AIMs Project Outcomes: Aims Fulfilled and Next Steps

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Background

The AIMs project arose out of the realization that hybrid chestnuts occur in naturally regenerated forests and in the orchards of chestnut growers. The hybrids in forests may occur due to sympatry with the native chinquapin species in the southern region of the previous native range of American chestnut. Hybrids in forests may also occur due to the naturalization and subsequent introgression of "intentional" hybrids, those made by chestnut growers hoping to improve the germplasm, and those made by USDA, University, and nonprofit organization scientists to study the host range of chestnut blight and to introgress the naturally occurring resistance in most Chinese chestnuts in the American chestnut. The low species barriers in *Castanea* could also have resulted in "unintentional" hybrids, those resulting from outcrossing with species and hybrids in chestnut orchards to natural forest settings. These factors plus the loss or lack of records on the location of intentional hybrids could have resulted in admixed descendants in orchards and natural settings. An additional complication is the difficulty of recognizing admixed trees, hybrid trees, or even species atypical trees by morphology alone. In the context of this report, "hybrid" means admixture consistent with F_1 hybrid. All other admixtures are simply reported as "admixed".

Aims of the AIMs Project

1. Identify and develop a set of markers, each of which are polymorphic across all *Castanea* species, reproducible, accurate, scalable, and platform independent.
2. Collect and genotype enough samples from putative "pure species" to detect admixture of species in any *Castanea* individual, at 5% or higher, for any combination of possible species.
3. Collect and genotype samples of naturally occurring American chestnut, other American chestnuts of paramount importance (e.g., Ellis), and chestnuts of interest to growers.
4. Optimize the approach to maintain accuracy, precision, and scalability while at the same time lowering the fully loaded cost per sample.

Results

The final dataset consisted of genotypes of 42 sequenced EST-SSR markers on each of 192 samples. The sample set included, as identified by the contributors, 42 *C. mollissima* (Chinese chestnut), 6 *C. henryi*, 3 *C. sequinii*, 22 *C. crenata* (Japanese chestnut), 18 *C. sativa* (European chestnut), 55 *C. dentata* (American chestnut), 13 *C. pumila* (Allegheny chinquapin), 33 *C. ozarkensis* (Ozark chinquapin), the chestnut cultivar hybrid 'Paragon' (*C. dentata*/*C. sativa*) and complex hybrid 'Luvall's Monster', of unknown ancestry. The samples included 3 sets of technical replicates and 2 sets of biological replicates.

The analysis method employed was Prichard's STRUCTURE[1], a Bayesian approach that is agnostic to human-assigned species labels. The method is not sensitive to the order of the data. This method tests the likelihood of a series of possible priors. The prior is how many groups there are (1 group, 2 groups, etc.). The likelihood of each prior is tested, then compared with the others. The analysis detects the group composition of individual samples, given the prior. Thus, admixture estimates arise directly from the analysis without regard to what the humans think. The data were scored by repeatedly sequencing

(~50x) through a simple sequence repeat (SSR) embedded in an expressed sequence to obtain accurate sequence and then counting the number of repeats.

The variation in technical and biological reps was due to missing data, not differences in allele calls. Missing data can generate "ghost admixture" estimates, the magnitude of which depend on the context of the entire dataset. In this dataset, based on the replicate data, any admixture below 3% is likely to be spooky (i.e., unlikely to reappear again).

How the groups change as K goes from 8 to 6: Examining which grouping merges or splits at different values for the number of groups reveals how "robust" a group designation is. The groups shown (p1-6) are for K = 8, the current understanding of the number of putative species the data set includes. As K goes down (p7), the only groups to disappear are *C. seguinii*, which merges into the *C. henryi* group at K = 7, then both *C sequinii* and *C. henryi* merge into admixtures of *C. mollissima* with either *C. ozarkensis* or *C. crenata*, at K = 6. The Evanno method (a method of selecting at which K value the data are most likely) chooses K = 6.[2] This result is most likely driven by the small number of *C. henryi* and *C. sequinii* samples. Alternative interpretations are premature until the sample size of these two species is increased. Note that most of admixtures detected, including the Cape Elizabeth, Maine samples, do not change across these 3 groupings. *To see figures associated with this report, visit the member page at chestnutgrowers.org.*

Aims Fulfillment

The first aim is fulfilled in all respects except the scalability. The method requires 100 samples to be cost-effective, given the next-gen sequencing approach. The second aim is fulfilled with respect to *C. mollissima* and *C. dentata*. The current collection of *C. crenata* and *C. sativa* are sufficient for the purpose of this analysis but require 10 to 20 more unrelated trees of each species for the accurate estimate of ancestry involving 3 or more species. This aim is not fulfilled with respect to *C. henryi* and *C. sequinii*. This aim is also inadequately fulfilled for *C. pumila* and *C. ozarkensis*. Ten to 15 more unrelated individuals of the Chinese chinquapins and *C. pumila* are needed. The third aim is not fulfilled in that not enough *C. dentata* could be included given the cost of the analysis. The fourth aim is unfulfilled.

Next Steps

Ron Revord at the University of Missouri and I at Notre Dame are funded to lead a participatory breeding program for chestnut growers in the central U.S. My part of this project will include the completion of aims two, three, and four above, followed by extensive genotyping of the germplasm available from growers. The latter activity will include generation of pedigrees as well as ascertainment of admixtures.

Conclusion

The results shown clearly show that unsuspected admixed *Castanea* occur in naturally regenerated forests, in the orchards of chestnut growers and in the orchards of breeding programs. Admixtures of American chestnuts and the native chinquapins are likely to be a long-standing natural result of range overlap. Some admixture with non-native *Castanea* may have preceded the appearance of ink disease and chestnut blight, at least in certain locations. Thus, consideration of what is "native", for the purpose of restoration, may be less important than consideration of ecological equivalence, at least under certain circumstances.

[1]Falush D, Stephens M, Pritchard J: Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics 2003, 164:1567-1587.

[2]Earl DA, vonHoldt BM: STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 2012, 4(2):359-361.

This report was originally submitted May 18, 2020.

Table 1. Some notable admixtures (by sample number)

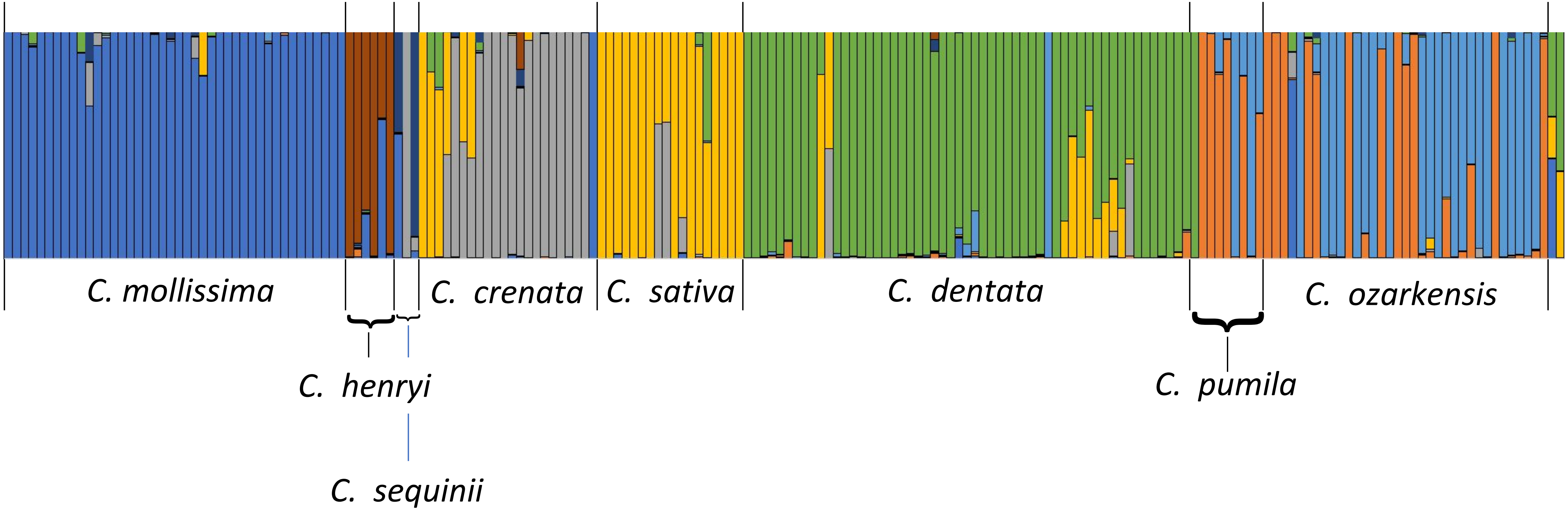
Under presumed *C. mollissima*		
11	Schmucki timber type	Admixed with *C. crenata* and *C. seguinii*/*C. henryi*
25	Chestnut cultivar Heritage	Admixed with *C. sativa*
Under presumed *C. sequinii*		
2	Tree possibly from Mo lut tsz, from China via S. Anagnostokis	Unadmixed *C. crenata*
Under presumed *C. crenata*		
22	Tree thought to be possible *C. crenata*/*C. sativa* hybrid	Unadmixed *C. mollissima*
Under presumed *C. sativa*		
8, 9	These are identical	*C. sativa*/*C. crenata* hybrid
14	Berlin sativa	*C. sativa*/*C. dentata* hybrid
Under presumed *C. dentata*		
10	Nursery stock tree	*C. sativa* admixed with *C. dentata*
11	Nursery stock tree	*C. sativa*/*C. crenata* hybrid
24	Naturally occurring tree	Evidence of admixture with *C. henryi*/*C. sequinii* (requires confirmation)
27	TACF breeding program tree	Slight admixture with *C. mollissima*
28	TACF breeding program tree	Evidence of admixture with *C. ozarkensis* (requires confirmation)
29	TACF breeding program tree	Admixed with *C. ozarkensis*
38	TACF chapter breeding program tree	Unadmixed *C. ozarkensis*
40	Cape Elizabeth, Maine	Admixed with *C. sativa*
41	Cape Elizabeth, Maine	*C. dentata*/*C. sativa* hybrid
42	Cape Elizabeth, Maine	*C. dentata*/*C. sativa* hybrid
43	Cape Elizabeth, Maine	*C. dentata*/*C. sativa* hybrid
44	Cape Elizabeth, Maine	Admixed with *C. sativa*
45	Cape Elizabeth, Maine	Admixed with *C. sativa*
46	Cape Elizabeth, Maine	Admixed with *C. sativa* and *C. crenata*
47	Cape Elizabeth, Maine	Admixed with *C. sativa*
48	Cape Elizabeth, Maine	Admixed with *C. crenata*
54	Naturally occurring progeny of native tree	Admixed with *C. pumila*
Under presumed *C. pumila* or *C. ozarkensis*		
1	Tree near Marshall, VA, presumed *C. pumila*	Unadmixed *C. dentata*
13	Progeny of *C. pumila*/Johnson *C. ozarkensis*	*C. mollissima* admixed with *C. sativa* and *C. crenata*

Overview

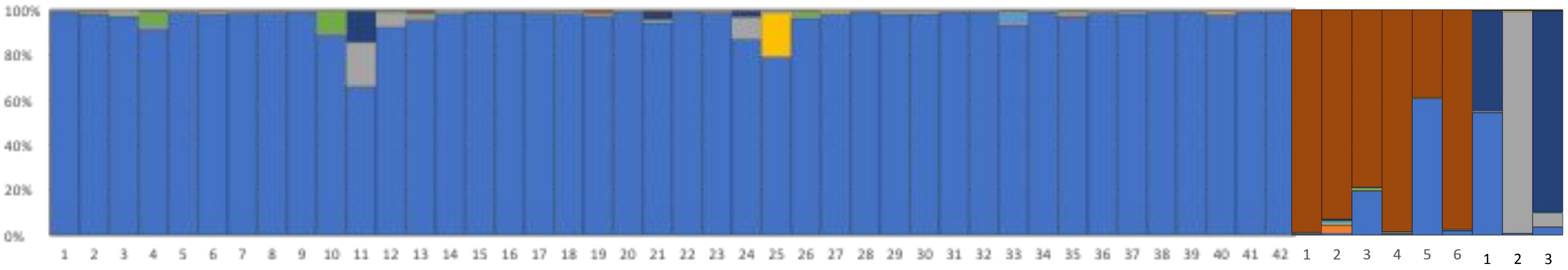
The AIMS project: 192 individuals, 42 qualified and sequenced EST-SSR markers

The individuals are grouped by putative species, detail by group follows overview

The species labels indicated on this page and in the spreadsheets on the following pages are those the collectors designated.

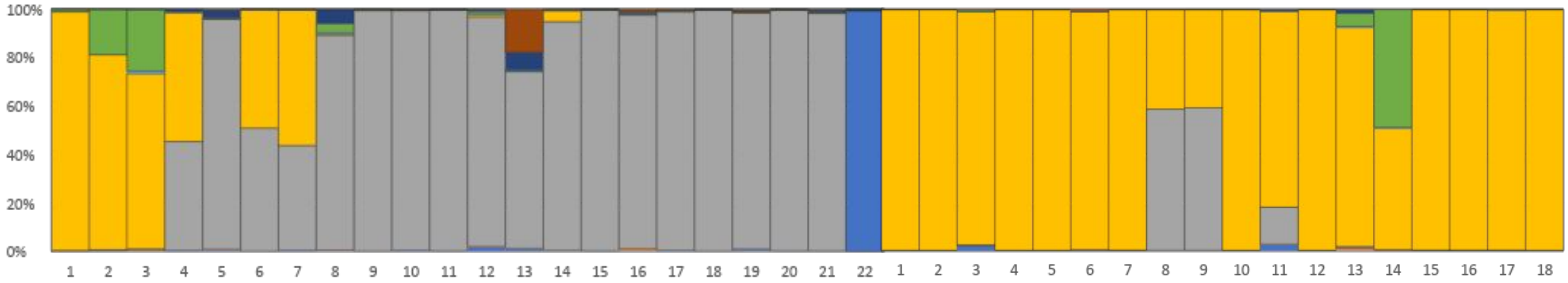


The colors indicate the groups STRUCTURE detected at $K = 8$.



1	CHC2150	Cm			Mike Nave
2	CHC2153	Cm		F1 seedling of Kintzel	Mike Nave
3	CHC2157	Cm			Mike Nave
4	CHC2162	Cm		sport from Bess	Mike Nave
5	CHC2163	Cm		seedling from nut from China	Mike Nave
6	CHC2171	Cm		F1 seedling of Dunstan Revival	Mike Nave
7	CHC2172	Cm		Seedling of nut from Yixian China	Mike Nave
8	CHC2173	Cm		Seedling of nut from Yixian China	Mike Nave
9	CHC2174	Cm		Seedling of Duanza	Mike Nave
10	CHC2201	Cm		Schmucki timber-type,	Greg Miller
11	CHC2209	Cm		N China (2001) timber-type 'Xin-T',	Greg Miller
12	CHC2211	Cm		N China (2001), NC2 blight suscept,	Greg Miller
13	CHC2213	Cm		N China (2001), NC6 blight res, vigorous,	Greg Miller
14	CHC2219	Cm		Old planting 65-4,	Greg Miller
15	CHC2220	Cm		Norris, weevil resistant,	Greg Miller
16	CHC2221	Cm		Chandler (from South Carolina),	Greg Miller
17	CHC2233	Cm		Liyuan (from China 2003),	Greg Miller
18	CHC2234	Cm		Liyuan (from China 2003),	Greg Miller
19	CHC2244	Cm		SC4 (from Nanjing Bot. Garden),	Greg Miller
20	CHC2245	Cm		Red bur B66 (from Nanjing Bot. Garden),	Greg Miller
21	CHC2259	Cm		Old orchard, largest tree 72-138,	Greg Miller
22	CHC2260	Cm		Old orchard, Gideon ortet,	Greg Miller
23	CHC2264	Cm		Old orchard, 72-226,	Greg Miller
24	CHC2266	Cm		Old orchard, Amy ortet,	Greg Miller
25	CHC2329	Cm/Cd	Dunstan Heritage	Chestnut Hill Tree Farm	R.D. Wallace
26	CHC2330	Cm/Cd	Dunstan Revival	Chestnut Hill Tree Farm	R.D. Wallace
27	CHC2331	Cm/Cd	Dunstan Willamette	Chestnut Hill Tree Farm	R.D. Wallace
28	CHC2352	Cm		Seedling of Chinese cultivar Honglizi	Mike Nave
29	CHC2355	Cm		Seedling of Chinese cultivar Jiandingyouli	Mike Nave
30	CHC2364	Cm		Seedling from nut from Hong Kong	Mike Nave
31	CHC2375	Cm		Seedling of Chinese cultivar Jiaoza	Mike Nave
32	CHC2376	Cm		Seedling of Chinese cultivar Duanza aka Duanzha	Mike Nave
33	CHC2377	Cm		Seedling of Chinese cultivar Maobauhong	Mike Nave
34	CHC2380	Cm			Mike Nave
35	CHC2420	Cm		graft	England/Miller
36	CHC2421	Cm		sdlg	Greg Miller
37	CHC2430	Cm	Vanuxem		
38	CHC2435	Cm	Nanking		
39	CHC2569	Cm		Belaire, MI	
40	NDCHC	Cm	ND2018	Biological replicate	
41	NDCHC	Cm	ND2017	Biological replicate	
42	NDCHC	Cm	ND2014	Biological replicate	
1	CHC3126	Ch		Leaves have yellow polka dots	Hill Craddock
2	CHC3127	Ch			Hill Craddock
3	CHC3128	Ch			Hill Craddock
4	CHC3130	Ch			Hill Craddock
5	CHC3134	Ch			Hill Craddock
6	CHC3135	Ch			Hill Craddock
1	CHC2229	Cg		Source may be Szego	Greg Miller
2	CHC2394	Cg	Mo lut tsz	Chiuhywashaan, Anhwei, China,	S Anagnostakis
3	CHC2395	Cg		1998 of SL R8T4 x SL R2T16, planted 1994	S Anagnostakis

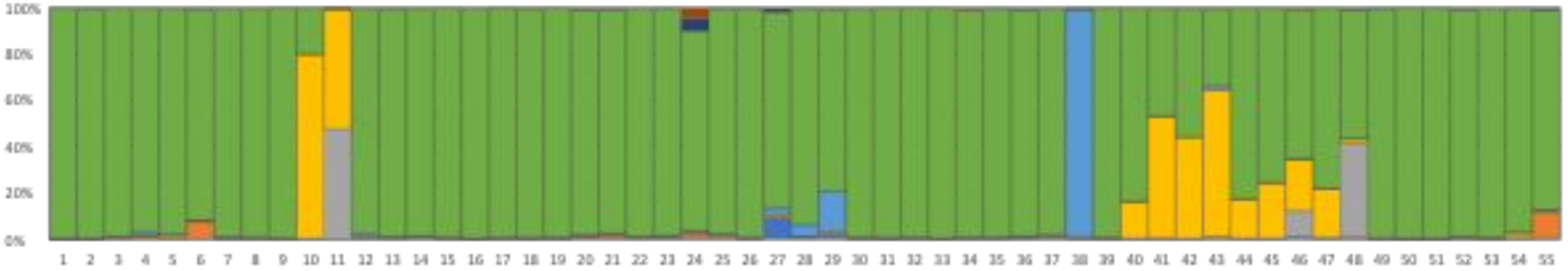
C. mollissma (Cm) *C. henryi* (Ch) *C. seguinii* (Cg) *C. crenata* (Cc) *C. sativa* (Cs) *C. dentata* (Cd) *C. pumila* (Cp) *C. ozarkensis* (Co)



1	CHC1632	Cc		Milwaukie, OR 40-60y medium-sized tree	Todd Birzer
2	CHC1633	Cc		Milwaukie, OR 40-60y medium-sized tree	Todd Birzer
3	CHC1635	Cc		Milwaukie, OR 40-60y medium-sized tree	Todd Birzer
4	CHC2100	Cc			Michael Dolan
5	CHC2102	Cc			Michael Dolan
6	CHC2114	Cc			Michael Dolan
7	CHC2115	Cc			Michael Dolan
8	CHC2183	Cc			Perkins, Miller
9	CHC2185	Cc	Gibson'	Mahn-Jo Kim 'Gibson'	Greg Miller
10	CHC2187	Cc		Mahn-Jo Kim unknown	Greg Miller
11	CHC2189	Cc		Anagnostakis “Bee & Thistle”	Greg Miller
12	CHC2191	Cc	'Ibuki'		Mahn-Jo Kim
13	CHC2194	Cc		Ok Kwang seedling	
14	CHC2369	Cc			Michael Nave
15	CHC2402	Cc		Humphrey Hill R1T7Sandy Anagnostakis	S Anagnostakis
16	CHC2404	Cc		Cheshire church, original 1876 tree	S Anagnostakis
17	CHC2408	Cc		Beatie 1929 wild trees Chitose Mura, Japan	S Anagnostakis
18	CHC2409	Cc		USDA #104016, FP 'GO', Ippoimura, Japan	S Anagnostakis
19	CHC2410	Cc		Bee and Thistle Inn, original 1876 tree	S Anagnostakis
20	CHC2412	Cc		Old Lyme Inn	S Anagnostakis
21	CHC2566	Cc			
22	CHC2161	Cc/Cs		crenata/sativa?	Michael Nave

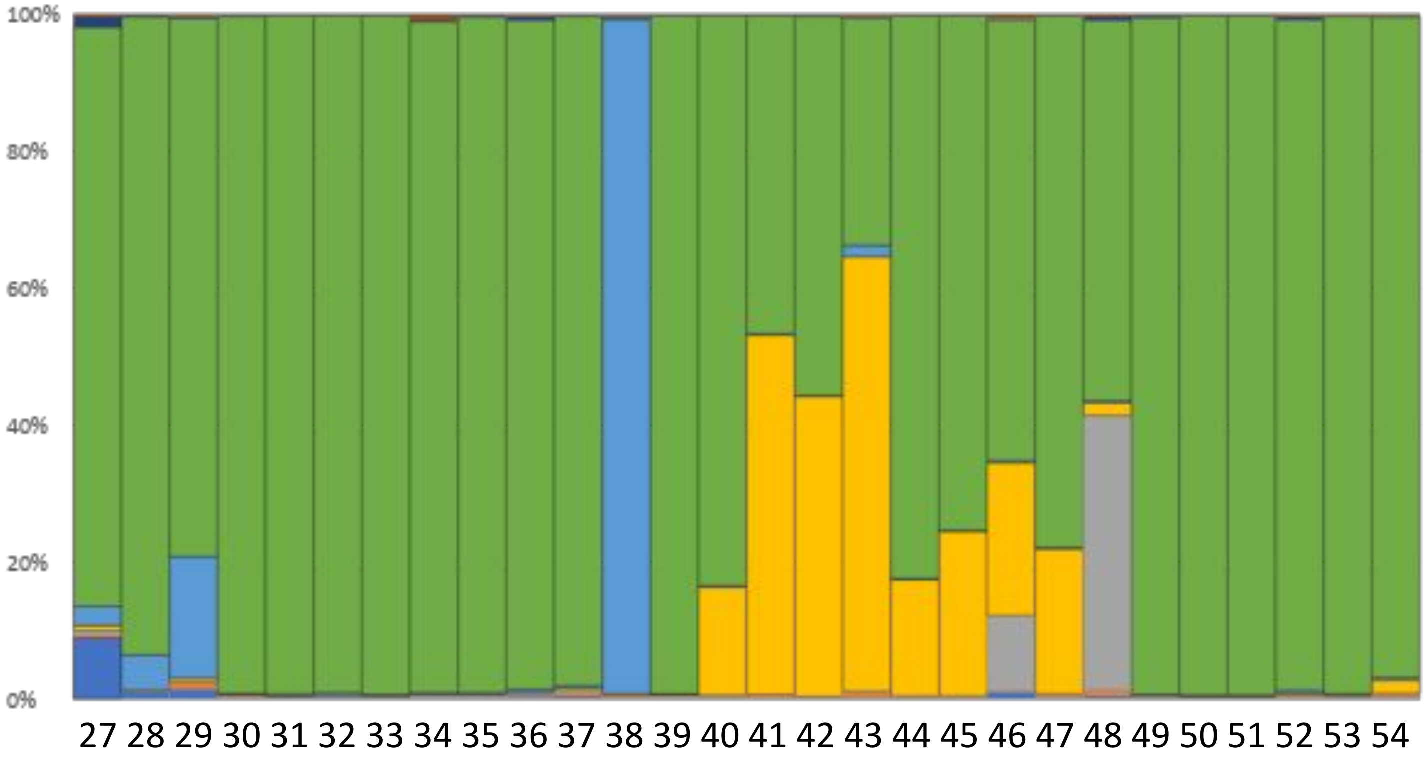
1	CHC1628	Cs		Canby, OR 50-70y old large and tall tree	Todd Bizer
2	CHC1631	Cs		Canby, OR 50-70y old large and tall tree	Todd Bizer
3	CHC2094	Cs			Michael Dolan
4	CHC2095	Cs			Michael Dolan
5	CHC2099	Cs			Michael Dolan
6	CHC2104	Cs			Michael Dolan
7	CHC2110	Cs			Michael Dolan
8	CHC2119	Cs			Michael Dolan
9	CHC2120	Cs			Michael Dolan
10	CHC2122	Cs			Michael Dolan
11	CHC2123	Cs			Michael Dolan
12	CHC2160	Cs			Michael Nave
13	CHC2179	Cs			Michael Nave
14	CHC2239	Cs		Berlin sativa	Greg Miller
15	CHC2305	Cs	marrone	Cascadia Chestnuts	Chris Foster
16	CHC2306	Cs	marrone	Cascadia Chestnuts	Chris Foster
17	CHC2307	Cs	marrone	Cascadia Chestnuts	Chris Foster
18	CHC2361	Cs			Michael Nave

C. mollissma (Cm)   *C. henryi* (Ch)   *C. seguinii* (Cg)   *C. crenata* (Cc)   *C. sativa* (Cs)   *C. dentata* (Cd)   *C. pumila* (Cp)   *C. ozarkensis* (Co)



1	CHC1660	Cd		Roxbury tree 6 (R6), Roxbury, CT	S Anagnostakis
2	CHC1664	Cd		Cornwall CT, tree C1	S Anagnostakis
3	CHC1681	Cd		Tree N3, Nepaug Reservoir, Litchfield Co., CT	S Anagnostakis
4	CHC1698	Cd		Tree G10, Goodwin State Forest, Hampton, CT	S Anagnostakis
5	CHC1706	Cd		Tree 4, Long Island, NY	S Anagnostakis
6	CHC2077	Cd		Bob Minor tree	Chuck Wilson
7	CHC2078	Cd			Gary Fernald
8	CHC2080	Cd			Gary Fernald
9	CHC2118	Cd		Burnt Ridge Nursery	Michael Dolan
10	CHC2147	Cd		Burnt Ridge Nursery	Michael Dolan
11	CHC2148	Cd		Burnt Ridge Nursery	Michael Dolan
12	CHC2199	Cd		Snedden native dentata	Greg Miller
13	CHC2240	Cd		WV state nursery dentata	Greg Miller
14	CHC2243	Cd		Rosati (from NJ)	Greg Miller
15	CHC2326	Cd		Amherst, NH	Curt Laffin
16	CHC2328	Cd		Hudson, NH	Curt Laffin
17	CHC2332	Cd		Atkinson, ME Champion tree from the site	Curt Laffin
18	CHC2333	Cd		Wilton, NH	Curt Laffin
19	CHC2336	Cd		Colchester, VT	Curt Laffin
20	CHC2336-2	Cd		Colchester, VT	Curt Laffin
21	CHC2381	Cd		Ada (Marshall), VA	Jack LaMonica
22	CHC2385	Cd		Delaplane, VA. Some Chinquapin traits	Jack LaMonica
23	CHC2389	Cd		Delaplane, VA. Near VOS and Little Cobbler (BCMT)	Jack LaMonica
24	CHC2416	Cd		Morgan County, KY	Scott Freidhof
25	CHC2417	Cd		Bath County, KY Terry Stamper	Terry Stamper
26	CHC2425	Cd			
27	CHC2605	Cd	Parent	MM12/MeadowviewBC: GL104/Meadowview: BH1B1C	TACF
28	CHC2609	Cd	Parent	Myco 10-8/MeadowviewBC: GL104/Meadowview: BH1B1C	TACF
29	CHC2619	Cd	Parent	Maddox Lilac/MeadowviewBC: GL403/Meadowview: BH1D3C	TACF
30	CHC3119	Cd		Non-transgenic C. dentata: NY	Powell
31	CHC3120	Cd		Non-transgenic C. dentata related to Ellis 1	Powell
32	CHC3121	Cd		Non-transgenic C. dentata: near Pond 1 site	Powell
33	CHC3123	Cd	Transgenic	Green Stem Tissue from transgenic C. dentata	Powell
34	CHC3125	Cd		Non-transgenic C. dentata: NY	Powell
35	CHC3125-2	Cd		Technical replicate	
36	CHC3140	Cd		Region = PA/NJ, Mother = PA-LLPi, Father = GL104	
37	CHC3156	Cd		Region = PA/NJ, Mother = PA-OrYo, Father = CL287	
38	CHC3207	Cd		Region = PA/NJ, Mother = GR-171 , Father = opAm	
39	CHC3255	Cd		Kennebunk ME	Mainers
40	CHC3256	Cd		Cape Elizabeth Maine	Mainers
41	CHC3257	Cd		Cape Elizabeth Maine	Mainers
42	CHC3258	Cd		Cape Elizabeth Maine	Mainers
43	CHC3259	Cd		Cape Elizabeth Maine	Mainers
44	CHC3260	Cd		Cape Elizabeth Maine	Mainers
45	CHC3261	Cd		Cape Elizabeth Maine	Mainers
46	CHC3263	Cd		Cape Elizabeth Maine	Mainers
47	CHC3264	Cd		Cape Elizabeth Maine	Mainers
48	CHC3265	Cd		Cape Elizabeth Maine	Mainers
49	CHC3266	Cd		Bremen ME	Mainers
50	CHC3267	Cd		Bremen ME	Mainers
51	CHC3268	Cd		Bremen ME	Mainers
52	CHC3269	Cd		Bremen ME	Mainers
53	CHC3270	Cd	Ellis	Biological replicate	Powell
54	CHC3270-2	Cd	Ellis	Biological replicate	Powell
55	CHC2204	Cd		Lee native dentata offspring	Greg Miller

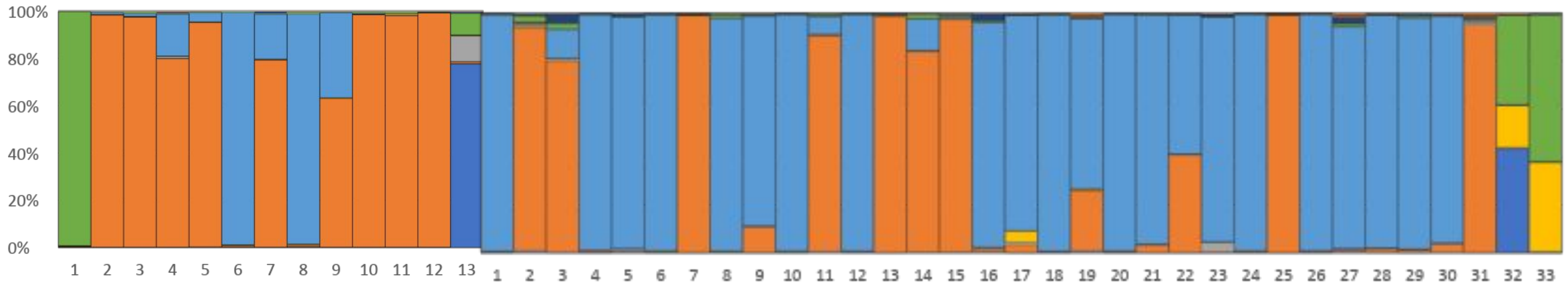
C. mollissma (Cm) *C. henryi* (Ch) *C. seguinii* (Cg) *C. crenata* (Cc) *C. sativa* (Cs) *C. dentata* (Cd) *C. pumila* (Cp) *C. ozarkensis* (Co)



"C. dentata" detail

27CHC2605	Parent	MM12/MeadowviewBC: GL104/Meadowview: BH1B1C
28CHC2609	Parent	Myco 10-8/MeadowviewBC: GL104/Meadowview: BH1B1C
29CHC2619	Parent	Maddox Lilac/MeadowviewBC: GL403/Meadowview: BH1D3C
30CHC3119		Non-transgenic C. dentata: NY
31CHC3120		Non-transgenic C. dentata related to Ellis 1
32CHC3121		Non-transgenic C. dentata: near Pond 1 site
33CHC3123	Transgenic	Green Stem Tissue from transgenic C. dentata
34CHC3125		Non-transgenic C. dentata: NY
35CHC3125		Technical replicate of 34
36CHC3140		Region = PA/NJ, Mother = PA-LLPi, Father = GL104
37CHC3156		Region = PA/NJ, Mother = PA-OrYo, Father = CL287
38CHC3207		Region = PA/NJ, Mother = GR-171 , Father = opAm
39CHC3255		Kennebunk ME
40CHC3256		Cape Elizabeth Maine
41CHC3257		Cape Elizabeth Maine
42CHC3258		Cape Elizabeth Maine
43CHC3259		Cape Elizabeth Maine
44CHC3260		Cape Elizabeth Maine
45CHC3261		Cape Elizabeth Maine
46CHC3263		Cape Elizabeth Maine
47CHC3264		Cape Elizabeth Maine
48CHC3265		Cape Elizabeth Maine
49CHC3266		Bremen ME
50CHC3267		Bremen ME
51CHC3268		Bremen ME
52CHC3269		Bremen ME
53CHC3270	Ellis	Biological replicate
54CHC3270	Ellis	Biological replicate

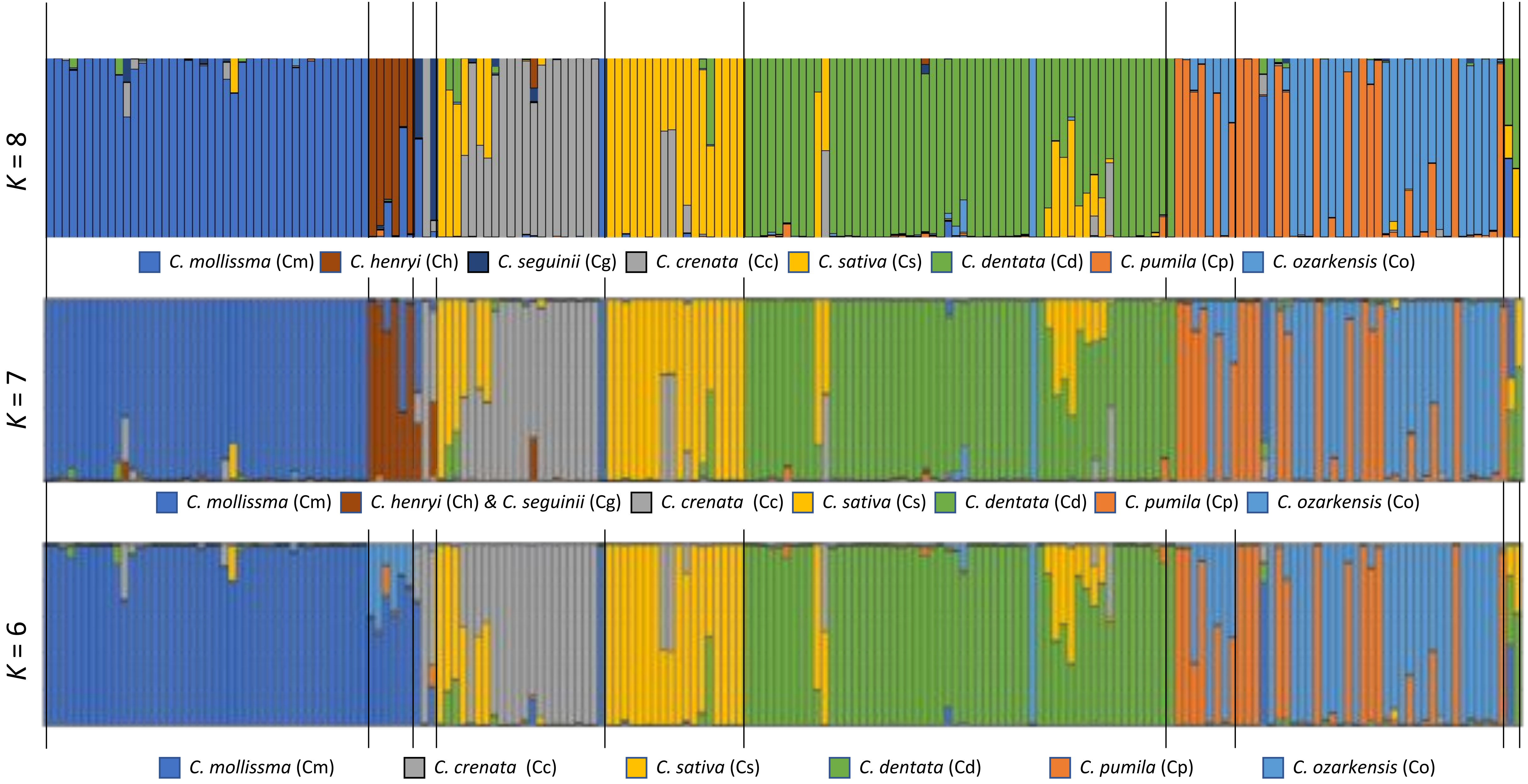
C. mollissma (Cm) *C. henryi* (Ch) *C. seguinii* (Cg) *C. crenata* (Cc) *C. sativa* (Cs) *C. dentata* (Cd) *C. pumila* (Cp) *C. ozarkensis* (Co)



1	CHC2091	Cp	Near Marshall, VA	Jack LaMonica
2	CHC2225	Cp	2nd gen local pumila, seedling	Greg Miller
3	CHC2246	Cp	1st gen local pumila, seedling	Greg Miller
4	CHC2288	Cp	C. pumila from VA, seedling	Greg Miller
5	CHC2289	Cp	“ACF” (from KY?), seedling	Greg Miller
6	CHC2295	Cp	Shibig from KY, seedling	Greg Miller
7	CHC2297	Cp	Taylor from VA, seedling	Greg Miller
8	CHC2298	Cp	Chinkapin cove unknown	Greg Miller
9	CHC2393	Cp	Delaplane, VA. Near Galanis C. dentata	Jack LaMonica
10	CHC2227	Cp/Co	George Johnson (from AR/LA)	Greg Miller
11	CHC2228	Cp/Co	George Johnson (from AR/LA)	Greg Miller
12	CHC2290	Cp/Co	Orman (from MS)	Greg Miller
13	CHC2226	Cp/Co	local x Johnson ozarkensis	Greg Miller
1	CHC2293	Co	Barnes from MO, seedling	Greg Miller
2	CHC3273	Co	JW78	MO/OCF
3	CHC3274	Co	JW77	MO/OCF
4	CHC3275	Co	3PLO	MO/OCF
5	CHC3276	Co	4K03	MO/OCF
6	CHC3277	Co	S3CC6WW	MO/OCF
7	CHC3278	Co	G16MET	MO/OCF
8	CHC3279	Co	24851CMM	MO/OCF
9	CHC3280	Co	9JLBS	MO/OCF
10	CHC3281	Co	CW366SCC	MO/OCF

11	CHC3282	Co	H28-R	MO/OCF
12	CHC3283	Co	1T0SS	MO/OCF
13	CHC3284	Co	KS3GLA	MO/OCF
14	CHC3285	Co	C2D5	MO/OCF
15	CHC3286	Co	1JDG	MO/OCF
16	CHC3288	Co	C38M	MO/OCF
17	CHC3289	Co	2J3B	MO/OCF
18	CHC3290	Co	T46WLEA	MO/OCF
19	CHC3291	Co	4E1E	MO/OCF
20	CHC3292	Co	3R7AOR	MO/OCF
21	CHC3292-2	Co	Technical replicate	MO/OCF
22	CHC3293	Co	O54BO	MO/OCF
23	CHC3294	Co	4M2FF	MO/OCF
24	CHC3295	Co	S4S6	MO/OCF
25	CHC3297	Co	L8BJD	MO/OCF
26	CHC3298	Co	1TOS	MO/OCF
27	CHC3299	Co	4D4T	MO/OCF
28	CHC3300	Co	2JB4	MO/OCF
29	CHC3301	Co	T45C	MO/OCF
30	CHC3302	Co	D76H	MO/OCF
31	CHC3303	Co	JH-2CM	MO/OCF
32	CHC3271	H	Luvall's Monster	Greg Miller
33	CHC3272	H	Paragon	Greg Miller

C. mollissma (Cm) *C. henryi* (Ch) *C. seguinii* (Cg) *C. crenata* (Cc) *C. sativa* (Cs) *C. dentata* (Cd) *C. pumila* (Cp) *C. ozarkensis* (Co)


K = 8
C. mollissma (Cm) C. henryi (Ch) C. seguinii (Cg) C. crenata (Cc) C. sativa (Cs) C. dentata (Cd) C. pumila (Cp) C. ozarkensis (Co)
K = 7
C. mollissma (Cm) C. henryi (Ch) & C. seguinii (Cg) C. crenata (Cc) C. sativa (Cs) C. dentata (Cd) C. pumila (Cp) C. ozarkensis (Co)
K = 6
C. mollissma (Cm) C. crenata (Cc) C. sativa (Cs) C. dentata (Cd) C. pumila (Cp) C. ozarkensis (Co)