Exploration of Multiple Treatments on the Metabolic Efficiency of the Mitochondria

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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

We explored patterns of VO_2 production across genotype, substrate, and dose to assess whether systematic differences exist.

Looking at graph 1 we can see that overall the genotype has a clear effect on VO_2 production. Across nearly all substrates we can see the transgenic mice display higher VO_2 production than natural mice. For substrates of $PMOc$ and $PMPc$ we can see different slopes for VO_2 production vs Dose and as the doses become higher, the effects of genotype becomes more significant. So the effect is most pronounced when the doses are higher in $PMPc$ and $PMOc$

and for OcM and PcM we just see a clear higher VO_2 production for all doses as the slopes for OcM and PcM are similar.

We also can see that substrates involving Octanoyl Carnitine Oc and Palmitoyl-Carnitine Pc has a more pronounced separation between transgenic vs natural mouse. So these results suggest transgenic mice have enhanced respiratory capacity

OcM and PcM show a relatively flat dose-response curves for both genotypes which suggests limited sensitivity to dose changes and $PMOc$ and $PMPc$ substrates highlight a stronger genotype effect as the transgenic mice has a more pronounced effect to dose. This points to an interaction between substrate and genotype where certain substrates amplify the genotype-specific differences in the VO_2 production efficiency.

Pair - Level Variation

We next examined the effects of pairs.

Conclusions

1. Genotype matters: transgenic mice had higher VO_2 than nautral mice across most conditions
2. Substrate seems to matter too: We saw certain substrates had a larger effect in the VO_2 production as $PMOc$ and $PMPc$ tended to effect the transgenic mice more while OcM and PcM tended to have an equal effect on transgenic and natural mice.
3. Pairs

3 Modeling

We explored 3 different models whose equations are shown below.

$$Model\ 1:\ VO_2\ natural * Dose * Substrate + (1|pair)$$
$$Model\ 2:\ VO_2\ natural * Dose * Substrate$$
$$Model\ 3:\ VO_2\ Dose * Substrate + (1|pair)$$

Model 1 is the model we ended up choosing and this is the fixed model including a random intercept for pair. Model 2 is just the fixed effects with no random intercept and Model 3 is just the mixed model without genotype to test if genotype contributes beyond the dose and substrate.

Looking at the log likelihoods for model 1 (shown in table _____) we can see a log likelihood value of -2489.7 and for model 2 and 3 they are -2759.2 and -2724.9 respectively. Since a higher

log likelihood means a better fit we can see model 1 has the highest log likelihood and fits better than model 2 and 3. Looking at the chi squared and the p value which compare the model fit of model 2 vs 1 and model 3 vs 1 we can see that the p values are very small (close to 0). Since the p values are very small the improvement in model 1 is not by chance so in model 2 it shows the random intercept for pair is essential. The p value comparing model 3 and 1 is also very small (close to 0) which suggests that genotype is also significant. Since the p values are very small the worse fit of model 2 and 3 is statistically significant which indicates their ommitted terms are necessary.

Looking at the ICC values shown in (table _____) which is the ratio of within pair variability to total variability, the value of 0.644 means about 64% of the remaining variability is due to which pair is sampled and this confirms a strong within pair clustering.

The conditional R^2 value is the variance explained by design and the pair difference. Looking at the results shown in (table _____) letting each pair have its own baseline we explain about 96.5% of the variaibility

The Marginal R^2 is variance explained by design factors and this only captures about 90% of the variability across all observations as shown in (table_____)

## 4 Analysis

5 Conclusion and Future Work

Our analysis showed strong evidence that genotype significantly influences VO_2 production conditional on both substrate and dose. Across nearly all experimental conditions, transgenic mice displayed a higher VO_2 production relative to natural mice with some substrates amplifying this effect more than others. These findings show that transgenic geotype is associated with enhanced metabolic efficiency

However, some limitiations to keep in mind is that first we excluded the observations with the 12.95 dosage…..

Future work should therefore focus on extending the analysis to higher doses, exploring a broader range of substrates, and leveraging larger samples to better account for variability across experimental pairs. These extensions would help clarify the extent to which the observed genotype effects generalize across different biological and experimental contexts.
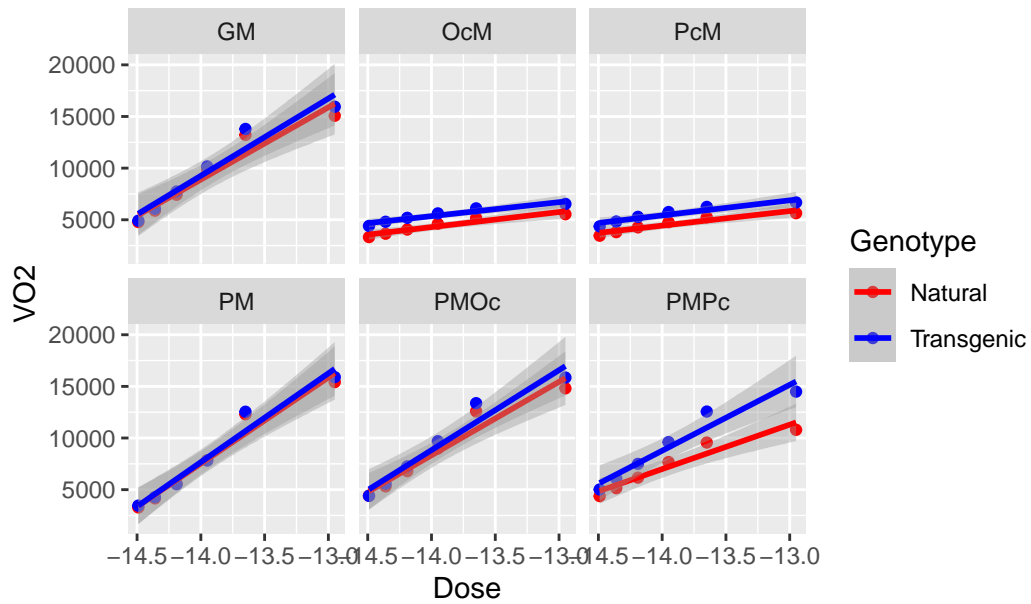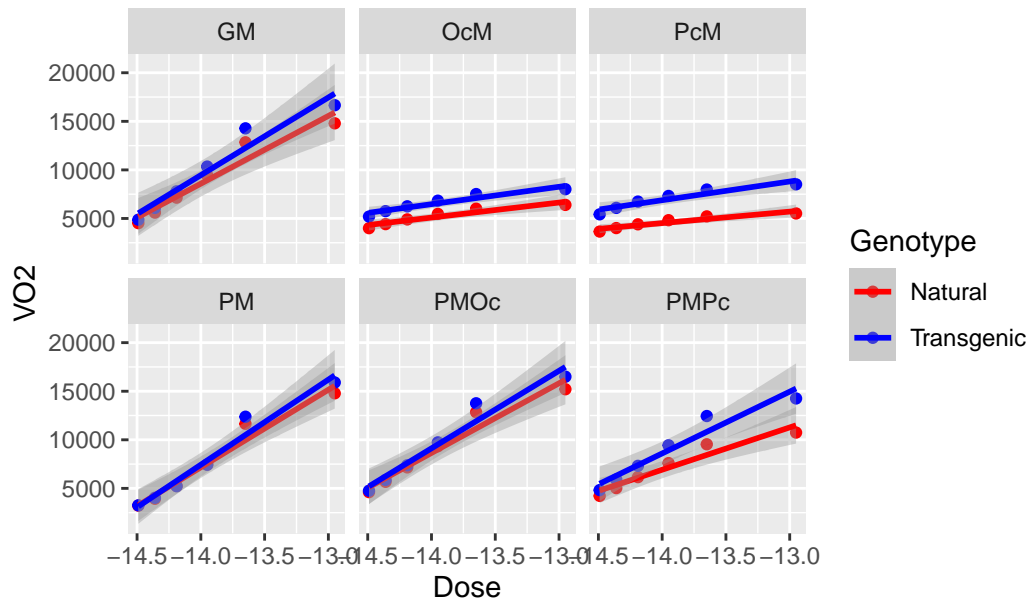
6 Appendix

Exploratory Data Analysis
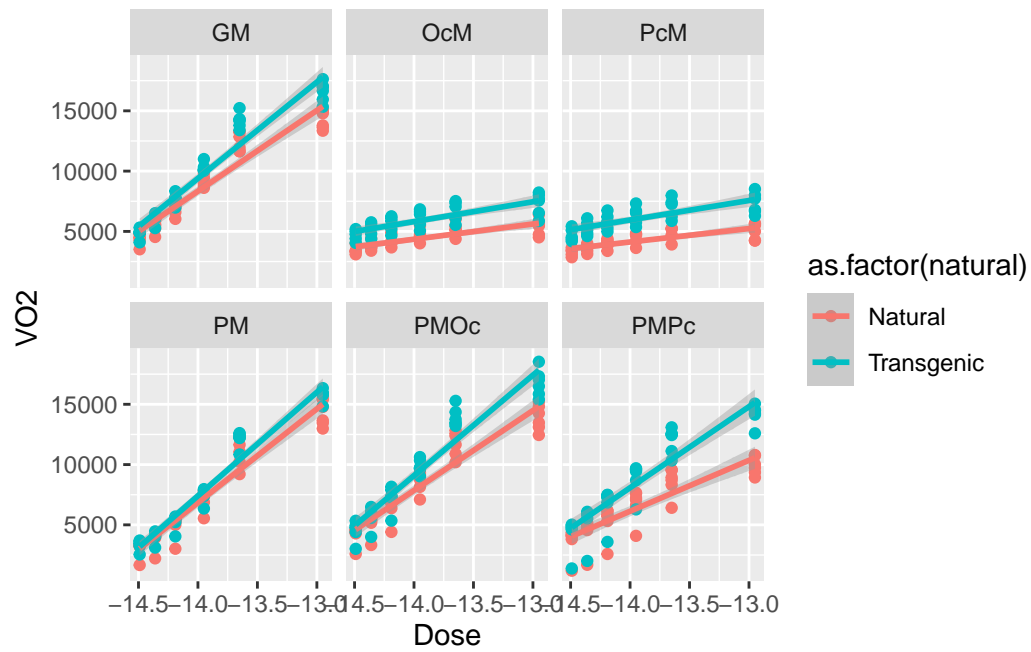
VO2 vs. Dose for pair 1
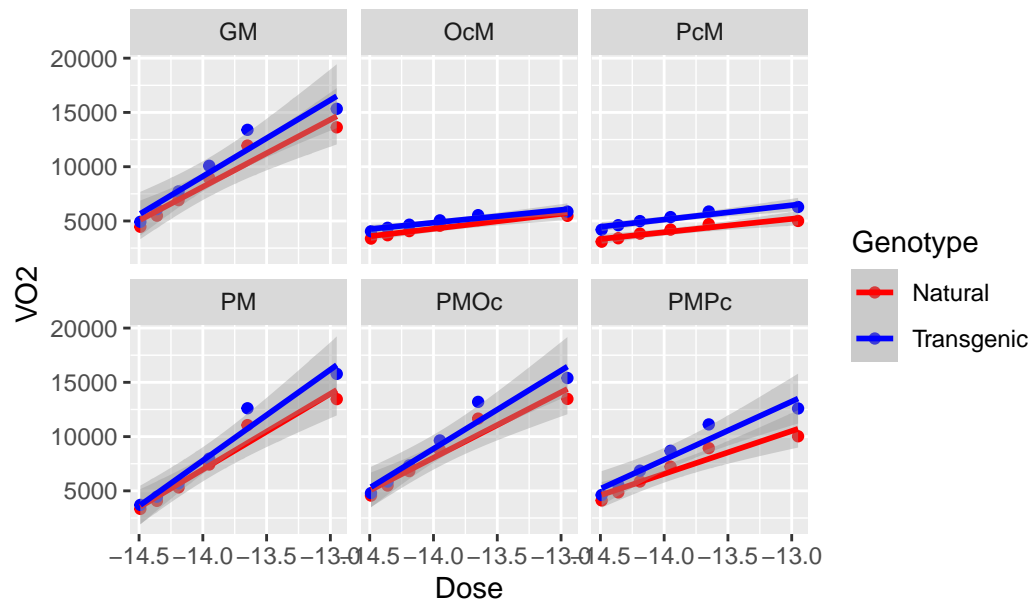
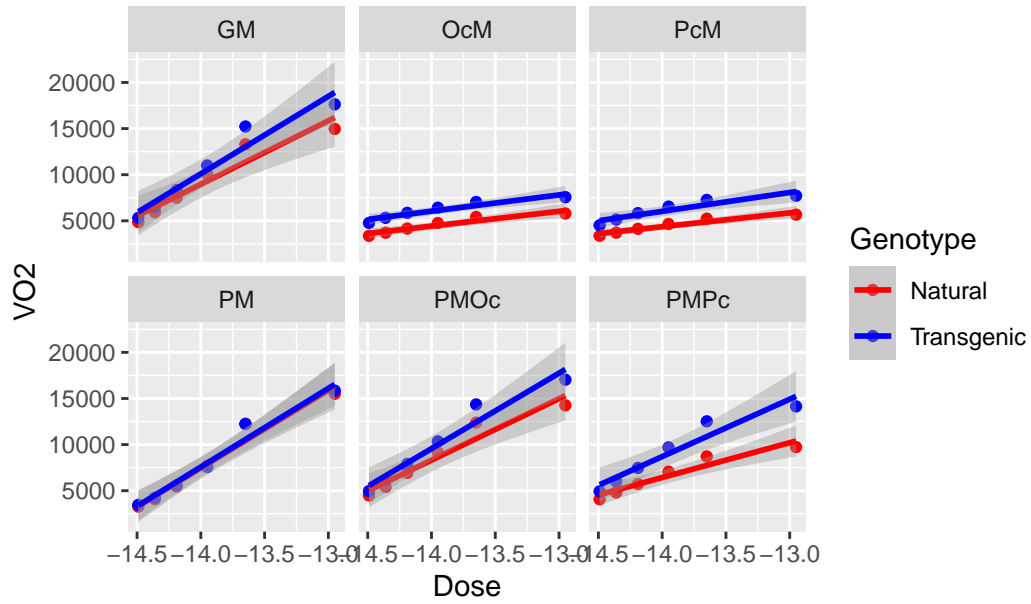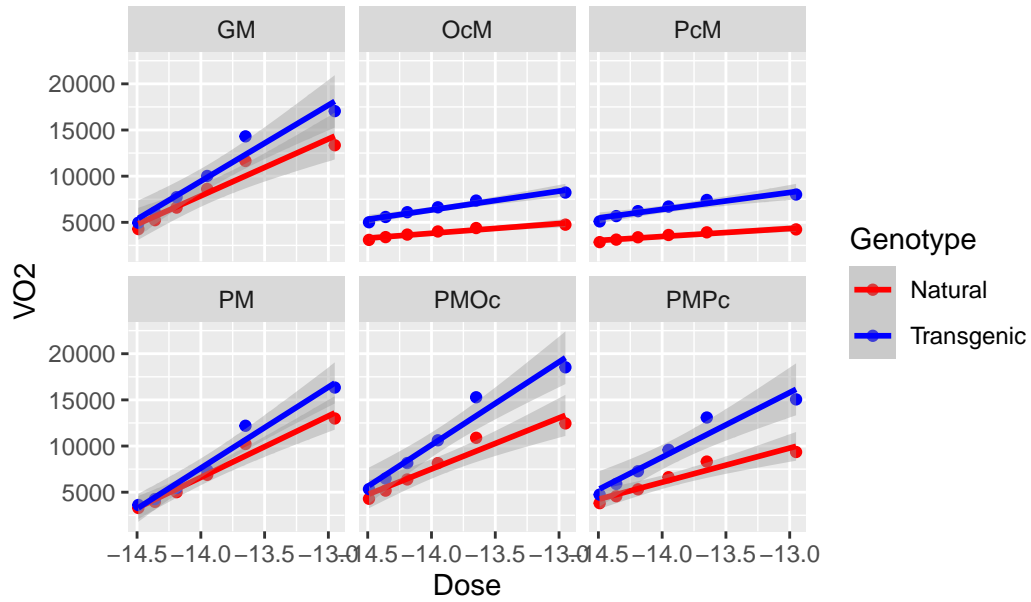VO2 vs. Dose for pair 2

VO2 vs. Dose for pair 3

VO2 vs. Dose for pair 4

VO2 vs. Dose for pair 5

VO2 vs. Dose for pair 6
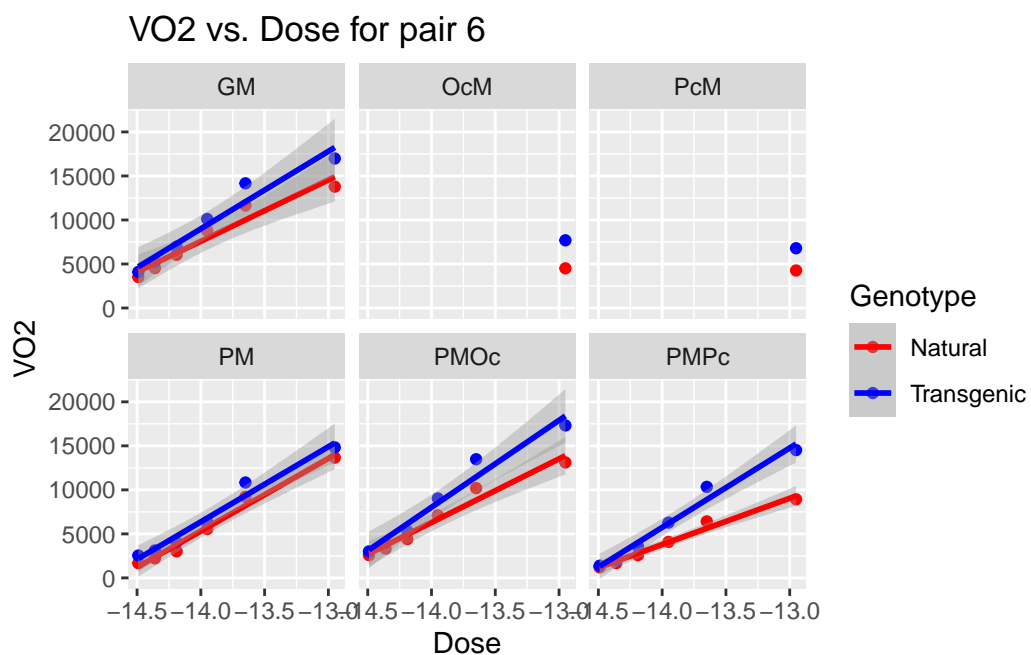

Table 1: Model comparison using AIC and Adjusted R²

Model	df	AIC	Adjusted_R2
Amino Acids	8	7439.921	0.721
Substrate	9	7386.290	0.755

Modeling

```
Call:
lm(formula = VO2 ~ 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 = VO2 ~ 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


Call:
lm(formula = VO2 ~ 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: VO2 ~ 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) 582981   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:SubstratePMOc       0.41945
naturalTransgenic:SubstratePMPc       0.00617 **
Dose:SubstrateOcM                     < 2e-16 ***
Dose:SubstratePcM                     < 2e-16 ***
Dose:SubstratePM                      0.95753
Dose:SubstratePMOc                    0.27757
Dose:SubstratePMPc                    4.23e-13 ***
naturalTransgenic:Dose:SubstrateOcM   0.07934 .
naturalTransgenic:Dose:SubstratePcM   0.28083
naturalTransgenic:Dose:SubstratePM    0.25451
naturalTransgenic:Dose:SubstratePMOc  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  4701.9665 < 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: VO2 ~ Dose * Substrate + (1 | pair)
lmm1: VO2 ~ natural * Dose * Substrate + (1 | pair)
     npar    AIC    BIC  logLik -2*log(L)  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: VO2 ~ natural * Dose * Substrate + (1 | pair)
Model 2: VO2 ~ Dose * Substrate + (1 | pair)
  #Df  LogLik  Df  Chisq Pr(>Chisq)
1  26 -2489.7
```
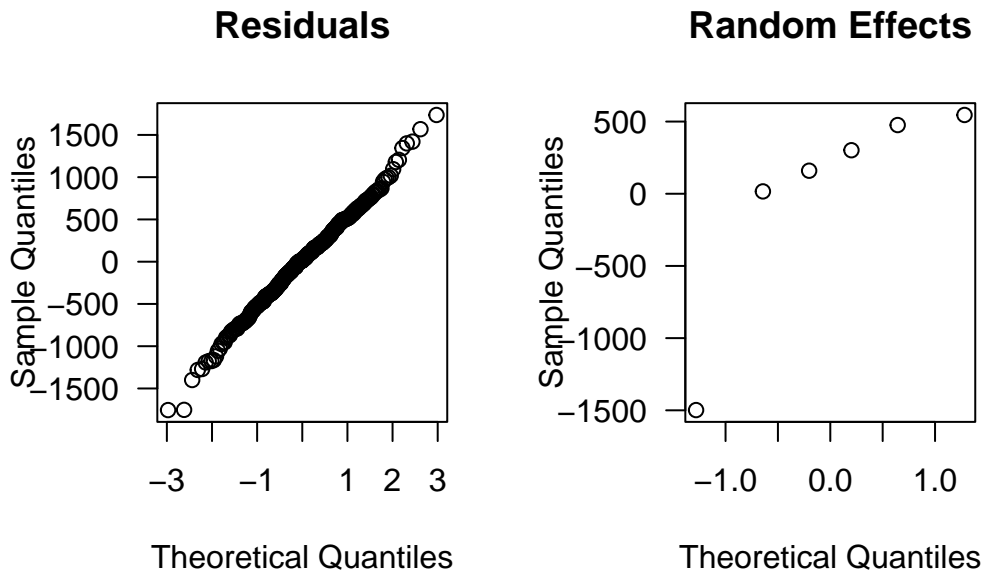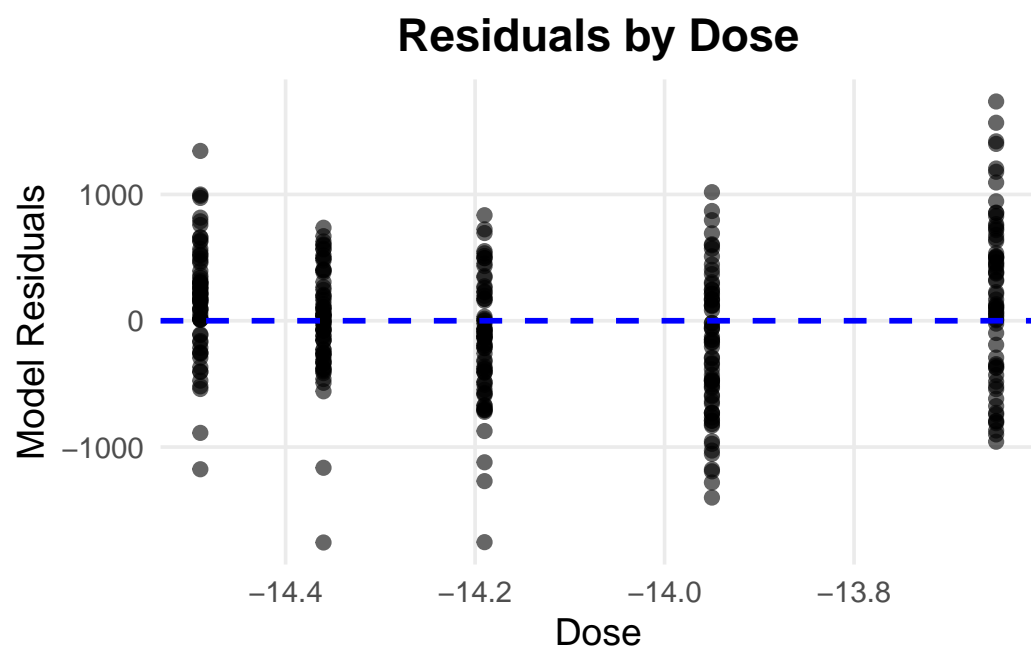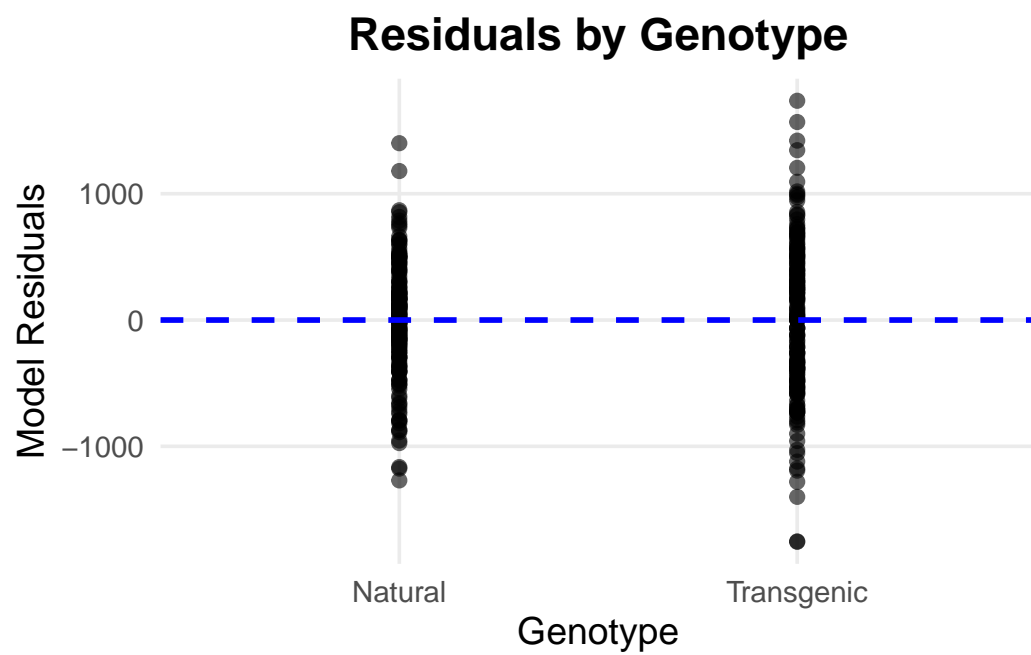
```
2  14 -2724.9 -12 470.45  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1


# R2 for Mixed Models

  Conditional R2: 0.965
    Marginal R2: 0.901


# Intraclass Correlation Coefficient

    Adjusted ICC: 0.644
  Unadjusted ICC: 0.064



Call:
lm(formula = VO2 ~ 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 *
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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
```
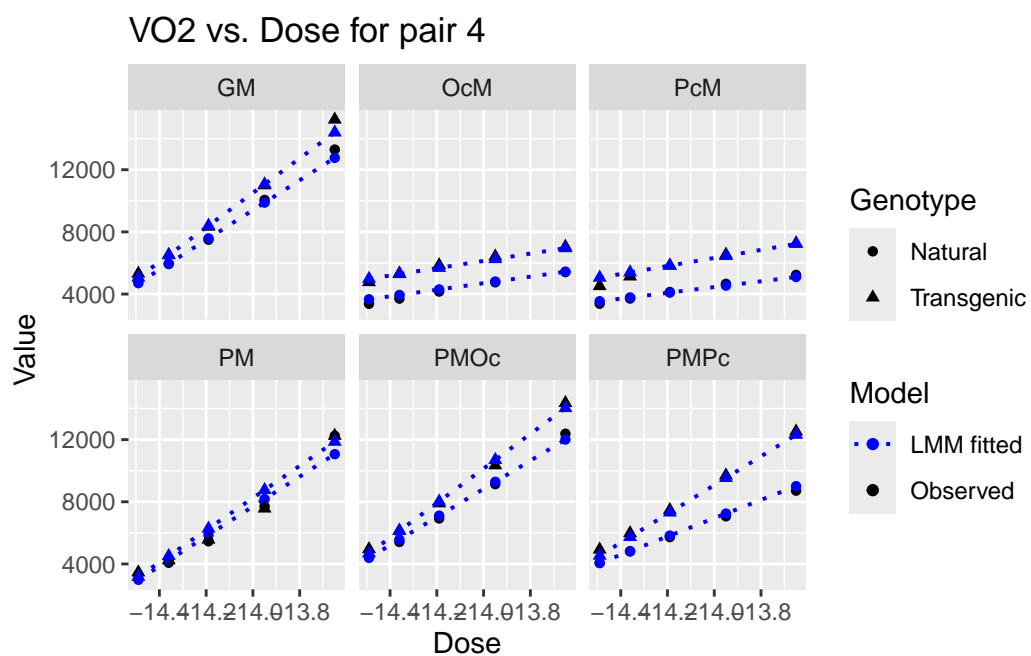
```
Dose:SubstratePMPc                        -3706.61      723.63  -5.122 5.26e-07 ***
naturalTransgenic:Dose:SubstrateOcM       -1278.14     1073.31  -1.191   0.2346
naturalTransgenic:Dose:SubstratePcM        -784.42     1073.31  -0.731   0.4654
naturalTransgenic:Dose:SubstratePM         -790.30     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: VO2 ~ natural * Dose * Substrate + (1 | pair)
Model 2: VO2 ~ 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   ## Random Effects

Theoretical Quantiles   Theoretical Quantiles

14

Residuals by Genotype

Residuals by Dose

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
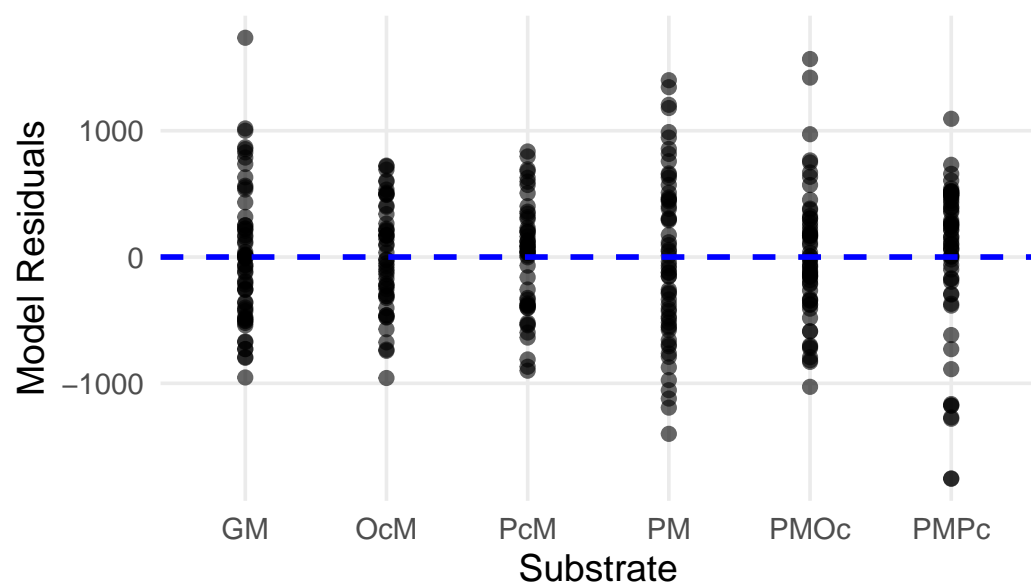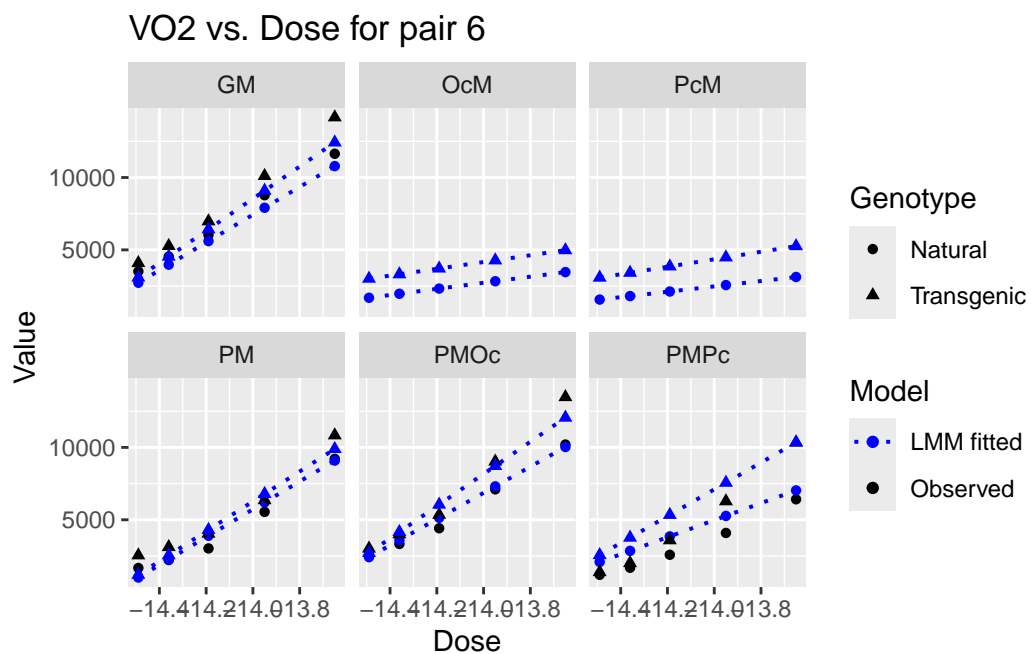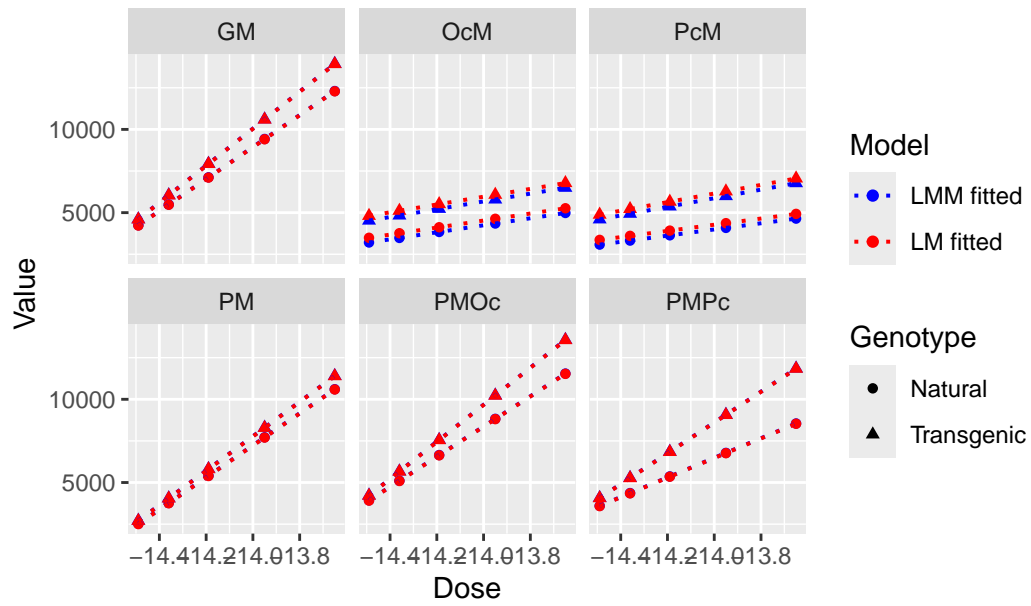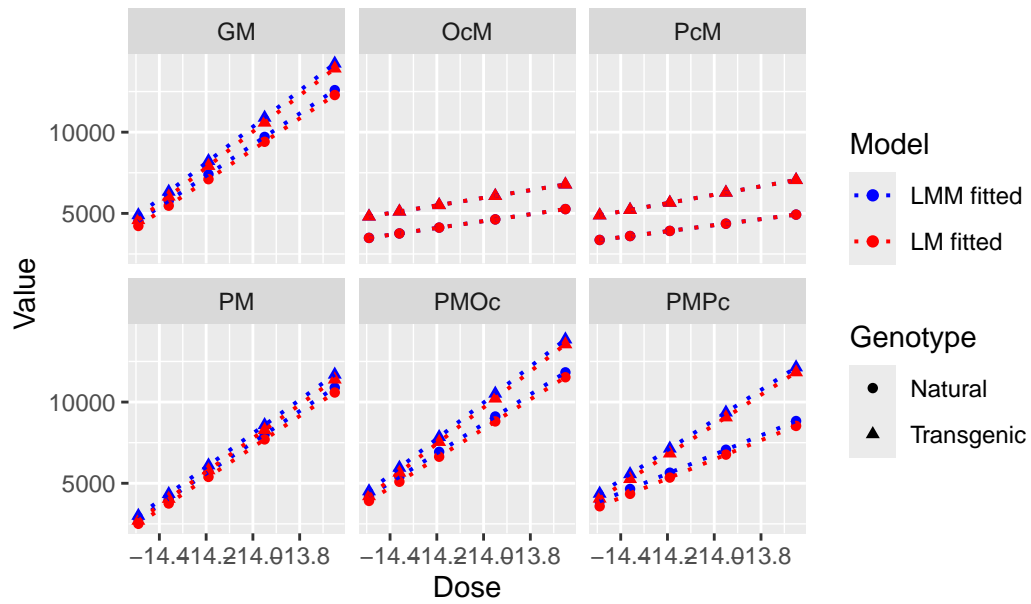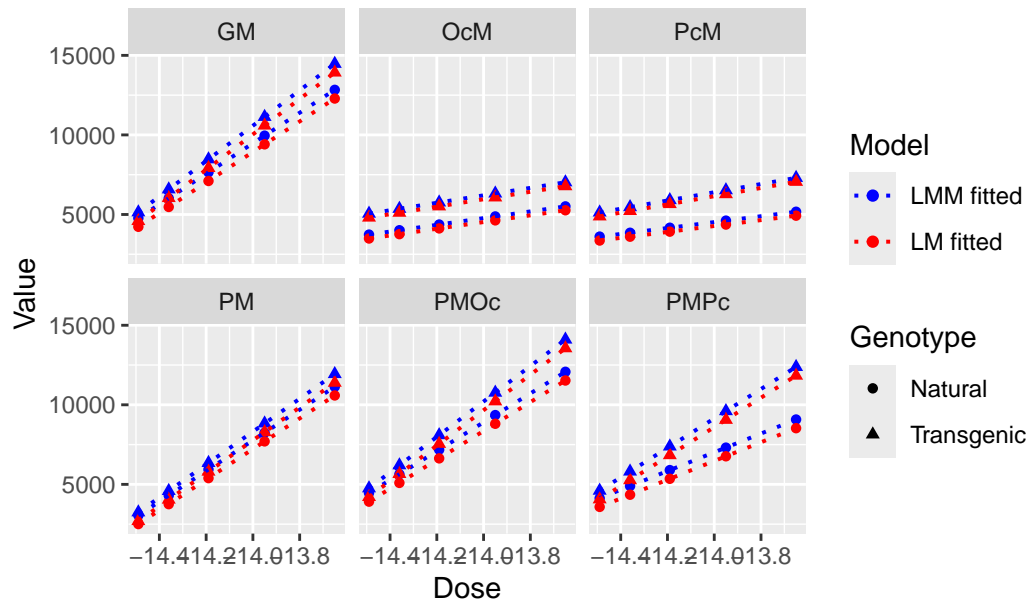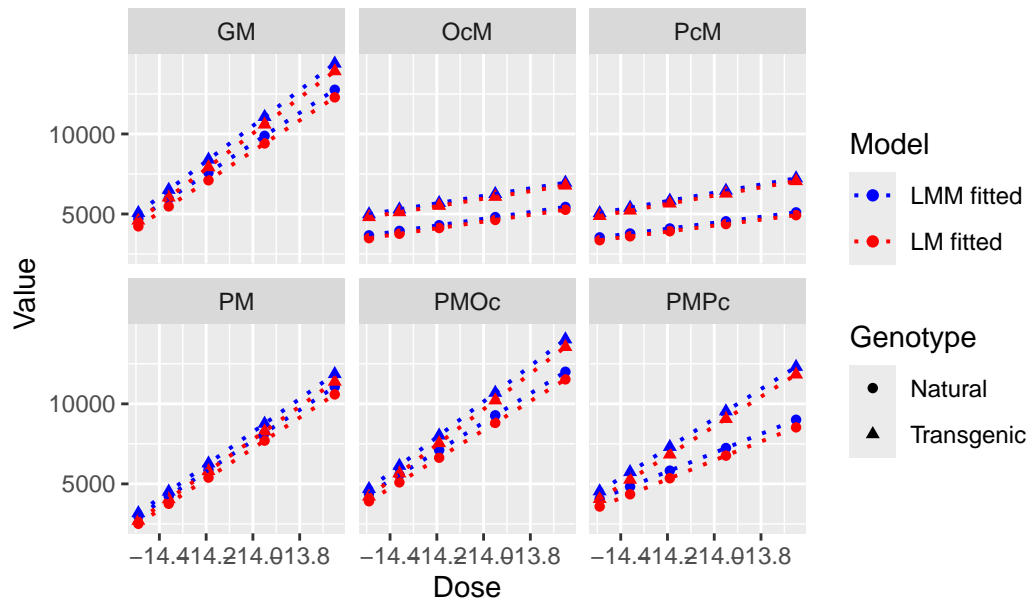
VO2 vs. Dose for pair 5
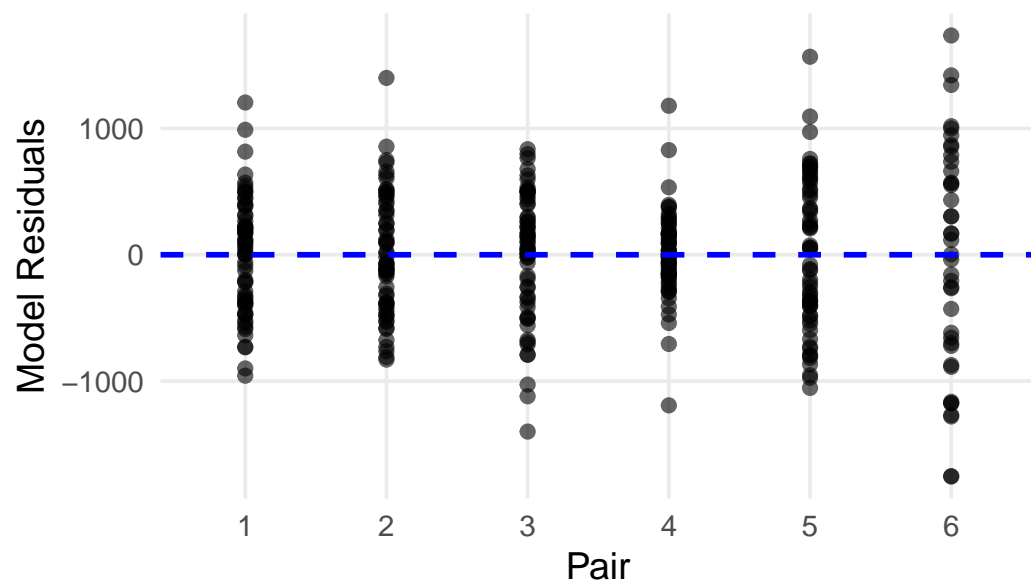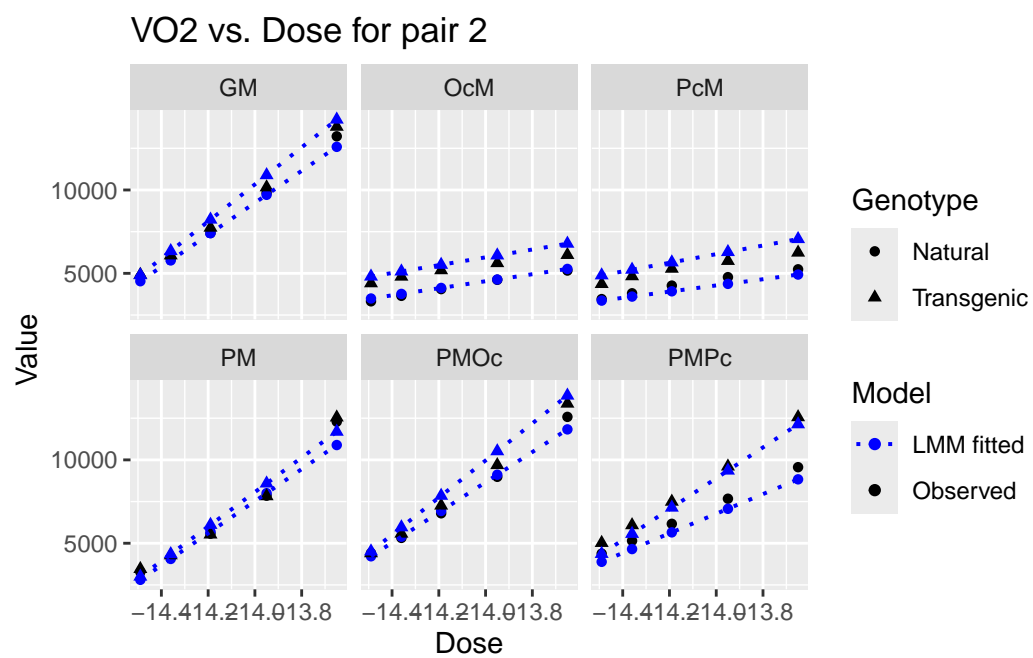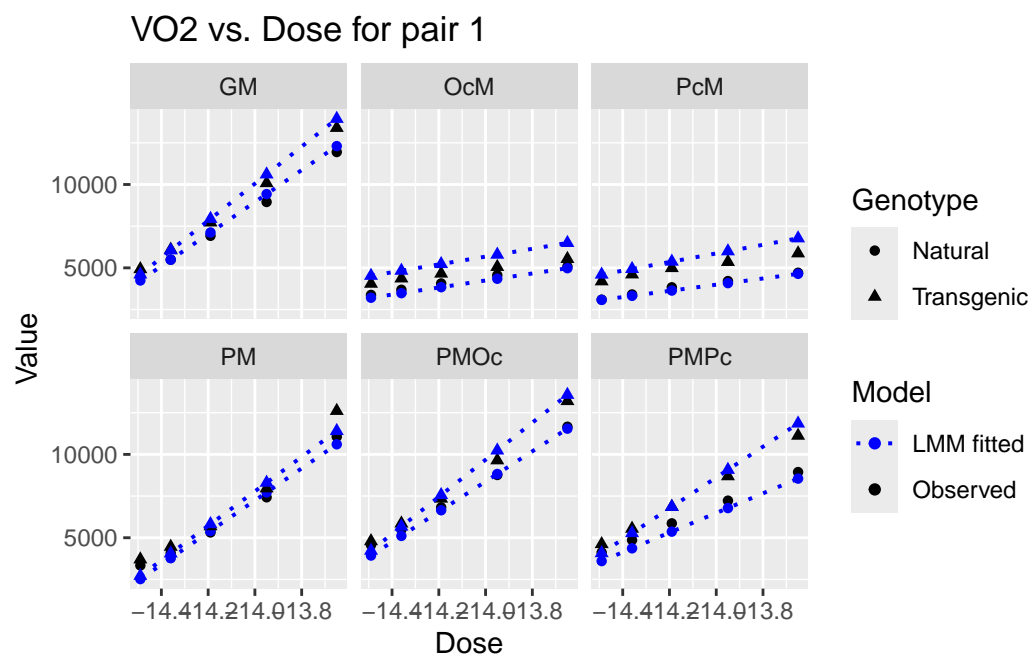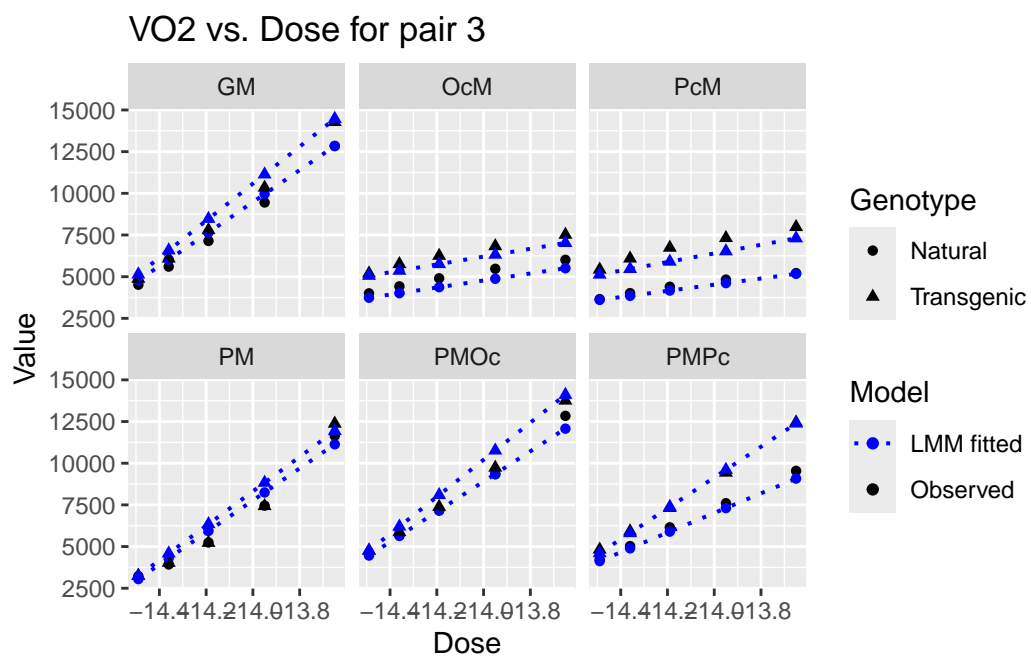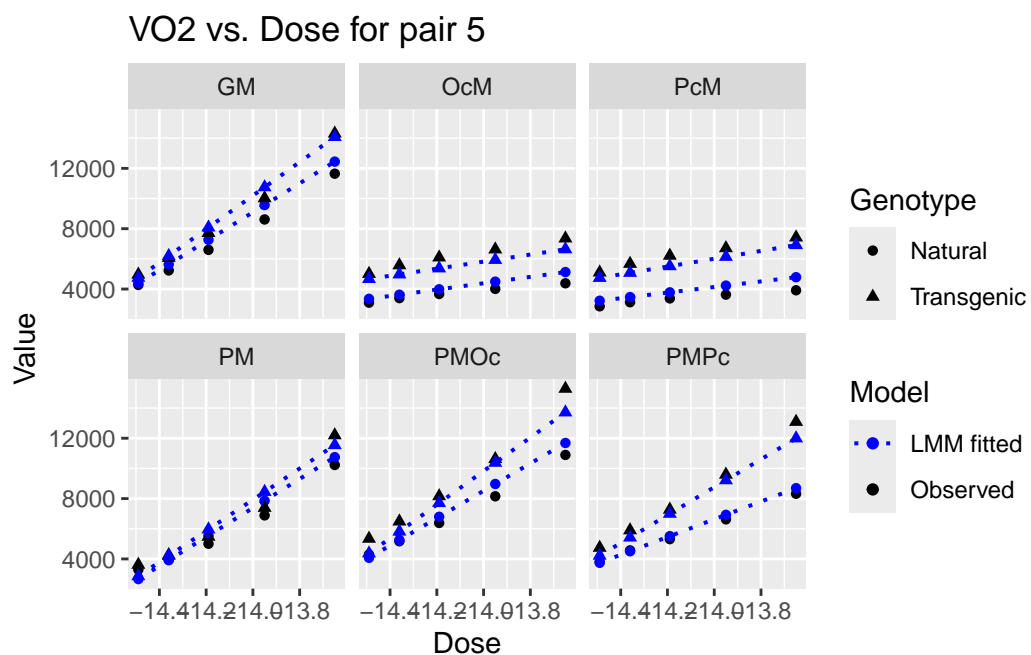
Warning: Removed 20 rows containing missing values or values outside the scale range
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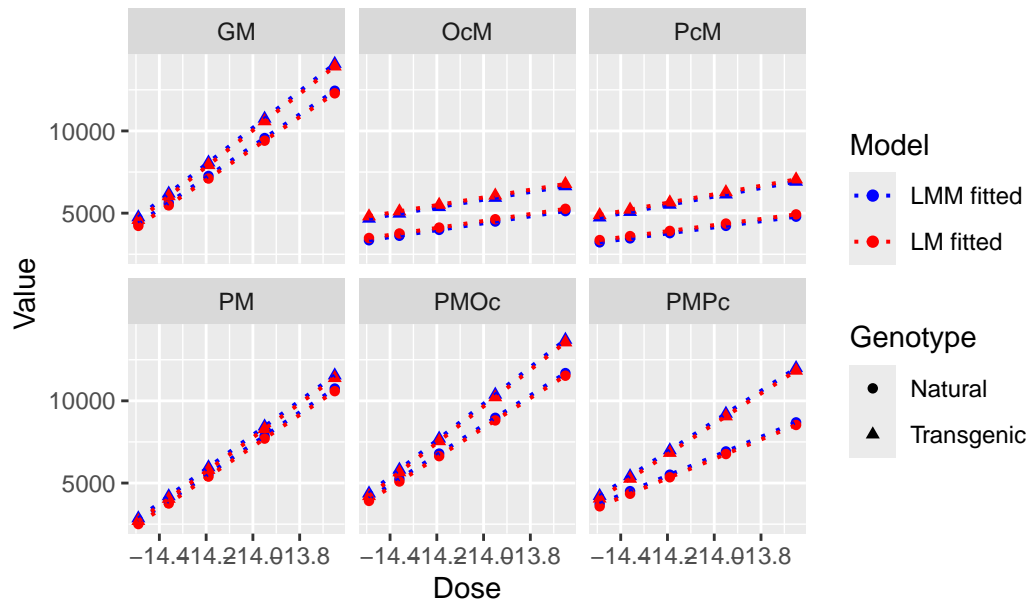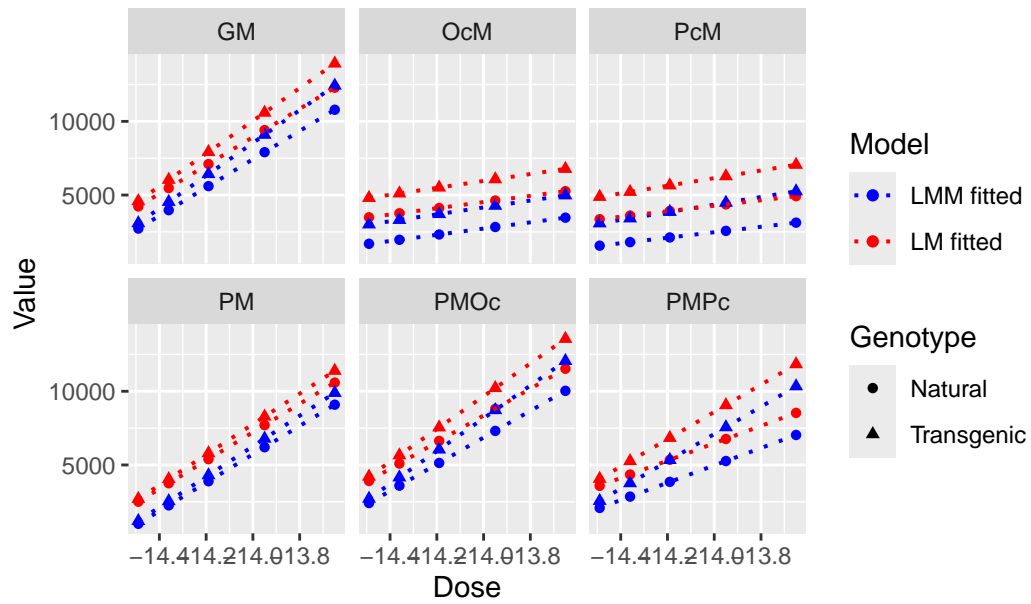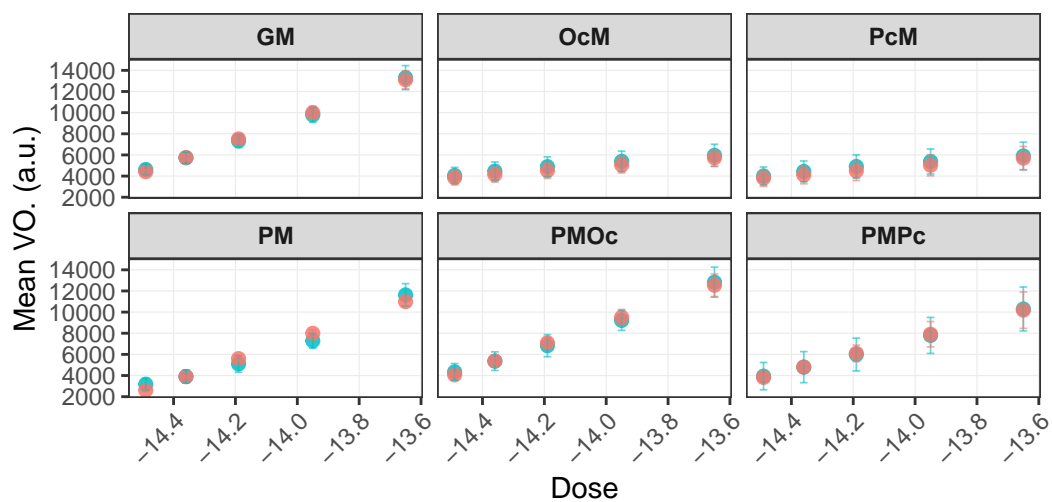## VO2 vs. Dose for pair 6

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.

Predictions vs. Observed VO.