Chemotype vs. Genotype in Retail *Cannabis* Strains

Anna Schwabe, School of Biological Sciences, University of Northern Colorado

Introduction

The recent legalization of medical *Cannabis* in 25 states and the District of Columbia, and decriminalization in many states, has led to a surge of new strains that vary widely in appearance, taste, smell, psychotropic effects, and cannabinoid levels. As more people look to *Cannabis* for medical and recreational purposes, it is important to further explore the inconsistencies seen in the genotypes within *Cannabis* strains to determine if there are similar inconsistencies observed in the chemotypes. Research on the chemical constituents of *Cannabis*, including cannabinoids and terpenes, is abundant. However, relatively few genetic studies have been conducted and the origins and genetic identities of most *Cannabis* strains are largely unknown. This study aims to investigate genetic and chemical aspects of multiple *Cannabis* strains concomitantly to determine the relationship between genotype and chemotype.

Methods

Sampling

- 122 samples
- 30 widely available strains
- 20 dispensaries
- 8 cities across
- 3 states

Genotype

- CTAB DNA extractio
- 'Purple Kush' genome from GENBANK to identify 10 variable microsatellites
- PCR and fragment analysis (Figure 1)
- STRUCTURE assigned each individual proportions of genetic identity(Figure 2)
- STRUCTURE HARVESTER determined the number of genetic groups (K=2)
- A Principle component analysis (PCoA) show clustering for all samples with 14.29%, 9.56% and 7.07% of the variation on axes 1, 2 and 3 (not shown) respectively (Figure 3).

Chemotype

- Cannabinoids: THC, CBD, CBN, CBG, THCV, CBC, CBL
- Terpenes : Linalool, β-Myrcene, a-Pinene, D-Limonene, and β- Caryophyllene
- Sonification of flower tissue samples
- Gas Chromotography (GC)
- High Performance Gas Chromatography (HPLC)
- Cannabinoid and terpene compared to known standards using mass spectrometry

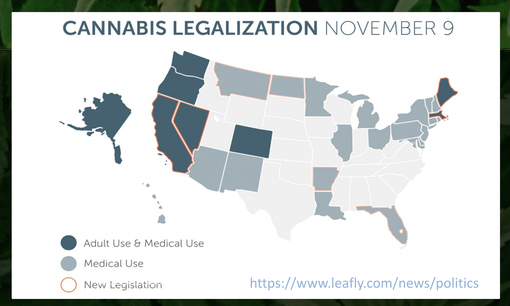


Table 1 *Cannabis* samples (122) from 30 strains with the reported proportion of Sativa retrieved from Wikileaf (2016). Strains considered pure Sativa, 50:50 Hybrid, and pure Indica are color coded with the brightest colors (red= Sativa, green= Hybrid, purple= Indica) to align with colors that Leafly (2016) uses to express the pure types of.. The proportions of membership for Genotype 1 from the STRUCTURE expressed as a percentage.

Strain	# samples	Sativa	% Genotype 1 (average)	Standard Deviation
Durban Poison*	9	100	86	9.9
Hawaiian	2	100	61	27.58
Sour Diesel*	7	90	14	53.74
Trainwreck❖	2	90	59	21.92
Island Sweet Skunk	3	80	93	9.19
AK-47	3	65	55	7.07
Golden Goat*❖	7	65	68	2.12
Green Crack❖	3	65	60	3.54
Bruce Banner*	6	60	19	28.99
Flo*	4	60	38	15.56
Jillybean	3	60	73	9.19
Pineapple Express*	5	60	62	1.41
Purple Haze	3	60	77	12.02
Tangerine	2	60	53	4.95
Jack Herer	3	55	66	7.78
OG Kush*❖	4	55	28	19.09
Blue Dream*❖	9	50	80	21.21
Tahoe OG	4	50	26	16.97
Chemdawg*	7	45	9	25.46
Headband	2	45	57	8.49
Banana Kush*	4	40	52	8.49
Girl Scout Cookies*❖	8	40	25	10.61
Jack Flash	2	40	96	39.6
Larry OG	3	40	7	23.33
G-13	3	30	50	14.14
Lemon Diesel❖	2	30	85	38.89
Hash Plant	4	20	37	12.02
Pre98-Bubba Kush	2	15	7	5.66
Grape Ape	2	0	55	38.89
Purple Kush*❖	4	0	29	20.51

*Popular Strains
❖Clone only strains

References

[1] ProCon25 Legal Medical Marijuana States and DC – Medical Marijuana – ProCon.org. 2016; 8: 1. Available from: http://medicalmarijuana.procon.org [2] Hillig KW. A chemotaxonomic analysis of terpenoid variation in Cannabis. Biochemical systematics and ecology. 2004 Oct 31;32(10):875-91 [3] de Meijer EP, Bagatta M, Carboni A, Crucitti P, Moliterni VC, Ranalli P, Mandolino G. The inheritance of chemical phenotype in Cannabis sativa L. Genetics. 2003 Jan 1;163(1):335-46 [4] Elzinga S, Fischedick J, Podkolinski R, Raber JC. Cannabinoids and terpenes as chemotaxonomic markers in cannabis. Natural Products Chemistry & Research. 2015 Aug 5;2015 [5 Schlichting CD. The evolution of phenotypic plasticity in plants. Annual review of ecology and systematics. 1986 Jan 1:667-93 [6] Wikileaf. Cannabis Strain Research Center. 2016. Available from: http://www.wikileaf.com [7] Earl DA. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation genetics resources. 2012 Jun 1;4(2):359-61 [8] Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular ecology. 2005 Jul 1;14(8):2611-20 [9] Peakall RO, Smouse PE. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular ecology notes. 2006 Mar 1;6(1):288-95 [10] Leafly. Cannabis Strain and Infused Product Explorer. 2016. Available from: https://www.leafly.com

Genotype Analyses

Cannabis strains are propagated through cloning or from stable seed stockand should therefore be essentially genetically identical no matter the source of origin. These results clearly show this is often not the case.

'Purple Kush'
Garden City 4

'Purple Kush'
Denver 1

'Purple Kush'
Garden City 3

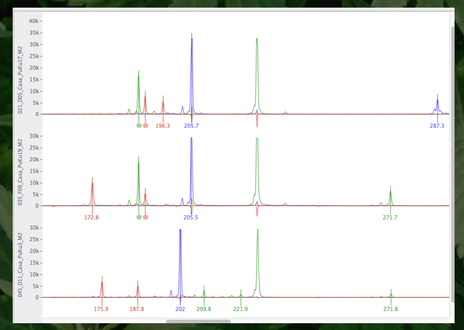


Figure 1. Fragment analysis of microsatellites in Geneious showing variability in the sizes of microsatellites amplified by PCR.

Figure 1 Bar plot graphs generated from STRUCTURE for 122 individuals from 30 strains. Each strain includes reported proportion of Sativa in parentheses (Wikileaf 2016) and each individual includes the location from where it was acquired. Each bar indicates proportion of assignment for K=2 to Genotype 1 (teal) and Genotype 2 (mustard).

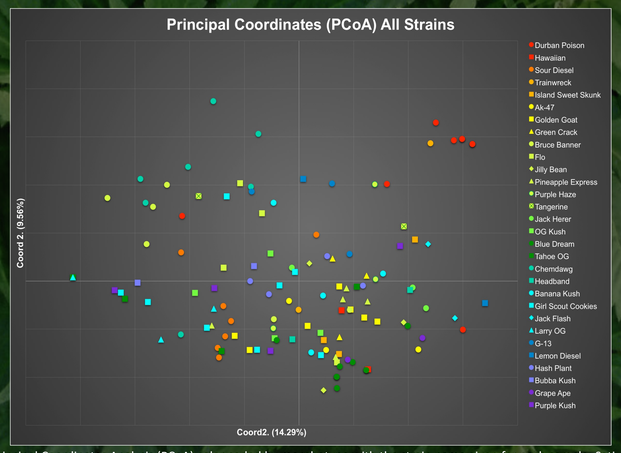
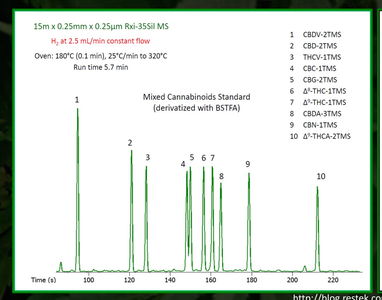
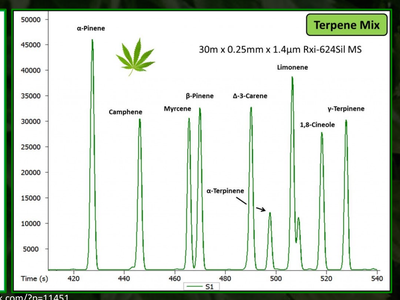


Figure 3 Principal Coordinates Analysis (PCoA) color coded by morphotype with the strain name given for each sample: Sativa type (red: 100% Sativa characteristics), Hybrid-type (dark green: 50% Sativa characteristics), and Indica-type (purple: 0% Sativa characteristics). Different symbols are used to indicate different strains within reported morphotype.

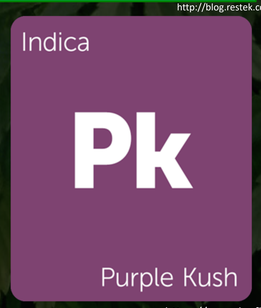
Proposed Chemotype Analyses

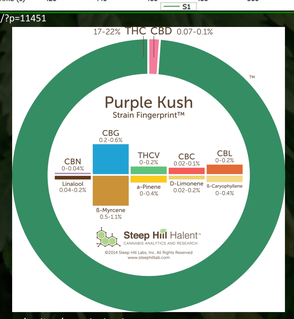
- Chemotypes of *Cannabis* strains may be similar within strains
- *Cannabis* contains hundreds of unique chemical compounds
- 66 cannabinoids and 140 terpenes
- *Cannabis* phenotypes, including chemotype can fluctuate under varying growing conditions
- Chemical profiles of each sample within a strain may be similar
- Cannabinoids (Δ^9THC, Δ^9THCA, CBD, CBDA, CBN, CBG, THCV, CBC)
- Terpenes (Linalool, β-Myrcene, a-Pinene, D-Limonene, and β-Caryophyllene).
- Determine Dif there is a relationship between the genetic identity and chemical profile
- PCoA to determine if there is clustering based on chemotype within strains
- Compare chemotypes to available online chemical profiles





http://blog.restek.com/?p=11451





https://www.leafly.com/indica/purple-kush

Aims

This study will use previously gathered genetic data from samples of popular *Cannabis* strains acquired from different sources in concert with new chemical data from Gas Chromatography (GC) and High Profile Liquid Chromatography (HPLC) analysis to examine:

(1) clustering patterns of genotype and chemotype
(2) chemical profiles of strains acquired from different sources
(3) strain assignment by combining chemotype and genotype data
(4) chemotypes of publicly available strains compared to online descriptions
(5) the accuracy of major cannabinoids (THCA, THC, CBDA and CBD) reported on retail *Cannabis*