3 Tables

Introduction: 1,126

Materials and Methods: 2,051 – 1 Figure

Results: 2,129 – 4 Figures – 3 Tables

Discussion: 2,007

Supporting Information: 1,679 – 10 Figures – 5 Tables

31 **Summary**

32

33

- 34 • We recently described, in *Cannabis sativa*, the oldest sex chromosome system documented
35 so far in plants (12-28 Myo). Based on the estimated age, we predicted that it should be
36 shared by its sister genus *Humulus*, which is known to also possess XY chromosomes.
- 37 • Here, we used transcriptome sequencing of a F1 family of *Humulus lupulus* to identify and
38 study the sex chromosomes in this species using the probabilistic method SEX-DETECTOR.
- 39 • We identified 265 sex-linked genes in *H. lupulus*, which preferentially mapped to the *C.*
40 *sativa* X chromosome. Using phylogenies of sex-linked genes, we showed that a region of
41 the sex chromosomes had already stopped recombining in an ancestor of both species.
42 Furthermore, as in *C. sativa*, Y-linked gene expression reduction is correlated to the position
43 on the X chromosome, and highly Y degenerated genes showed dosage compensation.
- 44 • We report, for the first time in Angiosperms, a sex chromosome system that is shared by two
45 different genera. Thus, recombination suppression started at least 21-25 My ago, and then
46 (either gradually or step-wise) spread to a large part of the sex chromosomes (~70%),
47 leading to a degenerated Y chromosome.

48

49

50

51 **Keywords**52 Cannabaceae; dioecy; dosage compensation; *Humulus lupulus*; sex chromosomes; Y degeneration

53

54

55

56

57

58

59 Introduction

60 Among more than 15,000 dioecious angiosperm species (*i.e.* species with separate sexes; Renner,
61 2014), less than twenty sex chromosome systems have been studied with genomic data (Ming *et al.*,
62 2011; Baránková *et al.*, 2020). Most plants with sex chromosomes exhibit male heterogamety, with
63 XY chromosomes in males, and XX chromosomes in females (Westergaard, 1958; Charlesworth,
64 2016). The portion of the Y chromosome that never recombines with the X experiences reduced
65 selection, which results in an accumulation of deleterious mutations and transposable elements
66 (Charlesworth & Charlesworth, 2000). This accumulation of transposable elements initially leads to
67 an increase of the size of the Y chromosome, which becomes larger than the X (Ming *et al.*, 2011).
68 When Y degeneration progresses, genetic material can be lost without fitness costs and the Y may
69 shrink (Ming *et al.*, 2011). Therefore, after sufficient time of divergence, we may observe
70 chromosome heteromorphy, *i.e.* a Y chromosome larger or smaller than the X chromosome,
71 depending on the progress of degeneration (Ming *et al.*, 2011). Classically, heteromorphy was
72 determined using light microscopy, which is rather imprecise and size differences of about 10%
73 could be considered homomorphic (see Divashuk *et al.*, 2014). While heteromorphy often
74 corresponds to the later stages of sex chromosome evolution, it is nevertheless possible that sex
75 chromosomes are homomorphic despite a large non-recombining region and strong degeneration of
76 the Y chromosome (*e.g.* Prentout *et al.*, 2020). Moreover, some systems do not evolve large non-
77 recombining region and stay homomorphic (Renner & Muller, 2021).

78 In plants, dioecy is often of recent origin (Renner, 2014; Käfer *et al.*, 2017), thus limiting the age of
79 the sex chromosomes. Indeed, several rather recently evolved (less than 10 million years (My) old)
80 homomorphic sex chromosome systems with small non-recombining regions have been described,
81 as in *Carica papaya* and *Asparagus officinalis* (Wu & Moore, 2015; Harkess *et al.*, 2017).
82 Heteromorphic sex chromosome systems are also found, with the Y being larger than the X, but
83 recombination suppression happened also relatively recently (less than 20 My ago), as in *Silene*
84 *latifolia* and *Coccinia grandis* (Sousa *et al.*, 2013; Krasovec *et al.*, 2018; Fruchard *et al.*, 2020).

85 A few cases in which dioecy evolved longer ago also exist (Käfer *et al.*, 2017), but no strongly
86 degenerated sex chromosomes have been described so far (Renner & Muller, 2021). Pucholt *et al.*
87 (2017) described very young sex chromosomes in *Salix viminalis* despite ancestral dioecy for the
88 sister genera *Salix* and *Populus*. Thus, either the sex chromosomes evolved independently in
89 different species, or there have been frequent turnovers. In the fully dioecious palm tree genus

90 *Phoenix*, a sex-linked region evolved before the speciation of the fourteen known species (Cherif *et al.*
91 *et al.*, 2016; Torres *et al.*, 2018). These sex chromosomes might be old, but do not appear to be
92 strongly differentiated. A similar situation has been reported in the grapevine (*Vitis*) genus (Badouin
93 *et al.*, 2020; Massonet *et al.*, 2020), possibly because sex chromosome evolution is slowed down in
94 such perennials with long generation time (Muyle *et al.*, 2017).

95 Thus, while homologous sex chromosomes are sometimes shared between species belonging to the
96 same genus (e.g. *Silene* sect. *Melandrium*, *Phoenix*) (Cherif *et al.*, 2016; Bacovsky *et al.*, 2020),
97 homologous sex chromosomes between different genera have never been described in plants so far.
98 This situation is in stark contrast to the situation in animals, for which several systems are more
99 than 100 My old and are shared by whole classes, e.g. birds and mammals (Ohno, 1969; Fridolfsson
100 *et al.*, 1998; Cortez *et al.*, 2014). Thus, although undoubtedly sex chromosomes have been less
101 intensively studied in plants, there seem to be fundamental differences in the evolution of sex
102 chromosomes in plants and animals (e.g. lack of strong sexual dimorphism in plants, discussed in
103 Renner & Muller, 2021). However, the extent of the differences needs to be clarified and more plant
104 sex chromosomes need to be studied.

105 Dioecy very likely evolved before the genera *Cannabis* and *Humulus* split, and might even be
106 ancestral in the Cannabaceae family (Yang *et al.*, 2013; Zhang *et al.*, 2018). *Cannabis sativa*
107 (marijuana and hemp) is a dioecious species with nearly homomorphic XY chromosomes (with
108 homomorphy defined as above). These sex chromosomes have a large non-recombining region and
109 are estimated to have started diverging between 12 and 28 My ago (Peil *et al.*, 2003; Divashuk *et al.*
110 *et al.*, 2014, Prentout *et al.*, 2020).

111 As for *C. sativa*, cytological analyses of *Humulus lupulus* (hop) found a XY chromosome system
112 with a large non-recombining region, but the Y chromosome is smaller than the X (Shephard *et al.*,
113 2000; Karlov *et al.*, 2003; Divashuk *et al.*, 2011). The *H. lupulus* and *C. sativa* lineages split
114 between 21 and 25 My old (Divashuk *et al.*, 2014; Jin *et al.*, 2020), which is more recently than our
115 higher bound estimate of the age of the *C. sativa* sex chromosomes (28 Mya; Prentout *et al.*, 2020).
116 It is thus possible that the sex chromosomes of *C. sativa* and *H. lupulus* evolved from the same pair
117 that already stopped recombining in their common ancestor, a question we address here.

118 As in many cultivated dioecious species, only female hop plants are harvested. Hop is used in beer
119 brewing for its bitterness, and its production is increasing worldwide (Neve, 1991; King &
120 Pavlovic, 2017), mostly because of the craft beer revolution (Barth-Haas, 2019; Mackinnon &
121 Pavlovic, 2019). The molecule responsible for hop flower bitterness, lupulin, is concentrated in

122 female ripe inflorescences, called cones (Okada & Ito, 2001). In pollinated cones, the presence of
123 seed reduces their brewing quality; since *H. lupulus* is wind pollinated, a single male plant in the
124 hop field or its vicinity can cause broad scale damage to the crop (Thomas & Neve, 1976).

125 Usually, hop is not grown from seeds, so female-only cultures are easy to obtain, and there is no
126 need for large-scale early sexing as in *Cannabis sativa* (cf. Prentout *et al.*, 2020). However, for
127 varietal improvement where controlled crosses are needed, knowing the sex early might be
128 beneficial. In *H. lupulus*, sexing is reliable 1-2 years after the sewing (Conway and Snyder, 2008;
129 Patzak *et al.*, 2002). A few markers have been developed, but the use of Y-specific coding
130 sequences may increase marker quality (Patzak *et al.*, 2002, Cerenak *et al.*, 2019).

131 Here we sequenced the transcriptome of fourteen *H. lupulus* individuals. These individuals came
132 from a cross, from which we sequenced the parents and six offspring of each sex. We used the
133 probabilistic approach SEX-DETECTOR, which is based on allele segregation analysis within a cross,
134 to identify sex-linked sequences (Muyle *et al.*, 2016). From these analyses on *H. lupulus* and our
135 previous results on *C. sativa* (Prentout *et al.*, 2020) we describe for the first time well-differentiated
136 sex chromosomes shared by two different genera in plants.

137

138 **Materials and Methods**

139 **Biological material and RNA-sequencing**

140 As indicated in Fig. 1a, we conducted a controlled cross for sequencing. The *H. lupulus* parents,
141 cultivar 'Wye Target' (WT; female) and the Slovenian male breeding line 2/1 (2/1), as well as 6
142 female and 6 male F1 siblings (Jakše *et al.*, 2013) were collected in July 2019 in the experimental
143 garden of Slovenian Institute of Hop Research and Brewing, Žalec.

144 All offspring were phenotypically confirmed to carry either male or female reproductive organs and
145 showed no anomalies in microsatellite genotyping data (Jakše *et al.*, 2013). Young leaves from the
146 laterally developing shoots were picked, wrapped in aluminium foil and flash frozen *in situ* in liquid
147 nitrogen. Later they were pulverized and stored at -80°C until RNA isolation.

148 Total RNA was isolated from 100 mg frozen tissue pulverized in liquid nitrogen according to the
149 protocol of Monarch Total RNA Miniprep Kit, including removal of DNA from the column with
150 DNase I (New England Biolabs). Total RNA was quantified with Qbit 3.0, and quality was verified
151 with the Agilent RNA Nano 6000 Kit to confirm appropriate sample RIN numbers. The total RNA
152 samples were sent to Novogen for mRNA sequencing using Illumina's 100 bp paired end service.

153 The data were submitted to the SRA database of the NCBI (BioSample accession
154 SAMN17526021).

155

156 **Mapping, genotyping and SEX-DETECTOR**

157 The bioinformatic pipeline is schematically described in Fig. **1b**. First, the RNA-seq data were
158 mapped to two different references: (1) the transcriptome *H. lupulus* (obtained from the annotated
159 genome; Padgitt-Cobb *et al.*, 2019) and (2) the transcriptome assembly of *C. sativa* that we also
160 used for our previous *C. sativa* sex chromosome analysis (Supporting Information; Van Bakel *et al.*,
161 2011; Prentout *et al.*, 2020). For the mapping, we ran GSNAP (version 2019-09-12; Wu and Nacu,
162 2010; Wu *et al.*, 2016), an aligner that enables SNP-tolerant mapping, with 10% mismatches
163 allowed. This approach, already used for *C. sativa* analysis, was iterated several times by adding Y-
164 specific SNPs to the references (and *H. lupulus* specific SNPs while mapping on *C. sativa*
165 reference; see Prentout *et al.*, 2020), which increased the number of mapped reads.

166 Then, SAMTOOLS (version 1.4; Li *et al.*, 2009) was used to remove unmapped reads and sort
167 mapping output files for the genotyping. We genotyped individuals with reads2snp (version 2.0.64;
168 Gayral *et al.*, 2013), as recommended for SEX-DETECTOR (Muyle *et al.*, 2016), *i.e.*, by accounting
169 for allelic expression biases, without filtering for paralogous SNPs, and only conserving SNPs
170 supported by at least three reads for subsequent analysis.

171 We ran the XY model of SEX-DETECTOR on the genotyping data, using the SEM algorithm and a
172 threshold for an assignment of 0.8. SEX-DETECTOR computes a posterior probability of being
173 autosomal (P_A), XY (P_{XY}) and X-hemizygous (P_{X-hemi}) for each SNP and for each gene (Fig. **1c**).
174 Thus, a gene with a P_A greater than or equal to 0.8 and at least one autosomal SNP without
175 genotyping error is classified as “autosomal”; a gene with $P_{XY} + P_{X-hemi}$ greater than or equal to 0.8
176 and at least one sex-linked SNP without genotyping error is classified as “sex-linked”; otherwise,
177 the gene is classified as “lack-of-information”. Among the sex-linked genes, we classified a gene as
178 X-hemizygous if it fulfilled one of these two criteria: (1) the gene carried only X-hemizygous SNPs
179 and at least one SNP without genotyping error, (2) the Y expression of the gene is detected only
180 from positions with genotyping errors. A parameter that is important to optimize with SEX-
181 DETECTOR is the Y specific genotyping error rate (p ; see Muyle *et al.*, 2016). However, the quantity
182 of Y-linked reads that map on a female reference diminishes with X-Y divergence, therefore, old
183 and highly divergent sex chromosomes are more susceptible to mapping errors and thus genotyping
184 errors. p is expected to be close to the whole transcriptome genotyping error rate (ϵ), but could be

185 higher due to weak expression (resulting in less reads) of the Y-linked copies or to mapping on a
 186 divergent X reference. To reduce the gap between these two error rates, we ran 4 iterations with
 187 GSNAP, using at each time the SNPs file generated by SEX-DETECTOR. This SNPs file contains *H.*
 188 *lupulus* specific polymorphisms, initially absent from the *C. sativa* reference transcriptome, and
 189 increased the quantity of mapped reads by adding these SNPs to the reference, and thus, fitting it
 190 with the *H. lupulus* RNA-seq.

191 As detailed in the Supporting Information, we retained the mapping on *C. sativa* transcriptome
 192 assembly for downstream analysis. Indeed, the mapping of Y-linked reads and SEX-DETECTOR
 193 results obtained with a mapping on *C. sativa* reference transcriptome were more robust than those
 194 obtained with a mapping on *H. lupulus* reference transcriptome (Supporting Information).

195

196

197

198

199

200

201

202

203

204

205

206

207

208

209

210

211

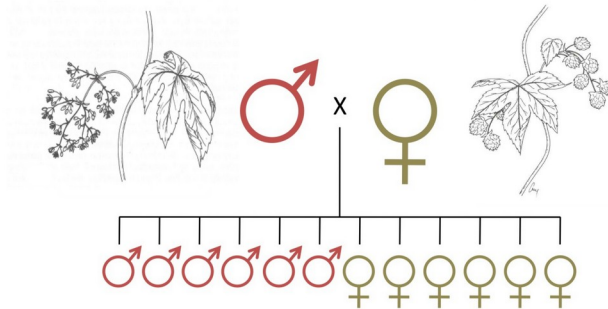
212

213

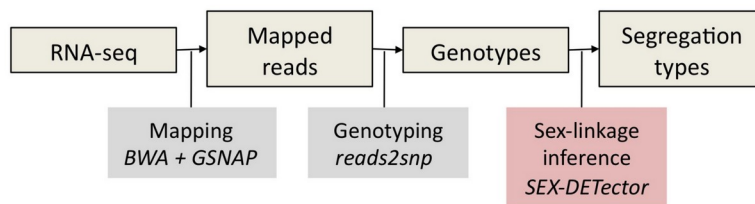
214

215

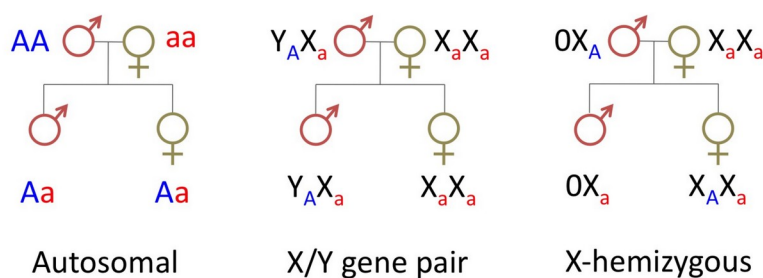
(a)



(b)



(c)



217

218 Figure 1. Schematic representation of the workflow used to detect sex-linkage. **(a)** Experimental design: six
219 females and six males were obtained by a controlled cross, and all individuals (14) were sequenced.
220 **(b)** Bioinformatic pipeline for the treatment of RNA-seq data. **(c)** Illustration of the underlying principles of the
221 SEX-DETECTOR segregation analysis.

222

223 Sex-linked gene positions on *C. sativa* genome

224 As the *H. lupulus* RNA-seq data were mapped on *C. sativa* transcriptome we determined the
225 position of the transcript sequences from the *C. sativa* transcriptome assembly (van Bakel *et al.*,
226 2011) on a chromosome-level assembly of the *C. sativa* genome (Grassa *et al.*, 2018) with blast
227 (version 2.2.30+; Altschul *et al.*, 1990). We selected the best hit with an e-value lower than 10^{-4} to
228 determine the position of the transcript on the genome. Then, we split each chromosome in
229 windows of 2 Mb and computed the density of sex-linked genes and non-sex-linked genes per
230 window using BEDTOOLS (version 2.26.0; Quinlan & Hall, 2010). Proportions of sex-linked
231 genes were computed by dividing the number of sex-linked genes by the total number of genes
232 (sex-linked, autosomal, and undetermined) in the same window. For *C. sativa*, densities were
233 already available from our previous analysis (Prentout *et al.*, 2020).

234

235 Molecular clock and age of sex chromosomes

236 We used the translated reference transcripts (van Bakel *et al.*, 2011) to determine the X and Y Open
237 Reading Frame (ORF) of nucleotide reference transcripts. For each XY gene pair, the *dS* values
238 were estimated with codeml (PAML version 4.9; Yang, 2007) in pairwise mode. Then, we used two
239 molecular clocks, derived from *Arabidopsis* species, to estimate the age of *H. lupulus* sex
240 chromosomes (Koch *et al.*, 2000; Ossowski *et al.*, 2010). In the wild, *H. lupulus* flowers in the
241 second or third year of development (Patzak *et al.*, 2002; Polley *et al.*, 1997), therefore, we took a
242 generation time (GT) of 2 years, and used the molecular clocks as follows:
243 $(dS)/rate = dS / (1.5 \times 10^{-8})$ using the molecular clock from Koch *et al.* (2000);
244 $(GT \times dS) / (2 \times \mu) = dS / (7 \times 10^{-9})$ using the clock from Ossowski *et al.* (2010). Three different
245 estimates of *dS* were used: the maximum *dS* value, the mean of the 5% highest *dS* values, and the
246 mean of the 10% highest *dS* values.

247

248

249

250 X and Y allele-specific expression analysis

251 In addition to identifying X and Y alleles, SEX-DETECTOR estimates their expression based on the
252 number of reads (Muyle *et al.*, 2016). These estimates rely on counting reads spanning XY SNPs
253 only and were normalized using the total read number in a library for each individual. We further
254 normalized them by the median autosomal expression for each individual. *C. sativa* results
255 presented here were generated in our previous analysis on *C. sativa* sex chromosomes (Prentout *et*
256 *al.*, 2020).

257

258 Correction of Y read mapping bias.

259 The use of a female reference for the mapping of the reads might create mapping biases, resulting in
260 the absence of Y reads in the most diverging parts of the genes. This issue may reduce the
261 divergence detected and change the phylogenetic signal (Dixon *et al.*, 2019). If, within a same gene,
262 regions that lack Y reads coexist with regions where the Y reads correctly mapped, we expect to see
263 a signature similar to gene conversion, *i.e.* region-wise variation in divergence. Therefore, we ran
264 geneconv (version 1.81a; Sawyer, 1999) in pairwise and group mode with the multiple alignments
265 used for the phylogeny (on 85 gene alignments before Gblock filtering, see below) in order to
266 identify and remove regions with reduced divergence. We defined two groups, one for X and Y
267 sequences in *H. lupulus* and the other one for X and Y sequences in *C. sativa*. Then, we conserved
268 only inner fragments and split the gene conversion regions from regions without gene conversion to
269 obtain two subsets per gene. Thus, we obtained a subset of sequences corrected for the mapping
270 bias, in addition to the set of genes not filtered with geneconv.

271

272 Phylogenetic analysis

273 We reconstructed gene families for genes identified as sex-linked in both *C. sativa* and *H. lupulus*.
274 Then, we used blastp, filtering for the best hit (with an e-value threshold fixed at 10^{-4}), to find
275 homologous sequences between *C. sativa* reference transcripts (the query sequence in blastp) (van
276 Bakel *et al.*, 2011) and 4 outgroup transcriptomes (the subject sequence in blastp): *Trema orientalis*
277 (Cannabaceae; van Velzen *et al.*, 2018), *Morus notabilis* (Moraceae; He *et al.*, 2013), *Fragaria*
278 *vesca ssp. vesca* (Rosaceae; Shulaev *et al.*, 2011), and *Rosa chinensis* (Rosaceae; Raymond *et al.*,
279 2018). Gene families for which at least two outgroup sequences have been identified were kept,
280 other gene families were discarded from subsequent analysis. Then, we added X and Y sequences

281 reconstructed by SEX-DETECTOR to each gene family. To distinguish potential paralogous sequences
282 or variants from alternative splicing, a blast of all sequences vs all sequences was realized. If two
283 sequences from two distinct gene families matched with each other (with an e-value threshold fixed
284 at 10^{-4}), then both families were removed from the dataset. Finally, we retrieved the corresponding
285 nucleotide sequence of each protein, which constituted the dataset used for the phylogenetic
286 analysis.

287 Using Macse (version 2.03; Ranwez *et al.*, 2011), and before alignment, non-homologous segments
288 of at least 60 nucleotides within or 30 nucleotides at the extremity of a nucleotide sequence were
289 trimmed if they displayed less than 30% of similarity with other sequences from the gene family.
290 This step allowed to remove misidentified outgroup sequences. Then, gene families with no
291 remaining outgroup sequences were discarded. Finally, remaining families were aligned with
292 Macse, allowing sequences to be removed and realigned, one sequence at a time and over multiple
293 iterations, to improve local alignment.

294 Nucleotide alignments were cleaned at the codon level using Gblocks (with default parameters) to
295 conserve only codons shared by all sequences (version 0.91b; Castresana, 2000). For maximum-
296 likelihood (ML) phylogenetic tree reconstruction, we used ModelFinder in IQ-TREE (version
297 1.639; Nguyen *et al.*, 2015; Kalyaanamoorthy *et al.*, 2017) to select the best-fit substitution model
298 for each alignment. Those models were then used in RAxML-NG (version 1.0.0; Kozlov *et al.*,
299 2019) to reconstruct gene family trees. The number of bootstrap replicates was estimated using
300 autoMRE (Pattengale *et al.*, 2010) criterion (maximum 2,000 bootstraps). The ML phylogenetic tree
301 reconstruction was run on two datasets, one without removing potential mapping biases, and one
302 with the potential mapping bias removed, as described above.

303 Bayesian phylogenies were built using Phylobayes (version 3.4; Lartillot *et al.*, 2009) with the site-
304 specific profiles CAT and the CAT-GTR models with a gamma distribution to handle across site
305 rate variations. Two chains were run in parallel for a minimum of 500 cycles. The convergence
306 between the two chains was checked every 100 cycles (with a burn-in equal to one fifth of the total
307 length of the chains). Chains were stopped once all the discrepancies were lower or equal to 0.1 and
308 all effective sizes were larger than 50 and used to build a majority rule consensus tree.

309

310 **Statistics and linear chromosome representations**

311 The statistical analyses have been conducted with R (version 3.4.4; R Core Team, 2013). We report
312 exact p-values when they are larger than 10^{-5} . The representation of phylogenetic topologies, *dS*

313 values on the first chromosome and the dosage compensation graphics have been done with ggplot2
314 (Wickham, 2011). For the circular representation of the sex-linked gene density along the *C. sativa*
315 genome we used Circlize package in R (GU *et al.*, 2014). We calculated confidence intervals for the
316 median of a dataset of n observations by resampling 5000 times n values from the dataset (with
317 replacement). The confidence intervals are then given by the quantiles of the distribution of median
318 values obtained by resampling.

319

320 Results

321 Identification of sex-linked genes in *H. lupulus*

322 As mentioned in the Materials and Methods section, we used the mapping of the *H. lupulus* RNA-
323 seq data on the *C. sativa* transcriptome assembly for downstream analysis. Of the 30,074 genes in
324 the *C. sativa* reference transcriptome, 21,268 had detectable expression in our *H. lupulus*
325 transcriptome data. The difference of properly-paired mapped reads between males (mean: 32.3%)
326 and females (mean: 34.9%) is slightly significant (Wilcoxon's test two-sided p -value = 0.038, see
327 Supporting Information Table S1), which may be explained by a reduced mapping efficiency of Y-
328 linked reads on the female reference.

329 The sex-linked sequences from *H. lupulus* transcriptome data were identified with SEX-DETECTOR
330 (Muyle *et al.*, 2016). It is important that genotyping error rate parameters ϵ and p have similar
331 values (ϵ : whole transcriptome; p : Y chromosome) to obtain reliable SEX-DETECTOR outputs. At the
332 fourth iteration of GSNAP mapping on *C. sativa* reference transcriptome ϵ and p stabilized at 0.06
333 and 0.20, respectively (Supporting Information Table S2). Upon closer inspection, one *H. lupulus*
334 male (#3) appeared to have many genotyping errors, as for some XY genes, this male was
335 genotyped both heterozygous (XY) and homozygous (XX), which increased the error rate p . The
336 identification of Y SNPs with this individual RNA-seq data discarded the hypothesis of a
337 mislabelled female or a XX individual that developed male flowers. A particularly strong Y reads
338 mapping bias in this male may explain these observations. After removal of this male, the error rate
339 p dropped to 0.10 (Supporting Information Table S2). A total of 265 sex-linked genes were
340 identified in *H. lupulus*, which represents 7.8% of all assigned genes (autosomal genes + sex-linked
341 genes; Table 1).

342

343

344

345 Table 1. Summary of the SEX-DETECTOR results.

	Number
All genes*	30,074
Expressed genes	21,268
Genes with SNPs used by SEX-DETECTOR	4,472
Genes with undetermined segregation type class 1 **	462
Genes with undetermined segregation type class 2 ***	354
Autosomal genes	3391
Sex-linked genes	265
XY genes	265
X-hemizygous genes	0

346 *transcripts from gene annotation of the *C. sativa* reference genome (van Bakel *et al.*, 2011).

347 ** Posterior probabilities < 0.8

348 ***Posterior probabilities > 0.8 but absence of SNPs without error.

349

350

351

352 ***H. lupulus* and *C. sativa* sex chromosomes are homologous**

353 Among 265 *H. lupulus* XY genes from the *C. sativa* transcriptome assembly (van Bakel *et al.*,
 354 2011), 254 genes are present on the *C. sativa* chromosome-level genome assembly (Grassa *et al.*,
 355 2018). As shown in Fig. **2a**, 192 of these genes (75.6%) map on *C. sativa* chromosome number 1,
 356 which is the chromosome we previously identified as the X chromosome in *C. sativa* (Prentout *et*
 357 *al.*, 2020). Of the 265 sex-linked genes in *H. lupulus*, 112 were also detected as sex-linked in *C.*
 358 *sativa*, while 64 were detected as autosomal and 89 had unassigned segregation type (Prentout *et*
 359 *al.*, 2020).

360 The synonymous divergence (*dS*) between X and Y copies of the sex-linked genes of *H. lupulus* is
 361 distributed similarly along the *C. sativa* sex chromosome as the values for this latter species, as
 362 shown in Fig. **2b**. While the sampling variation of these *dS* values is large, as expected (cf Takahata
 363 & Nei, 1985), it can be observed that the larger values occur in the region beyond 65 Mb.

364

365

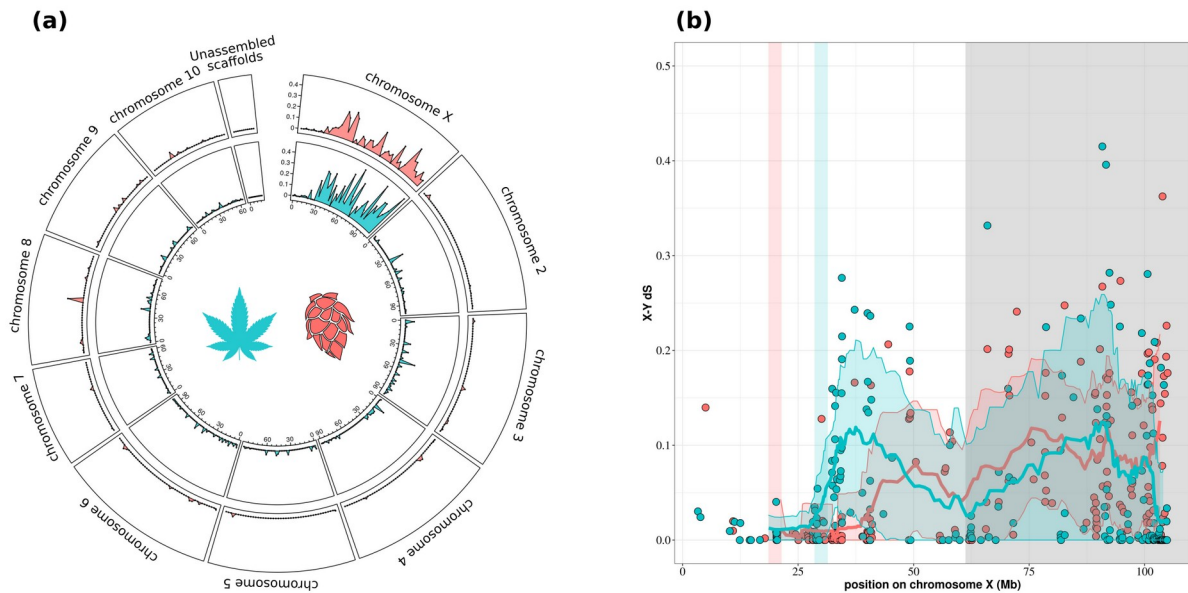
366

367

368

369

370



372 Figure 2. **(a)** *H. lupulus* sex-linked genes mapped on the *C. sativa* genome (Grassa *et al.*, 2018). Inner graphs (in
 373 blue): *C. sativa* sex-linked gene density corrected by the total gene density in 2-Mb windows (from Prentout *et al.*,
 374 2020). Outer graphs (in red): *H. lupulus* sex-linked gene density corrected by the total gene density in 2-Mb
 375 windows. Chromosome positions are given in Megabases. **(b)** Synonymous divergence (d_S) between X and Y
 376 copies of *H. lupulus* sex-linked genes (red) and those of *C. sativa* (blue) along the X chromosome of *C. sativa*.
 377 The curves represent the average d_S with sliding windows (windows of 20 points), for *H. lupulus* (red) and *C.*
 378 *sativa* (blue). Confidence intervals (average \pm standard deviation) are indicated around the *H. lupulus* curve (red
 379 area) and the *C. sativa* curve (blue area). The vertical red bar represents the putative Pseudo-Autosomal Boundary
 380 (PAB) in *H. lupulus*, the vertical blue bar represents the putative PAB in *C. sativa*, the grey area represents the
 381 region that stopped recombining in a common ancestor.

382

383

384

385

386 **X-Y recombination likely stopped before the *Cannabis* and *Humulus* genera split**

387 We reconstructed phylogenetic trees of genes detected as sex-linked in both species, including
388 outgroup sequences from the order Rosales. For 27 out of the 112 sex-linked genes present in both
389 species, we could not identify any homologous sequences in the outgroup species and those genes
390 were excluded from further analysis. For the remaining 85, we determined the topology of the
391 gametologous sequences in the Cannabaceae, considering a node as well resolved when the
392 bootstrap support exceeded 95%, or Bayesian support exceeded 0.95.

393 The three different methods for phylogenetic reconstruction provided consistent phylogenies (Table
394 2). More precisely, we observed three major topologies, as shown in Figure 3: X copies of both
395 species form a clade separated from a clade of Y sequences (topology I, Fig. **3a**), the X and Y
396 sequences of each species group together (topology II, Fig. **3b**), or a paraphyletic placement of the
397 X and Y sequences of *H. lupulus*, relative to *C. sativa* sequences (topology III, Fig. **3c**). As shown
398 in Table 2, we found that most genes had topology II, corresponding to recombination suppression
399 after the split of the genera. A few genes, however, had topology I, which corresponds to genes for
400 which recombination was already suppressed in a common ancestor of both species. As shown in
401 Fig. **3d**, topologies I and III occurred mainly beyond 80 Mb, while topology II occurred all over the
402 chromosome. Topology I is associated with higher synonymous divergence.

403 We identified 42 genes, out of the 85 genes used for the phylogeny, with at least one fragment in at
404 least one species that displayed reduced divergence (with a p-value < 0.05 in geneconv output).
405 Because this reduction of divergence may be caused by a mapping bias of Y reads, we ran the ML
406 phylogenetic reconstruction method on regions with and without mapping bias (example in
407 Supporting Information Fig. S7). As shown in Table 2 and Fig. **3e**, after mapping bias filter with
408 geneconv, the proportion of genes displaying topology I, indicating recombination suppression in a
409 common ancestor, increased, while less genes with topology II were mainly found in a restricted
410 region corresponding to the region where recombination stopped independently in both species.

411

412

413

414

415

416

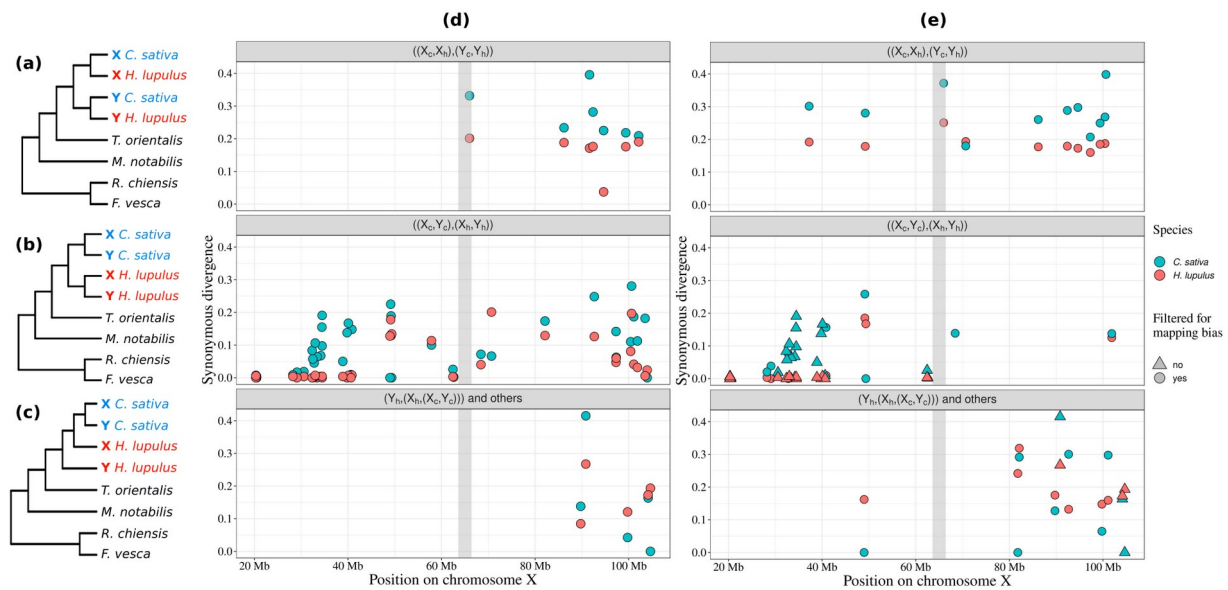
417

418

419

420 Table 2. Results of the phylogenetic reconstruction of sex-linked genes. Phylogenetic trees with a bootstrap value
 421 equal or greater than 95% (and posterior probabilities higher than 0.95 for Bayesian reconstructions) at the node
 422 separating *C. sativa* and *H. lupulus*, or Y and X sequences, are presented in the first four columns. Phylogenetic
 423 trees without such support are classified as “unresolved”.

	Topology I ((X _c ,X _h),(Y _c ,Y _h))	Topology II ((X _c ,Y _c),(X _h ,Y _h))	Topology III (Y _h ,(X _h ,X _c ,Y _c))	Other	Un- resolved	Total
Maximum Likelihood (ML)	7	44	7	1	26	85
GTR (bayesian)	4	45	4	8	24	85
CAT-GTR (bayesian)	4	44	7	7	23	85
ML after geneconv filtering	11	27	11	4	32	85



425 Figure 3. Distribution of the three topologies of the sex-linked genes on the X chromosome: (a) Topology I, XX-
 426 YY – arrest of recombination before the split of the two genera, (b) Topology II, XY-XY – arrest of
 427 recombination after the split of the two genera, (c) Topology III, Y-X-XY – *H. lupulus* X chromosome is closer to
 428 *C. sativa* sequences than its Y counterpart. (d) Distribution of the topologies along the *C. sativa* X chromosome
 429 (“other” topology is included in the Y-X-XY topology panel), using the full gene sequences. For each gene, dots
 430 represent the *dS* values in *C. sativa* (blue) and *H. lupulus* (red). (e) Distribution of the topologies after filtering out

431 possible mapping biases through geneconv. Triangles indicate that at least one segment was removed, dots
432 indicate sequences for which no mapping bias was detected.

433 The vertical grey bar (panels **(d)** and **(e)**) represents the putative boundary between the region that stopped
434 recombining in a common ancestor and the region that stopped recombining independently in the two species.

435

436 This leads us to define three regions on the X chromosomes of *C. sativa* and *H. lupulus* (with the *C.*
437 *sativa* X chromosome as a reference). A region from ~65Mb to the end of the X chromosome that
438 already stopped recombining in a common ancestor; from ~20-30Mb to ~65Mb, a part of the non-
439 recombining region that evolved independently in the two species; and from the beginning of the
440 chromosome to ~20-30Mb, the pseudo-autosomal region (PAR), where few sex-linked genes are
441 found.

442

443 **Age of *H. lupulus* sex chromosomes**

444 To estimate the age of the sex chromosomes, we used the maximum synonymous divergence
445 between X and Y sequences and two molecular clocks, which were both derived from *Arabidopsis*.
446 Because the sampling variance in *dS* values can be large, we used three ways to calculate the
447 maximum *dS* value: the single highest *dS* value; the average of the 5% highest values; and the
448 average of the 10% highest values. Furthermore, we calculated these on the raw alignments as well
449 as the alignments with possible mapping biases removed. The different estimates are given in Table
450 3, and yield values between 14.5 and 51.4 My. Minimum synonymous divergence between *C.*
451 *sativa* and outgroup species *Morus notabilis* and *Rosa chinensis* is ~0.45 and ~0.65, respectively
452 (Supporting Information Fig. S5 and Fig. S6), higher than the maximum synonymous divergence
453 between sex-linked gene copies, indicating that the sex chromosomes probably evolved in the
454 Cannabaceae family.

455

456

457

458

459

460

461

462

463

464 Table 3. Age estimates (in millions of years, My) with two molecular clocks and different maximum dS values.
 465 For each dS value, two ages were obtained using the molecular clocks of ¹Ossowski *et al.* (2010) and ²Koch *et al.*
 466 (2000). Two alignment datasets were used, with or without filtering for possible mapping bias.

	No filtering			Mapping bias filtering		
	dS	age (My) ¹	age (My) ²	dS	age (My) ¹	age (My) ²
Highest dS	0.362	51.4	24.0	0.362	51.4	24.0
Mean highest 5%	0.249	35.6	16.6	0.274	39.1	18.3
Mean highest 10%	0.217	31.0	14.5	0.214	34.4	16.1

467

468 **Y gene expression**

469 The Y over X expression ratio is a standard proxy for the degeneration of the Y chromosome. A Y/
 470 X expression ratio close to 1 means no degeneration, a Y/X expression ratio close to 0.5 or below
 471 means strong degeneration. In *H. lupulus*, the median Y/X expression ratio is equal to 0.637
 472 (Supporting Information Fig. S1), which is significantly different from 1 (99th percentile of median
 473 distribution with 5,000 samples in initial distribution = 0.673, see methods). The median is not
 474 different when considering all sex-linked genes (0.637) or only the sex-linked genes mapping on *C.*
 475 *sativa* X chromosome (0.639, p-value = 0.70, one-sided Wilcoxon rank sum test).

476 In both species, the reduced Y expression is correlated to the position on the X chromosome (linear
 477 regression: adjusted $R^2 = 0.134$, p-value < 10^{-5} for *H. lupulus*; and adjusted $R^2 = 0.278$, p-value <
 478 10^{-5} for *C. sativa*). As shown in Figure 4, the Y/X expression ratio decreases while moving away
 479 from the PAR in *H. lupulus*, and this is also confirmed in *C. sativa*.

480

481

482

483

484

485

486

487

488

489

490

491

492

493

494

495

496

497

498

499

500

501

502

503

504

505

506

507

508

509

510

511

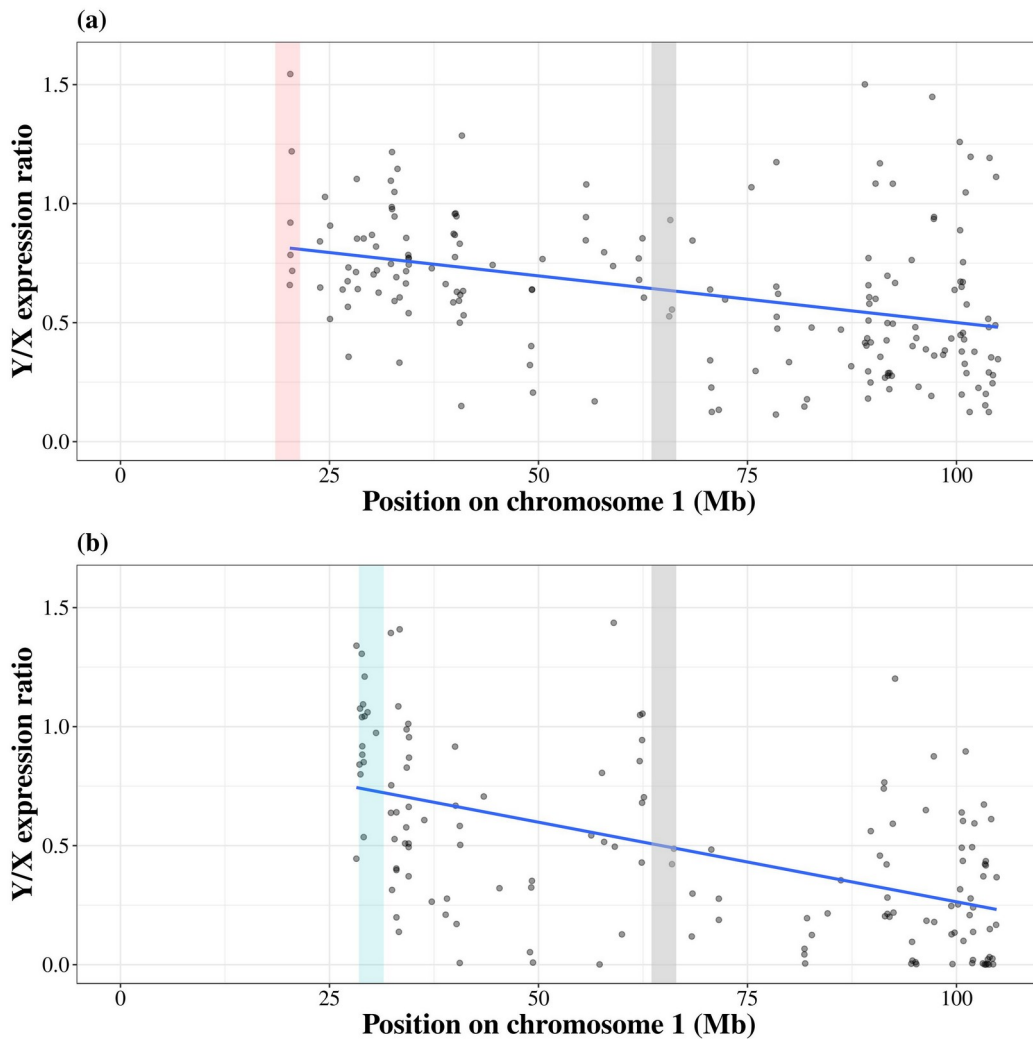
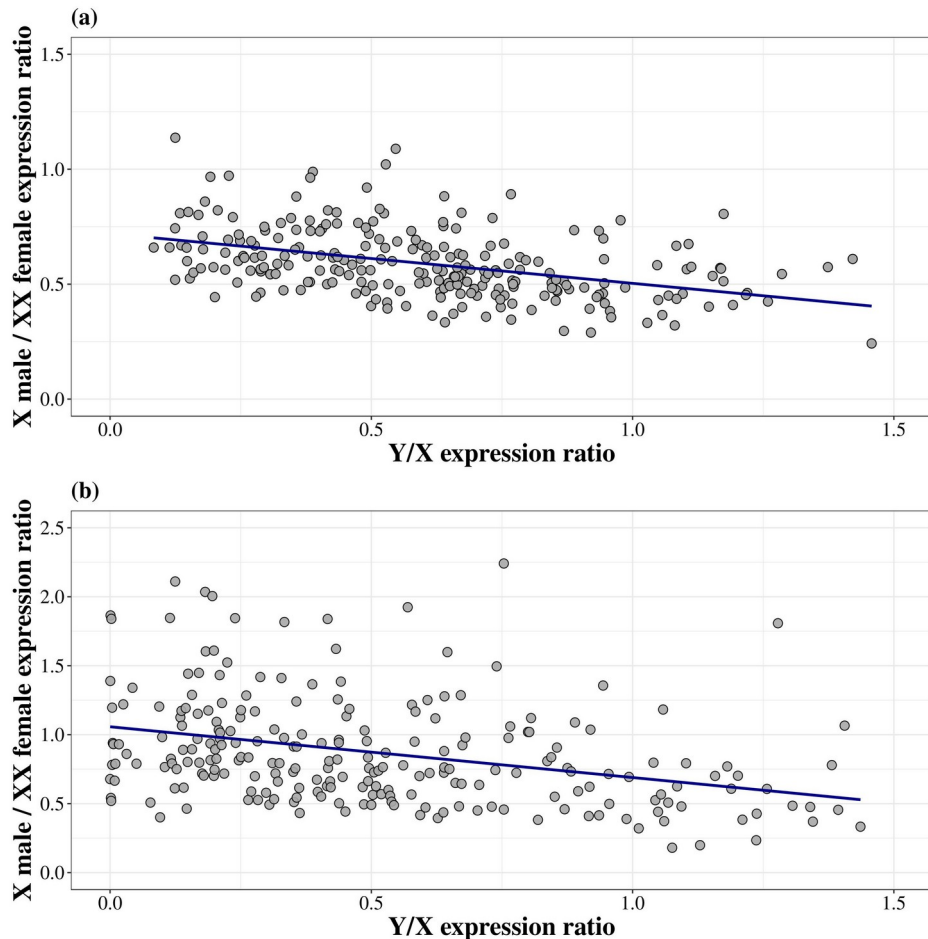


Figure 4. Y/X expression ratio along the X chromosome in *H. lupulus* (a), and *C. sativa* (b). Each dot is the Y/X expression ratio for one gene in the non-recombining region only (the linear regression result is indicated by the blue line). The vertical red bar represents the putative PAB in *H. lupulus*, the vertical blue bar represents the putative PAB in *C. sativa*, the vertical grey bar represents the putative boundary between the region that stopped recombining in a common ancestor and the region that stopped recombining independently in the two species.

Dosage compensation

We tested whether the expression of the X chromosome changed following degeneration of the Y chromosome, a phenomenon called dosage compensation (Muyle *et al.*, 2017). We used the ratio of the male X expression over the female XX expression as a proxy for dosage compensation (Muyle *et al.*, 2012) and Y/X expression ratio as a proxy for Y degeneration. Genes with strong degeneration (Y/X expression ratio close to zero) display an increased expression of the X in males

512 (linear regression: adjusted $R^2=0.179$, p-value $< 10^{-5}$ and adjusted $R^2=0.097$, p-value $< 10^{-5}$ for *H.*
 513 *lupulus* and *C. sativa* respectively), as shown in Figure 5. A dosage compensation pattern was
 514 found in both in *H. lupulus* and *C. sativa* in agreement with previous work (Prentout *et al.*, 2020).



528 Figure 5. The male X expression over female XX expression versus Y/X expression ratio for *H. lupulus* (a) and *C.*
 529 *sativa* (b). Each black dot represents one gene. The blue line represents a linear regression.

530

531

532

533

534

535

536

537 **Discussion**

538 We here identified the *H. lupulus* sex chromosomes, and found that they are homologous to those of
539 *C. sativa* (Prentout *et al.*, 2020), and that a part of these chromosomes had already stopped
540 recombining in a common ancestor of the two species. Performing a segregation analysis with SEX-
541 DETector (Muyle *et al.*, 2016), we identified 265 XY genes in *H. lupulus*, among which 112 are
542 also sex-linked in *C. sativa*. Mapping these genes on the chromosome-level assembly of *C. sativa*
543 (Grassa *et al.*, 2018) suggested that the non-recombining region is large in *H. lupulus*, as proposed
544 before, based on cytological studies (Divashuk *et al.*, 2011).

545 We identified three different regions on the sex chromosome, based on the distribution of sex-
546 linked gene phylogenetic topologies and synonymous divergence between the X and Y copies on
547 the *C. sativa* X chromosome: one region that had already stopped recombining in a common
548 ancestor of *C. sativa* and *H. lupulus*, a region that independently stopped recombining in both
549 species, and the pseudo-autosomal region. Our results suggest the pseudo-autosomal boundary
550 (PAB) in *H. lupulus* may be located around position 20Mb, whereas we estimated a PAB around
551 30Mb in *C. sativa* (Prentout *et al.*, 2020); the non-recombining region may thus be larger in *H.*
552 *lupulus* than in *C. sativa*. With this estimation of the size of the non-recombining region in *H.*
553 *lupulus*, among the 3469 genes present on the X chromosome, 2045 genes would be located in this
554 non-recombining region (which represents 59.1% of all the genes on the X chromosome). However,
555 a chromosome-level assembly of the *H. lupulus* genome would be needed to determine the exact
556 position of the PAB in this species, as synteny might not be fully conserved. In addition, because
557 we used one single cross, it is possible that we overestimated the size of the non-recombining
558 region due to linkage disequilibrium. Thus, genes around the PAB classified as sex-linked and for
559 which we estimated a low *dS* value may still be recombining. An accurate estimation of the PAB, as
560 has been done for example in *S. latifolia*, would require much more offspring and data from several
561 populations (Krasovec *et al.*, 2020).

562 Several sex-linked genes had topologies that were not compatible with either recombination
563 suppression in a common ancestor or in each of the species independently. Strikingly, most of these
564 topologies placed the *H. lupulus* Y sequence as an outgroup to the other sex-linked gene sequences.
565 Whether this is the result of errors (*e.g.* long branch attraction, mapping biases) remains to be
566 investigated. Interestingly, genes with these “unexpected” topologies all clustered (except for one
567 gene) in a region of ~25Mb. This region is located at the extremity of the X chromosome, which, as

568 we suggested, stopped recombining first. It is likely to observe a high rate of unexpected
569 phylogenetic results in the region that stopped recombining first since the X-Y divergence should be
570 the highest in this region, which could increase the mapping bias. Our approach to correct for the Y
571 read mapping relies on geneconv, which is known to have a high rate of false negatives (Lawson &
572 Zhang, 2009). This could also explain the unexpected presence of some of the XY-XY genes in the
573 older region.

574 X-Y gene conversion has been shown to affect only a few genes in animals (Katsura *et al.*, 2012;
575 Trombetta *et al.*, 2014; Peneder *et al.*, 2017). Although we don't expect gene conversion for half of
576 the genes that are sex-linked in both species, it is worth noting that a part of fragments identified by
577 geneconv may correspond to real gene conversion rather than mapping biases. Here again,
578 assemblies of Y and X chromosomes in both species are required to determine the presence of true
579 X-Y gene conversion.

580 The highest dS values and the genes with a topology indicating that recombination was already
581 suppressed in the common ancestor are located in the same region (65Mb to the end of the
582 chromosome). These results suggest the presence of at least two strata in these sex chromosomes.
583 We estimated that the youngest stratum in *H. lupulus* is 10.1-29.4 Myo, and is 15.9-19.8 Myo in *C.*
584 *sativa* (Supporting Information Table S4). However, while recombination suppression clearly did
585 not occur for all the sex-linked genes at the same time, we cannot determine the exact number of
586 strata in the sex chromosomes of *C. sativa* and *H. lupulus*. It is also possible that recombination was
587 suppressed gradually, with the recombination suppression starting before the split of both genera
588 and continuing afterwards. To clearly determine the number of strata, an identification of
589 chromosomal inversions or significant differences in dS values along the sex chromosomes are
590 required (Nicolas *et al.*, 2004; Lemaitre *et al.*, 2009; Wang *et al.*, 2012; reviewed in Wright *et al.*,
591 2016). Thus, X and Y chromosome assemblies for both *H. lupulus* and *C. sativa* are needed to
592 exactly determine the number (and location) of strata in both species. Moreover, a Y chromosome
593 assembly will allow the identification of Y-specific genes, which is not possible with SEX-
594 DETector and the data we used.

595 We did not find X-hemizygous genes in *H. lupulus*. This is striking as 218 X-hemizygous genes
596 (38% of all sex-linked genes) were found in *C. sativa* using the same methodology (Prentout *et al.*,
597 2020). A very low level of polymorphism could result in the inability of SEX-DETECTOR to identify
598 X-hemizygous genes (Muyle *et al.*, 2016), but in that case SEX-DETECTOR should also have
599 problems identifying autosomal genes, which was not the case here. Non-random X-inactivation in

600 females could be an explanation, as the non-random expression of a single X allele in females
601 would impede SEX-DETECTOR to identify X-linkage and X-hemizygous genes (Muyle *et al.*, 2016).
602 We ran an Allele-Specific Expression (ASE) analysis, which doesn't support this hypothesis
603 (Supporting Information Fig. S2, Fig. S3, Fig. S4). *H. lupulus* is probably an ancient polyploid that
604 reverted to the ancestral karyotype (Padgitt-Cobb *et al.*, 2019). It is thus possible, that the *H.*
605 *lupulus* X chromosome is made of two copies of the ancestral X as some cytological data seem to
606 suggest (Divashuk *et al.*, 2011). In this case, SEX-DETECTOR would manage to identify the XY gene
607 pairs, but would fail to identify the X-hemizygous genes as these genes would exhibit unexpected
608 allele transmission patterns (Supporting Information Fig. S10).

609 *H. lupulus* is a rare case of XY systems in plants in which the Y is smaller than the X (cf Ming *et al.*
610 *et al.*, 2011). In *C. sativa*, both sex chromosomes have similar sizes (Divashuk *et al.*, 2014). If the size
611 difference is caused by deletions of parts of the *H. lupulus* Y chromosome, which is the
612 hypothesized mechanism in many species (cf Ming *et al.*, 2011), we expect to observe that many
613 XY gene pairs in *C. sativa* have missing Y copies in *H. lupulus*. As explained above, we did not
614 detect any X-hemizygous genes. Furthermore, the XY gene pairs of *H. lupulus* are distributed
615 uniformly on the *C. sativa* X chromosome, and no region appeared to be depleted in XY genes,
616 which is not what we would observe if large deletions were present on the *H. lupulus* Y
617 chromosome. The sex chromosome size differences observed in *H. lupulus* probably reflect
618 complex dynamics, different from that of old animal systems with tiny Y chromosome due to large
619 deletions (*e.g.* Skaletsky *et al.*, 2003; Ross *et al.*, 2005). The large size of the X chromosome in *H.*
620 *lupulus* may be due to a full-chromosome duplication followed by a fusion (see above), whereas the
621 Y chromosome has remained unchanged. Assemblies of the *H. lupulus* sex chromosomes will be
622 needed to test these hypotheses.

623 Our estimates of the age of the *H. lupulus* sex chromosomes are larger than the estimates for *C.*
624 *sativa*, although we found very similar X-Y maximum divergence in both species (higher bound age
625 estimates are ~50My and ~28My; highest *dS* values are 0.362 and 0.415 in *H. lupulus* and *C. sativa*
626 respectively, see Prentout *et al.*, 2020). Of course, the molecular clocks that we used are known to
627 provide very rough estimates as they derive from the relatively distant *Arabidopsis* genus and are
628 sensitive to potential differences in mutation rates between the annual *C. sativa* and the perennial *H.*
629 *lupulus* (Neve, 1991; Petit & Hampe, 2006; Small, 2015; but see Krasovec *et al.*, 2018). Indeed,
630 only one of these molecular clocks (which is based on the mutation rate) takes into account the
631 generation time, (two years in *H. lupulus* vs. one year in *C. sativa*). This produced age estimates

632 approximately twice as high as the other clock (based on the substitution rate), which was not the
633 case with *C. sativa* (Prentout *et al.*, 2020). It is not known, however, if the generation time
634 influences the substitution rate (Petit and Hampe, 2006). Furthermore, the short generation time in
635 *C. sativa* is probably a derived trait, not reflecting the long-term generation time of the *Cannabis*-
636 *Humulus* lineage, as the *Cannabis* genus is the only herbaceous genus in the Cannabaceae family
637 (Yang *et al.*, 2013). Thus, the remarkable similarity between the highest dS values in both species
638 indicates that the *C. sativa* and *H. lupulus* sex chromosomes have a similar age, as expected if they
639 derive from the same common ancestor. Although it's not possible to estimate their age exactly with
640 the current data, initial recombination suppression at least predates the split between the genera, that
641 occurred between 21 and 25 My ago (Divashuk *et al.*, 2014; Jin *et al.*, 2020), and might even be 50
642 My old. We thus confirmed here that the XY system shared by *C. sativa* and *H. lupulus* is among
643 the oldest plant sex chromosome systems documented so far (Prentout *et al.*, 2020).

644 Dioecy was inferred as the ancestral sexual system for the Cannabaceae, Urticaceae and Moraceae
645 (Zhang *et al.*, 2018; note however that many monoecious Cannabaceae were not included). We
646 found that the synonymous divergence between the Cannabaceae species and *Morus notabilis* was
647 about 0.45, higher than the maximum divergence of the X and Y copies in the Cannabaceae. It
648 remains possible that the sex chromosomes evolved before the split of the Cannabaceae and
649 Moraceae families, because the oldest genes might have been lost or were not detected in our
650 transcriptome data. There is however no report of whether or not sex chromosomes exist in
651 Urticaceae and Moraceae (Ming *et al.*, 2011).

652 To estimate the Y expression, we counted the number of reads with Y SNPs. Therefore, the impact
653 of a potential Y reads mapping bias should be weaker on Y expression analysis than on X-Y
654 divergence analysis. We validated this assumption by removing genes with detected mapping bias
655 from the analysis, which didn't change the signal of Y expression reduction and dosage
656 compensation (Supporting Information Fig. S8, Fig. S9). Dosage compensation is a well-known
657 phenomenon in animals (*e.g.* Gu & Walters, 2017). It has only been documented quite recently in
658 plants (reviewed in Muyle *et al.*, 2017). Here we found evidence for dosage compensation in *H.*
659 *lupulus*. This is not surprising as previous work reported dosage compensation in *C. sativa* and we
660 showed here that both systems are homologous. *C. sativa* and *H. lupulus* add up to the list of plant
661 sex chromosome systems with dosage compensation (see Muyle *et al.*, 2017 for a review and
662 Prentout *et al.*, 2020; Fruchard *et al.*, 2020 for the latest reports of dosage compensation in plants).

663 Further analyses are needed to determine whether this dosage compensation has been selected or is
664 an outcome of regulatory feedback (Malone *et al.*, 2012; Krasovec *et al.*, 2019).

665 *H. lupulus* sex chromosomes, as those of *C. sativa*, are well-differentiated, with a large non-
666 recombining region. Both species show similar patterns of Y degeneration and dosage
667 compensation, despite the fact that a large part of the non-recombining region evolved
668 independently in both species. These similarities, as well as the age of the chromosomes and the
669 fact that they have been conserved since the most recent common ancestor of the two genera, a
670 unique situation in plants so far, provide an exciting opportunity to test and elaborate hypotheses on
671 sex chromosome evolution in plants.

672

673 **Acknowledgments**

674 We thank Roberto Bacilieri for his help in setting up this collaboration and for discussions, Aline
675 Muyle for advice on SEX-DETECTOR and Florian Bénétière for helpful suggestions regarding
676 graphical representations. This work was performed using the computing facilities of the CC LBBE/
677 PRABI; we thank Bruno Spataro and Stéphane Delmotte for cluster maintenance. Virtual machines
678 from the Institut Français de Bioinformatique were also used to perform this work. This work
679 received financial support from P4-0077 grant by ARRS (Slovenian Research Agency) to JJ and
680 from an ANR grant (ANR-14-CE19-0021-01) to GABM.

681

682

683 **Author Contribution**

684 Conceptualization of the study: G.A.B.M., J.K. and D.P.; methodology: G.A.B.M., J.K., D.P., N.S.
685 and J.J.; software: D.P., T.T. and C.B.A.; formal analysis: D.P., T.T. and C.B.A.; investigation:
686 D.P., N.S., T.T., C.B.A., J.J., J.K., and G.A.B.M.; resources: A.C., N.S. and J.J.; writing—original
687 draft: D.P., G.A.B.M., J.K. and T.T.; writing—review and editing: all authors; visualization: D.P.
688 and T.T.; supervision: G.A.B.M., J.K.; project administration: G.A.B.M.; funding acquisition: N.S.,
689 J.J. and G.A.B.M.

690

691 **Data Availability**

692 The sequence data were deposited under the Bioproject with accession number PRJNA694508,
693 BioSample SAMN17526021 (SRR13528971 ; SRR13528970; SRR13528969; SRR13528968;
694 SRR13528966; SRR13528965; SRR13528964; SRR13528967; SRR13528963; SRR13528962;
695 SRR13528961; SRR13528960; SRR13528959; SRR13528958)

696

697 **References**

698 • **Regular research articles:**

699 **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990.** Basic local alignment search
700 tool. *Journal of Molecular Biology* **215**: 403–410.

701

- 702 **Bačovský V, Čegan R, Šimoníková D, Hřibová E, Hobza R. 2020.** The Formation of Sex
703 Chromosomes in *Silene latifolia* and *S. dioica* Was Accompanied by Multiple Chromosomal
704 Rearrangements. *Frontiers in Plant Science* 11.
705
- 706 **Badouin H, Velt A, Gindraud F, Flutre T, Dumas V, Vautrin S, Marande W, Corbi J, Sallet
707 E, Ganofsky J, et al. 2020.** The wild grape genome sequence provides insights into the transition
708 from dioecy to hermaphroditism during grape domestication. *Genome Biology* 21: 223.
709
- 710 **van Bakel H, Stout JM, Cote AG, Tallon CM, Sharpe AG, Hughes TR, Page JE. 2011.** The
711 draft genome and transcriptome of *Cannabis sativa*. *Genome Biology* 12: R102.
712
- 713 **Baránková S, Pascual-Díaz JP, Sultana N, Alonso-Lifante MP, Balant M, Barros K,
714 D’Ambrosio U, Malinská H, Peska V, Lorenzo IP, et al. 2020.** Sex-chrom, a database on plant
715 sex chromosomes. *New Phytologist* 227: 1594–1604.
716
- 717 **Bergero R, Charlesworth D. 2009.** The evolution of restricted recombination in sex chromosomes.
718 *Trends in Ecology & Evolution* 24: 94–102.
719
- 720 **Castresana J. 2000.** Selection of Conserved Blocks from Multiple Alignments for Their Use in
721 Phylogenetic Analysis. *Molecular Biology and Evolution* 17: 540–552.
722
- 723 **Čerenak A, Kolenc Z, Sehur P, Whittock SP, Koutoulis A, Beatson R, Buck E, Javornik B,
724 Škof S, Jakše J. 2019.** New Male Specific Markers for Hop and Application in Breeding Program.
725 *Scientific Reports* 9: 14223.
726
- 727 **Charlesworth B, & Charlesworth D. 2000.** The degeneration of Y chromosomes. *Philosophical
728 Transactions of the Royal Society of London. Series B: Biological Sciences* 355: 1563–1572.
729
- 730 **Charlesworth D. 2016.** Plant Sex Chromosomes. *Annual Review of Plant Biology* 67: 397–420.
731
- 732 **Cherif E, Zehdi-Azouzi S, Crabos A, Castillo K, Chabrillange N, Pintaud J-C, Salhi-
733 Hannachi A, Glémin S, Aberlenc-Bertossi F. 2016.** Evolution of sex chromosomes prior to
734 speciation in the dioecious *Phoenix* species. *Journal of Evolutionary Biology* 29: 1513–1522.
735

- 736 **Cortez D, Marin R, Toledo-Flores D, Froidevaux L, Liechti A, Waters PD, Grützner F,**
737 **Kaessmann H. 2014.** Origins and functional evolution of Y chromosomes across mammals. *Nature*
738 **508:** 488–493.
739
- 740 **Divashuk MG, Alexandrov OS, Kroupin PY, Karlov GI. 2011.** Molecular Cytogenetic Mapping
741 of *Humulus lupulus* Sex Chromosomes. *Cytogenetic and Genome Research* **134:** 213–219.
742
- 743 **Divashuk MG, Alexandrov OS, Razumova OV, Kirov IV, Karlov GI. 2014.** Molecular
744 Cytogenetic Characterization of the Dioecious *Cannabis sativa* with an XY Chromosome Sex
745 Determination System. *PLOS ONE* **9:** e85118.
746
- 747 **Dixon G, Kitano J, Kirkpatrick M. 2019.** The Origin of a New Sex Chromosome by Introgression
748 between Two Stickleback Fishes. *Molecular Biology and Evolution* **36:** 28–38.
749
- 750 **Fridolfsson A-K, Cheng H, Copeland NG, Jenkins NA, Liu H-C, Raudsepp T, Woodage T,**
751 **Chowdhary B, Halverson J, Ellegren H. 1998.** Evolution of the avian sex chromosomes from an
752 ancestral pair of autosomes. *Proceedings of the National Academy of Sciences* **95:** 8147–8152.
753
- 754 **Fruchard C, Badouin H, Latrasse D, Devani RS, Muyle A, Rhoné B, Renner SS, Banerjee AK,**
755 **Bendahmane A, Marais GAB. 2020.** Evidence for Dosage Compensation in *Coccinia grandis*, a
756 Plant with a Highly Heteromorphic XY System. *Genes* **11:** 787.
757
- 758 **Gayral P, Melo-Ferreira J, Glémin S, Bierne N, Carneiro M, Nabholz B, Lourenco JM, Alves**
759 **PC, Ballenghien M, Faivre N, et al. 2013.** Reference-Free Population Genomics from Next-
760 Generation Transcriptome Data and the Vertebrate–Invertebrate Gap. *PLOS Genetics* **9:** e1003457.
761
- 762 **Gu L, Walters JR. 2017.** Evolution of Sex Chromosome Dosage Compensation in Animals: A
763 Beautiful Theory, Undermined by Facts and Bedeviled by Details (K Makova, Ed.). *Genome*
764 *Biology and Evolution* **9:** 2461–2476.
765
- 766 **Gu, Z., Gu, L., Eils, R., Schlesner, M., & Brors, B. (2014).** circlize implements and enhances
767 circular visualization in R. *Bioinformatics*, **30(19):** 2811-2812.
768

- 769 **Harkess A, Zhou J, Xu C, Bowers JE, Van der Hulst R, Ayyampalayam S, Mercati F,**
770 **Riccardi P, McKain MR, Kakrana A, et al. 2017.** The asparagus genome sheds light on the origin
771 and evolution of a young Y chromosome. *Nature Communications* **8**: 1279.
772
- 773 **He N, Zhang C, Qi X, Zhao S, Tao Y, Yang G, Lee T-H, Wang X, Cai Q, Li D, et al. 2013.**
774 Draft genome sequence of the mulberry tree *Morus notabilis*. *Nature Communications* **4**: 2445.
775
- 776 **Jakse J, Cerenak A, Radisek S, Satovic Z, Luthar Z, Javornik B. 2013.** Identification of
777 quantitative trait loci for resistance to *Verticillium* wilt and yield parameters in hop (*Humulus*
778 *lupulus* L.). *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik* **126**:
779 1431–1443.
780
- 781 **Jin J-J, Yang M-Q, Fritsch PW, Velzen R van, Li D-Z, Yi T-S. 2020.** Born migrators: Historical
782 biogeography of the cosmopolitan family Cannabaceae. *Journal of Systematics and Evolution* **58**:
783 461–473.
784
- 785 **Käfer J, Marais GAB, Pannell JR. 2017.** On the rarity of dioecy in flowering plants. *Molecular*
786 *Ecology* **26**: 1225–1241.
787
- 788 **Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. 2017.** ModelFinder:
789 fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**: 587–589.
790
- 791 **Karlov GI, Danilova TV, Horlemann C, Weber G. 2003.** Molecular cytogenetics in hop
792 (*Humulus lupulus* L.) and identification of sex chromosomes by DAPI-banding. *Euphytica* **132**:
793 185–190.
794
- 795 **Katsura Y, Iwase M, Satta Y. 2012.** Evolution of Genomic Structures on Mammalian Sex
796 Chromosomes. *Current Genomics* **13**: 115–123.
797
- 798 **Kejnovsky E, Vyskot B. 2010.** *Silene latifolia*: The Classical Model to Study Heteromorphic Sex
799 Chromosomes. *Cytogenetic and Genome Research* **129**: 250–262.
800
- 801 **Koch MA, Haubold B, Mitchell-Olds T. 2000.** Comparative Evolutionary Analysis of Chalcone
802 Synthase and Alcohol Dehydrogenase Loci in *Arabidopsis*, *Arabis*, and related genera
803 (Brassicaceae). *Molecular Biology and Evolution* **17**: 1483–1498.

804

805 **Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. 2019.** RAxML-NG: a fast, scalable
806 and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**: 4453–
807 4455.

808

809 **Krasovec M, Chester M, Ridout K, Filatov DA. 2018.** The Mutation Rate and the Age of the Sex
810 Chromosomes in *Silene latifolia*. *Current Biology* **28**: 1832-1838.e4.

811

812 **Krasovec M, Kazama Y, Ishii K, Abe T, Filatov DA. 2019.** Immediate Dosage Compensation Is
813 Triggered by the Deletion of Y-Linked Genes in *Silene latifolia*. *Current Biology* **29**: 2214-2221.e4.

814

815 **Krasovec M, Zhang Y, Filatov DA. 2020.** The Location of the Pseudoautosomal Boundary in
816 *Silene latifolia*. *Genes* **11**: 610.

817

818 **Lartillot N, Lepage T, Blanquart S. 2009.** PhyloBayes 3: a Bayesian software package for
819 phylogenetic reconstruction and molecular dating. *Bioinformatics* **25**: 2286–2288.

820

821 **Lawson MJ, Zhang L. 2009.** Sexy gene conversions: locating gene conversions on the X-
822 chromosome. *Nucleic Acids Research* **37**: 4570–4579.

823

824 **Lemaitre C, Braga MDV, Gautier C, Sagot M-F, Tannier E, Marais GAB. 2009.** Footprints of
825 Inversions at Present and Past Pseudoautosomal Boundaries in Human Sex Chromosomes. *Genome*
826 *Biology and Evolution* **1**: 56–66.

827

828 **Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin**
829 **R, 1000 Genome Project Data Processing Subgroup. 2009.** The Sequence Alignment/Map
830 format and SAMtools. *Bioinformatics* **25**: 2078–2079.

831

832 **Mackinnon D, Pavlovič M. 2019.** Global Hop Market Analysis Within the International Hop
833 Growers' Convention. *GLOBALNA ANALIZA HMELJSKEGA TRGA V OKVIRU SVETOVNE*
834 *HMELJARSKE ORGANIZACIJE.*: 99–108.

835

836 **Malone JH, Cho D-Y, Mattiuzzo NR, Artieri CG, Jiang L, Dale RK, Smith HE, McDaniel J,**
837 **Munro S, Salit M, et al. 2012.** Mediation of *Drosophila* autosomal dosage effects and
838 compensation by network interactions. *Genome Biology* **13**: R28.

839

840 **Massonnet M, Cochetel N, Minio A, Vondras AM, Lin J, Muyle A, Garcia JF, Zhou Y,**
841 **Delledonne M, Riaz S, et al. 2020.** The genetic basis of sex determination in grapes. *Nature*
842 *Communications* **11**: 2902.

843

844 **Ming R, Bendahmane A, Renner SS. 2011.** Sex Chromosomes in Land Plants. *Annual Review of*
845 *Plant Biology* **62**: 485–514.

846

847 **Muyle A, Zemp N, Deschamps C, Mousset S, Widmer A, Marais GAB. 2012.** Rapid De Novo
848 Evolution of X Chromosome Dosage Compensation in *Silene latifolia*, a Plant with Young Sex
849 Chromosomes. *PLOS Biology* **10**: e1001308.

850

851 **Muyle A, Käfer J, Zemp N, Mousset S, Picard F, Marais GA. 2016.** SEX-DETECTOR: A
852 Probabilistic Approach to Study Sex Chromosomes in Non-Model Organisms. *Genome Biology and*
853 *Evolution* **8**: 2530–2543.

854

855 **Muyle A, Shearn R, Marais GA. 2017.** The Evolution of Sex Chromosomes and Dosage
856 Compensation in Plants. *Genome Biology and Evolution* **9**: 627–645.

857

858 **Natri HM, Shikano T, Merilä J. 2013.** Progressive Recombination Suppression and
859 Differentiation in Recently Evolved Neo-sex Chromosomes. *Molecular Biology and Evolution* **30**:
860 1131–1144.

861

862 **Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015.** IQ-TREE: A Fast and Effective
863 Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. *Molecular Biology and*
864 *Evolution* **32**: 268–274.

865

866 **Nicolas M, Marais G, Hykelova V, Janousek B, Laporte V, Vyskot B, Mouchiroud D,**
867 **Negrutiu I, Charlesworth D, Monéger F. 2004.** A Gradual Process of Recombination Restriction
868 in the Evolutionary History of the Sex Chromosomes in Dioecious Plants. *PLOS Biology* **3**: e4.

869

870 **Ohno S. 1969.** Evolution of Sex Chromosomes in Mammals. *Annual Review of Genetics* **3**: 495–
871 524.

872

- 873 **Okada Y, Ito K. 2001.** Cloning and Analysis of Valerophenone Synthase Gene Expressed
874 Specifically in Lupulin Gland of Hop (*Humulus lupulus L.*). *Bioscience, Biotechnology, and*
875 *Biochemistry* **65**: 150–155.
876
- 877 **Ossowski S, Schneeberger K, Lucas-Lledó JI, Warthmann N, Clark RM, Shaw RG, Weigel D,**
878 **Lynch M. 2010.** The Rate and Molecular Spectrum of Spontaneous Mutations in *Arabidopsis*
879 *thaliana*. *Science* **327**: 92–94.
880
- 881 **Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. 2010.** How
882 Many Bootstrap Replicates Are Necessary? *Journal of Computational Biology* **17**: 337–354.
883
- 884 **Patzak J, Nesvadba V (Chmelarsky I, Vejl P, Skupinova S. 2002.** Identification of sex in F1
885 progenies of hop (*Humulus lupulus*) by molecular marker. *Rostlinna Vyroba - UZPI (Czech*
886 *Republic)*.
887
- 888 **Peil A, Flachowsky H, Schumann E, Weber WE. 2003.** Sex-linked AFLP markers indicate a
889 pseudoautosomal region in hemp (*Cannabis sativa L.*). *TAG. Theoretical and applied genetics.*
890 *Theoretische und angewandte Genetik* **107**: 102–109.
891
- 892 **Peneder P, Wallner B, Vogl C. 2017.** Exchange of genetic information between therian X and Y
893 chromosome gametologs in old evolutionary strata. *Ecology and Evolution* **7**: 8478–8487.
894
- 895 **Petit RJ, Hampe A. 2006.** Some Evolutionary Consequences of Being a Tree. *Annual Review of*
896 *Ecology, Evolution, and Systematics* **37**: 187–214.
897
- 898 **Polley A, Ganal MW, Seigner E. 2011.** Identification of sex in hop (*Humulus lupulus*) using
899 molecular markers. *Genome*.
900
- 901 **Prentout D, Razumova O, Rhoné B, Badouin H, Henri H, Feng C, Käfer J, Karlov G, Marais**
902 **GAB. 2020.** An efficient RNA-seq-based segregation analysis identifies the sex chromosomes of
903 *Cannabis sativa*. *Genome Research* **30**: 164–172.
904
- 905 **Pucholt P, Wright AE, Conze LL, Mank JE, Berlin S. 2017.** Recent Sex Chromosome
906 Divergence despite Ancient Dioecy in the Willow *Salix viminalis*. *Molecular Biology and Evolution*
907 **34**: 1991–2001.

908

909 **Quinlan AR, Hall IM. 2010.** BEDTools: a flexible suite of utilities for comparing genomic
910 features. *Bioinformatics* **26**: 841–842.

911

912 **Ranwez V, Harispe S, Delsuc F, Douzery EJP. 2011.** MACSE: Multiple Alignment of Coding
913 SEquences Accounting for Frameshifts and Stop Codons. *PLOS ONE* **6**: e22594.

914

915 **Raymond O, Gouzy J, Just J, Badouin H, Verdenaud M, Lemainque A, Vergne P, Moja S,**
916 **Choisne N, Pont C, et al. 2018.** The Rosa genome provides new insights into the domestication of
917 modern roses. *Nature Genetics* **50**: 772–777.

918

919 **Renner SS. 2014.** The relative and absolute frequencies of angiosperm sexual systems: Dioecy,
920 monoecy, gynodioecy, and an updated online database. *American Journal of Botany* **101**: 1588–
921 1596.

922

923 **Renner SS, Müller NA. 2021.** Plant sex chromosomes defy evolutionary models of expanding
924 recombination suppression and genetic degeneration. *Nature Plants*: 1–11.

925

926 **Ross MT, Grafham DV, Coffey AJ, Scherer S, McLay K, Muzny D, Platzer M, Howell GR,**
927 **Burrows C, Bird CP, et al. 2005.** The DNA sequence of the human X chromosome. *Nature* **434**:
928 325–337.

929

930 **Shephard HL, Parker JS, Darby P, Ainsworth CC. 2000.** Sexual development and sex
931 chromosomes in hop. *New Phytologist* **148**: 397–411.

932

933 **Shulaev V, Sargent DJ, Crowhurst RN, Mockler TC, Folkerts O, Delcher AL, Jaiswal P,**
934 **Mockaitis K, Liston A, Mane SP, et al. 2011.** The genome of woodland strawberry (*Fragaria*
935 *vesca*). *Nature Genetics* **43**: 109–116.

936

937 **Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, Repping S,**
938 **Pyntikova T, Ali J, Bieri T, et al. 2003.** The male-specific region of the human Y chromosome is a
939 mosaic of discrete sequence classes. *Nature* **423**: 825–837.

940

941 **Small E. 2015.** Evolution and Classification of *Cannabis sativa* (Marijuana, Hemp) in Relation to
942 Human Utilization. *The Botanical Review* **81**: 189–294.

943

944 **Sousa A, Fuchs J, Renner SS. 2013.** Molecular Cytogenetics (FISH, GISH) of *Coccinia grandis*:
945 A ca. 3 myr-Old Species of Cucurbitaceae with the Largest Y/Autosome Divergence in Flowering
946 Plants. *Cytogenetic and Genome Research* **139**: 107–118.

947

948 **Takahata N, Nei M. 1985.** Gene genealogy and variance of interpopulational nucleotide
949 differences. *Genetics* **110**: 325–344.

950

951 **Team, R. C. 2013.** R: A language and environment for statistical computing.

952

953 **Thomas GG, Neve RA. 1976.** Studies on the Effect of Pollination on the Yield and Resin Content
954 of Hops (*humulus Lupulus L.*). *Journal of the Institute of Brewing* **82**: 41–45.

955

956 **Torres MF, Mathew LS, Ahmed I, Al-Azwani IK, Krueger R, Rivera-Nuñez D, Mohamoud
957 YA, Clark AG, Suhre K, Malek JA. 2018.** Genus-wide sequencing supports a two-locus model
958 for sex-determination in *Phoenix*. *Nature Communications* **9**: 3969.

959

960 **Trombetta B, Sellitto D, Scozzari R, Cruciani F. 2014.** Inter- and intraspecies phylogenetic
961 analyses reveal extensive X-Y gene conversion in the evolution of gametologous sequences of
962 human sex chromosomes. *Molecular Biology and Evolution* **31**: 2108–2123.

963

964 **Velzen R van, Holmer R, Bu F, Rutten L, Zeijl A van, Liu W, Santuari L, Cao Q, Sharma T,
965 Shen D, et al. 2018.** Comparative genomics of the nonlegume *Parasponia* reveals insights into
966 evolution of nitrogen-fixing rhizobium symbioses. *Proceedings of the National Academy of
967 Sciences* **115**: E4700–E4709.

968

969 **Wang J, Na J-K, Yu Q, Gschwend AR, Han J, Zeng F, Aryal R, VanBuren R, Murray JE,
970 Zhang W, et al. 2012.** Sequencing papaya X and Yh chromosomes reveals molecular basis of
971 incipient sex chromosome evolution. *Proceedings of the National Academy of Sciences* **109**:
972 13710–13715.

973

974 **Westergaard M. 1958.** The Mechanism of Sex Determination in Dioecious Flowering Plants. In:
975 Demerec M, ed. *Advances in Genetics*. Academic Press, 217–281.

976

- 977 **Wickham, H. 2011.** *ggplot2*. *Wiley Interdisciplinary Reviews: Computational Statistics*, 3(2), 180-
978 185.
979
- 980 **Wright AE, Dean R, Zimmer F, Mank JE. 2016.** How to make a sex chromosome. *Nature*
981 *Communications* 7: 12087.
982
- 983 **Wu TD, Reeder J, Lawrence M, Becker G, Brauer MJ. 2016.** GMAP and GSNAP for Genomic
984 Sequence Alignment: Enhancements to Speed, Accuracy, and Functionality. In: Mathé E, Davis S,
985 eds. *Methods in Molecular Biology. Statistical Genomics: Methods and Protocols*. New York, NY:
986 Springer, 283–334.
987
- 988 **Yang Z. 2007.** PAML 4: Phylogenetic Analysis by Maximum Likelihood. *Molecular Biology and*
989 *Evolution* 24: 1586–1591.
990
- 991 **Yang M-Q, Velzen R van, Bakker FT, Sattarian A, Li D-Z, Yi T-S. 2013.** Molecular
992 phylogenetics and character evolution of Cannabaceae. *TAXON* 62: 473–485.
993
- 994 **Zhang Q, Onstein RE, Little SA, Sauquet H. 2019.** Estimating divergence times and ancestral
995 breeding systems in *Ficus* and *Moraceae*. *Annals of Botany* 123: 191–204.
996
- 997 • **Preprint repository:**
998 **Padgitt-Cobb, L. K., Kingan, S. B., Wells, J., Elser, J., Kronmiller, B., Moore, D., et al. 2019.**
999 A phased, diploid assembly of the Cascade hop (*Humulus lupulus*) genome reveals patterns of
1000 selection and haplotype variation. *BioRxiv*, 786145.
1001
- 1002 **Grassa, C. J., Wenger, J. P., Dabney, C., Poplawski, S. G., Motley, S. T., Michael, T. P., et al**
1003 **2018.** A complete Cannabis chromosome assembly and adaptive admixture for elevated cannabidiol
1004 (CBD) content. *BioRxiv*, 458083.
1005
- 1006 • **Web Document:**
1007 **Barth-Haas GmbH & Co. KG. 2019.** Barth Report (1950-2019). Nuremberg
1008
- 1009 **Conway Sean and Snyder Reid 2008.** *Humulus lupulus*– Hops. College Seminar 235 Food for
1010 Thought: The Science, Culture, & Politics of Food.
1011
- 1012 **King M, Pavlovic M. 2018.** Analysis of Hop Use in Craft Breweries in Slovenia. 3: 21–26.
1013

1014 **Neve, R. A. 1991.** Hops. Chapman and Hall. *London, England.*

1015

1016 **Supporting information legends**

1017

1018 Table S1.

1019 Statistics of mapping on *H. lupulus* and *C. sativa* references.

1020

1021 Table S2.

1022 Summary of SEX-DETECTOR genotyping errors and inferences

1023

1024 Table S3.

1025 Expression analysis statistics summary.

1026

1027 Table S4.

1028 Age estimates of the youngest strata in *H. lupulus* and *C. sativa*.

1029

1030 Table S5.

1031 Summary of chromosome name in Fig. 2A and assembly fasta file.

1032

1033 Table S6

1034 SEX-DETECTOR assignment file output

1035

1036 Figure S1.

1037 Histogram of the Y/X expression ratio.

1038

1039 Figure S2.

1040 Histogram of the Allele-specific expression analysis for the parents.

1041

1042 Figure S3.

1043 Histogram of the Allele-specific expression analysis for the daughters.

1044

1045 Figure S4.

1046 Histogram of the Allele-specific expression analysis for the sons.

1047

1048 Figure S5.

1049 Histogram of synonymous divergence (dS) between *C. sativa* and *M. notabilis*.

1050

1051 Figure S6.

1052 Histogram of synonymous divergence (dS) between *C. sativa* and *R. chinensis*.

1053

1054 Figure S7.

1055 Example of genes which topology changed with the mapping bias filtering.

1056

1057 Figure S8.

1058 Y/X expression ratio along the sex chromosome without genes with a detected mapping bias.

1059

1060 Figure S9.
1061 Dosage compensation analysis without genes with a detected mapping bias.
1062
1063 Figure S10.
1064 SEX-DETECTOR inference errors due to Whole Genome Duplication in *H. lupulus*.
1065
1066