Examining population structure across multiple collections of Cannabis

Anna Halpin-McCormick[1]
Karolina Heyduk[3]
Michael B. Kantar[1*]
Nicholas L.Batora[2]
Rishi R. Masalia[2]
Kerin Law[2]
Eleanor J. Kuntz[2*]

[1]Department of Tropical Plant and Soil Sciences, University of Hawai‘i at Mānoa, Honolulu, HI 96822
[2]LeafWorks Inc. 130 S. High St, Sebastopol, CA 95472
[3]School of Life Sciences, University of Hawai‘i at Mānoa, Honolulu, HI 96822

*corresponding authors: Eleanor J. Kuntz - eleanor@leafworks.com and Michael Kantar – mbkantar@hawaii.edu

Abstract

Population structure of *Cannabis sativa* L. was explored across nine independent collections that each contained a unique sampling of varieties. Hierarchical Clustering of Principal Components (HCPC) identified a range of three to seven genetic clusters across datasets with inconsistent structure based on use type indicating the importance of sampling particularly when there is limited passport data. There was broader genetic diversity in modern cultivars relative to landraces. Further, in a subset of geo-referenced landrace accessions, population structure was observed based on geography. The inconsistent structure across different collections shows the complexity within *Cannabis*, and the importance of understanding any particular collection which could then be leveraged in breeding programs for future crop improvement.

Key Words: Population Structure, Genome Scan, Public Data, Medicinal Plants, Fiber Plants

Introduction

Cannabis sativa L. is an annual flowering herb which has been domesticated multiple times for food, fiber and medicine over the last twelve thousand years (Hillig, 2005; Clarke & Merlin, 2013; Clarke & Merlin, 2016; Ren et al., 2021). *Cannabis* is popularly known for its psychoactive effects; however, it is its medicinal capacity is driving increased production (Punja & Holmes 2020). The compounds tetrahydrocannabinol (THC) and cannabidiol (CBD) are the most studied due to their potential in pain management (Walker & Huang, 2002; Alexander, 2020; Bicket et al., 2023), as a multiple sclerosis treatment (Svendsen et al., 2004), for epilepsy management (Charlotte's Web (CW2A) US Plant Patent No. PP30,639 P2; Perucca, 2017), for reduction in nausea (Parker et al., 2011) and as an appetite stimulant (Badowski & Perez, 2016). Today, *Cannabis* is broadly divided into non-drug and drug-type cultivars (**Table 1**).

Due to the classification of *Cannabis* as a Schedule I narcotic in the United States, research during the 20th century was largely restricted (Hurgobin et al., 2021). However, the 2018 United States Farm Bill reduced these restrictions, with many states now having reduced regulations (Mead, 2019). In 2020 the Drug Enforcement Administration expanded research licenses (Ryan et al., 2021) leading to increased *Cannabis* research. However, due to past restrictions C*annabis* has not fully benefited from scientific tool developments (e.g. molecular marker tools, heterotic pattern development) of the last century. Further, drug control laws and prohibition have constrained formal documentation often resulting in unverifiable and anecdotal cultivar origins (Duvall, 2016). However, there has been recent work to develop tools and initiate breeding (Toth et al., 2020; Petit et al., 2021; Woods et al., 2021; Toth et al., 2022; Woods et al., 2023).

While there is still some debate, the taxonomy of *Cannabis* has moved towards a monotypic description of the genus (McPartland 2018; McPartland & Small 2020). *Cannabis*

58 populations have been partitioned using different methods (e.g., genetic, chemical and
59 phenotype) with different populations showing different patterns (de Meijer et al., 2003; Lynch
60 et al., 2016). There are also examples of studies using regional, ecotype, and use-type to
61 understand population partitions (Soorni et al., 2017; Zhang et al., 2020; Carlson et al., 2021;
62 Ren et al., 2021). These studies have found contrasting results due to contested definitions and
63 different samples. In addition to understanding species and population delineation, previous
64 genetic work has explored the cannabinoid metabolic pathways (Guerriero et al. 2017; Guerriero
65 et al., 2019; Allen et al., 2019; McKernan et al., 2020; van Velzen & Schranz 2021).
66 Understanding population structure provides insight into evolutionary relationships and
67 facilitates the identification of cultivars that have value for breeding practices. Further,
68 understanding genetic relationships can help reconstruct pedigrees and genetic relationships
69 which have been lost due to a century of prohibition. Clarification of cultivar relationships could
70 provide more concrete reproducible results in addition to the ethnohistorical information and
71 spoken accounts that underpin current research. The molecular genetic profiles of *Cannabis*
72 cultivars will enhance our understanding of them, providing a valuable tool to confirm marketing
73 claims independently of relying solely on visual characteristics. This, in turn, can contribute to a
74 more reliable and sustainable industry.
75 Previous work has used a range of sequencing methods, reference genomes, and sampling
76 schemes (Small & Cronquist, 1976; Clarke, 1987; van Bakel et al., 2011; Duvall 2016; Soorni et
77 al. 2017; Soler et al., 2017; Maoz 2020; Hurgobin et al. 2021; Grassa et al. 2021). In an attempt
78 to understand previous studies (eight publicly available datasets) as well as a newly generated
79 dataset we used a common single nucleotide polymorphism (SNP) calling pipeline and the same
80 reference genome (Grassa et al., 2021) to explore population structure present across different

germplasm collections and to identify potential samples that can be explored as the basis for breeding.

Materials and Methods

Sequence Data Acquisition

Raw sequence data from Soorni et al. 2017 (PRJNA419020), Lynch et al., 2016 (PRJNA317659), Phylos Biosciences (PRJNA347566 & PRJNA510566), Courtagen Life Sciences (PRJNA297710), and Sunrise Genetics (PRJNA350539) were downloaded from National Center for Biotechnology Information (NCBI: https://www.ncbi.nlm.nih.gov/) using the SRA toolkit (https://hpc.nih.gov/apps/sratoolkit.html). Data from Medicinal Genomics, where 61 paired samples were provided for bulk download (Medicinal Genomics 61 - https://www.medicinalgenomics.com/kannapedia-fastq/) and an additional 289 samples (Medicinal Genomics StrainSEEK v1) were individually downloaded from each cultivar page. The last data source used here was developed by LeafWorks Inc., consisting of 498 individuals. Full dataset descriptions are available in **Table 2**.

Sample Name Acquisition

Sample names were assigned to individual samples as supplied by authors in supplemental materials of publication or through the metadata supplied through NCBI. All individual line assignments can be seen in **Tables S1-S9**. For Phylos Biosciences datasets, each SRR number was searched in NCBI in the SRA database. For the n=845 and the n=1,378 datasets this facilitated the association of SRR numbers with cultivar names from the “Sample” section and aided in matching the sample to the genotype information sheet on the Phylos Biosciences website (https://phylos.bio/). The links to the matching genotype report page for each SRA sample have been included in the metadata of the supplemental tables (N=845 **Table S2** and n=1,378 **Table S3**).

Use-type Category Assignment

Different meta-data for each dataset was used to identify the use-type (**Table 1**). For Phylos Biosciences (https://phylos.bio/) and Medicinal Genomics (https://www.kannapedia.net). For the Medicinal Genomics dataset, where no information was reported in the "Plant Type" section on individual strain pages, the cannabinoid section on strain pages which reports percentages of THC and CBD as well as other cannabinoids was used to assign type to individual samples, with well-known hemp variety names facilitated by the EU Plant variety database https://ec.europa.eu/ (eg. Santhica, Carmagnola, Fedora, Felina). For the LeafWorks Inc. dataset, type associations were provided for 101 landrace samples and 44 hemp samples with remaining use-type associations assigned through searching sample names on https://www.leafly.com or https://www.wikileaf.com. For Soorni et al. 2017 dataset a recent publication used chemistry of these same accessions to determine use-type (Mostafaei Dehnavi et al. 2022). For the remaining datasets (Sunrise Genetics, Lynch et al., 2016, and Courtagen Life Sciences) sample names were searched on https://www.leafly.com or https://www.wikileaf.com for assignment to a category of use-type (**Tables S1-S9**). Use-types were not evenly represented and this uneven representation of different use-types may influence conclusions related to the genus overall (**Tables S1-S9**).

Sequence Data Processing

Where demultiplexing was required, barcodes were acquired from the supplemental materials and removed using the software SABRE (version 1.0 - https://github.com/najoshi/sabre). All dataset fastq files were checked for adapter sequence content using the FASTQC (version 0.11.8-Andrews, 2010). Datasets were examined post FASTQC using MULTIQC (Ewels et al., 2016). Where adapters were present, TRIMMOMATIC (version 0.39 - Bolger et al., 2014) was used to remove these sequence elements. The software SKEWER (Jiang et al. 2014) was used to trim adaptors from Phylos Biosciences n=1,378 dataset. Some data from Lynch et al. 2016

129 (PRJNA310948) was also not included as PRJNA310948 appears to contain duplicates of
130 samples from PRJNA317659 both released in 2016. Therefore, only PRJNA317659 was used.
131 The Medicinal Genomics' Kannapedia site contains samples that have been sequenced across a
132 variety of platforms, for consistency here we used the samples from StrainSEEK v1 (n=289).
133 Reads were then aligned to the CBDRx genome (Grassa et al. 2021) using BWA-MEM
134 (version 0.7.17 - Li, 2013). SAMTOOLS (version 1.9 - Li et al., 2009) was used to convert SAM
135 files to BAM files and mapped reads were sorted for a mapping quality of 30 or above.
136 BCFTOOLS (version 1.9 - Danecek & McCarthy, 2017) using the mpileup function was used to
137 generate SNPs and create VCF files. Samples were filtered using VCFtools (version 0.1.16 -
138 Danecek et al., 2011) for a minor allele frequency of 0.05, Hardy-Weinberg Equilibrium (0.05),
139 and a maximum missingness of 10%. After filtering, data were analyzed using the SNPRelate
140 (Zheng et al. 2012), FactoMineR (Lê et al., 2008) and factoextra (Kassambara & Mundt, 2017)
141 packages in RStudio (version 1.4.1106 - R Core Team, 2013).

142 Nucleotide Diversity Calculation

143 VCF files for known modern cultivars and landraces were separately merged into a single VCF
144 file. Nucleotide diversity (π) was calculated using VCFtools with a 10,000 bp sliding window
145 across the strictly filtered files for each dataset. Changes in π across chromosomes were plotted
146 in RStudio using the ggplot package (Wickham, 2011).

147 Population Structure and Phylogenetic Analysis

148 VCFtools was used to generate MAP and PED files. These were then used to generate BED,
149 BIM, and FAM files in the software PLINK (version 1.9 - Purcell et al., 2007). For each dataset
150 population structure across a range of population partitions was assessed in fastSTRUCTURE
151 (version 1.0 - Raj et al., 2014). The optimal number of K was also examined for each dataset
152 using the elbow and silhouette methods in the FactoMineR and factoextra packages. In addition,

153 each dataset was examined using Principal Component Analysis (PCA) in SNPRelate (Zheng et
154 al., 2012). Only bi-allelic SNPs further filtered for linkage disequilibrium (0.2) were used for the
155 PCA and Hierarchical Clustering on Principal Components (HCPC) (**Fig. 1-2 and Fig. S2-6**). A
156 Maximum Likelihood (ML) phylogenetic tree was constructed for the LeafWorks Inc. dataset, a
157 VCF file was converted to NEXUS and FASTA format using the software package
158 VCF2PHYLIP (version 2.6 - https://github.com/edgardomortiz/vcf2phylip). Ambiguities were
159 changed to "N" where observed. Multiple sequence alignment was performed using MAFFT
160 (version 7.475- Katoh & Standley 2013) and this was submitted to the software ModelTest-NG
161 (version 0.1.6 - Darriba et al. 2020) to best evaluate the substitution model to be used.
162 Phylogenetic trees were constructed in IQ-TREE (version 2.0.7 - Minh et al., 2020) with the -B
163 1000 flag for bootstrap support. Trees were visualized in FigTree (Version1.4.4 -
164 http://tree.bio.ed.ac.uk/software/figtree/).

165 **Assembly of 126 whole chloroplast genomes**

166 Chloroplast DNA was assembled using the Fast-Plast program, with default parameters -
167 https://github.com/mrmckain/Fast-Plast. To explore haplo-group assignment a maximum
168 likelihood phylogeny was constructed on 126 whole chloroplast sequences which were provided
169 by LeafWorks Inc. Multiple sequence alignment was performed using MAFFT (version 7.475 -
170 Katoh and Standley 2013) and using the ModelTest-NG software (Darriba et al. 2020) the
171 GTR+G4 model was selected as the best substitution model. A phylogenetic tree was generated
172 using IQ-TREE (version 2.0.7 - Minh et al., 2020) with the -B 1000 flag for bootstrap support.
173 Trees were visualized in FigTree (Version1.4.4 - http://tree.bio.ed.ac.uk/software/figtree/).
174

Results

Commonalities across Datasets

Cannabis genetic diversity and population structure were explored using independent data sources, all of which were analyzed using the same pipeline (**Table 2**). All datasets were aligned to the same CBDRx reference genome (Grassa et al., 2021). Reanalysis allows for a cleaner comparison, as previous studies have used multiple reference genomes (e.g., Laverty et al., 2019; McKernan et al., 2020; van Bakel et al., 2011; Gao et al., 2020). There were not common SNPs across all datasets, when joint SNP calling was attempted. Therefore, each dataset was analyzed independently and each had a different number of SNPs (**Table 3-5)**. As sample sizes are robust, this suggests that the type of sequencing approach taken, library prep, sequencing depth, chromosomal coverage, and/or sample properties may bias the genetic diversity.

Population Structure

Hierarchical Clustering of Principal Components (HCPC) identified three to seven clusters across datasets (**Fig. 1-2 and Fig. S1-6**). In the LeafWorks Inc. dataset, four groups were identified. When use-type was used to interpret the clusters there was some partitioning, with Group 1 being predominantly Hemp, but Groups 2/3 being largely type I (**Fig. 1A**). In the LeafWorks Inc. fastSTRUCTURE analysis there were large amounts of admixture regardless of use-type/market-class (**Fig. 1B**). Within the Phylos Biosciences datasets, hierarchical clustering identified five groups in the n=845 dataset (**Fig. 1C**) and three clusters in the n=1378 dataset (**Fig. S2B**). In the small Phylos Biosciences dataset (n=845) hierarchical clustering shows a concentrated number of Landrace (95 of 127), Hemp (14 of 17) and type I (11 of 48) samples in Group 1 (**Fig. 1C**). In Group 2 the majority of the type III samples are observed (30 of 48 – **Fig. 1C**). .fastSTRUCTURE analysis indicated less admixture in Landrace and Hemp samples as compared to type I samples (K=3/4/5), with some differentiation based on use-types observed

200 (**Fig. 1D**). In the large dataset from Phylos Biosciences (n=1378 - **Fig. S2**), the hierarchical
201 clustering shows a concentration of samples which have the designation of "Kush" in Group 1
202 (34 of 88 -**Fig. S2B**). The subsequent fastSTRUCTURE analysis for the Phylos Biosciences
203 (n=1378) shows a similar pattern where Landrace samples show less admixture as compared to
204 type I samples (K=4/5) (**Fig. S2C**). In the HCPC for the Phylos Biosciences dataset (n=1378)
205 there is a concentration of samples with the designation "OG" (49/115 in Group 1 of HCPC) -
206 **Fig. S2B**). In the Soorni et al dataset there was clear clustering by use-type in both analyses (**Fig.**
207 **2A-B**). The Medicinal Genomics StrainSEEK v1 dataset was partitioned into five groups (**Fig.**
208 **2C**). There was some clustering of specific genotypes (e.g. Blue Dream (n=11) in Group 1 of
209 HCPC – **Fig. 2C**), but in fastSTRUCTURE analysis there were no clear trends in clustering
210 observed across use-type (**Fig 2D**). For the Sunrise Genetics dataset (n=25) HCPC shows
211 grouping of samples with the same names but no clear pattern in the fastSTRUCTURE clustering
212 analysis (**Fig. S3C**). In the Lynch et al., 2016 dataset (n=162) there was some evidence of use-
213 type but the pattern was not consistent (**Fig. S4B)**. For the Courtagen Life Sciences dataset
214 (n=58), there was clustering by cultivar name (e.g. Kandy Kush (n=5) in Group 5 of HCPC) but
215 not by use-type (**Fig. S5**). Within the Medicinal Genomics dataset (n=61) there was no clear
216 clustering by use-type (**Fig. S6**). Across datasets there is no clear partitioning pattern based on
217 use-type or based on accession name, this lack of pattern does not indicate a lack of population
218 structure, but rather confirms the inconsistency in definitions of use-type and the fact that
219 cultivar naming conventions do not reflect pedigrees.

220 **Phylogenetic Relationships**

221 A Maximum Likelihood (ML) phylogeny was assembled for the new LeafWorks Inc. (n
222 = 498) dataset which partitioned accessions into ten clades (**Fig. S7**). Clades 1 to 7 and clade 9
223 have bootstrap support of over 90, with clades 8 and 10 having low support (63 and 52,

224 respectively). The majority of accessions (454 of 498) were in four clades (clades 4, 6, 9 and 10).
225 There is not a clear pattern to which clade landrace samples are in (Clades 5=11 of 101; clade
226 9=24 of 101; clade10=52 of 101) with remaining individuals spread across the remaining clades
227 - **Fig. S7)**. There did not appear to be clear use-type partitioning in the phylogeny. Within the
228 chloroplast data, two clades were identified, clade 1 (n= 2) and clade 2 (n=124) (**Fig. S8**).
229 However, with low support for the majority of samples it is possible that additional groupings
230 within this might be possible.

231 Exploration of nucleotide diversity and geographic partitioning Landraces

232 The LeafWorks Inc. (n=498) and Phylos Biosciences (n=845) datasets both contained
233 known landrace and modern cultivars (**Table 1; Table S2-S3**). The LeafWorks Inc. dataset
234 contained 101 landrace samples and 397 known modern accessions (**Table S1**) and the Phylos
235 Biosciences dataset contained 127 landrace samples and 718 modern accessions (**Table S2**).
236 Clustering patterns were similar in the two datasets (**Fig. 3**). Within both datasets nucleotide
237 diversity differences were explored between landrace and domesticated samples using a 10 kb
238 sliding window (**Fig. 4**), revealing many genomic regions that differed between modern cultivars
239 and landraces. A subset of landraces in the LeafWorks Inc. dataset contained geo-references,
240 allowing for an exploration of structure based on geography. There was geographic clustering
241 with the Lolab Valley and Hindu Kush samples (**Fig. 5C**). There were low levels of admixture
242 based on the three geographic regions (**Fig. 5E**), indicating that despite being geographically
243 close populations remained isolated. Differences in nucleotide diversity in these geographically
244 distinct populations were observed on chromosomes 5, 6, and 7 (**Fig. 5D)**, and these genomic
245 regions may hold locally adaptive genes and may be useful sources of variation for breeding.

246 Core collection identification

247 Plant breeding relies on the available genetic variation within a given germplasm
248 collection or breeding program. A core collection is a representative subset of a germplasm
249 collection which attempts to capture the majority of the genetic diversity in that collection
250 (Frankel 1984). Using genetic distance, the 25 most diverse samples were selected from each
251 dataset (with the exception of the Sunrise dataset where 10 samples were selected). These
252 samples represent a core collection for each specific dataset (**Table S10**).

253 Discussion

254 Species descriptions in the *Cannabis* genus have been based on morphology and
255 chemistry (Clarke & Merlin, 2013; Onofri & Mandolino, 2017; Lewis et al., 2018; McPartland,
256 2018; Garfinkel et al., 2021; Smith et al., 2022). While three putative species have been
257 described in the *Cannabis* genus, genetic studies have not supported these delineations, instead
258 observing a monotypic genus (Clarke & Merlin, 2013; Sawler et al., 2015; Lynch et al., 2016;
259 Schwabe & McGlaughlin, 2019). Much effort has been made exploring use-type/marketing class
260 as markers of population stratification (Clarke & Merlin, 2013; Small, 2015). Here we continue
261 this tradition, by exploring population structure in nine different collections of *Cannabis* that
262 consisted of privately bred THC-dominant, public hemp samples and landrace accessions.
263 Understanding genetic diversity within each individual collection aides in understanding
264 population history and helps in developing strategies for future breeding. In particular,
265 establishing the number of distinct populations may help reduce the number of individuals that
266 need to be tested for the development of hybrid cultivars (Carlson et al., 2021).

267 Understanding population structure

268 Previous work has used various reference genomes (Lynch et al., 2016; Soorni et al.,
269 2017; Laverty et al., 2019; Jin et al., 2021) and this reflects the current predicament within the
270 industry where standards are still in development. Here a single reference genome (CBDRx -

271 Grassa et al. 2021) was used to facilitate comparison; however, it is acknowledged that this can
272 create reference bias impacting the examination of some questions. Reference limitations are
273 being addressed through the utilization of pangenomes and are increasingly becoming available
274 for many crop species (Hübner et al., 2019; Li et al., 2021; Della Coletta et al., 2021). Future
275 work to develop a *Cannabis* pangenome would be of great utility to the community.

276 *Cannabis* has historically used morphological and ethnographic data to delineate
277 populations not genetic data. Creating genetic profiles to cluster accessions and conduct
278 phylogenetic analysis facilitates using classic use-type to understand accession relationships (**Fig**
279 **3 and Fig. S7**) and offers a perspective on how *Cannabis* populations may have been influenced
280 by human mediated selection for important traits (**Fig. S2-6**). The LeafWorks Inc. and Soorni et
281 al., 2017 datasets exhibited more use-type separation (**Fig. 3A/3C**) than other datasets. A broader
282 distribution of genetic variation in Type I cultivars was observed in multiple datasets (**Fig. 3A-**
283 **B**). This may be indicative of the purported large-scale hybridization that is thought to have
284 occurred in Type I cultivars in the United States after 1960 (Clarke & Merlin, 2016).
285 Alternatively, this higher genetic diversity in Type I cultivars could represent convergent
286 selection, with each lineage being bred in isolation and now released back to the market as
287 regulations relax. However, the lack of available pedigree records makes it difficult to reconcile
288 these two alternative hypotheses. The other datasets did not show clear relationships with use-
289 type.

290 While the *Cannabis* genus has been described with the presence of one, two, three, and
291 even up to seven proposed species and subspecies (Linnaeus, 1753; Lamarck, 1785; Vavilov &
292 Bukinich, 1929; Schultes et al., 1974; Small & Cronquist, 1976; Hillig, 2004; Clarke & Merlin,
293 2013; McPartland & Guy 2014), contemporary genetic studies have not supported these

294 polytypic classifications delineations which are primarily rooted in morphological and
295 geographical data. While modern genetic studies consistently do not support previously
296 suggested species delineation (Gilmore et al., 2007; Sawler et al., 2015; Lynch et al., 2016;
297 Small, 2015; Zhang et al., 2018; Schwabe & McGlaughlin, 2019; McPartland & Small 2019;
298 Roman et al., 2019; Henry et al. 2020; Ren et al 2021; Schwabe et al., 2021; Vergara et al. 2021;
299 Woods et al., 2023), they do support multiple potential populations within the genus. Despite
300 these findings, the prevailing taxonomic treatment of the *Cannabis* genus tends to favor a
301 monotypic classification. Several publications have proposed delineations in the relationship
302 between use-type and population structure (Gilmore et al., 2007; Roman et al., 2019; Zhang et
303 al., 2018; Henry et al., 2020; Ren et al., 2021; Woods et al., 2023). This suggests that collection
304 origin and accurate passport data greatly impact the population structure observed. High levels of
305 hybridization and shared ancestry may all contribute to the relatively shallow population
306 structure observed in some datasets (**Fig. 1D**) and hamper the ability to clearly differentiate
307 populations.

308 Landrace samples are distributed throughout population clusters and across the
309 phylogenies, however the number of landrace samples in a particular partition appear to be
310 affected by the germplasm sampling (**Fig. 1 and Fig. S7**). When landraces were analyzed with
311 modern cultivars, they were broadly distributed across clades suggesting that modern cultivars in
312 the same clade share the most ancestry with the landraces in the same clade (**Fig. 1A-B**).
313 Landraces did not cluster with a particular use-type. In a subset of georeferenced samples (n=26)
314 hierarchical clustering revealed three geographically discrete landrace populations appear to be
315 quite distinct from one another with minimal admixture (**Fig. 5B/E**). The subset landraces with
316 georeferences and which were geographically separated, clustered distinctly when analyzed

separately (**Fig. 5E**). While landraces were defined based on metadata and a history of being grown in a specific geography, limitations on passport data cloud inference. Without new collections and legitimate chain of custody documentation, this likely cannot be addressed.

Inconsistent Naming

The naming problem in *Cannabis* refers to the unreliable naming of cultivars which frequently do not reflect accession pedigree causing problems for both the producer and consumer. Name fidelity was explored using the twelve 'Blue Dream' samples in the LeafWorks Inc. dataset (**Fig. S7**). Of these, 7/12 placed in clade 1 (blue_dream samples #1, 3, 4, 6, 7, 9 & 10), 1 sample in clade 6 (blue_dream_5), clade 9 (blue_dream_11) and clade 10 (blue_dream_2). The remaining two samples (blue_dream # 8 & 12) were unplaced in the phylogenetic tree. Cultivars that show consistent placement within a phylogeny have a high likelihood of name accuracy. This exemplifies the naming problem, where only 58% of the samples appeared to be similar. This data further supports previous work demonstrating misconceptions in strain reliability and which further showed that the marketing varieties of *Cannabis* as "indica" and "sativa" does not appear to have genetic support (Schwabe & McGlaughlin, 2019). Further work is needed to determine how pervasive the naming problem is. This work also highlights the importance of genetics to inform label claims, which will be particularly crucial in the event of legalization when the Federal Drug Administration would require accurate plant label claims as it does for all other natural products sold in the United States. As sequencing costs continue to decrease, genomic approaches for understanding *Cannabis* naming will likely become standard practice and could overcome the challenge of clone and cultivar misidentification.

Strategic use of Germplasm for Breeding

Variation in cannabinoid content is genetically complex and potentially affected by the environment (Lydon et al., 1987; Campbell et al., 2020; Caplan et al., 2019; Toth et al., 2021).

341 Breeding with a focus on a particular use-type could help to ensure consistency in secondary
342 chemistry and incorporating an assessment of admixture or hybridization in this selection may
343 expedite the time taken to reach population stability. Coupling plant phylogeny with
344 metabolomics could facilitate the identification of plants with unique genetic and secondary
345 chemistries and would provide unique market classes (Stone et al. 2020).

346 Breeding targets in the future will likely focus on the common traits of disease and pest
347 resistance but will also likely need to maintain certain metabolite content to ensure use-type.
348 There is potential value in exploring if specific SNP markers can be identified to differentiate
349 use-type as this can inform parental choice in plant breeding programs. Expanding the use of
350 genome wide markers will not only help to characterize populations but can also help establish
351 preliminary partitioning of samples into potential heterotic groups. Population stratification and
352 use-type categorization have already found applications in hybrid breeding efforts (Carlson et al.,
353 2021). To establish new patterns of heterosis, it has been proposed that a practical starting point
354 could involve segregating individuals based on genetic distance, with a threshold set at >0.4
355 (Govindaraju 2019). This approach can be empirically tested within any germplasm collection,
356 whether it's publicly or privately held, to identify effective patterns for achieving improved
357 breeding outcomes.

358 Another option will be to use evolutionary plant breeding (EPB) to help maintain
359 diversity and stability of a crop in a specific environment leveraging natural selection (Merrick et
360 al. 2020). This has been used to aid in hybridization (Dreiseitl, 2020). Here landrace sampling
361 was limited (**Tables S1-9**), but a more thorough characterization of *Cannabis* landrace
362 populations would facilitate use of this approach. The history of prohibition and local cultivar
363 development suggests that there is a large possibility of biopiracy (e.g. unauthorized exploitation

364 or theft of valuable genetic resources or traditional knowledge) with respect to the developing
365 industry. It will be important to develop equitable distribution and ensure that local communities
366 benefit from the work their communities have done in the past and be in compliance with The
367 International Treaty on Plant Genetic Resources for Food and Agriculture (Cooper, 2002).

368 **Public Data Implications**

369 The relaxing of governmental regulations and decrease in sequencing costs technologies
370 have made it possible to genotype many different germplasm collections over the last decade
371 (**Table 2**). Public-private partnerships offer a route to harness the diverse resources and expertise
372 present in both sectors and provide a useful mechanism to advance *Cannabis* science (Ferroni &
373 Castle, 2011). Ensuring data standards are upheld and that metadata are available will make the
374 increasing amount of data available useful to many different researchers (Chao, 2014). The
375 ability to analyze the data requires accurate metadata and while this problem is not unique to
376 *Cannabis*, it is acutely problematic in any species that has high economic value and limited
377 foundational genomic resources. When working with public data sources care must be taken in
378 the cross comparison of specific datasets as the amount of shared germplasm and data quality
379 can influence the breadth and inference potential of the analysis (Williamson et al. 2021).
380 Additionally, when expanding these observations to conclusions about the genus as whole, it is
381 important to carefully consider germplasm sampling bias which limits the direct comparison,
382 which may result in limited or no shared SNPs across datasets (Zimmerman et al., 2020). It is
383 evident that the selection of sequencing technology, such as short-read amplicon sequencing or
384 genome-wide sequencing, can substantially alter the capacity for making inferences and can have
385 a notable impact on the value of some genomic statistics (Evangelou and Ioannidis, 2013;
386 Marchi et al., 2021). In this analysis it is very likely that due to the high numbers of potential
387 Type I plants, sampling of male *Cannabis* plants has been largely unobserved. This is because

388 Type I plants typically consist of female flowers exclusively, with males often being removed
389 from cultivation.

390 **Future perspectives**

391 Nine datasets were explored to understand population structure in *Cannabis*, identifying
392 inconsistent genetic clustering with use-type. The inconsistency of use-type as a predictor of
393 relatedness implies that it may be a collection specific association, or that the relatively simple
394 inheritance of tetrahydrocannabinolic acid synthase (THCAS) may obfuscate background genetic
395 relationships. With the legal status of *Cannabis* now shifting, researchers can begin to examine
396 the effects of prohibition on extant *Cannabis* varieties and keep better records while developing
397 new cultivars. In the United States, prohibition may have created closed gene pools through the
398 breeding of limited germplasm facilitated by limiting plant exchange. Limited genetic diversity
399 in breeding may have had a role to play in the increased potency of *Cannabis* varieties over time,
400 with increases in THC content from ~4% in 1995 to ~12% in 2014 reported (El Sohly et al.
401 2016). Analogous to this in the wild, repeated range contractions during the Holocene are
402 thought to have resulted in repeated genetic bottlenecks and likely initiated incomplete allopatric
403 speciation which has led to differences between European (CBD-dominant - Type III) and Asian
404 (THC-Dominant - Type I) *Cannabis* populations (McPartland 2018).

405 The study of *Cannabis* genetics and its population structure is influenced by historical
406 factors like prohibition and contemporary breeding practices. Genome sequencing technologies
407 play a pivotal role in shaping our understanding of *Cannabis* genetic diversity. The debate over
408 species and subspecies classifications persists, with genetic research consistently challenging
409 traditional delineations. Importantly, the identification of population stratification and genetic
410 markers holds promise for enhancing breeding efforts, particularly in developing new heterotic
411 patterns. Additionally, while the industry burgeons, concerns regarding biopiracy and the

preservation of genetic diversity remain salient. Despite the challenges posed by inconsistent naming conventions and limited sampling, ongoing research efforts continue to shed light on the intricate genetic landscape of *Cannabis,* with significant implications for its future cultivation, medicinal use, and industrial applications.

Acknowledgements

We would like to thank Mr. Robert Connell Clarke for his curation of the use-type associations for the Phylos Biosciences (n=1378) dataset as well as for valuable discussions and insights.

Funding: This manuscript was prepared without external financial support or funding.

Conflicts of interest/Competing interests: LeafWorks Inc. is a for profit company

Availability of data and material: data are available upon reasonable request to the corresponding author.

Code availability: code are available at https://github.com/ahmccormick and at https://figshare.com/authors/Anna_H_McCormick/17741367

Author contributions: *Conceptualization:* AHMC, KH, MBK, NB, RRM, KL, EJK, *Formal Analysis:* AHMC, RRM, *Figure Preparation:* AHMC, *Manuscript Drafting:* AHMC, *Writing and Reviewing Manuscript:* AHMC, KH, MBK, NB, RRM, KL, EJK.

Main Figure Legends

Fig. 1 Examining hierarchical clustering on principal components (HCPC) and population structure in the LeafWorks Inc. (n=498) and Phylos Biosciences (n=845) datasets. In each case population genetic clustering was conducted based only on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** Hierarchical cluster dendrogram from 520 nuclear SNPs for the LeafWorks Inc. dataset with use-type indicated below. Use-type are pictured below (Type I=288, Type II =5, Type III=16, Hemp=44, Landrace=101 and Unknown=44) **(B)** Visualization of population structure and admixture from 1,405 nuclear SNPs for the LeafWorks Inc. dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 4 using the silhouette method **(Fig. S9-10) (C)** Hierarchical cluster dendrogram from 292 nuclear SNPs for the Phylos Biosciences dataset with use-type indicated below. Use-type accessions include Type I=479, Type II=8, Type III=46, Landrace=127, Hemp=143 and Unknown=42 **(D)** Visualization of population structure and admixture from 385 nuclear SNPs for the Phylos Biosciences dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the Silhouette method **(Fig. S9-10)**.

Fig. 2 Examining hierarchical clustering and population structure in the Soorni et al. 2017 (n=94) and the Medicinal Genomics StrainSEEK V1 (n=289) datasets. In each case clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** Hierarchical cluster dendrogram from 6,865 nuclear SNPs for the Soorni et al. 2017 dataset with use-type of each accession indicated below. Use-type are pictured below (Type I=20, Type III=10, Type II=1, Landrace=78 and Unknown=63) **(B)** Visualization of population structure and admixture from 33,629 nuclear SNPs for the Soorni et al. 2017 dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method **(Fig. S9-10)** (**C)** Hierarchical cluster dendrogram from 5,045 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset with use-type indicated below. Use-type of accessions include Type I=108, Type III=9, Type II=17 and Unknown=155 **(D)** Visualization of population structure and admixture from 20,566 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method **(Fig. S9-10)**.

Fig. 3 Examination of use-type association across datasets **(A)** Principal component analysis (PCA) from 520 nuclear SNPs for the LeafWorks Inc. dataset **(B)** PCA from 213 SNPs Phylos Biosciences(n=845) dataset **(C)** PCA from 6,865 nuclear SNPs for the Soorni et al. 2017 dataset where cannabinoid content could be determined due to recent publication for 31/94 samples. (**D)** PCA from 5,045 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset.

Fig. 4 Nucleotide diversity as examined by a 10kb sliding window for landrace and domesticated partitions for the LeafWorks Inc. and Phylos Biosciences datasets **(A)** Nucleotide diversity by chromosome and **(B)** across chromosome length for Domesticated (n=397, 2,096 SNPs) and Landrace (n=101, 2,131 SNPs) samples for the LeafWorks Inc. dataset **(C)** Nucleotide diversity by chromosome and (D) across chromosome length for Domesticated (n=718, 749 SNPs) and Landrace (n=127, 566 SNPs) samples for the Phylos Biosciences dataset.

Fig. 5 Landrace accessions from the LeafWorks Inc. dataset show separation between Indian and Myanmar populations **(A)** Map detailing the locations of landrace accessions, highlighted are the Hindu Kush Mountains, Lolab Valley and Myanmar **(B)** Hierarchical cluster dendrogram based on 304 SNPs (LD 0.2) across 26 samples of known and trusted origin (**C)** PCA based on 304 SNPs with geographical locations of samples as indicated **(D)** Nucleotide diversity comparison between Hindu Kush Mountains (n=6, 4,304 SNPs), Lolab Valley (n=4, 853 SNPs) and Myanmar (n=4, 2,204 SNPs) as examined by a 10kb sliding window **(E)** Visualization of population structure and admixture using the fastSTRUCTURE

software (k=3) with the optimal number of K being 3 using the silhouette method.

Supplemental Figure Legends

Fig. S1 Dataset overview **(A)** Nucleotide diversity examined by a 10kb sliding window for all 9 genomic datasets for *Cannabis sativa* L. **(B)** Nucleotide diversity across the length of the 10 chromosomes for all 9 genomic datasets.

Fig. S2 Nuclear SNP analysis for the Phylos Biosciences (n=1,378) dataset. Clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** PCA by use-type based on 269 nuclear SNPs. Use-type associations include THC-Dominant (Type I) (n=996) CBD-Dominant (Type III) (n=87), Hemp (n=215), Landrace (n=78) and Unknown (n=2) **(B)** Hierarchical cluster dendrogram with use-type indicated below (C) Visualization of population structure and admixture using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method.

Fig. S3 Nuclear SNP analysis for the Sunrise Genetics dataset for 25 samples. Clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** PCA by use-type based on 1,604 nuclear SNPs. Use-type associations include THC-Dominant (Type I) (n=38) and Unknown (n=12) **(B)** Hierarchical cluster dendrogram with use-type indicated below **(C)** Visualization of population structure and admixture using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method.

Fig. S4 Nuclear SNP analysis for the Lynch et al., 2016 dataset for 162 samples. Clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** PCA by use-type for 162 samples from 2,223 SNPs. Type associations include Hemp (n=1), Landrace (n=1), THC-Dominant (Type I) (n=162), CBD-Dominant (Type III) (n=11), THC:CBD (Type II) (n=2) and Unknown (n=21) **(B)** Hierarchical cluster dendrogram with use-type indicated below **(C)** Visualization of population structure and admixture using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 2 using the silhouette method.

Fig. S5 Nuclear SNP analysis for the Courtagen Life Sciences dataset for 58 samples. Clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** PCA by use-type based on 119 nuclear SNPs. Use-type associations include Hemp (n=1), THC-Dominant (Type I) (n=41), CBD-Dominant (Type III) (n=11) and Unknown (n=5) **(B)** Hierarchical cluster dendrogram with use-type indicated below **(C)** Visualization of population structure and admixture using the fastSTRUCTURE software (k=2-5).

Fig. S6 Nuclear SNP analysis for the Medicinal Genomics 61 dataset for 61 samples. Clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** PCA by use-type based on 2,267 nuclear SNPs. Use-type associations include Hemp (n=1), THC-Dominant (Type I) (n=47), CBD-Dominant (Type III) (n=5) and Unknown (n=9) (**B)** Hierarchical cluster dendrogram with use-type indicated below **(C)** Visualization of population structure and admixture using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method.

Fig. S7 Maximum Likelihood tree for the LeafWorks Inc. dataset constructed from 1,405 nuclear SNPs from 498 samples. Modeltest-ng revealed the TIM2+G4 as the best fit substitution model and IQ-Tree software was used for phylogenetic inference. Blue Dream samples (n=12) are highlighted in blue at the branch tips. Use-type for individual samples is additionally indicated.

Fig. S8 Maximum Likelihood phylogenetic tree for 126 whole chloroplast assemblies. Individuals were aligned using MAFFT. Modeltest-NG revealed the GTR+G4 as the best fit substitution model and IQ-Tree software was used for phylogenetic inference. The resultant tree was visualized using FigTree (Version 1.4.4).

Figure S9 Examining optimal K number across the datasets using the Elbow Method **(A)** LeafWorks Inc. dataset **(B)** Phylos Biosciences dataset (n=845) **(C)** Soorni dataset (n=94) **(D)** Medicinal Genomics StrainSEEK V1 (n=289) **(E)** Phylos Biosciences dataset (n=1378) **(F)** Sunrise Genetics (n=25) **(G)** Colorado dataset (n=162) **(H)** Courtagen dataset (n=58) **(I)** Kannapedia 61 dataset (n=61) **(J)** LeafWorks Inc. landrace samples (n=14).

Figure S10 Examining optimal K number across the datasets using the Silhouette Method **(A)** LeafWorks Inc. dataset **(B)** Phylos Biosciences dataset (n=845) **(C)** Soorni dataset (n=94) **(D)** Medicinal Genomics StrainSEEK V1 (n=289) **(E)** Phylos Biosciences dataset (n=1378) **(F)** Sunrise Genetics (n=25) **(G)** Colorado dataset (n=162) **(H)** Courtagen dataset (n=58) **(I)** Kannapedia 61 dataset (n=61) **(J)** LeafWorks Inc. landrace samples (n=14).

547 # Table Legends
548 **Table 1.** Definitions related to the different types of germplasm that were used in this study.
549 **Table 2.** Data sources used for this project.
550 **Table 3.** SNP count per dataset pre and post filtering.
551 **Table 4.** SNP counts for each dataset by chromosome following biallelic sorting and Linkage
552 Disequilibrium prune at 0.2 and mapped to CBDRx (cs10) genome.
553 **Table 5.** Partition specific (Landrace and Domesticates) SNP count per dataset pre and post filtering.
554 **Table S1.** Cultivar name, use-type, clade association and domestication classifications for the LeafWorks
555 Inc. data set.
556 **S2.** SSR ID, Cultivar name, use-type and domestication classifications for the Phylos Biosciences
557 (n=845) data set.
558 **Table S3.** SSR ID, Cultivar name, use-type, clade association and domestication classifications for the
559 Phylos Biosciences (n=1,378) data set.
560 **Table S4.** SSR ID, Cultivar name, Chemistry Type and and HCPC group for the Soorni et al. 2017 data
561 set.
562 **Table S5.** Sample ID, RSP ID, Cultivar name and use-type association for Medicinal Genomics (n=753)
563 data set.
564 **Table S6.** SSR ID, Cultivar name and use-type association for the Sunrise Genetics data set.
565 **Table S7.** SSR ID, Cultivar name and use-type association for Lynch et al., 2016 data set.
566 **Table S8. S**SR ID, Cultivar name and use-type association for the Courtagen Life Sciences data set.
567 **Table S9.** Sample ID, Cultivar name and use-type association for Medicinal Genomics (n=61) data set.
568 **Table S10.** Core collections for the nine datasets.

References

Alexander, SP. 2020. Barriers to the wider adoption of medicinal Cannabis. *British Journal of Pain*, *14*(2), 122-132.

Allen, KD, McKernan, K, Pauli, C, Roe, J, Torres, A, Gaudino, R. 2019. Genomic characterization of the complete terpene synthase gene family from Cannabis sativa. *PloS one*, *14*(9), e0222363.

Andrews, S. 2010. FastQC: a quality control tool for high throughput sequence data.

Badowski, ME, Perez, SE. 2016. Clinical utility of dronabinol in the treatment of weight loss associated with HIV and AIDS. *HIV/AIDS-Research and Palliative Care*, 37-45.

Van Bakel, H, Stout, JM, Cote, AG, Tallon, CM, Sharpe, AG, Hughes, TR, Page, JE. 2011. The draft genome and transcriptome of Cannabis sativa. *Genome biology*, *12*(10), 1-18.

Bicket, MC, Stone, EM, McGinty, EE. 2023. Use of cannabis and other pain treatments among adults with chronic pain in US states with medical cannabis programs. *JAMA network open*, *6*(1), e2249797-e2249797.

Bolger, AM, Lohse, M, Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114-2120.

Campbell, LG, Dufresne, J, Sabatinos, SA. 2020. Cannabinoid inheritance relies on complex genetic architecture. *Cannabis and Cannabinoid Research*, *5*(1), 105-116.

Caplan, D, Dixon, M, Zheng, Y. 2019. Increasing inflorescence dry weight and cannabinoid content in medical cannabis using controlled drought stress. *HortScience*, *54*(5), 964-969.

Carlson CH, Stack GM, Jiang Y, Taşkıran B, Cala AR, Toth JA, Philippe G, Rose JK, Smart CD, Smart LB. 2021. Morphometric relationships and their contribution to biomass and cannabinoid yield in hybrids of hemp (Cannabis sativa). Journal of Experimental Botany;72(22):7694-709.

Chao, TC. 2014. Enhancing metadata for research methods in data curation. *Proceedings of the American society for information science and technology*, *51*(1), 1-4.

Clarke, RC, Merlin, MD. 2013. Cannabis. Evolution and Ethnobotany, University of Cali.

Clarke RC. 1987 Cannabis evolution. MS thesis, Indiana University, Bloomington, IN.

Clarke, RC, Merlin, MD. 2016. Cannabis domestication, breeding history, present-day genetic diversity, and future prospects. *Critical reviews in plant sciences*, *35*(5-6), 293-327.

Della Coletta, R, Qiu, Y, Ou, S, Hufford, MB, Hirsch, CN. 2021. How the pan-genome is changing crop genomics and improvement. *Genome biology*, *22*(1), 1-19.

Cooper, HD. 2002. The international treaty on plant genetic resources for food and agriculture. *Rev. Eur. Comp. & Int'l Envtl. L.*, *11*, 1.

Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T. McVean, G. 2011. The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156-2158.

Danecek, P., McCarthy, S.A. 2017. BCFtools/csq: haplotype-aware variant consequences. *Bioinformatics*, *33*(13), 2037-2039.

Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T. 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Molecular biology and evolution*, *37*(1), 291-294.

De Meijer, E.P., Bagatta, M., Carboni, A., Crucitti, P., Moliterni, V.C., Ranalli, P., Mandolino, G. 2003. The inheritance of chemical phenotype in Cannabis sativa L. *Genetics*, *163*(1), 335-346.

De Meijer, E.P.M., Hammond, K.M., Micheler, M. 2009. The inheritance of chemical phenotype in Cannabis sativa L.(III): variation in cannabichromene proportion. *Euphytica*, *165*(2), 293-311.

Dreiseitl, A. 2020. Specific resistance of barley to powdery mildew, its use and beyond: A concise critical review. *Genes*, *11*(9), 971.

Duvall, C.S. 2017. Drug laws, bioprospecting and the agricultural heritage of Cannabis in Africa. In *Drugs, Law, People, Place and the State,* 10-25. Routledge.

El Sohly, M.A., Mehmedic, Z., Foster, S., Gon, C., Chandra, S., Church, J.C. 2016. Changes in cannabis potency over the last 2 decades (1995–2014): analysis of current data in the United States. *Biological psychiatry*, *79*(7), 613-619.

Evangelou, E., Ioannidis, J. P. 2013. Meta-analysis methods for genome-wide association studies and beyond. Nature Reviews Genetics, 14(6), 379-389.

Ewels, P., Magnusson, M., Lundin, S., Käller, M. 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047-3048.

Ferroni, M., Castle, P. 2011. Public-private partnerships and sustainable agricultural development. *Sustainability*, *3*(7), 1064-1073.

Frankel, O.H. 1984. Genetic perspectives of germplasm conservation. *Genetic manipulation: impact on man and society*, *161*, 170.

Gao, S., Wang, B., Xie, S., Xu, X., Zhang, J., Pei, L., Yu, Y., Yang, W., Zhang, Y.,2020. A high-quality reference genome of wild Cannabis sativa. *Horticulture research*, *7*.

Garfinkel, A.R., Otten, M. and Crawford, S. 2021. SNP in potentially defunct tetrahydrocannabinolic acid synthase is a marker for cannabigerolic acid dominance in Cannabis sativa L. *Genes*, *12*(2), 228.

Gilmore, S., Peakall, R., Robertson, J., 2007. Organelle DNA haplotypes reflect crop-use characteristics and geographic origins of Cannabis sativa. *Forensic Science International*, *172*(2-3), 179-190.

Govindaraju, D.R., 2019. An elucidation of over a century old enigma in genetics—Heterosis. *PLoS Biology*, *17*(4), e3000215.

Grassa, C.J., Weiblen, G.D., Wenger, J.P., Dabney, C., Poplawski, S.G., Timothy Motley, S., Michael, T.P., et al. 2021. A new Cannabis genome assembly associates elevated cannabidiol (CBD) with hemp introgressed into marijuana. *New Phytologist*, *230*(4), 1665-1679.

Guerriero, G., Behr, M., Legay, S., Mangeot-Peter, L., Zorzan, S., Ghoniem, M., et al. 2017. Transcriptomic profiling of hemp bast fibres at different developmental stages. *Scientific reports*, *7*(1), 4961.

Guerriero, G., Deshmukh, R., Sonah, H., Sergeant, K., Hausman, J.F., Lentzen, E., Valle, N., Siddiqui, K.S., et al. 2019. Identification of the aquaporin gene family in Cannabis sativa and evidence for the accumulation of silicon in its tissues. *Plant Science*, *287*, 110167.

Henry, P., Khatodia, S., Kapoor, K., Gonzales, B., Middleton, A., Hong, K., Hilyard, A., Johnson, S., Allen, D., Chester, Z., et al. 2020. A single nucleotide polymorphism assay sheds light on the extent and distribution of genetic diversity, population structure and functional basis of key traits in cultivated north American cannabis. *Journal of Cannabis Research*, *2*(1), 1-11.

Hillig, KW. 2005. Genetic evidence for speciation in Cannabis (Cannabaceae). *Genetic Resources and Crop Evolution*, *52*, 161-180.

Hübner, S., Bercovich, N., Todesco, M., Mandel, J.R., Odenheimer, J., Ziegler, E., Lee, J.S., Baute, G.J., Owens, G.L., Grassa, C.J., et al. 2019. Sunflower pan-genome analysis shows that hybridization altered gene content and disease resistance. *Nature plants*, *5*(1), 54-62.

Hurgobin, B., Tamiru-Oli, M., Welling, M.T., Doblin, M.S., Bacic, A., Whelan, J., Lewsey, M.G. 2021. Recent advances in Cannabis sativa genomics research. *New Phytologist*, *230*(1), 73-89.

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 bioinformatics*, *15*, 1-12.

Jin, D., Henry, P., Shan, J., Chen, J. 2021. Classification of cannabis strains in the Canadian market with discriminant analysis of principal components using genome-wide single nucleotide polymorphisms. *Plos one*, *16*(6), e0253387.

Kassambara, A., Mundt, F. 2017. factoextra: Extract and visualize the results of multivariate data analyses (Version 1.0. 5). *URL https://www. rdocumentation. org/packages/factoextra/versions/1.0*, *5*.

Katoh, K., Standley, D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular biology and evolution*, *30*(4), 772-780.

Lamarck, J.B. 1785. Encyclopédie méthodique. Botanique. Panckoucke, Paris.

Laverty, K.U., Stout, J.M., Sullivan, M.J., Shah, H., Gill, N., Holbrook, L., Deikus, G., Sebra, R., Hughes, T.R., Page, J.E., Van Bakel, H. 2019. A physical and genetic map of Cannabis sativa identifies extensive rearrangements at the THC/CBD acid synthase loci. *Genome research*, *29*(1), 146-156.

Lewis, M.A., Russo, E.B., Smith, K.M. 2018. Pharmacological foundations of cannabis chemovars. *Planta medica*, *84*(04), 225-233.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R. 2009. 1000 Genome Project Data Processing Subgroup, 2009. The sequence alignment/map format and SAMtools. *bioinformatics*, *25*(16), 2078-2079.

Li, H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv preprint arXiv:1303.3997*.

Li, J., Yuan, D., Wang, P., Wang, Q., Sun, M., Liu, Z., Si, H., Xu, Z., Ma, Y., Zhang, B., Pei, L. 2021. Cotton pan-genome retrieves the lost sequences and genes during domestication and selection. *Genome biology*, *22*(1), 1-26.

Linnaeus, C. 1753. Species Plantarum. Laurentius Salvius, Stockholm, 1200.

Lydon, J., Teramura, A.H., Coffman, C.B. 1987. UV-B radiation effects on photosynthesis, growth and cannabinoid production of two Cannabis sativa chemotypes. *Photochemistry and Photobiology*, *46*(2), 201-206.

Lynch, R.C., Vergara, D., Tittes, S., White, K., Schwartz, C.J., Gibbs, M.J., Ruthenburg, T.C., DeCesare, K., Land, D.P., Kane, N.C. 2016. Genomic and chemical diversity in Cannabis. *Critical Reviews in Plant Sciences*, *35*(5-6), 349-363.

Maoz, T.Y. 2020. Making Cannabis History in 2020. *WWW document] URL https://www. nrgene. com/blog/making-cannabis-history-in-2020*.

Marchi, N., Schlichta, F., Excoffier, L. 2021. Demographic inference. Current Biology, 31(6), R276-R279.

McKernan, K.J., Helbert, Y., Kane, L.T., Ebling, H., Zhang, L., Liu, B., Eaton, Z., McLaughlin, S., Kingan, S., Baybayan, P., Concepcion, G. 2020. Sequence and annotation of 42 cannabis

genomes reveals extensive copy number variation in cannabinoid synthesis and pathogen resistance genes. *BioRxiv*, 2020-01.

McPartland, J.M., Guy, G.W. 2017. Models of Cannabis taxonomy, cultural bias, and conflicts between scientific and vernacular names. The botanical review, 83, 327-381.

McPartland, J.M. 2018. Cannabis systematics at the levels of family, genus, and species. *Cannabis and cannabinoid research*, *3*(1), 203-212.

McPartland, J.M., Hegman, W., Long, T. 2019. Cannabis in Asia: its center of origin and early cultivation, based on a synthesis of subfossil pollen and archaeobotanical studies. *Vegetation history and archaeobotany*, *28*, 691-702

McPartland, J.M., Small, E. 2020. A classification of endangered high-THC cannabis (*Cannabis sativa subsp. indica*) domesticates and their wild relatives. *PhytoKeys*, *144*, 81.

Mead, A. 2017. The legal status of cannabis (marijuana) and cannabidiol (CBD) under US law. *Epilepsy & Behavior*, *70*, 288-291.

Mead, A. 2019. Legal and regulatory issues governing cannabis and cannabis-derived products in the United States. *Frontiers in plant science*, *10*, 697.

Merrick, L.F., Lyon, S.R., Balow, K.A., Murphy, K.M., Jones, S.S., Carter, A.H.,\ 2020. Utilization of evolutionary plant breeding increases stability and adaptation of winter wheat across diverse precipitation zones. *Sustainability*, *12*(22), 9728.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., Lanfear, R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, *37*(5), 1530-1534.

Mostafaei Dehnavi, M., Ebadi, A., Peirovi, A., Taylor, G., Salami, S.A. 2022. THC and CBD Fingerprinting of an Elite Cannabis Collection from Iran: Quantifying Diversity to Underpin Future Cannabis Breeding. *Plants*, *11*(1), 129.

Murovec, J., Eržen, J.J., Flajšman, M., Vodnik, D. 2022. Analysis of Morphological Traits, Cannabinoid Profiles, THCAS Gene Sequences, and Photosynthesis in Wide and Narrow Leaflet High-Cannabidiol Breeding Populations of Medical Cannabis. *Frontiers in plant science*, *13*, 786161.

Onofri, C., Mandolino, G. 2017. Genomics and Molecular Markers in Cannabis sativa L. *Cannabis sativa L.-botany and biotechnology*, 319-342.

Onofri, C., de Meijer, E.P., Mandolino, G. 2015. Sequence heterogeneity of cannabidiolic-and tetrahydrocannabinolic acid-synthase in Cannabis sativa L. and its relationship with chemical phenotype. *Phytochemistry*, *116*, 57-68.

Parker, L.A., Rock, E.M., Limebeer, C.L. 2011. Regulation of nausea and vomiting by cannabinoids. *British journal of pharmacology*, *163*(7), 1411-1422.

Perucca, E. 2017. Cannabinoids in the treatment of epilepsy: hard evidence at last?. *Journal of epilepsy research*, *7*(2), 61.

Petit, J., Salentijn, E.M., Paulo, M.J., Thouminot, C., van Dinter, B.J., Magagnini, G., Gusovius, H.J., Tang, K., Amaducci, S., Wang, S., Uhrlaub, B. 2020. Genetic variability of morphological, flowering, and biomass quality traits in hemp (Cannabis sativa L.). *Frontiers in plant science*, *11*, 102.

Punja, Z.K., Holmes, J.E. 2020. Hermaphroditism in marijuana (Cannabis sativa L.) inflorescences–impact on floral morphology, seed formation, progeny sex ratios, and genetic variation. *Frontiers in Plant Science*, 718.

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller, J., Sklar, P., De Bakker, P.I., Daly, M.J., Sham, P.C. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American journal of human genetics*, *81*(3), 559-575.

Raj, A., Stephens, M., Pritchard, J.K. 2014. fastSTRUCTURE: variational inference of population structure in large SNP data sets. *Genetics*, *197*(2), 573-589.

Ren, G., Zhang, X., Li, Y., Ridout, K., Serrano-Serrano, M.L., Yang, Y., Liu, A., Ravikanth, G., Nawaz, M.A., Mumtaz, A.S., Salamin, N. 2021. Large-scale whole-genome resequencing unravels the domestication history of Cannabis sativa. *Science advances*, *7*(29), eabg2286.

Roman, M.G., Gangitano, D., Houston, R. 2019. Characterization of new chloroplast markers to determine biogeographical origin and crop type of Cannabis sativa. *International journal of legal medicine*, *133*, 1721-1732.

Ryan, J. E., McCabe, S. E., Boyd, C. J. 2021. Medicinal cannabis: policy, patients, and providers. *Policy, Politics, & Nursing Practice*, *22*(2), 126-133.

Sawler, J., Stout, J.M., Gardner, K.M., Hudson, D., Vidmar, J., Butler, L., Page, J.E., Myles, S. 2015. The genetic structure of marijuana and hemp. *PloS one*, *10*(8), e0133292.

Schultes, R.E., Klein, W.M., Plowman, T., Lockwood, T.E. 1974. Cannabis: an example of taxonomic neglect. Botanical Museum Leaflets, Harvard University, 23(9), 337-367.

Schwabe, A.L., McGlaughlin, M.E. 2019. Genetic tools weed out misconceptions of strain reliability in Cannabis sativa: implications for a budding industry. *Journal of Cannabis Research*, *1*(1), 1-16.

Schwabe, A.L., Hansen, C.J., Hyslop, R.M., McGlaughlin, M.E. 2021. Comparative genetic structure of Cannabis sativa including federally produced, wild collected, and cultivated samples.

Frontiers in Plant Science, 2098.

Scott, M.F., Ladejobi, O., Amer, S., Bentley, A.R., Biernaskie, J., Boden, S.A., Clark, M., Dell'Acqua, M., Dixon, L.E., Filippi, C.V., Fradgley, N. 2020. Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. *Heredity*, *125*(6), 396-416.

Small, E., Cronquist, A. 1976. A practical and natural taxonomy for Cannabis. *Taxon*, 405-435.

Small, E. 2015. Evolution and classification of Cannabis sativa (marijuana, hemp) in relation to human utilization. *The botanical review*, *81*, 189-294.

Smith, C.J., Vergara, D., Keegan, B., Jikomes, N. 2022. The phytochemical diversity of commercial cannabis in the United States. *PLoS one*, *17*(5), e0267498.

Soler, S., Gramazio, P., Figàs, M.R., Vilanova, S., Rosa, E., Llosa, E.R., Borràs, D., Plazas, M. and Prohens, J. 2017. Genetic structure of Cannabis sativa var. indica cultivars based on genomic SSR (gSSR) markers: implications for breeding and germplasm management. *Industrial crops and products*, *104*, 171-178.

Soorni, A., Fatahi, R., Haak, D.C., Salami, S.A., Bombarely, A. 2017. Assessment of genetic diversity and population structure in Iranian cannabis germplasm. *Scientific reports*, *7*(1), 15668.

Stone, N.L., Murphy, A.J., England, T.J., O'Sullivan, S.E. 2020. A systematic review of minor phytocannabinoids with promising neuroprotective potential. *British Journal of Pharmacology*, *177*(19), 4330-4352.

Svendsen, K.B., Jensen, T.S., Bach, F.W. 2004. Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. *Bmj*, *329*(7460), 253.

Toth, J.A., Stack, G.M., Cala, A.R., Carlson, C.H., Wilk, R.L., Crawford, J.L., Viands, D.R., Philippe, G., Smart, C.D., Rose, J.K., Smart, L.B. 2020. Development and validation of genetic markers for sex and cannabinoid chemotype in Cannabis sativa L. *Gcb Bioenergy*, *12*(3), 213-222.

Toth, J.A., Smart, L.B., Smart, C.D., Stack, G.M., Carlson, C.H., Philippe, G., Rose, J.K. 2021. Limited effect of environmental stress on cannabinoid profiles in high-cannabidiol hemp (Cannabis sativa L.). *GCB Bioenergy*, *13*(10), 1666-1674.

Toth, J.A., Stack, G.M., Carlson, C.H., Smart, L.B. 2022. Identification and mapping of major-effect flowering time loci Autoflower1 and Early1 in Cannabis sativa L. *Frontiers in Plant Science*, *13*, 991680.

van Velzen, R., Schranz, M.E. 2021. Origin and evolution of the cannabinoid oxidocyclase gene family. *Genome Biology and Evolution*, *13*(8), evab130.

Vavilov N.I., Bukinich D.D. 1929. Agricultural Afghanistan. Bull. Appl. Bot. Genet. Plant Breeding Supp. 33: 378–382, 474, 480, 584–585, 604

Vergara, D, Huscher, EL, Keepers, KG, Pisupati, R, Schwabe, AL, McGlaughlin, ME, Kane, N. C. 2021. Genomic evidence that governmentally produced Cannabis sativa poorly represents genetic variation available in state markets. Frontiers in plant science, 12, 668315.

Walker, J.M., Huang, S.M. 2002. Cannabinoid analgesia. *Pharmacology & therapeutics*, *95*(2), 127-135.

Wickham, H. 2011. ggplot2. *Wiley interdisciplinary reviews: computational statistics*, *3*(2), 180-185.

Williamson, H. F., Brettschneider, J., Caccamo, M., Davey, R. P., Goble, C., Kersey, P. J., ... & Leonelli, S. 2021. Data management challenges for artificial intelligence in plant and agricultural research. F1000Research, 10.

Woods, P., Campbell, B.J., Nicodemus, T.J., Cahoon, E.B., Mullen, J.L., McKay, J.K. 2021. Quantitative trait loci controlling agronomic and biochemical traits in Cannabis sativa. *Genetics*, *219*(2), iyab099.

Woods, P., Price, N., Matthews, P., McKay, J.K. 2023. Genome-wide polymorphism and genic selection in feral and domesticated lineages of Cannabis sativa. *G3*, *13*(2), ikac209.

Zimmerman, S. J., Aldridge, C. L., Oyler-McCance, S. J. 2020. An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. BMC genomics, 21, 1-16.

Zhang, Q., Chen, X., Guo, H., Trindade, L.M., Salentijn, E.M., Guo, R., Guo, M., Xu, Y., Yang, M. 2018. Latitudinal adaptation and genetic insights into the origins of Cannabis sativa L. *Frontiers in plant science*, *9*, 1876.

Zhang, J., Yan, J., Huang, S., Pan, G., Chang, L., Li, J., Zhang, C., Tang, H., Chen, A., Peng, D. and Biswas, A. 2020. Genetic diversity and population structure of cannabis based on the genome-wide development of simple sequence repeat markers. *Frontiers in Genetics*, *11*, 958.

Zheng, X., Levine, D., Shen, J., Gogarten, S.M., Laurie, C., Weir, B.S. 2012. A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*(24), 3326-3328.

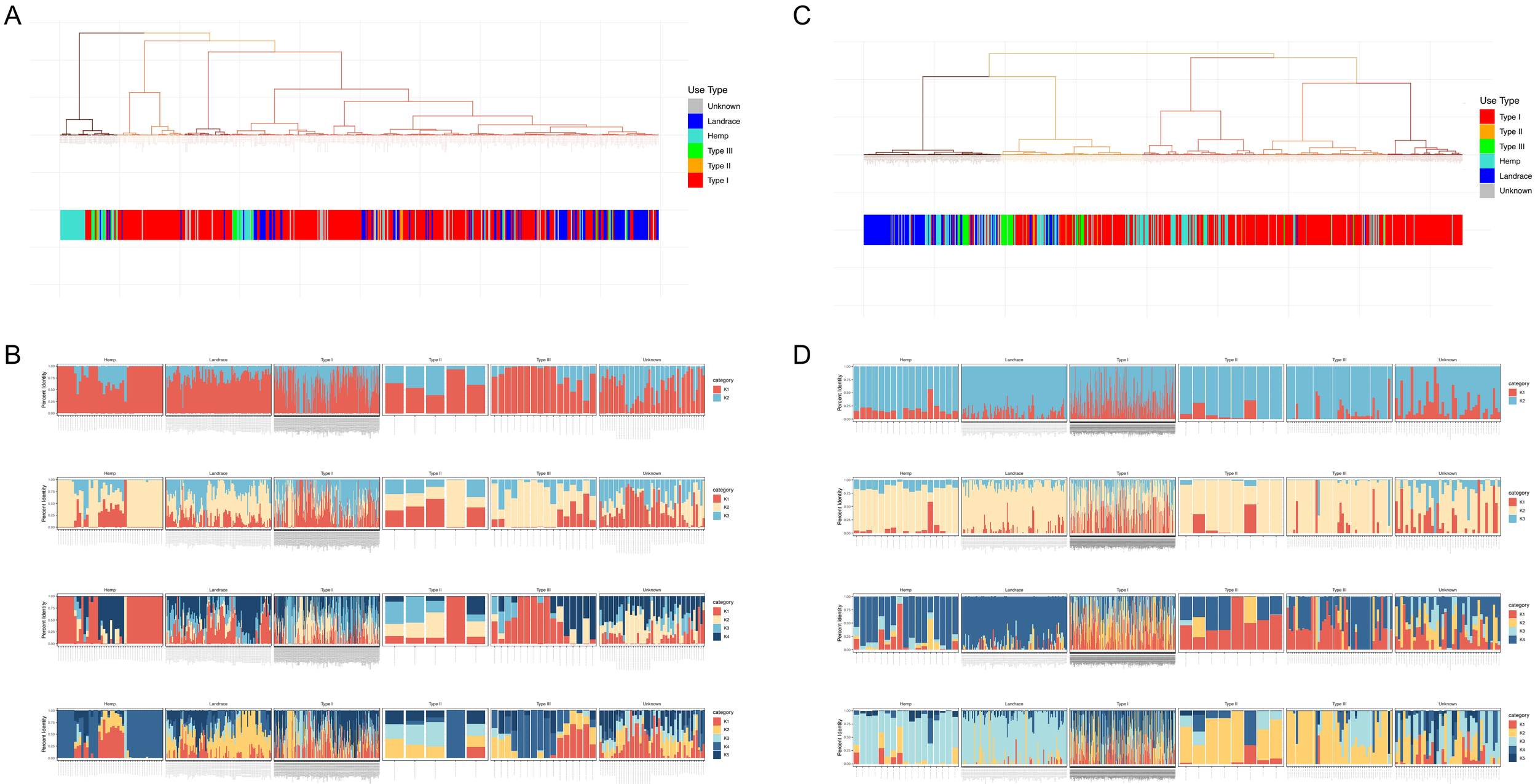

Figure 1 Examining hierarchical clustering on principal components (HCPC) and population structure in the LeafWorks Inc. (n=498) and Phylos Biosciences (n=845) datasets. In each case population genetic clustering was conducted based only on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** Hierarchical cluster dendrogram from 520 nuclear SNPs for the LeafWorks Inc. dataset with use-type indicated below. Use-type are pictured below (Type I=288, Type II =5, Type III=16, Hemp=44, Landrace=101 and Unknown=44) **(B)** Visualization of population structure and admixture from 1,405 nuclear SNPs for the LeafWorks Inc. dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 4 using the silhouette method **(Fig. S9-10) (C)** Hierarchical cluster dendrogram from 292 nuclear SNPs for the Phylos Biosciences dataset with use-type indicated below. Use-type accessions include Type I=479, Type II=8, Type III=46, Landrace=127, Hemp=143 and Unknown=42 **(D)** Visualization of population structure and admixture from 385 nuclear SNPs for the Phylos Biosciences dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the Silhouette method **(Fig. S9-10)**.

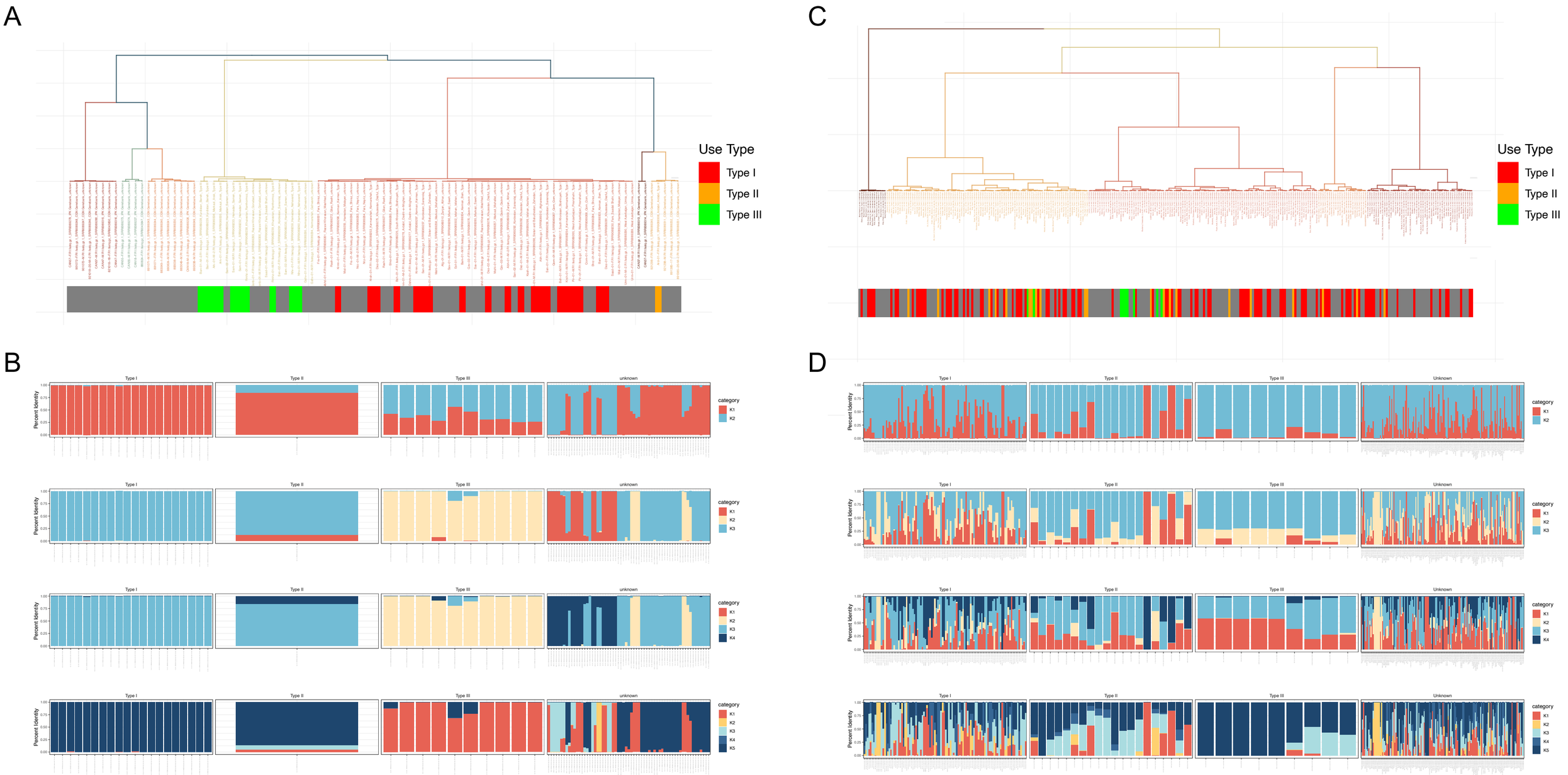

Figure 2 Examining hierarchical clustering and population structure in the Soorni et al. 2017 (n=94) and the Medicinal Genomics StrainSEEK V1 (n=289) datasets. In each case clustering was conducted based on nuclear genetic SNPs while reported use-type within the dataset is below in solid bars to facilitate interpretation based upon community standards **(A)** Hierarchical cluster dendrogram from 6,865 nuclear SNPs for the Soorni et al. 2017 dataset with use-type of each accession indicated below. Use-type are pictured below (Type I=20, Type III=10, Type II=1, Landrace=78 and Unknown=63) **(B)** Visualization of population structure and admixture from 33,629 nuclear SNPs for the Soorni et al. 2017 dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method **(Fig. S9-10)** **(C)** Hierarchical cluster dendrogram from 5,045 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset with use-type indicated below. Use-type of accessions include Type I=108, Type III=9, Type II=17 and Unknown=155 **(D)** Visualization of population structure and admixture from 20,566 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset using the fastSTRUCTURE software (k=2-5) with the optimal number of K being 3 using the silhouette method **(Fig. S9-10)**.

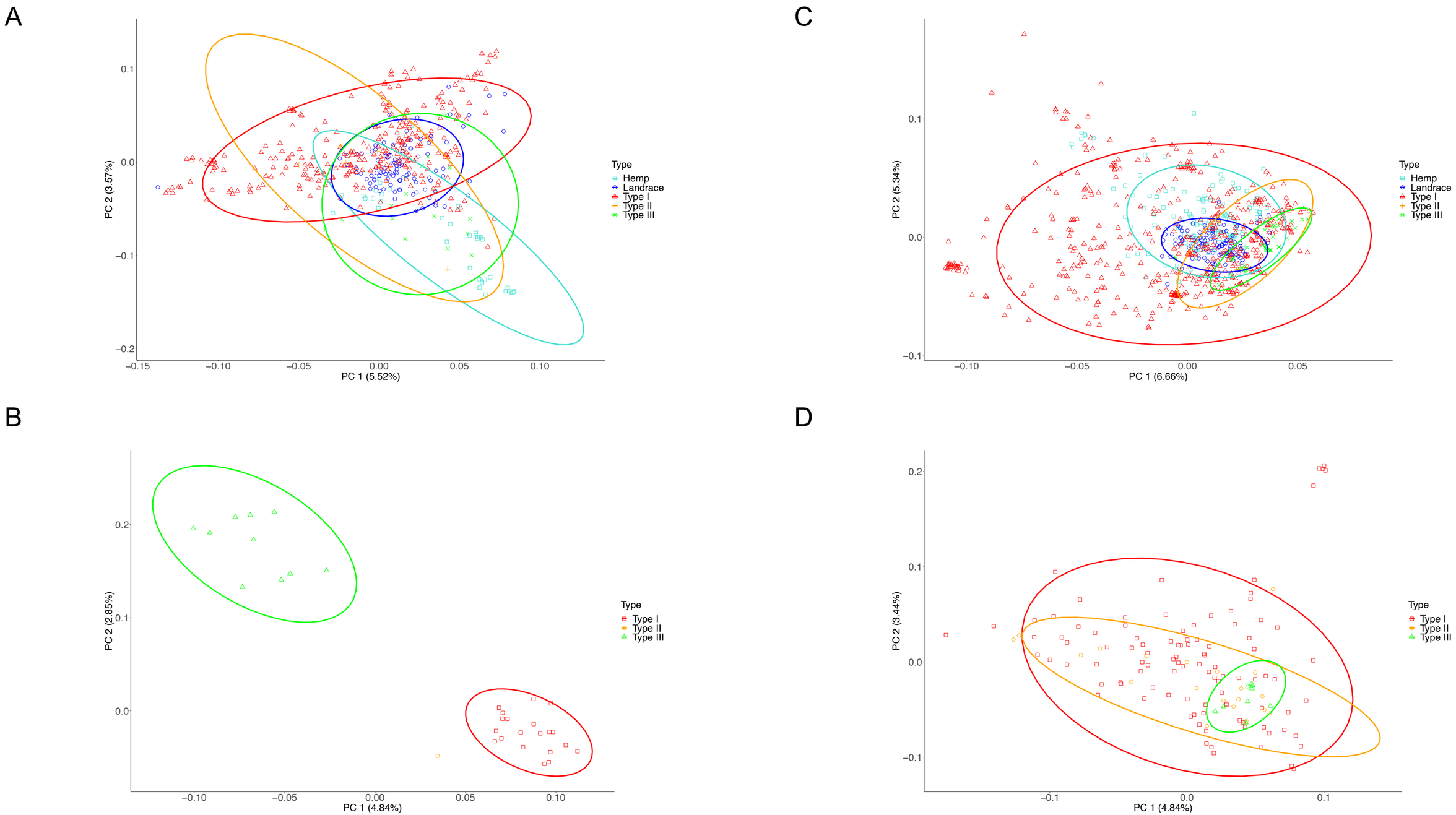

Figure 3 Examination of use-type association across datasets **(A)** Principal component analysis (PCA) from 520 nuclear SNPs for the LeafWorks Inc. dataset **(B)** PCA from 213 SNPs Phylos Biosciences (n=845) dataset **(C)** PCA from 6,865 nuclear SNPs for the Soorni et al. 2017 dataset where cannabinoid content could be determined due to recent publication for 31/94 samples. **(D)** PCA from 5,045 nuclear SNPs for the Medicinal Genomics StrainSEEK V1 dataset.

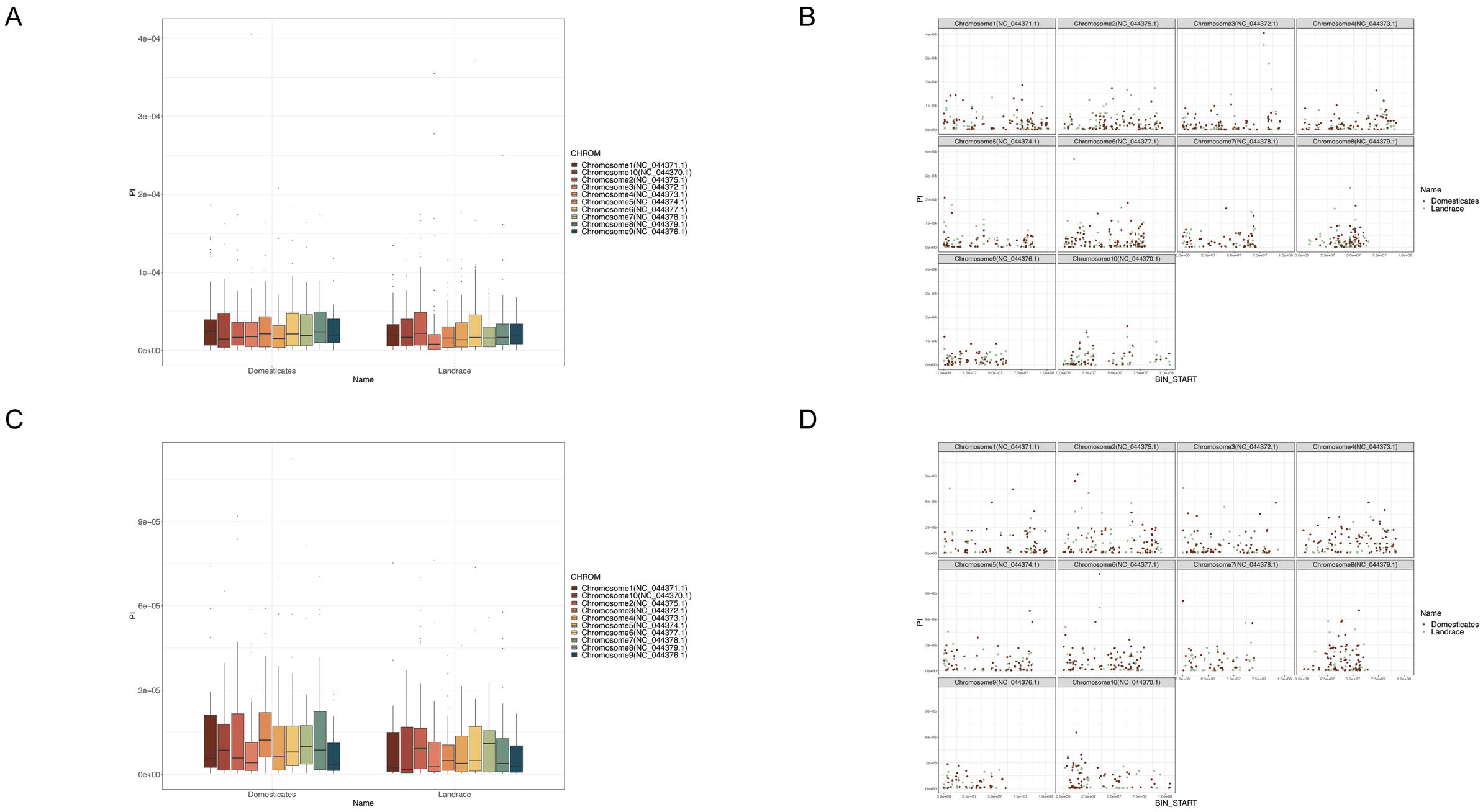

Figure 4 Nucleotide diversity as examined by a 10kb sliding window for landrace and domesticated partitions for the LeafWorks Inc. and Phylos Biosciences datasets **(A)** Nucleotide diversity by chromosome and **(B)** across chromosome length for Domesticated (n=397, 2,096 SNPs) and Landrace (n=101, 2,131 SNPs) samples for the LeafWorks Inc. dataset **(C)** Nucleotide diversity by chromosome and (D) across chromosome length for Domesticated (n=718, 749 SNPs) and Landrace (n=127, 566 SNPs) samples for the Phylos Biosciences dataset.

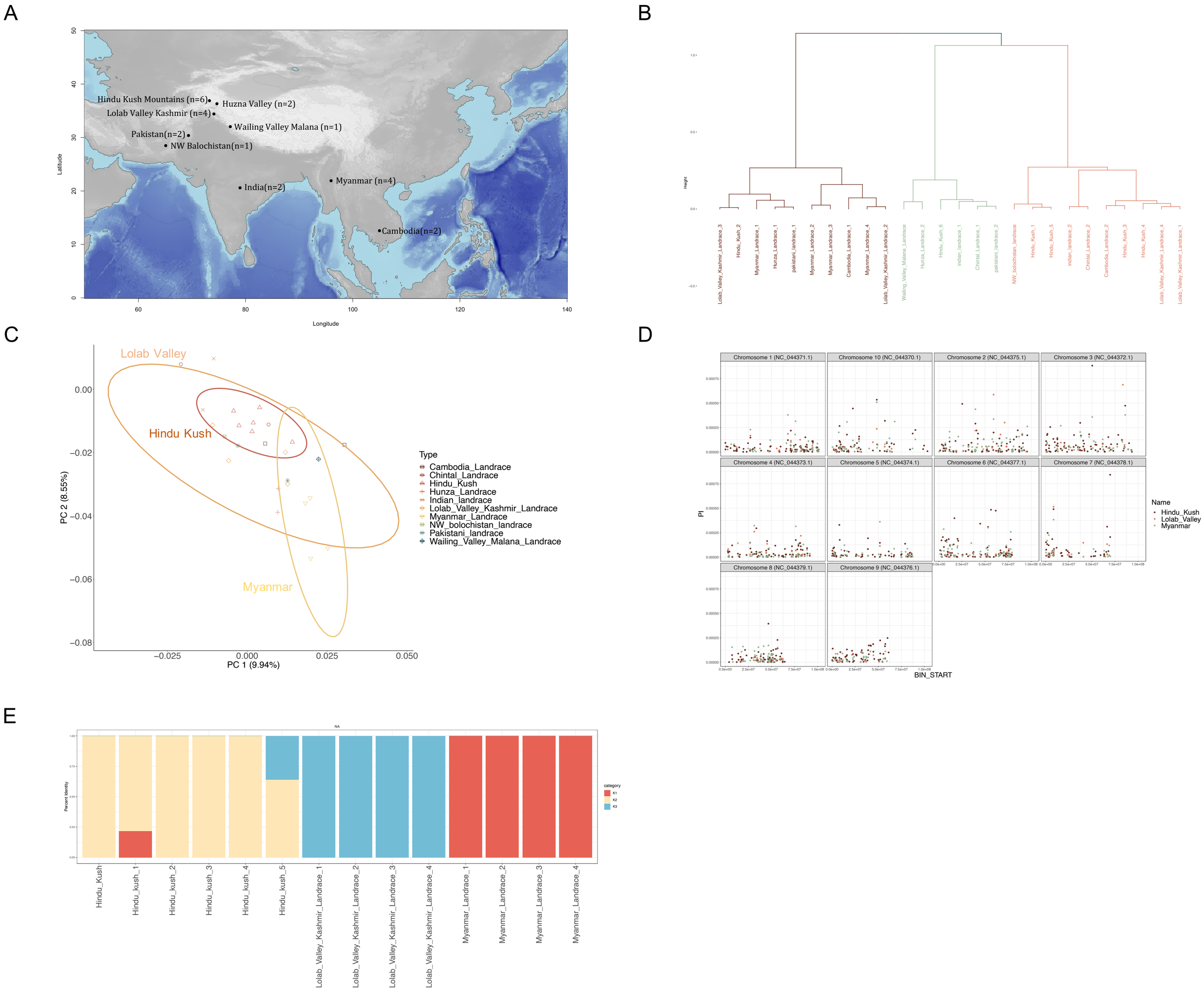

Figure 5 Landrace accessions from the LeafWorks Inc. dataset show separation between Indian and Myanmar populations **(A)** Map detailing the locations of landrace accessions, highlighted are the Hindu Kush Mountains, Lolab Valley and Myanmar **(B)** Hierarchical cluster dendrogram based on 304 SNPs (LD 0.2) across 26 samples of known and trusted origin **(C)** PCA based on 304 SNPs with geographical locations of samples as indicated **(D)** Nucleotide diversity comparison between Hindu Kush Mountains (n=6, 4,304 SNPs), Lolab Valley (n=4, 853 SNPs) and Myanmar (n=4, 2,204 SNPs) as examined by a 10kb sliding window **(E)** Visualization of population structure and admixture using the fastSTRUCTURE software (k=3) with the optimal number of K being 3 using the silhouette method.

Table 1. Definitions related to the different types of germplasm that were used in this study.

Type	Class	Definition
Feral	Used as both Non-drug and Drug type	Plants that have escaped cultivation and are now growing in the wild without human intervention. These accessions have no influence of human selection.
Landrace	Used as both Non-drug and Drug type	Cultivars are introduced to a region by humans and then become locally adapted to a specific geography over time mostly through indirect selection by farmers and natural selection.
Modern	Used as both Non-drug and Drug type	Cultivars that have been intentionally bred and selected by humans using advanced breeding techniques (genetics and statistics) with the goal of enhancing specific traits. These cultivars have been developed in recent years or decades and may not have the same regional or historical ties as landrace strains.
Hemp	This material is used for fiber - Non-drug type	Samples that had names of hemp used for grain and fiber or wild collected feral plants (no chemical analysis to confirm hemp or marijuana)
Type III	Non-drug type	(CBD-dominant): cannabis flower defined as hemp in the U.S with <0.3% THC with a wide range of CBD (average 12% 30:1 CBD:THC)
Type II	Drug-type	(CBD:THC): balanced ratio of THC:CBD (1:1)
Type I	Drug-type	(THC-dominant): modern cannabis strains found in the legal U.S. medical and adult use market (generally >10% THC, average 21% THC, usually 30:1 THC:CBD ratio)

Table 2. Data sources used for this project. Light grey indicates other public datasets which were not utilized in this study.

Data source	Dataset reference in this text	Bioproject	Number of individuals	Sequencing Platform	Type	Citation
Phylos Biosciences	Phylos Biosciences	PRJNA347566	845		Paired read	https://phylos.bio
Phylos Biosciences	Phylos Biosciences	PRJNA510566	1,378	1 ILLUMINA (NextSeq 500)	Paired read	NA
LeafWorks Inc	LeafWorks	NA	498	Illumina NovaSeq	Paired read	This manuscript
University of Tehran	Soorni *et al.*	PRJNA419020	94	Illumina HiSeq 2500	Single read	Soorni *et al*., 2017
Sunrise Genetics	Sunrise Genetics	PRJNA350539	25	Illumina HiSeq 4000	Paired read	NA
Courtagen Life Sciences	Courtagen Life Sciences	PRJNA297710	58	2 ILLUMINA (Illumina MiSeq)	Paired read	NA
University of Colorado Boulder	University of Colorado Boulder	PRJNA317659	162	1 ILLUMINA (Illumina HiSeq 2000)	Single read	Lynch *et al*., 2016
Medicinal Genomics	Medicinal Genomics (n=61)	NA	61		Paired read	www.medicinalgenomics.com/kannapedia-fastq/
Medicinal Genomics	Medicinal Genomics (strainSEEK v1)	NA	289		Paired read	www.kannapedia.net
Total			3347			

Table 3. SNP count per dataset pre and post filtering.

Dataset	Sample (n)	Total # SNPs	# SNPs post filter (0.9)	# Bi-allelic SNPs	LD (0.2)	PC 1-6 (%)
Phylos Biosciences	845	1,620,202	385	383	292	[1] 6.66 5.34 4.14 3.17 2.77 2.28
Phylos Biosciences	1,378	2,175,027	363	362	269	[1] 8.14 4.61 4.18 3.41 2.91 2.76
LeafWorks	498	10,911,876	1,405	1400	520	[1] 5.52 3.57 3.02 2.67 2.32 2.12
Soorni *et al.*	94	37,615,406	33,629	33,346	6,865	[1] 4.84 2.85 2.12 1.96 1.83 1.68
Sunrise Genetics	25	7,502,178	6,329	6,284	1,604	[1] 15.66 8.45 6.85 6.35 5.65 5.28
Courtagen Life Sciences	58	470,780,334	311	310	119	[1] 6.16 4.83 4.59 4.47 4.26 3.86
University of Colorado Boulder	162	139,508,383	5,999	5,946	2,223	[1] 5.40 3.64 3.12 2.57 2.45 2.24
Medicinal Genomics 61	61	246,261,943	8,716	8,709	2,267	[1] 4.95 3.89 2.75 2.53 2.39 2.32
Medicinal Genomics StrainSEEK V1	289	121,471,853	20,566	20,454	5,045	[1] 4.84 3.44 2.61 2.17 1.95 1.81

Table 4. SNP counts for each dataset by chromosome following biallelic sorting and Linkage Disequilibrium prune at 0.2 and mapped to CBDRx (cs10) genome.

Dataset	SNPs CHR1	SNPs CHR2	SNPs CHR3	SNPs CHR4	SNPs CHR5	SNPs CHR6	SNPs CHR7	SNPs CHR8	SNPs CHR9	SNPs CHRX	SNPs Total
Phylos Biosciences (n=845)	32	43	22	38	26	37	19	32	17	26	292
Phylos Biosciences (n=1,378)	30	41	21	43	15	31	18	28	13	29	269
LeafWorks (n=498)	65	63	51	43	51	83	38	32	46	48	520
Soorni *et al.* (n=94)	917	797	658	780	593	670	552	667	642	589	6,865
Sunrise Genetics (n=25)	191	184	176	174	136	178	138	158	117	152	1,604
Courtagen Life Sciences (n=58)	13	15	18	5	25	15	7	12	2	7	119
Lynch *et al.* (n=162)	336	338	327	304	249	291	264	114	0	0	2,223
Kannapedia 61	215	209	239	196	329	289	198	166	129	297	2,267
Medicinal Genomics StrainSEEK V1	436	528	578	526	545	543	550	367	359	613	5,045

Table 5. Partition specific (Landrace and Domesticates) SNP count per dataset pre and post filtering.

	Accession #	Total # SNPs	# SNPs post filter	# Bi-allelic SNPs	LD (0.2)
LeafWorks (Landrace_101)	101	4,761,034	2138	2131	1919
LeafWorks (Domesticates_397)	397	10,183,788	2096	2090	711
LeafWorks (Hindu_Kush_6)	6	835,656	4,304	4265	640
LeafWorks (Lolab_Valley_4)	4	502,884	853	850	170
LeafWorks (Myanmar_Burma_4)	4	617,666	2,204	2,186	384
Phylos Biosciences (Landrace_107)	107	1,027,670	267	266	219
Phylos Biosciences (Domesticates_679)	679	3,342,423	704	704	478