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Genetic Basis for High Limonene - Cineole Content of Exceptional *Mentha citrata* Hybrids

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Summary. Most *Mentha* species have 1–25% 1-limonene and 0.5–8% 1,8-cineole, but 19 individuals having more than 50% limonene-cineole were found in a progeny of 10,000 Strain 2 *M. citrata*–*M. crispa* F_1 hybrids. When the same strain of *M. citrata* (2n = 96) having the genotype $I_1I_1\ i_2i_2$, a lavender herbage odor with oil assaying 30% linalool and 58.5% linalyl acetate, is hybridized with the closely related octoploid species *M. aquatica* ($2n = 96$) having the genotype $i_1i_1\ i_2i_2$, a menthofuran herbage odor with oil assaying 65–80% menthofuran, the fertile F_1 hybrids should have the genotype $I_1i_1\ i_2\ i_2$ and a lavender odor with oil assaying 84–90% linalool–linalyl acetate. In addition to 111 normal lavender-odored hybrids, this cross gave one individual (Strain 38) having 20.4% limonene and 36.4% cineole and one individual (Strain 625) having 67.5% limonene and 23.6% cineole. Since *M. aquatica* is homozygous for menthofuran production, and since Strain 38–*M. aquatica* backcross progenies had the disomic ratio of 1 limonene and cineole-odored: 1 methofuran-odored, it is evident that the 57% limonene–cineole content of Strain 38 is due to a single dominant gene *Lm*. Strains 38 and 625 were hybridized with other tester species having known genotypes for other oil constituents to demonstrate that the gene *Lm* prevents the conversion of limonene to more advanced compounds, namely: carvone, pulegone, methofuran, menthone, menthol, and menthyl acetate which are normally developed in the oil of other species having the recessive gene *lm*. Strain 38 hybrids with *M. citrata* show that the dominant *I* gene interrupts oil biogenesis at an earlier stage than the *Lm* gene and largely prevents the synthesis of limonene and cineole. Nine of 21 strains having 57–94% limonene–cineole were investigated. Strains 38 and 62 had the genotype $i_1i_1\ i_2i_2\ Lm_1\ lm_1\ lm_2\ lm_2$ or $i_1i_1\ i_2i_2\ lm_1\ lm_1\ Lm_2\ lm_2$, whereas Strain 625 and six others had the genotype $i_1i_1\ i_2\ i_2\ Lm_1lm_1\ Lm_2\ lm_2$. These segregants from the segmental allopolyploids may be explained by assuming that *M. aquatica* has the genotype $\frac{lm\text{-}A\text{-}i}{lm\text{-}A\text{-}i}\ \frac{lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$ and Strain 2 of *M. citrata* the genotype $\frac{Lm\text{-}A\text{-}I}{Lm\text{-}A\text{-}I}\ \frac{lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$ with *A* and *a* designating the non-homologous centromere regions of the two chromosome pairs carrying the linked genes on different chromosome arms. Crossing over between the genes would not be detectible when there is normal autosyndetic bivalent pairing, whereas occasional quadrivalent pairing of the four chromosomes of Strain 2 of *M. citrata* could lead to gene interchanges between chromosomes non-homologous for the centromere region.

Introduction

Most strains of *Mentha* species have 1–25% 1-limonene and 0.5–8% 1,8-cineole with the total of two oil components seldom exceeding 25–30%, but 19 individuals having more than 50% limonene-cineole were found in a progeny of 10,000 Strain 2 *M. citrata*–*M. crispa* F_1 hybrids (Murray and Lincoln 1970). Two other high limonene–cineole individuals were found in a progeny of 113 Strain 2 *M. citrata*–Strain 1 *M. aquatica* F_1 hybrids. The purpose of the present research was to determine the genetic basis for the 57–94% limonene–cineole content of the 21 strains.

Materials and Methods

In the subgenus *Menthastrum* of the genus *Mentha*, the spike-flowered evolutionary series consists of the species *M. longifolia* (L.) Huds. ($2n = 24$), *M. rotundifolia* L. ($2n = 24$), *M. spicata* L. ($2n = 48$), *M. crispa* L. ($2n = 48 + 6$ "B" type chromosomes in certain strains, Ikeda 1961), *M. aquatica* L. ($2n = 96$), and *M. citrata* Ehrh. ($2n = 96$). These chromosome numbers were determined by Ruttle (1931) and have since been verified by others. Ikeda (1961) not only verified the above numbers but also showed that the species *M. japonica* Makino ($2n = 48$) and *M. arvensis* L. ($2n = 96$) in the axillary-flowered series of the subgenus have the basic chromosome number of 12. All of the above species are fertile, except for the segregation of a gene causing male sterility.

All crosses were made utilizing monogenic male-sterile individuals as seed parents to avoid the need for emasculation and all possibility of self-pollination. Strain 2 of *M. citrata* is male sterile. Fertile as well as male-sterile individuals were found in the Dutch and German races of *M. aquatica* and have been maintained by vegetative propagation as clonal strains. We are indebted to Professor R. Hegnauer for Strain 1 of *M. aquatica* from Leiden, Holland, and to S. R. Baquar of Pakistan for Strain 3 of *M. aquatica* from West Germany.

All individuals in the segregating progenies were classified for herbage odor with verification of the oil composition of 4–12 individuals per culture by gas chromatography. The strong lavender odor of the herbage and oil of *M. citrata* is due to the fact that 84–90% of the oil is linalool and linalyl acetate (Todd and Murray 1968). The herbage and oil odor of *M. aquatica* is primarily that of the principal constituent menthofuran. Arctander (1969) has described 1-limonene as having a refreshing, light, very clean odor which was not reminiscent of citrus fruits, mints, or any pine; and pure 1,8-cineole as having a fresh diffusive camphoraceous, cool odor with poor tenacity. The herbage odor of the strains having a high limonene-cineole content can be described as weakly camphoraceous, bland, with a slight spearmint aroma, and without any citrus odor. The weak spearmint-like odor is limonene, since commercial spearmint oil usually has 13–23% limonene, 2–8% cineole, and 60–68% carvone (Smith, Skakum, and Levi 1963). While the odor of the high limonene-cineole strains is due to both

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oil constituents, we shall hereafter, for the sake of brevity, refer to the combination odor as a limonene odor.

Strains with linalool and linalyl acetate often have an optical rotation of $-8°$ to $-14°$, whereas the oil from the high limonene strains has an optical rotation of $-54°$ to $-74°$. This is due to the fact that pure 1,8-cineole has no effect on optical rotation and gives a 0° reading whereas pure 1-limonene has the extremely high optical rotation of $-122°$. It is possible to approximate the 1-limonene content of these strains from optical rotation. Pure menthofuran has a $+95°$ rotation.

The two principal oil constituents found in these exceptional limonene-odored individuals were identified by F. J. Cramer using a gas chromatograph with (1) a 6.1 m × 3.2 mm O.D. stainless steel column packed with Amine 220, (2) a 61 m × .25 mm I.D. column coated with 5% solution of castorwax, and (3) a 61 m × .25 mm I.D. column coated with 6% solution of polyphenyl ether 6 rings. The fact that the three columns gave similar qualitative and quantitative data for eight strains is strong presumptive evidence that the major oil constituents of these strains are 1-limonene and 1,8-cineole.

The gas chromatographic quantitative assays reported in this paper were made by D. E. Lincoln using a 7.31 m × 3.2 mm O.D. stainless steel column coated with a 3% solution of silicone DC QF-1 plus .2% Co 880 on chromosorb W High Performance 100–120 mesh with the packing dissolved in acetone. A Beckman G.C. 4 instrument equipped with a 61 m × .25 mm I.D. capillary column using Ucon HB 2000 10% loading was operated at 3 ml/min flow with the temperature programmed from 120° to 155° in 32 minutes to determine whether menthone, menthol, and menthofuran occur in the high limonene strains.

Results and Discussion

Assay data for parental species and high limonene selections: Strain 2 of *M. citrata* has 0.4% limonene, 0.3% cineole, 30.0% linalool, and 58.5% linalyl acetate. These results are in agreement with those of Handa *et al.* (1964) who showed that a different strain of *M. citrata* had 1.1% limonene, 0.2% cineole, 0.1% menthofuran, 32.4% linalool, and 45.0% linalyl acetate.

The oil of Strain 1 of *M. aquatica* has 5.6% limonene, 4.3% cineole, 4.5% piperitone, 83.0% menthofuran, 0.8% 1-menthol, and no measurable quantity of 1-menthone, d-isomenthone, neomenthol, 3-octanol, menthyl acetate, or sabinene hydrate. Hefendehl (1967) has shown that Strain 3 of *M. aquatica* has 5.1% limonene, 7.9% cineole, 67.75% menthofuran, and small amounts of 16 other oil constituents.

Mentha crispa has 7.9% limonene, 4.8% cineole, with 38.4–60.5% carvone, and 11.4–17.9% dihydrocarvone dependent upon the time of harvest.

The 19 *M. citrata*–*M. crispa* F_1 hybrid individuals having high limonene were vegetatively propagated and grown in 1/100-hectare plots. The 2-year averages of total limonene and cineole were as follows: 58, 67, 68, 73, 75, 77, 78, 79, 82, 84, 85, 85, 88, 88, 90, 90, 90, 91, 94. The nine most vigorous strains vary in limonene content from 20–84% (Table 1). The cineole content varies from 7–36%. While there is 3–10% yearly biological variation in the amount of limonene produced by a strain, the clonal strains have retained their specific characteristics for six years. To illustrate, Strain 27 always has a very high limonene – low cineole content, whereas Strain 42 always has a limonene content that barely exceeds the cineole content.

Table 1. *Limonene and cineole content of the oil of nine selected high limonene strains and their parental species*

Strain No.	Origin	Limonene %	Cineole %
27	Strain 2 *M. citrata* × *M. crispa*	84.0	10.4
28	Strain 2 *M. citrata* × *M. crispa*	77.5	13.3
41	Strain 2 *M. citrata* × *M. crispa* S_1	63.1	19.3
42	Strain 2 *M. citrata* × *M. crispa*	37.4	25.6
62	Strain 2 *M. citrata* × *M. crispa* S_1	76.2	14.1
130	Strain 2 *M. citrata* × *M. crispa*	62.3	15.4
136	Strain 2 *M. citrata* × *M. crispa*	80.4	7.7
38	Strain 2 *M. citrata* × Strain 1 *M. aquatica*	20.4	36.4
625	Strain 2 *M. citrata* × Strain 1 *M. aquatica*	67.5	23.6
Parental species Strain 2 *M. citrata*		0.4	0.3
Parental species Strain 1 *M. aquatica*		5.6	4.3
Parental species Strain 3 *M. aquatica*		9.1*	9.4
Parental species *M. crispa*		7.9	4.8

* Slightly higher than Hefendehl (1967) found for this strain

The first seven strains in Table 1 were selections from the F_1 *M. citrata* ($2n = 96$)–*M. crispa* ($2n = 48$) hybrids and should have an allohexaploid chromosome number ($2n = 72$). These strains are highly sterile and difficult to use in a genetic study.

Strains 38 and 625 are F_1 hybrids between male-sterile, seed-fertile *M. citrata* ($2n = 96$) and wholly fertile *M. aquatica* ($2n = 96$). Strain 38 is completely fertile, whereas Strain 625 is seed fertile and male sterile. Strain 38 had the highest cineole (36.4%) content of all 21 strains. The 67.5% limonene content of Strain 625 is 16.5% below the 84% of Strain 27, but the total limonene–cineole content of Strain 625 is 91.1% and that of Strain 27 is 94.4%.

Genetic data for fertile strains: Male-sterile and fertile individuals of Strain 1 (Dutch origin) and Strain 3 (German origin) of *M. aquatica* are maintained by vegetative propagation. All parental individuals have a strong menthofuran herbage odor since menthofuran is the principal oil constituent. The S_1 progeny of fertile individuals, the sib crosses of male-sterile by fertile individuals, and strain intercrosses are alike in showing that *M. aquatica* is true breeding for a strong menthofuran herbage odor. No exceptions have been found in over 7,000 individuals.

Strain 2 *M. citrata*–Strain 1 *M. aquatica* F_1 hybrids in Cross 1 of Table 2 consisted of 111 lavender-odored individuals like the *M. citrata* parent and two high limonene–cineole individuals unlike either

Table 2. *Data to determine genetic basis for high limonene content of Strains 38 and 625*

Cross	Origin	Number of progeny with Limonene odor	Menthofuran odor	Ratio[++]
1	Strain 2 *M. citrata* ms* × Strain 1 *M. aquatica* ♂	2[+]	—	
2	Strain 1 *M. aquatica* ms × limonene Strain 38, an F_1 (Strain 2 *M citrata* ms × Strain 1 *M. aquatica* ♂)♂	666	648	1:1
3	Limonene Strain 625 ms, an F_1 (Strain 2 *M. citrata* ms × Strain 1 *M. aquatica* ♂) × Strain 1 *M. aquatica* ♂	519	176	3:1
4	Male-sterile limonene selections from Cross 3 × Strain 1 *M. aquatica* ♂ having 3:1 ratios (2 cultures)	100	31	3:1
5	Male-sterile limonene selections from Cross 3 × Strain 1 *M. aquatica* ♂ having 1:1 ratios (6 cultures)	615	622	1:1
6	Limonene Strain 625 ms, an F_1 (Strain 2 *M. citrata* ms × Strain 1 *M. aquatica* ♂) × Strain 3 *M. aquatica* ♂	370	123	3:1
7	Male-sterile limonene selections from Cross 6 × Strain 3 *M. aquatica* ♂ having 3:1 ratios (3 cultures)	618	200	3:1
8	Male-sterile limonene selections from Cross 6 × Strain 3 *M. aquatica* ♂ having 1:1 ratios (7 cultures)	336	334	1:1
9	Limonene Strain 625 ($Lm_1lm_1\ Lm_2lm_2$) ms × limonene Strain 38 ($Lm_1lm_1\ lm_2lm_2$) ♂	109	16	7:1

* ms = male sterile. [+] 111 individuals had linalool—linalyl acetate like *M. citrata*. The two limonene individuals are known as Strains 38 and 625.
[++] None of the *P* values for the individual cultures or their totals are significant.

parent. These two exceptional individuals have been maintained by vegetative propagation and are known as Strain 38 and Strain 625. These two limonene strains have less than 1% linalool, linalyl acetate, and menthofuran.

Strain 38 hybridized with *M. aquatica* gave a first backcross progeny of 666 limonene-odored individuals like Strain 38 and 648 menthofuran-odored like the *M. aquatica* parent as seen in Cross 2 of Table 2. The 1:1 ratio in the backcross progeny and the fact that the *M. aquatica* parent has a genotype homozygous for menthofuran production indicate that the high limonene content of Strain 38 is due to a single dominant gene *Lm*. Strain 625 hybridized with Strain 1 of *M. aquatica* gave a duplicate gene ratio of 3 limonene-odored to 1 menthofuran-odored in the first generation backcross progeny as seen in Cross 3 of Table 2. Strain 625 hybridized with Strain 3 of *M. aquatica* gave similar results in Cross 6 of Table 2. One may conclude that both strains of *M. aquatica* have the genotype $lm_1lm_1\ lm_2lm_2$ and that limonene Strain 625 has the genotype $Lm_1lm_1\ Lm_2lm_2$. The genotype of Strain 38 is either $Lm_1lm_1\ lm_2lm_2$ or $lm_1lm_1\ Lm_2lm_2$.

Eight limonene-odored first generation backcross individuals (of Cross 3) were hybridized to Strain 1 *M. aquatica* to determine their genotype. Two individuals summarized in Cross 4 gave duplicate gene ratios, whereas six individuals summarized in Cross 5 gave 1:1 ratios. Of the ten individuals tested from Cross 6, three gave duplicate gene ratios. To summarize, five individuals out of 18 tested had duplicate gene ratios, when the expectation is one third.

Strain 625 with the genotype $Lm_1lm_1\ Lm_2lm_2$ hybridized with Strain 38 with the genotype $Lm_1lm_1\ lm_2lm_2$ should give a 7:1 ratio and did in Cross 9 of Table 2.

Chemical verification of segregation based on herbage odor: The confirmation of ratios based on herbage odor may be illustrated by Cross 5 of Table 2. The six second backcross progenies having 1:1 ratios summarized together in Table 2 are given individually in Table 3 with typical assays cited for individuals from five cultures. The assays of the 14 second backcross individuals suffice to show that limonene-odored individuals may have a higher or lower limonene content than parental Strains 625 and that selection can increase or decrease the limonene content. The lower limonene content of individuals in Culture 69—1118 was evident from the herbage odor. There are no dosage effects of the gene *Lm* since all individuals in Table 3 have a single dominant *Lm* gene and 11 of the 14 assayed strains have more limonene than the parental Strain 625 with the genotype $Lm_1lm_1\ Lm_2lm_2$. Menthofuran-odored individuals from these cultures are not different from the *M. aquatica* parent and have 60 to 81.4% menthofuran.

Table 4 shows that the limonene content of the Strain 625—Strain 38 hybrids from Cross 9 of Table 2 varied from 31—62% and the cineole content from 13—21%. The average limonene content of 12 hybrids from the interstrain cross is 48% whereas the mean value of parental strains is 44%. A selection program to obtain strains having 80—90% limonene should use Strain 625—*M. aquatica* hybrids rather than Strain 625 — Strain 38 hybrids. While high

Table. 3 *Summary of the individual second backcross cultures of Cross 5 of Table 2 with typical assays of limonene-odored segregants*

Culture number	Principal odor of plants in 2nd backcross progenies		Assay data of selections		Total limonene and cineole
	Limonene	Menthofuran	Limonene	Cineole	
69-1114	149	142	72.4	6.6	79.0
			78.5	8.1	86.6
			70.2	12.2	82.4
69-1115	140	143	76.8	5.5	82.3
			68.8	12.6	81.4
			69.4	18.8	88.2
			71.7	15.0	86.7
69-1116	47	51	79.0	8.0	87.0
			80.2	7.1	87.3
69-1118	129	128	32.0	20.2	52.2
			44.5	10.5	55.0
			42.2	12.0	54.2
69-1119	139	146	71.4	3.8	75.2
			73.8	8.0	81.8
69-1120	11	12	—	—	—
Total 6 cultures	615	622			
Avg. 14 2 nd generation backcross individuals			66.5	10.6	77.1
Parental Strain 625			67.5	23.6	91.1

Table 4. *The limonene and cineole content of 12 randomly selected limonene-odored Strain 625—Strain 38 hybrids from Cross 9 in Table 2 and their parental strains*

	Limonene	Cineole	Total
Strain 625, ♀	67.5	23.6	91.1
Strain 38, ♂ parent	20.4	36.4	56.8
Average of parental strains	44.0	30.0	74.0
Strain 625 × Strain 38			
hybrid 1	61.9	13.3	75.2
2	57.2	18.8	76.0
3	56.7	20.9	77.6
4	56.3	16.8	73.1
5	54.9	15.3	70.2
6	49.6	20.1	69.7
7	47.4	18.1	65.5
8	44.1	14.7	58.8
9	44.0	14.5	58.5
10	39.7	21.3	61.0
11	32.9	16.4	49.3
12	31.1	15.6	46.7
Average of 12 hybrids	48.0	17.1	65.1

selections have no commercial value due to the low price of limonene, the effects of high-low selections on oil biogenesis are of interest.

Genetic data for seven sterile allohexaploid strains: Despite the sterility, the dominant *Lm* gene can be transferred into *M. aquatica* in a convergence program. Strain 62 definitely has the $Lm_1lm_1\ lm_2lm_2$ or $lm_1lm_1\ Lm_2lm_2$ genotype, since the Strain 62—*M. aquatica* backcross progeny consisted of 15 limonene-odored to 13 non-limonene-odored. Strain 130 has the genotype $Lm_1lm_1\ Lm_2lm_2$ since its test cross progeny had 61 limonene-odored to 19 non-limonene-odored, or a 3:1 ratio. Similar segregating progenies of 10—20 individuals for Strains 27, 28, 41, 42, and 136 indicate that these strains may have the genotype $Lm_1lm_1\ Lm_2lm_2$. To conclude, seven of the nine tested strains appear to have an $Lm_1lm_1\ Lm_2lm_2$ genotype.

Effects of dominant gene Lm on biogenesis: An original biogenetic design by Reitsema (1958) was modified and extended by Fujita (1960a, b, and 1961), but these designs based primarily on chemical structure need verification. The Fujita design assumes that the primitive acyclic constituent linalool was converted to its ester linalyl acetate or to geraniol (nerol) → alpha-terpineol with alpha-terpineol producing both cineole and limonene. He assumed on the basis of chemical structure that limonene was converted either to carvone → dihydrocarvone or to isopiperitenone → piperitenone → piperitone → pulegone → menthone with pulegone producing menthofuran and menthone producing menthol and menthyl acetate. If the dominant *Lm* gene affects an enzyme that prevents the conversion of limonene to more advanced compounds, limonene would be accumulated and the increase in cineole could be due to the fact that the antecedent compound alpha-terpineol actually produces both limonene and cineole. At least, strains with a high cineole content have less limonene. The data in Table 2 showed that the dominant gene *Lm* inhibits the formation of large amounts of menthofuran in hybrids with *M. aquatica*. The gene *Lm* should inhibit the formation of the major ketones piperitone, menthone, pulegone, and carvone found in other *Mentha* species, if limonene is a precursor compound of these ketones.

In Cross 1 of Table 5, one half of the progeny were limonene odored with 46.6—64.4% limonene, whereas the other half had a pulegone odor (*cc Aa*) rather than a musty piperitone odor (*cc aa*) like the piperitone tester strain. In a study of ketone inheritance Murray (1960) has shown that the double recessive *cc aa* produced 50—80% piperitone, the *cc AA* or *cc Aa* genotype pulegone and menthone, and the dominant *C* gene carvone. While this cross was made to determine whether the *Lm* gene prevented piperitone formation, the *cc AA* genotype of Strain 38 does not allow a direct test. Crosses 1 and 2 show that the *Lm* gene prevents the formation of the 3-oxygenated compounds pulegone and menthone, whereas Cross 3 shows that the gene prevents the formation of the 2-oxygenated compounds carvone and dihydrocarvone.

Murray and Lincoln (1970) published an abbreviated biogenetic diagram to illustrate that the dominant gene *I* almost completely prevents the conversion of linalool to more advanced oil constituents and results in the accumulation of linalool and its ester linalyl acetate. The conversion is not totally prevented since Table 1 shows that Strain 2 of *M. citrata* had 0.4% limonene and 0.3% cineole.

Table 5. *Effects of the gene Lm on chemical composition of F_1 hybrids with other species*

Cross	♀ parent × ♂ parent	Number of F_1 progeny with odor of					
		Lavender	Limonene	Carvone	Pulegone	Menthofuran	Isopinocamphone
1	High piperitone strain of *M. crispa* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ aa$ × Strain 38*		21		23		
2	High pulegone strain of *M. arvensis* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ AA$ × Strain 38		135		142		
3	High carvone strain of *M. spicata* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ Cc\ Aa$ × Strain 38		11	10	2		
4	Strain 1 *M. citrata* $I_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ AA$ × Strain 38	11	5			8	
5	Strain 2 *M. citrata* $I_1I_1\ i_2i_2\ Lm_1Lm_1\ lm_2lm_2\ cc\ AA$ × Strain 38	212	11				20
6	Strain 625⁺ × High piperitone *M. longifolia* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ aa$		19		6		
7	Strain 625 × High piperitone strain *M. crispa* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ aa$		4		1		
8	Strain 625 × High menthone strain *M. spicata* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ AA$		48		17		
9	Strain 625 × High carvone Line 1 *M. spicata* $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ Cc\ Aa$		91	21	15		
10	Strain 625 × *M. citrata* Strain 4 $I_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ cc\ AA$	20	15			8	

* Strain 38 $i_1i_1\ i_2i_2\ Lm_1lm_1\ lm_2lm_2\ cc\ AA$.

\+ Strain 625 $i_1i_1\ i_2i_2\ Lm_1lm_1\ Lm_2\ lm_2\ cc\ AA$.

While the gene I must be considered "leaky" as it allows some development of cyclic compounds, Cross 4 of Table 5 shows that the gene I prevents the development of large amounts of limonene and cineole that would otherwise be made.

In Cross 5, Strain 2 of *M. citrata* with the genotype $I_1I_1\ i_2i_2$ hybridized with Strain 38 would be expected to produce all lavender-odored F_1 hybrids. The progeny had 212 lavender-odored individuals, but 11 individuals definitely had limonene and cineole and 20 individuals had about 40% isopinocamphone with no measurable amounts of linalool and linalyl acetate. Shimizu, Karasawa, and Ikeda (1966) have described a strain of *M. aquatica* with 49% isopinocamphone. An explanation of these exceptional segregants is given later.

The genetic data for a similar series of crosses with Strain 625 substantiate the previous conclusions. Strain 38 crosses gave a total of 172 limonene-odored to 185 non-limonene-odored ($P = .5$) whereas Strain 625 crosses gave a total of 177 limonene-odored to 68 non-limonene-odored ($P = .3$).

To conclude, the gene Lm prevents the formation of 2-oxygenated compounds carvone and dihydrocarvone and the 3-oxygenated compounds pulegone, menthofuran the oxidation product of pulegone, and menthone the reduction product of pulegone. Due to the $cc\ AA$ genotype of the limonene strains, summarized tests did not determine that the Lm gene inhibited either piperitone or piperitenone. However, five limonene individuals from Cross 1 of Table 5 had 1—1.8% alpha-pinene, 2.9—10.4% beta-pinene, 46.6—64.4% limonene, 13.8—23.3% cineole, and less than 0.1% piperitenone, piperitone, pulegone, 1-menthone, 1-menthol, menthyl acetate, and menthofuran.

Postulated origin of high limonene strains: The duplicate genes found in this research indicate that the closely related species *M. aquatica* and *M. citrata* have two pairs of chromosomes with homologous areas. Ruttle (1931) and Ikeda (1961) have shown that *M. aquatica* may have bivalent chromosome pairing. The high fertility of the strains further indicates that quadrivalent association is rare. To explain the exceptional segregants in these segmental allopolyploids, we may assume that *M. aquatica* has the genotype $\frac{lm\text{-}A\text{-}i}{lm\text{-}A\text{-}i}\ \frac{lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$ and Strain 2 of *M. citrata* the genotype $\frac{Lm\text{-}A\text{-}I}{Lm\text{-}A\text{-}I}\ \frac{lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$ with A and a designating the non-homologous centromere regions of the two chromosome pairs carrying the linked genes on different chromosome arms. Subscripts need not be used to label the genes, since duplicate genes are alike except for linkage relations with other genes. Crossing over in these genotypes would not be detectible if there is autosyndetic bivalent pairing, whereas occasional quadrivalent pairing of the four chromosomes of Strain 2 of *M. citrata* could lead to gene interchanges between chromosomes non-homologous for the centromere region. Specifically, a crossover between unlike chromatids in the $A-I$ region could produce a *M. citrata* gamete having the chromosomes $Lm\text{-}A\text{-}i$ and $lm\text{-}a\text{-}i$. When this gamete was fertilized by an gamete from *M. aquatica* (or *M. crispa*), one would obtain the $\frac{Lm\text{-}A\text{-}i}{lm\text{-}A\text{-}i}\ \frac{lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$ genotype of limonene Strain 38. A crossover in the $Lm-A$ region between unlike chromatids and a second crossover in the $A-I$ region between the other unlike chromatids could produce a gamete having the chromosomes $Lm\text{-}A\text{-}i$ and $Lm\text{-}a\text{-}i$ which upon fertilization with any *M. aquatica* or *M. crispa* gamete would give the genotype of Strain 625 $\frac{Lm\text{-}A\text{-}i}{lm\text{-}A\text{-}i}\ \frac{Lm\text{-}a\text{-}i}{lm\text{-}a\text{-}i}$. If a crossover occurred in both regions between the same two unlike chromatids, a gamete could be obtained with *lm-A-i* and *lm-a-i* chromosomes. When this gamete was fertilized by a gamete from *M. crispa* having the genotype $i_1i_1\ i_2i_2\ lm_1lm_1\ lm_2lm_2\ Cc\ Aa$, F_1 hybrids could be obtained that had a carvone or a menthone—pulegone odor. One individual having the ketones carvone and dihydrocarvone and two individuals having the ketones menthone and pulegone were found in the 10,000 Strain 2 *M. citrata—M. crispa* hybrids.

A reciprocal crossover type having the gene combination *lm* and *I* would be expected, but our present knowledge of gene action does not indicate that the *lm—I* and *Lm—I* genotypes would differ greatly in oil composition. The possibility that the *lm—I* genotype causes the isopinocamphone segregants cannot be ignored, since Murray and Lincoln (1970) found 18 individuals of this kind to 19 high limonene—cineole individuals in the Strain 2 *M. citrata—M. crispa* F_1 hybrids. Cross 5 of Table 5 also had 11 limonene to 20 isopinocamphone exceptional segregants. Conversely, there does not seem to be any reasonable biogenetic explanation why the *lm—I* genotype would produce 20—40% isopinocamphone as a major oil constituent while the *Lm—I* genotype of Strain 2 of *M. citrata* produces 1—1.5% isopinocamphone, 30% linalool, and 58.5% linalyl acetate.

While this kind of segregation may appear to be due to chromosome aberration, the gene *Lm* has no noticeable effect on plant morphology after three backcrosses to *M. aquatica*. The genetic ratios also do not indicate that the *I* or *Lm* genes are associated with chromosome loss or duplication. Quadrivalent pairing could lead to a 3—1 distribution of chromosomes, but Murray and Lincoln (1970) have noted only one individual trisomic for the gene *I*. Four monosomic individuals identified by the loss of the gene *I* were severely depressed in vigor.

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