

Exploration of Multiple Treatments on the Metabolic Efficiency of the Mitochondria

Dom Fenoglio, Brian Kim, Kathleen Zhang

1 Background

The mitochondria are considered the “powerhouse” of the cell, responsible for generating the cell’s usable energy through oxidative phosphorylation, a process necessary for all biological processes, particularly in high-demand organs such as the heart, brain, and muscles. Their proper function is critical for overall health, as disruptions to their function are associated with various health issues, such as cancer, heart disease, and Alzheimer’s.

One way to examine mitochondrial function is by using the multiplexed assay platform, a laboratory method that allows researchers to measure multiple dimensions of mitochondrial activity across different substrates and energy demand conditions. By measuring respiration rates under different combinations of substrates across different experimental settings, such as genetic background and dose, researchers hope to better understand these effects on the metabolic and functional phenotypes of mitochondria.

The main motivation for our analysis is to quantitatively test hypotheses about genetic changes on mitochondrial efficiency and energy production, and whether there is evidence that genotype effects (transgenic vs. natural mice) depend on substrate and/or dose. By building a modeling framework, we hope to determine how mitochondrial efficiency varies by substrate, genotype, and dose while capturing both fixed and random sources of variation.

2 Exploratory Data Analysis

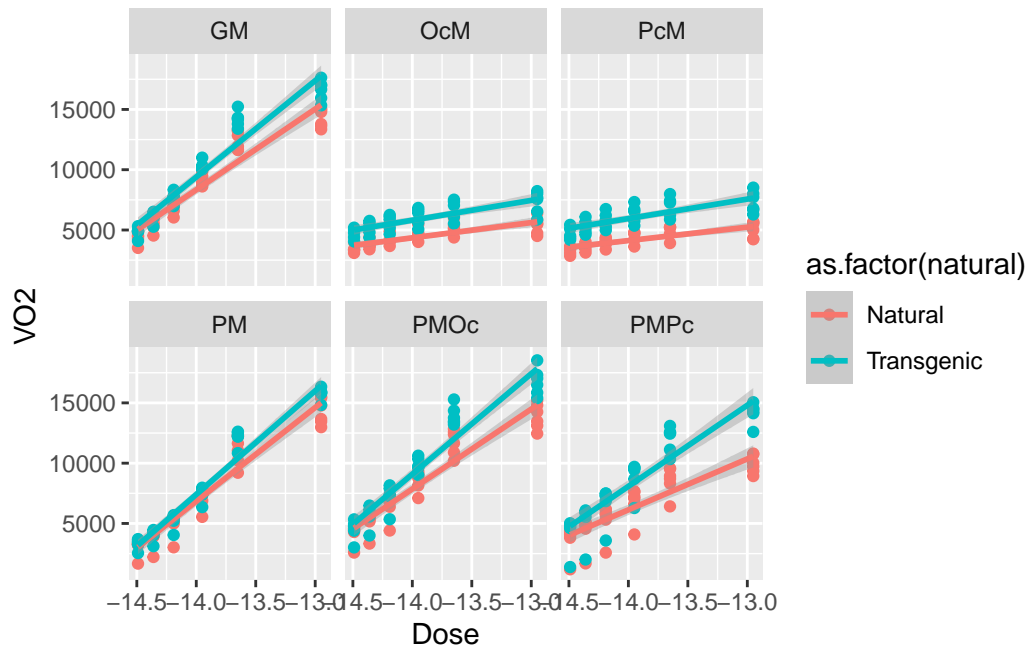
3 Modeling

4 Analysis

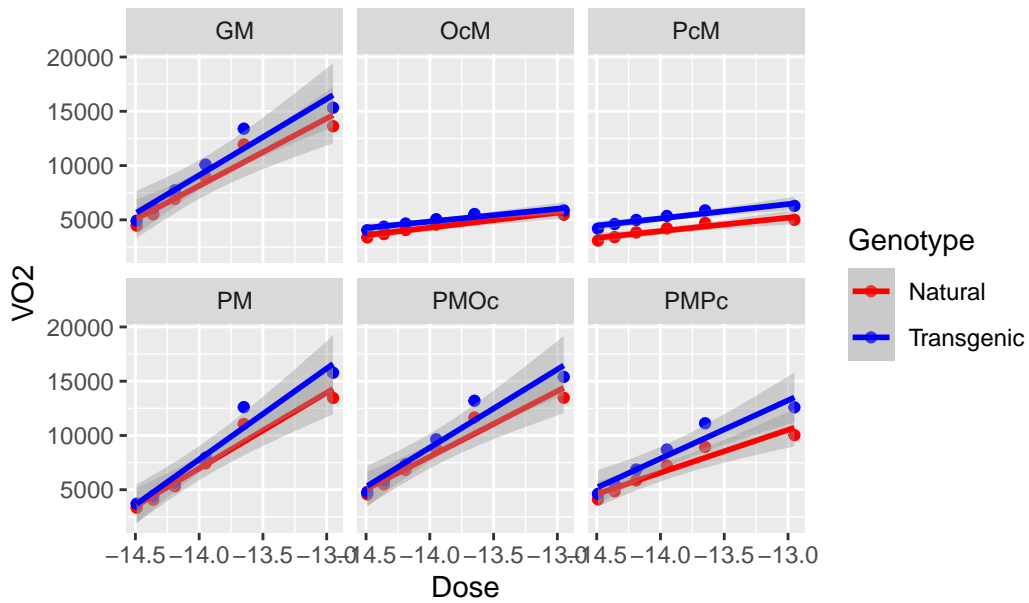
5 Conclusion and Future Work

6 Appendix

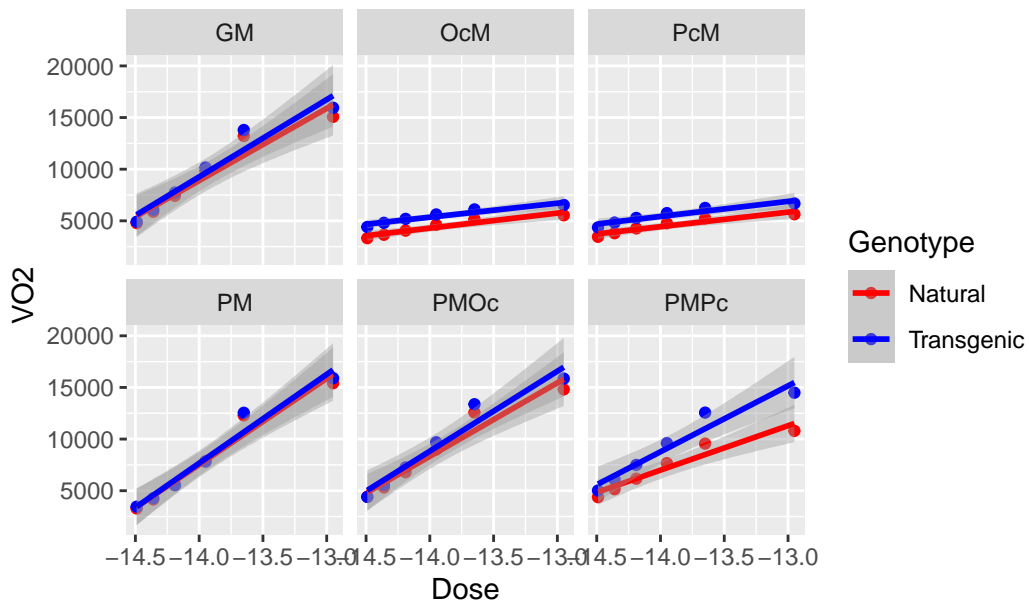
Exploratory Data Analysis



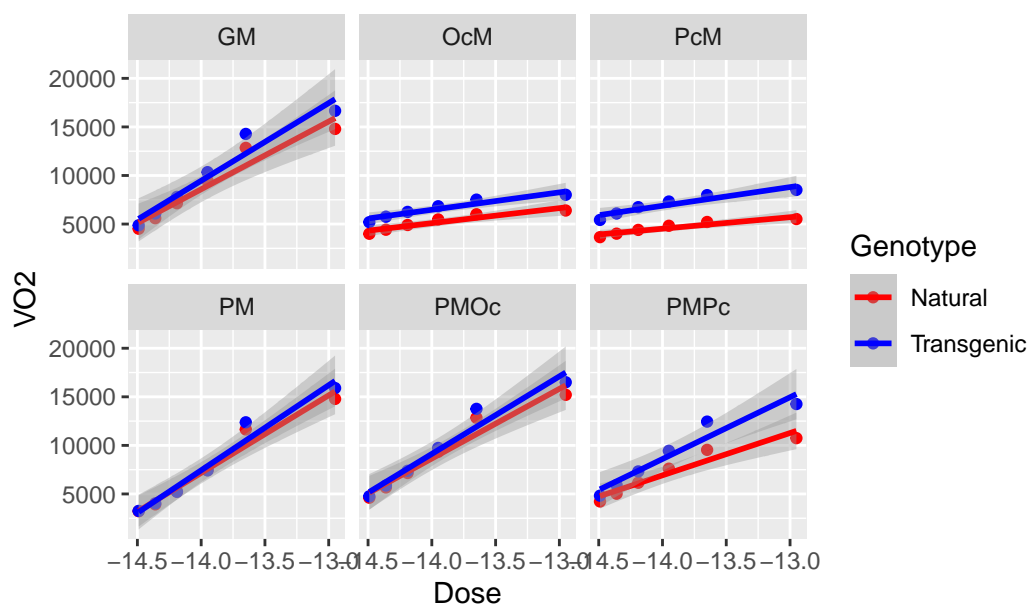
VO2 vs. Dose for pair 1



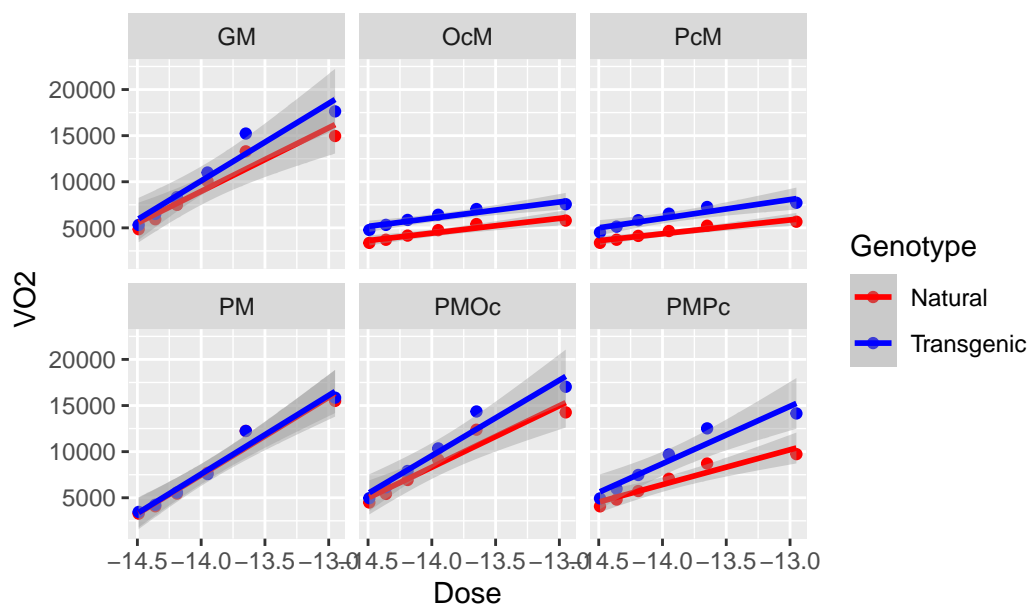
VO2 vs. Dose for pair 2



VO2 vs. Dose for pair 3



VO2 vs. Dose for pair 4



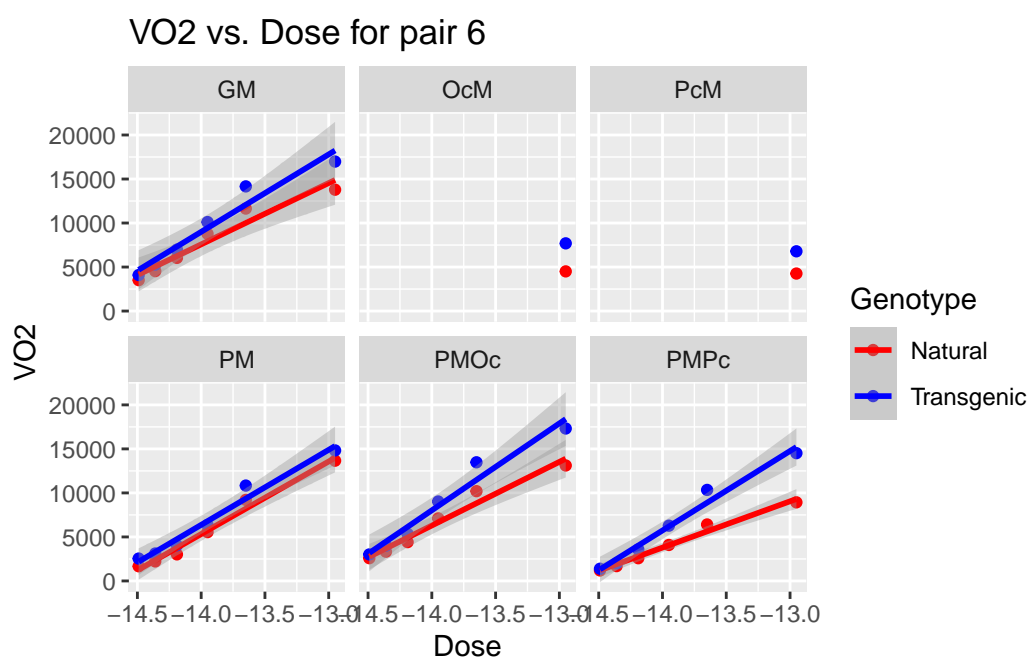
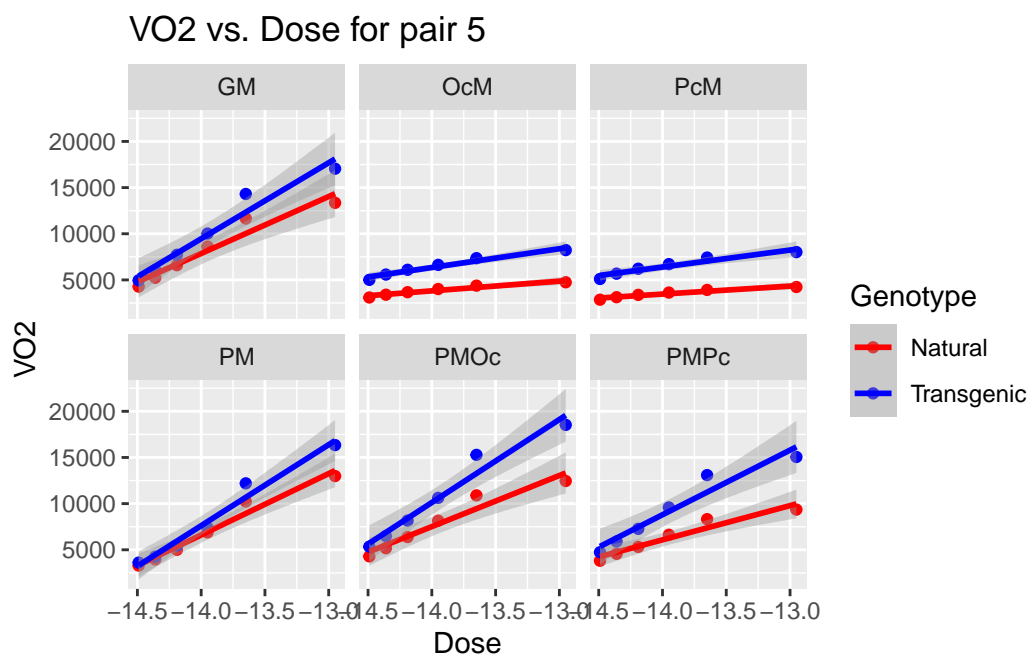


Table 1: Model comparison using AIC and Adjusted R^2

Model	df	AIC	Adjusted_R2
Amino Acids	8	7439.921	0.721

Substrate	9	7386.290	0.755
-----------	---	----------	-------

Modeling

Call:

```
lm(formula = V02 ~ Substrate:natural + Dose:natural:Substrate,
    data = data)
```

Residuals:

Min	1Q	Median	3Q	Max
-3254.1	-370.8	186.2	536.4	1727.3

Coefficients: (1 not defined because of singularities)

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	138201.0	7230.7	19.113	< 2e-16	***
SubstrateGM:naturalNatural	5077.4	10225.7	0.497	0.619863	
SubstrateOcM:naturalNatural	-104126.4	10724.8	-9.709	< 2e-16	***
SubstratePcM:naturalNatural	-107896.1	10724.8	-10.060	< 2e-16	***
SubstratePM:naturalNatural	3735.0	10225.7	0.365	0.715163	
SubstratePMOc:naturalNatural	-2949.2	10225.7	-0.288	0.773223	
SubstratePMPc:naturalNatural	-49273.5	10225.7	-4.819	2.25e-06	***
SubstrateGM:naturalTransgenic	27374.7	10225.7	2.677	0.007815	**
SubstrateOcM:naturalTransgenic	-99396.5	10724.8	-9.268	< 2e-16	***
SubstratePcM:naturalTransgenic	-95812.6	10724.8	-8.934	< 2e-16	***
SubstratePM:naturalTransgenic	14409.0	10225.7	1.409	0.159790	
SubstratePMOc:naturalTransgenic	27257.2	10225.7	2.666	0.008081	**
SubstratePMPc:naturalTransgenic	NA	NA	NA	NA	
SubstrateGM:naturalNatural:Dose	9596.5	511.7	18.755	< 2e-16	***
SubstrateOcM:naturalNatural:Dose	2110.8	560.5	3.766	0.000198	***
SubstratePcM:naturalNatural:Dose	1859.4	560.5	3.317	0.001015	**
SubstratePM:naturalNatural:Dose	9622.6	511.7	18.806	< 2e-16	***
SubstratePMOc:naturalNatural:Dose	9064.0	511.7	17.714	< 2e-16	***
SubstratePMPc:naturalNatural:Dose	5889.9	511.7	11.511	< 2e-16	***
SubstrateGM:naturalTransgenic:Dose	11110.1	511.7	21.713	< 2e-16	***
SubstrateOcM:naturalTransgenic:Dose	2346.2	560.5	4.186	3.69e-05	***
SubstratePcM:naturalTransgenic:Dose	2588.6	560.5	4.618	5.64e-06	***
SubstratePM:naturalTransgenic:Dose	10345.9	511.7	20.219	< 2e-16	***
SubstratePMOc:naturalTransgenic:Dose	11128.3	511.7	21.748	< 2e-16	***
SubstratePMPc:naturalTransgenic:Dose	9257.5	511.7	18.092	< 2e-16	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom

(20 observations deleted due to missingness)

Multiple R-squared: 0.9233, Adjusted R-squared: 0.9177

F-statistic: 165.4 on 23 and 316 DF, p-value: < 2.2e-16

Call:

lm(formula = V02 ~ natural * Dose * Substrate, data = data)

Residuals:

Min	1Q	Median	3Q	Max
-3254.1	-370.8	186.2	536.4	1727.3

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	143278.42	7230.68	19.815	< 2e-16 ***
naturalTransgenic	22297.32	10225.72	2.181	0.0300 *
Dose	9596.47	511.68	18.755	< 2e-16 ***
Substrate0cM	-109203.79	10724.83	-10.182	< 2e-16 ***
SubstratePcM	-112973.48	10724.83	-10.534	< 2e-16 ***
SubstratePM	-1342.39	10225.72	-0.131	0.8956
SubstratePM0c	-8026.61	10225.72	-0.785	0.4331
SubstratePMPc	-54350.93	10225.72	-5.315	2.02e-07 ***
naturalTransgenic:Dose	1513.62	723.63	2.092	0.0373 *
naturalTransgenic:Substrate0cM	-17567.40	15167.20	-1.158	0.2476
naturalTransgenic:SubstratePcM	-10213.84	15167.20	-0.673	0.5012
naturalTransgenic:SubstratePM	-11623.31	14461.35	-0.804	0.4221
naturalTransgenic:SubstratePM0c	7909.05	14461.35	0.547	0.5848
naturalTransgenic:SubstratePMPc	26976.20	14461.35	1.865	0.0631 .
Dose:Substrate0cM	-7485.71	758.95	-9.863	< 2e-16 ***
Dose:SubstratePcM	-7737.11	758.95	-10.195	< 2e-16 ***
Dose:SubstratePM	26.09	723.63	0.036	0.9713
Dose:SubstratePM0c	-532.46	723.63	-0.736	0.4624
Dose:SubstratePMPc	-3706.61	723.63	-5.122	5.26e-07 ***
naturalTransgenic:Dose:Substrate0cM	-1278.14	1073.31	-1.191	0.2346
naturalTransgenic:Dose:SubstratePcM	-784.42	1073.31	-0.731	0.4654
naturalTransgenic:Dose:SubstratePM	-790.30	1023.37	-0.772	0.4405
naturalTransgenic:Dose:SubstratePM0c	550.68	1023.37	0.538	0.5909
naturalTransgenic:Dose:SubstratePMPc	1854.02	1023.37	1.812	0.0710 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom

(20 observations deleted due to missingness)

Multiple R-squared: 0.9233, Adjusted R-squared: 0.9177

F-statistic: 165.4 on 23 and 316 DF, p-value: < 2.2e-16

Call:

lm(formula = V02 ~ Substrate - 1, data = data)

Residuals:

Min	1Q	Median	3Q	Max
-5366.1	-1939.5	-445.7	1327.1	7567.6

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
SubstrateGM	8155.9	346.4	23.55	<2e-16 ***
SubstrateOcM	4955.3	379.4	13.06	<2e-16 ***
SubstratePcM	4926.5	379.4	12.98	<2e-16 ***
SubstratePM	6215.9	346.4	17.95	<2e-16 ***
SubstratePMOc	7716.4	346.4	22.28	<2e-16 ***
SubstratePMPc	6563.2	346.4	18.95	<2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 2683 on 334 degrees of freedom

(20 observations deleted due to missingness)

Multiple R-squared: 0.8611, Adjusted R-squared: 0.8586

F-statistic: 345.1 on 6 and 334 DF, p-value: < 2.2e-16

Linear mixed model fit by REML. t-tests use Satterthwaite's method [lmerModLmerTest]

Formula: V02 ~ natural * Dose * Substrate + (1 | pair)

Data: data

REML criterion at convergence: 4979.4

Scaled residuals:

Min	1Q	Median	3Q	Max
-3.09075	-0.64782	0.02403	0.63668	3.05598

Random effects:

Groups	Name	Variance	Std.Dev.
pair	(Intercept)	582983	763.5
	Residual	322707	568.1

Number of obs: 340, groups: pair, 6

Fixed effects:

	Estimate	Std. Error	df	t value
(Intercept)	143278.42	4901.42	313.21	29.232
naturalTransgenic	22297.32	6917.62	310.97	3.223
Dose	9596.47	346.15	310.97	27.724
SubstrateOcM	-109503.46	7255.28	310.97	-15.093
SubstratePcM	-113273.15	7255.28	310.97	-15.613
SubstratePM	-1342.39	6917.62	310.97	-0.194
SubstratePMOc	-8026.61	6917.62	310.97	-1.160
SubstratePMPc	-54350.93	6917.62	310.97	-7.857
naturalTransgenic:Dose	1513.62	489.53	310.97	3.092
naturalTransgenic:SubstrateOcM	-17567.40	10260.49	310.97	-1.712
naturalTransgenic:SubstratePcM	-10213.84	10260.49	310.97	-0.995
naturalTransgenic:SubstratePM	-11623.31	9782.99	310.97	-1.188
naturalTransgenic:SubstratePMOc	7909.05	9782.99	310.97	0.808
naturalTransgenic:SubstratePMPc	26976.20	9782.99	310.97	2.757
Dose:SubstrateOcM	-7485.71	513.42	310.97	-14.580
Dose:SubstratePcM	-7737.11	513.42	310.97	-15.070
Dose:SubstratePM	26.09	489.53	310.97	0.053
Dose:SubstratePMOc	-532.46	489.53	310.97	-1.088
Dose:SubstratePMPc	-3706.61	489.53	310.97	-7.572
naturalTransgenic:Dose:SubstrateOcM	-1278.14	726.09	310.97	-1.760
naturalTransgenic:Dose:SubstratePcM	-784.42	726.09	310.97	-1.080
naturalTransgenic:Dose:SubstratePM	-790.30	692.30	310.97	-1.142
naturalTransgenic:Dose:SubstratePMOc	550.68	692.30	310.97	0.795
naturalTransgenic:Dose:SubstratePMPc	1854.02	692.30	310.97	2.678

	Pr(> t)
(Intercept)	< 2e-16 ***
naturalTransgenic	0.00140 **
Dose	< 2e-16 ***
SubstrateOcM	< 2e-16 ***
SubstratePcM	< 2e-16 ***
SubstratePM	0.84626
SubstratePMOc	0.24681
SubstratePMPc	6.48e-14 ***
naturalTransgenic:Dose	0.00217 **
naturalTransgenic:SubstrateOcM	0.08787 .

```

naturalTransgenic:SubstratePcM      0.32029
naturalTransgenic:SubstratePM        0.23570
naturalTransgenic:SubstratePM0c      0.41945
naturalTransgenic:SubstratePMPc      0.00617 **
Dose:Substrate0cM                    < 2e-16 ***
Dose:SubstratePcM                     < 2e-16 ***
Dose:SubstratePM                      0.95753
Dose:SubstratePM0c                   0.27757
Dose:SubstratePMPc                   4.23e-13 ***
naturalTransgenic:Dose:Substrate0cM  0.07934 .
naturalTransgenic:Dose:SubstratePcM  0.28083
naturalTransgenic:Dose:SubstratePM   0.25451
naturalTransgenic:Dose:SubstratePM0c 0.42697
naturalTransgenic:Dose:SubstratePMPc 0.00780 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Correlation matrix not shown by default, as $p = 24 > 12$.
 Use `print(x, correlation=TRUE)` or
`vcov(x)` if you need it

Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
natural	17606539	17606539	1	310.97	54.5589	1.397e-12
Dose	1517357360	1517357360	1	310.97	4701.9667	< 2.2e-16
Substrate	380517369	76103474	5	310.97	235.8284	< 2.2e-16
natural:Dose	15683854	15683854	1	310.97	48.6009	1.886e-11
natural:Substrate	8560933	1712187	5	310.97	5.3057	0.0001077
Dose:Substrate	362539320	72507864	5	310.97	224.6864	< 2.2e-16
natural:Dose:Substrate	8399558	1679912	5	310.97	5.2057	0.0001323

```

natural      ***
Dose         ***
Substrate    ***
natural:Dose ***
natural:Substrate ***
Dose:Substrate ***
natural:Dose:Substrate ***
---

```

```

Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

refitting model(s) with ML (instead of REML)

Data: data

Models:

lmm2: V02 ~ Dose * Substrate + (1 | pair)

lmm1: V02 ~ natural * Dose * Substrate + (1 | pair)

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)
lmm2	14	5632.8	5686.4	-2802.4	5604.8			
lmm1	26	5332.5	5432.0	-2640.2	5280.5	324.39	12	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Likelihood ratio test

Model 1: V02 ~ natural * Dose * Substrate + (1 | pair)

Model 2: V02 ~ Dose * Substrate + (1 | pair)

	#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	26	-2489.7			
2	14	-2724.9	-12	470.45	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

boundary (singular) fit: see help('isSingular')

R2 for Mixed Models

Conditional R2: 0.965

Marginal R2: 0.901

boundary (singular) fit: see help('isSingular')

Intraclass Correlation Coefficient

Adjusted ICC: 0.644

Unadjusted ICC: 0.064

Call:

lm(formula = V02 ~ natural * Dose * Substrate, data = data)

Residuals:

Min	1Q	Median	3Q	Max
-3254.1	-370.8	186.2	536.4	1727.3

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)	
(Intercept)	143278.42	7230.68	19.815	< 2e-16	***
naturalTransgenic	22297.32	10225.72	2.181	0.0300	*
Dose	9596.47	511.68	18.755	< 2e-16	***
SubstrateOcM	-109203.79	10724.83	-10.182	< 2e-16	***
SubstratePcM	-112973.48	10724.83	-10.534	< 2e-16	***
SubstratePM	-1342.39	10225.72	-0.131	0.8956	
SubstratePMOc	-8026.61	10225.72	-0.785	0.4331	
SubstratePMPc	-54350.93	10225.72	-5.315	2.02e-07	***
naturalTransgenic:Dose	1513.62	723.63	2.092	0.0373	*
naturalTransgenic:SubstrateOcM	-17567.40	15167.20	-1.158	0.2476	
naturalTransgenic:SubstratePcM	-10213.84	15167.20	-0.673	0.5012	
naturalTransgenic:SubstratePM	-11623.31	14461.35	-0.804	0.4221	
naturalTransgenic:SubstratePMOc	7909.05	14461.35	0.547	0.5848	
naturalTransgenic:SubstratePMPc	26976.20	14461.35	1.865	0.0631	.
Dose:SubstrateOcM	-7485.71	758.95	-9.863	< 2e-16	***
Dose:SubstratePcM	-7737.11	758.95	-10.195	< 2e-16	***
Dose:SubstratePM	26.09	723.63	0.036	0.9713	
Dose:SubstratePMOc	-532.46	723.63	-0.736	0.4624	
Dose:SubstratePMPc	-3706.61	723.63	-5.122	5.26e-07	***
naturalTransgenic:Dose:SubstrateOcM	-1278.14	1073.31	-1.191	0.2346	
naturalTransgenic:Dose:SubstratePcM	-784.42	1073.31	-0.731	0.4654	
naturalTransgenic:Dose:SubstratePM	-790.30	1023.37	-0.772	0.4405	
naturalTransgenic:Dose:SubstratePMOc	550.68	1023.37	0.538	0.5909	
naturalTransgenic:Dose:SubstratePMPc	1854.02	1023.37	1.812	0.0710	.

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 839.7 on 316 degrees of freedom

(20 observations deleted due to missingness)

Multiple R-squared: 0.9233, Adjusted R-squared: 0.9177

F-statistic: 165.4 on 23 and 316 DF, p-value: < 2.2e-16

Warning in modelUpdate(objects[[i - 1]], objects[[i]]): original model was of class "lmerModLmerTest", updated model is of class "lm"

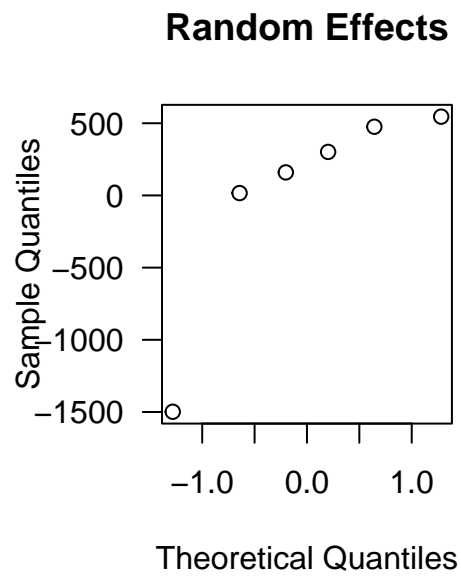
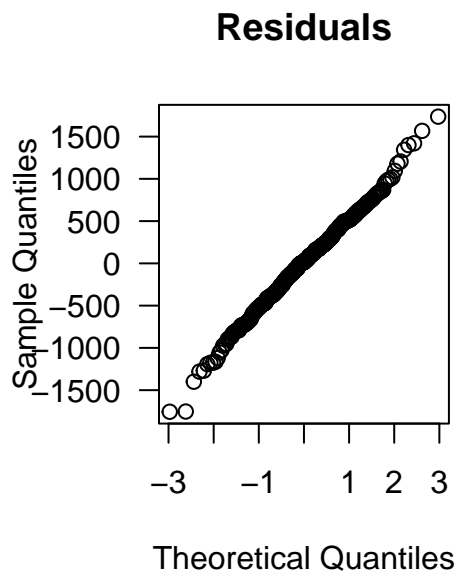
Likelihood ratio test

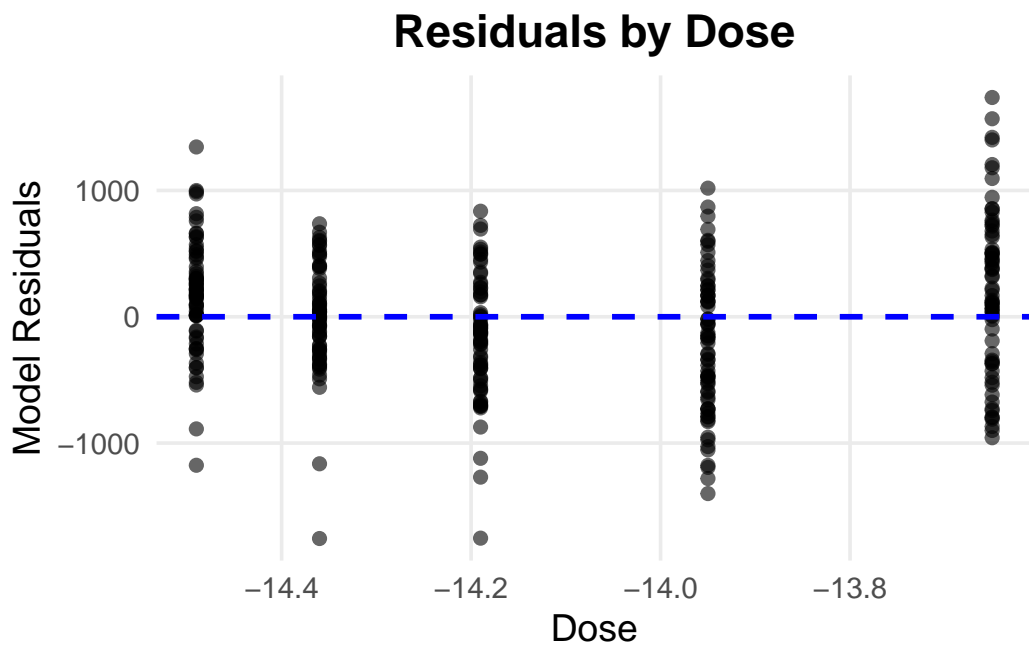
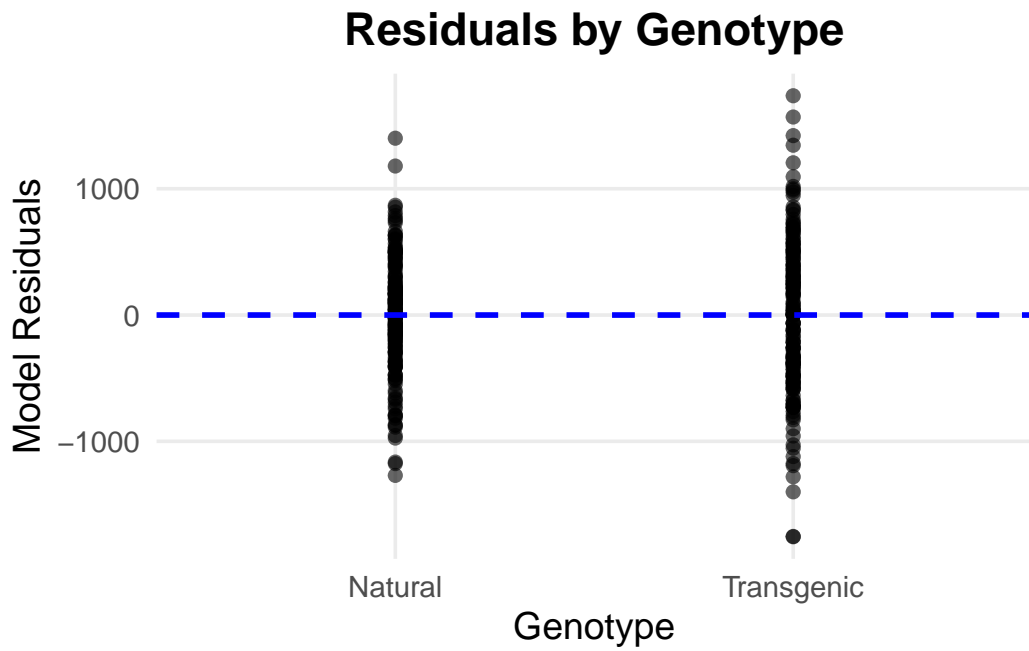
Model 1: V02 ~ natural * Dose * Substrate + (1 | pair)

Model 2: V02 ~ natural * Dose * Substrate

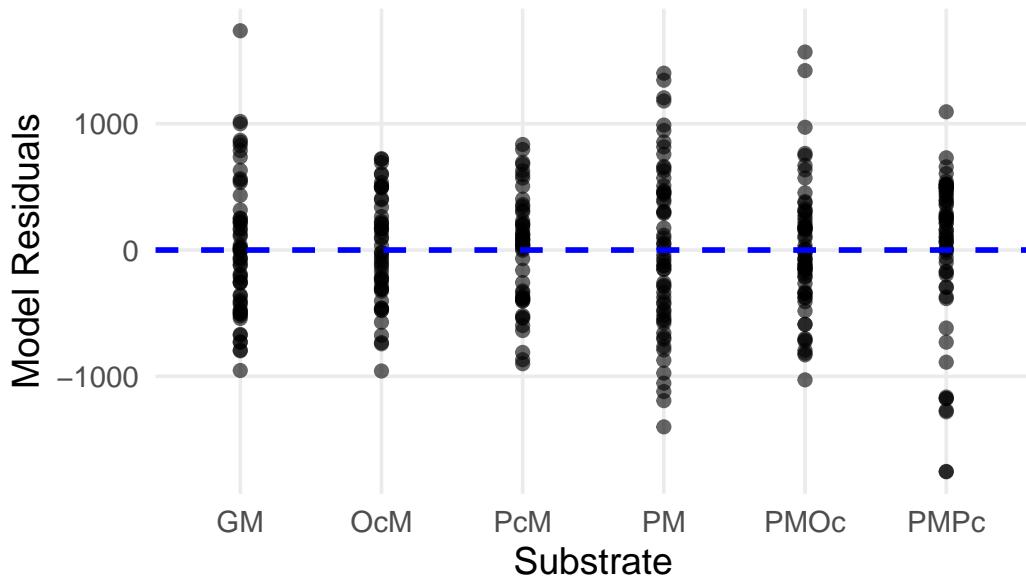
	#Df	LogLik	Df	Chisq	Pr(>Chisq)
1	26	-2489.7			
2	25	-2759.2	-1	539.1	< 2.2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

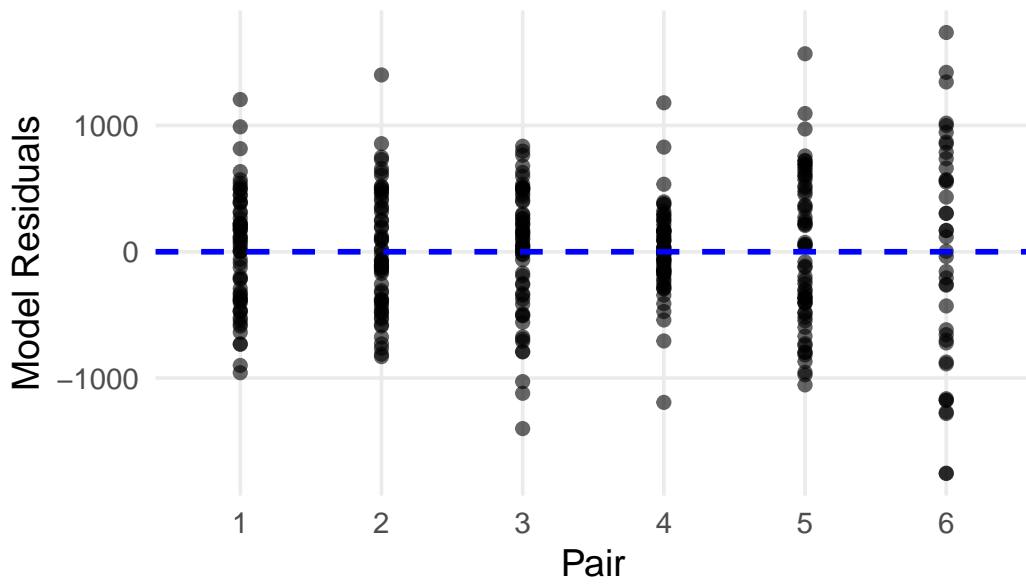




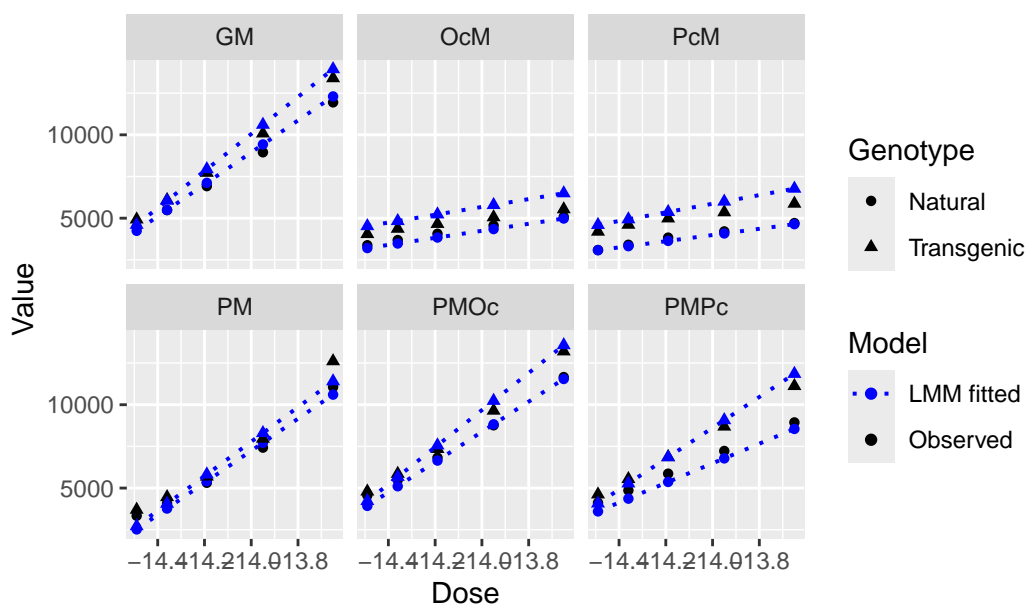
Residuals by Substrate



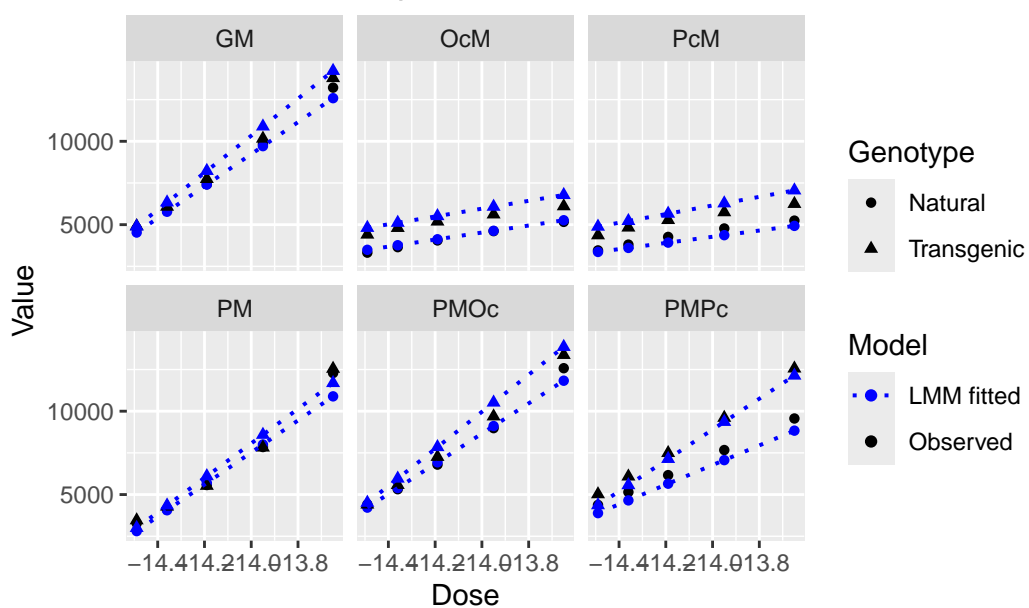
Residuals by Pair



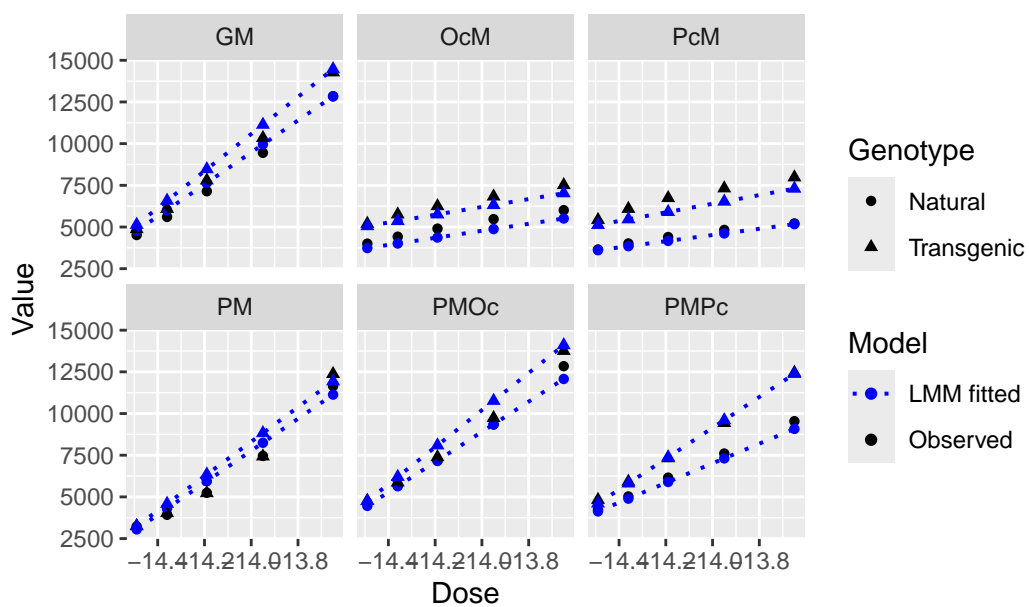
VO2 vs. Dose for pair 1



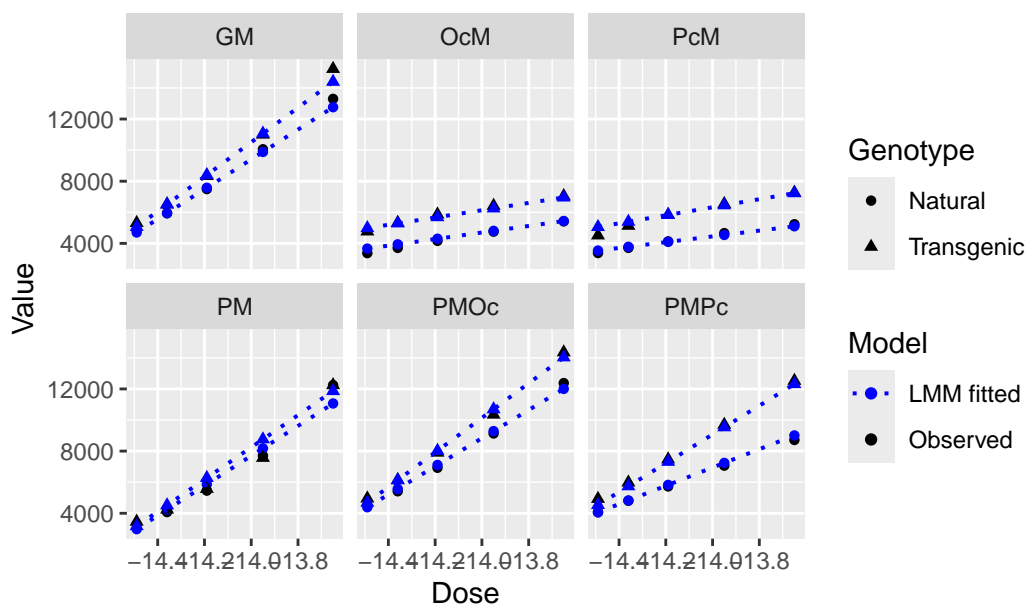
VO2 vs. Dose for pair 2

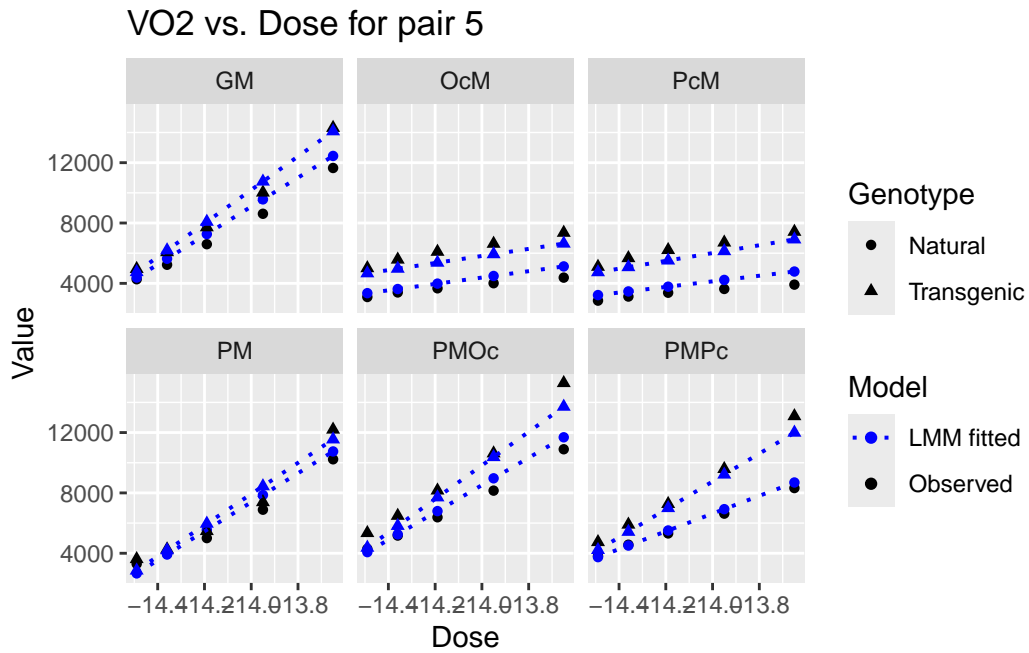


VO2 vs. Dose for pair 3

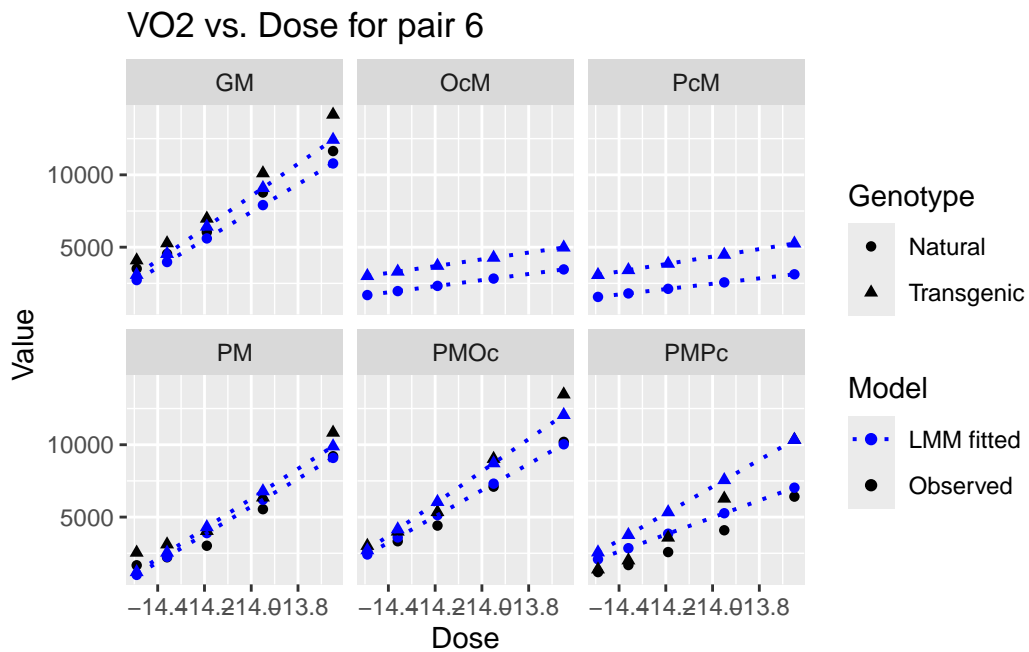


VO2 vs. Dose for pair 4

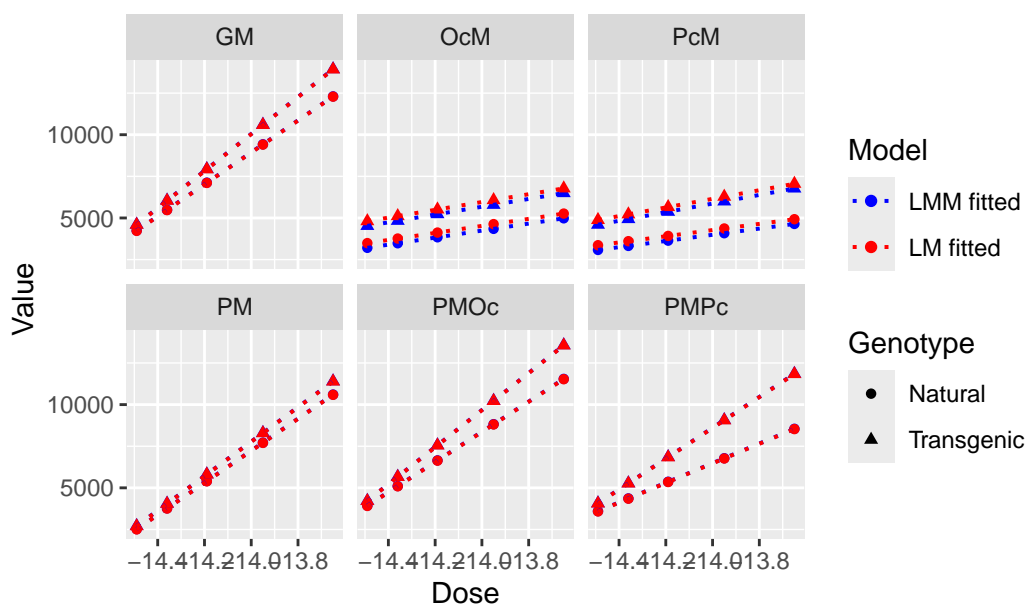




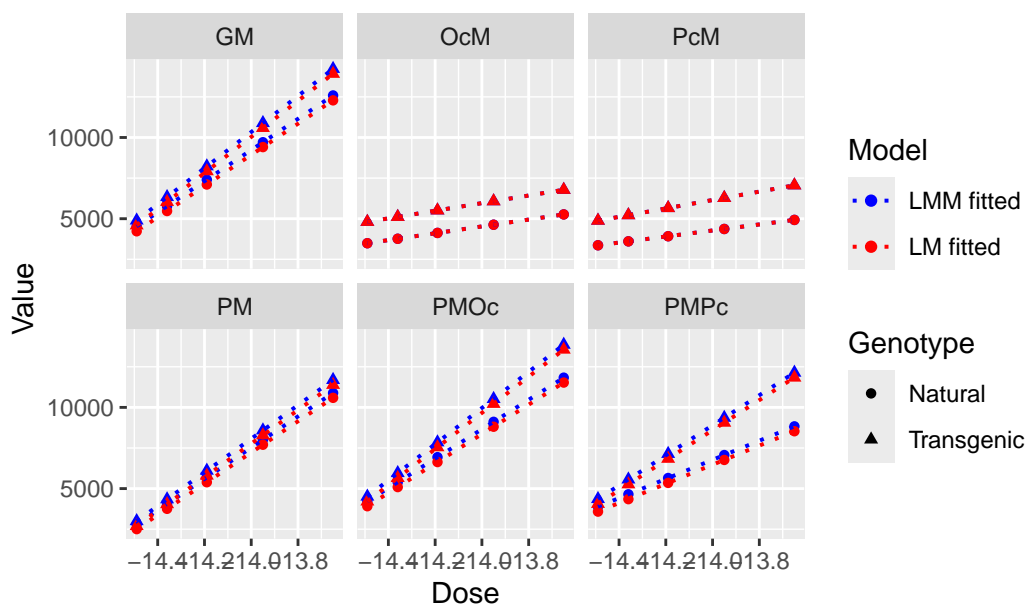
Warning: Removed 20 rows containing missing values or values outside the scale range (``geom_point()``).



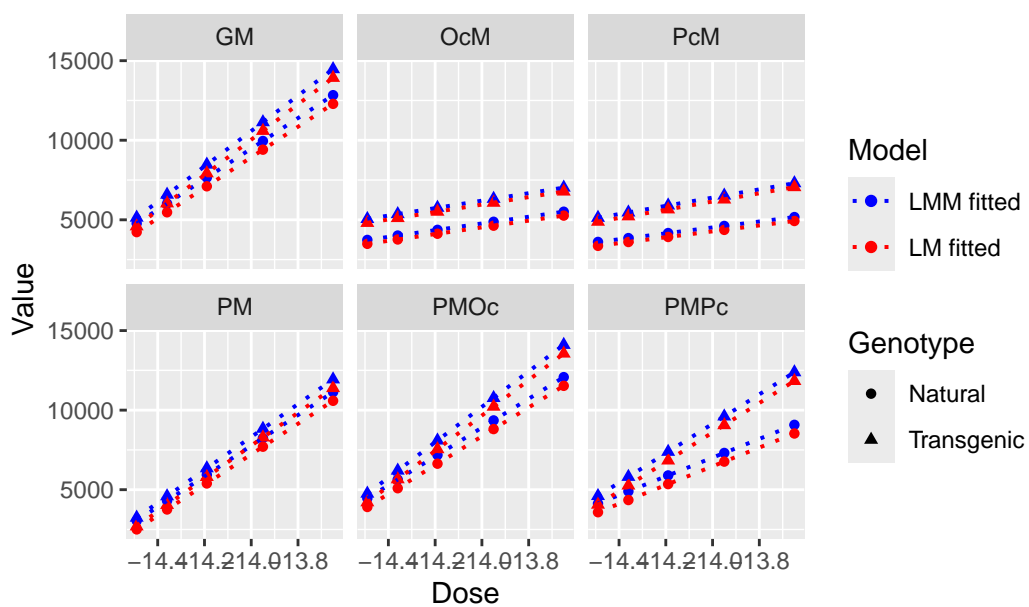
VO2 vs. Dose for pair 1



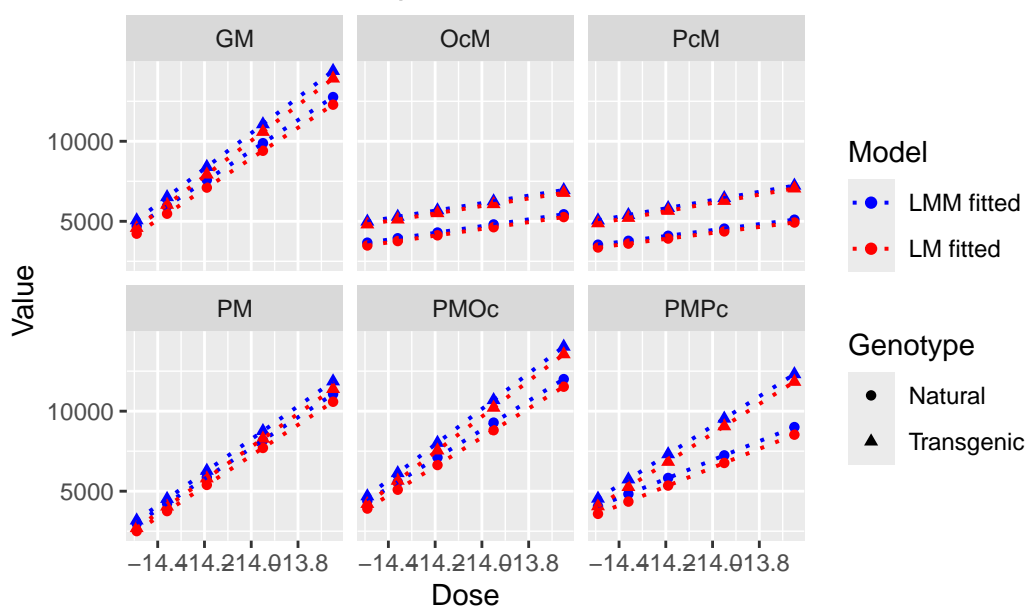
VO2 vs. Dose for pair 2



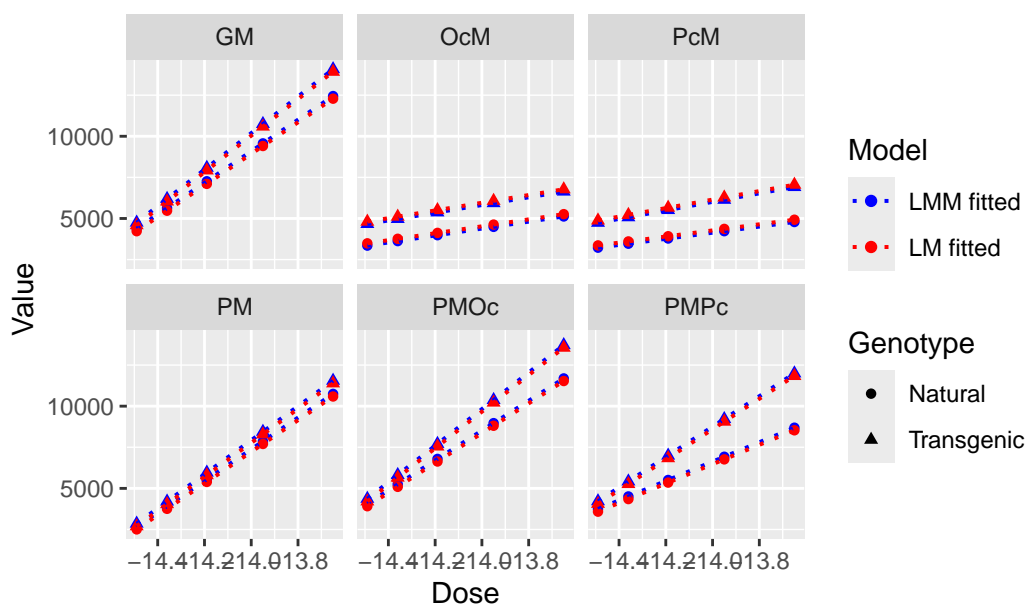
VO2 vs. Dose for pair 3



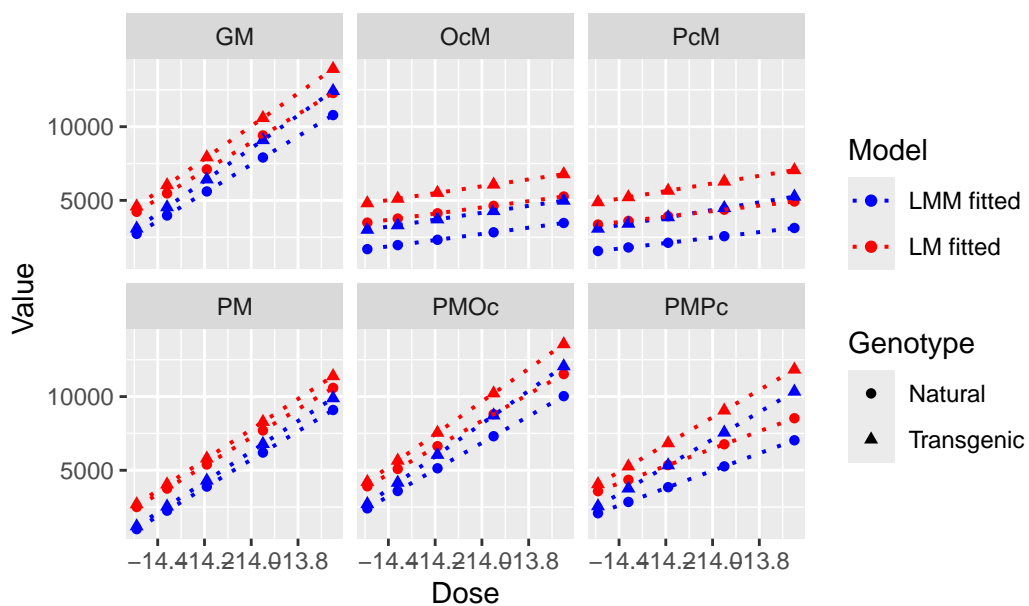
VO2 vs. Dose for pair 4



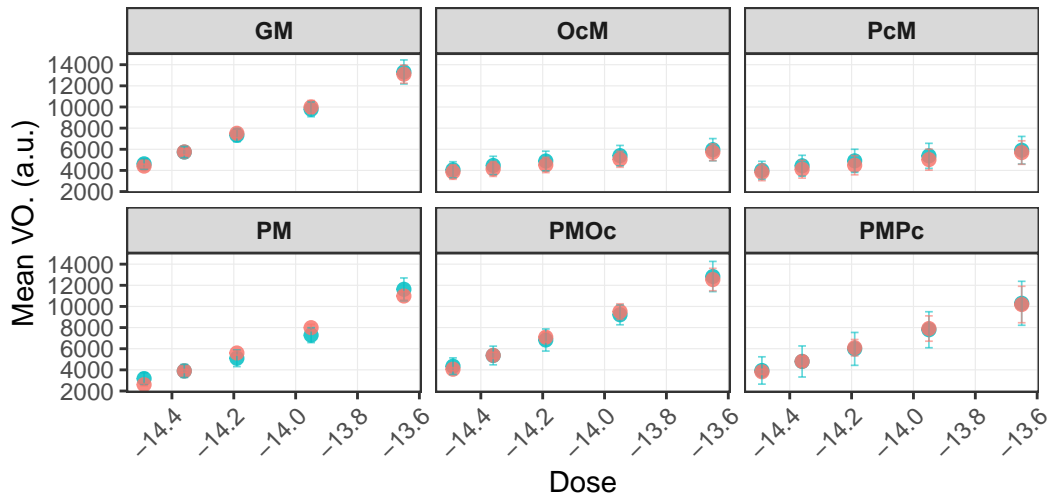
VO2 vs. Dose for pair 5



VO2 vs. Dose for pair 6

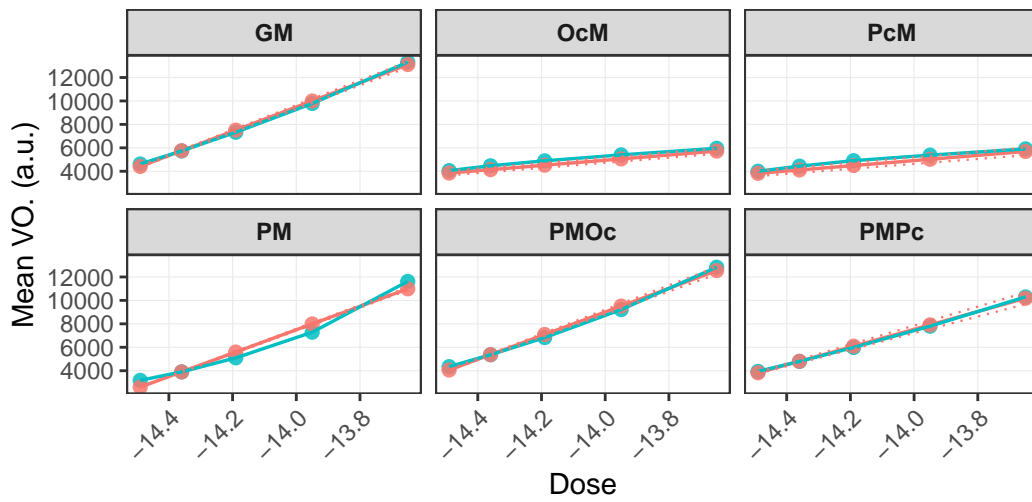


Dose vs. Mean VO.



Data Type — predictions — VO2

Predictions vs. Observed VO.



Data Type — predictions — VO2