# 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 figure 1 we can see that genotype has an 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. This suggests a need for an interaction between dose and substrate. The effect is most pronounced

when the doses are higher in $PMPc$ and $PMOc$, and for $OcM$ and $PcM$ we see a clear higher $VO_2$ production for all doses. We also can see that substrates involving Octanoyl Carnitine $Oc$ and Palmitoyl-Carnitine $Pc$ has a more pronounced separation between transgenic vs natural mouse.

$OcM$ 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

The researchers' experimental design, which matched a transgenic mouse with a natural type mouse and tested each pair on a different day, could induce some added variation that dose and substrate cannot account for. This is because the experimental setup could vary slightly day-to-day, influencing the measurement of our response variable $VO_2$.

Looking at Figure 2, we can see that there does in fact seem to be systematic differences on the pair level. For example, pair 5 exhibits a much larger gap between $VO_2$ production of transgenic and natural type mice for $OcM$ and $PcM$. Across every pair, we see enough variation between the genotype-$VO_2$ relationship to warrant consideration in our final modeling decisions.

### Conclusions

1. Genotype appears significantly correlated with $VO_2$ production: transgenic mice had higher $VO_2$ than nautral mice across most conditions
2. Substrate and dose seem to matter as well: 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. This relationship was highly dependent on dose.
3. Pairs: While researchers do not need to know the specific measurement predictions for each pair, we need to account for variation on the pair level in our model.

## 3 Modeling

To account for all of the points noted above, we chose to fit a fully interactive linear model regressing genotype, dose, and substrate on $VO_2$. Furthermore, we included a random intercept for pair, allowing us to include this added variation in the model while retaining the ability to predict on an unobserved pair.

Comparing this model to two other models – a model excluding genotype and a model excluding the random intercept – we see that our chosen equation is the best fit for the data.

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

From Table 1, we can see a log likelihood value of -2489.7 for our chosen model, and lower values for model 2 and 3 (-2759.2 and -2724.9, respectively). This supports the conclusion that our chosen model captures the behavior of the data the best.

NEED TO FIX p-values? 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.

Furthermore, Table 2 shows our models intraclass correlation (ICC), which is the ratio of pair variability to total variability. An ICC value of 0.644 is a strong piece of evidence in support of including a random intercept for pair, as it signifies that the variation across pairs is non-neglible.

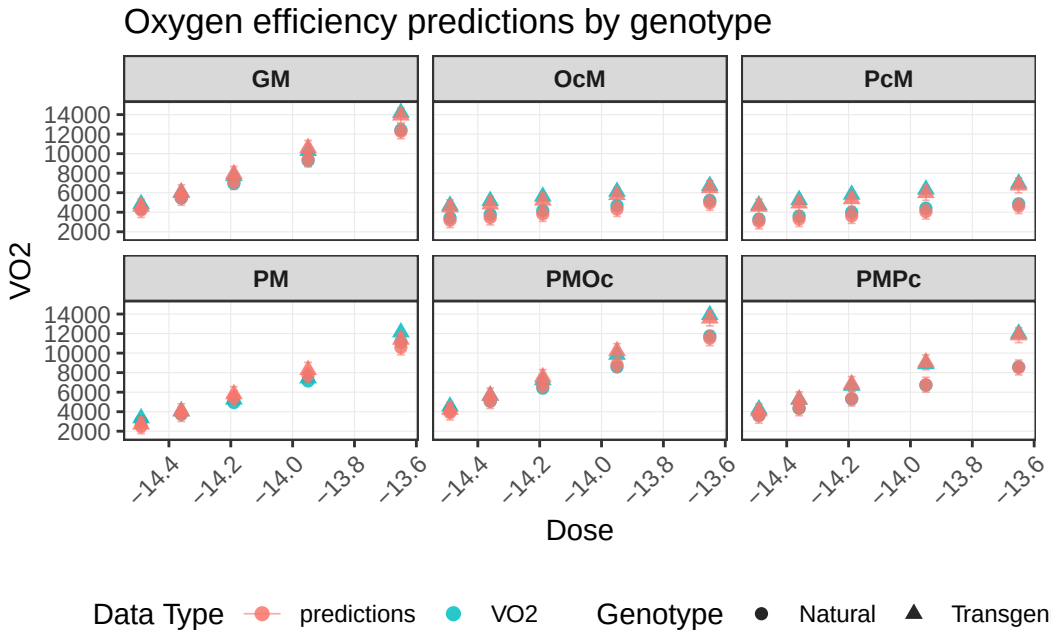
The conditional $R^2$ value in Table 2 represents the variance explained by our model. By allowing each pair have its own intercept we explain about 96.5% of the total variability in our data, higher than the 90% explained marginally by the fixed effects.

## 4 Analysis

TODO: bonferroni correction for p-values, include/analyze graph and give interpretation examples (see presentation).

```
preds <- predict(lmm1, data, allow.new.levels = TRUE)
graph_data <- data |>
  mutate(predictions = preds) |>
  tidyr::pivot_longer(cols = c(predictions, VO2),
                      names_to = "measure",
                      values_to = "value") |>
  dplyr::group_by(Substrate, measure, Dose,natural) |>
  dplyr::summarize(mean_value = mean(value, na.rm = TRUE),
                   standard_error = sd(value, na.rm = TRUE),
```

```
                    .groups = "drop")
graph_data |>
  ggplot(aes(x = Dose, y = mean_value, color = measure,shape = natural)) +
  geom_point(size = 2, alpha = 0.85) +
  geom_errorbar(data = filter(graph_data, measure == "predictions"),aes(ymin = mean_value - s
                    ymax = mean_value + standard_error),
                width = 0.025, linewidth = 0.3, alpha = 0.6) +
  facet_wrap(. ~ Substrate) +
  labs(title = "Oxygen efficiency predictions by genotype",
    x = "Dose",
    y = "VO2",
    color = "Data Type",
    shape = "Genotype"
  ) +
  scale_y_continuous(
    breaks = seq(0, 16000, 2000)
  ) +
  theme_bw() +
  theme(
    legend.position = "bottom",
    strip.text = element_text(face = "bold"),
    panel.grid.major = element_line(linewidth = 0.2),
    panel.grid.minor = element_blank(),
    axis.text.x = element_text(angle = 45, hjust = 1, vjust = 1)
  )
```




## 5 Conclusion and Future Work

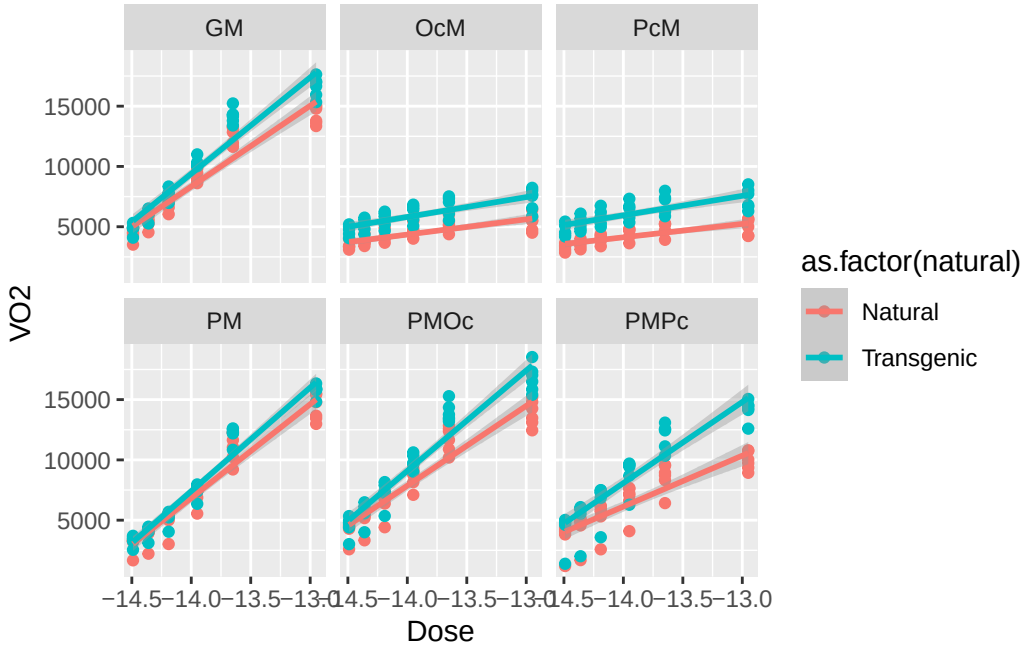
Our analysis showed strong evidence that genotype significantly influences $VO_2$ production conditional on both substrate and dose. Across nearly all experimental conditions, transgenic mice displayed a higher $VO_2$ production relative to natural mice with some substrates amplifying this effect more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

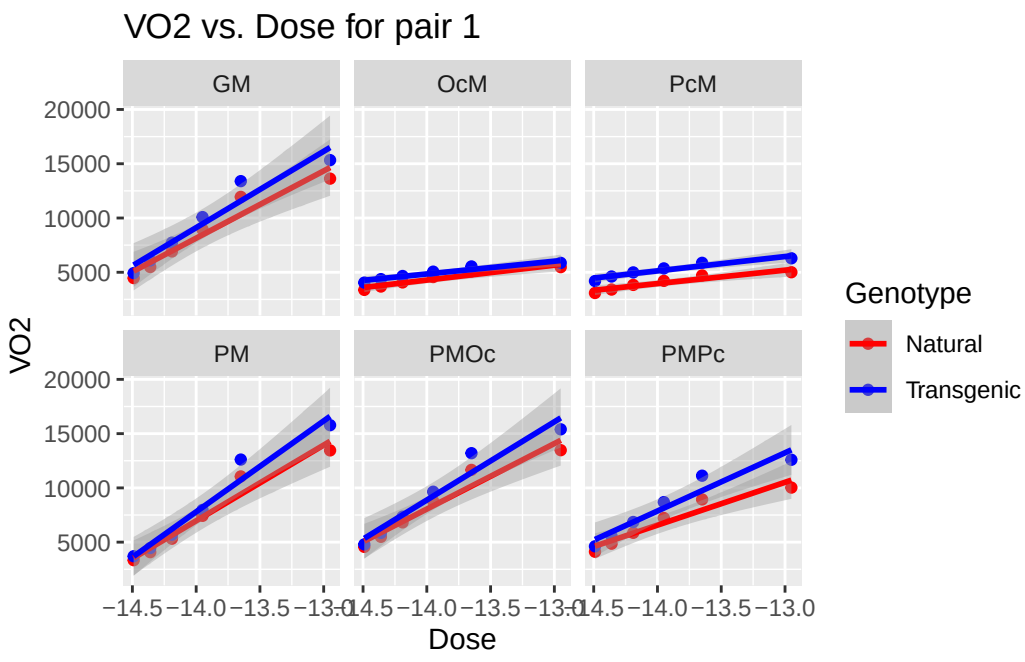
However, one major limitation can be seen in Figure 4, the residual plot across dosage levels. We see some evidence of a nonlinear relationship between dose and $VO_2$ production conditional on substrate and genotype, something that was not accounted for in our model. We chose to live the relationship linear because it was our understanding that the researchers' had some biological motivation behind this claim. Since our analysis shows evidence arguing against this claim, further work should include interrogating these assumptions, especially among higher dosage levels.

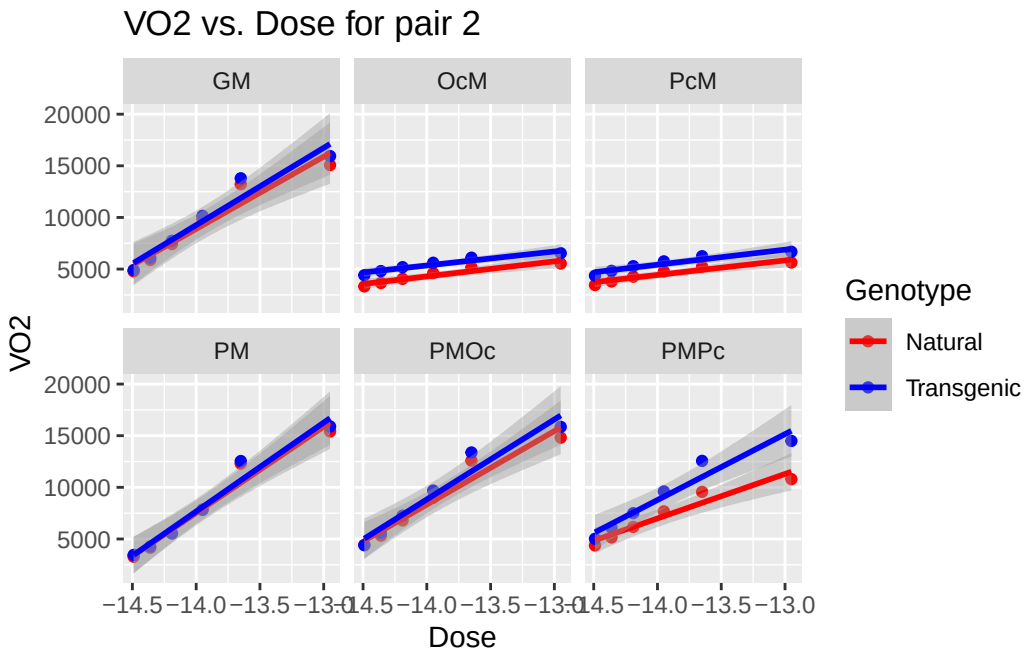
In addition, Future work should focus on exploring a broader range of substrates and leveraging larger samples to better account for variability across experimental pairs. These extensions would help clarify the extent to which the observed genotype effects generalize across different biological and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

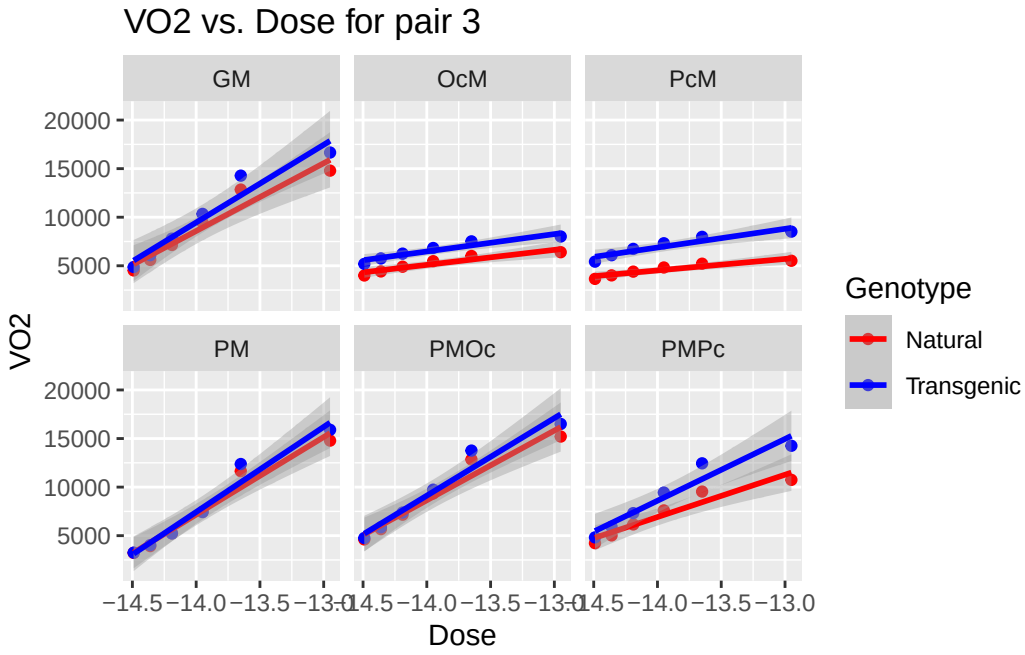
## 6 Appendix

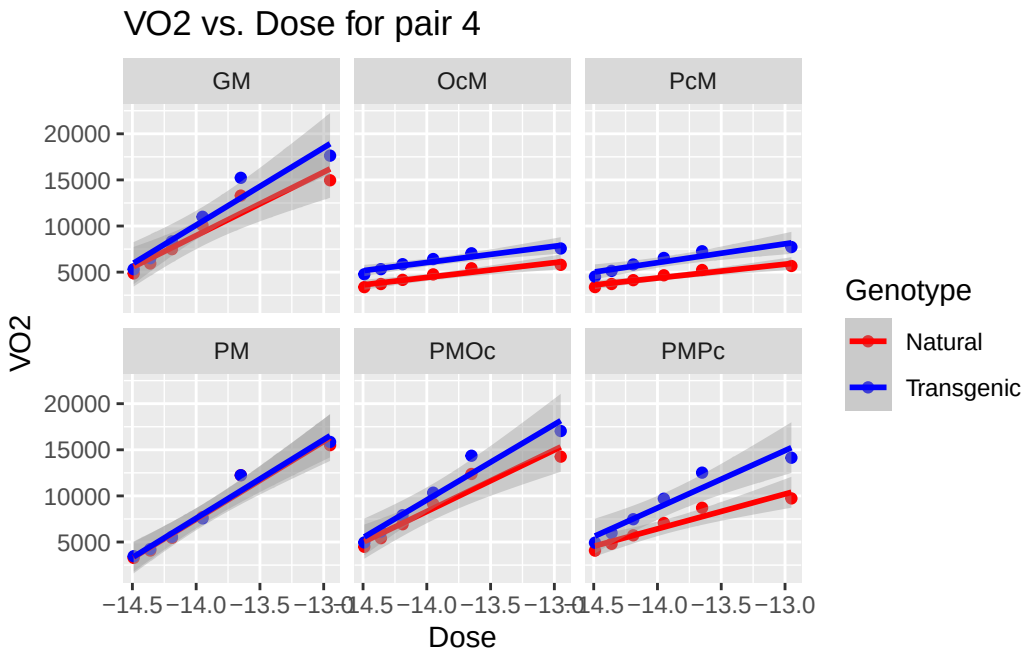
### Exploratory Data Analysis

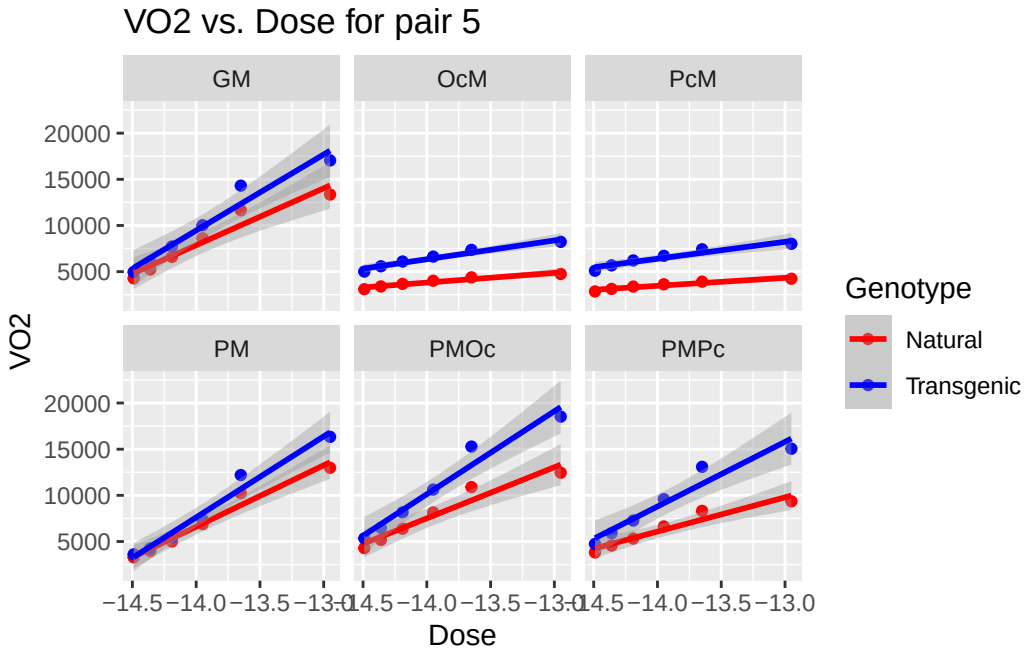



VO2 vs. Dose for pair 2
GM
OcM
PcM
PM
PMOc
PMPc
VO2
20000
15000
10000
5000
−14.5
−14.0
−13.5
−13.0
Dose
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 3
GM
OcM
PcM
PM
PMOc
PMPc
VO2
20000
15000
10000
5000
−14.5
−14.0
−13.5
−13.0
Dose
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 4
GM
OcM
PcM
PM
PMOc
PMPc
VO2
Dose
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 5
GM
OcM
PcM
PM
PMOc
PMPc
VO2
Dose
Genotype
Natural
Transgenic

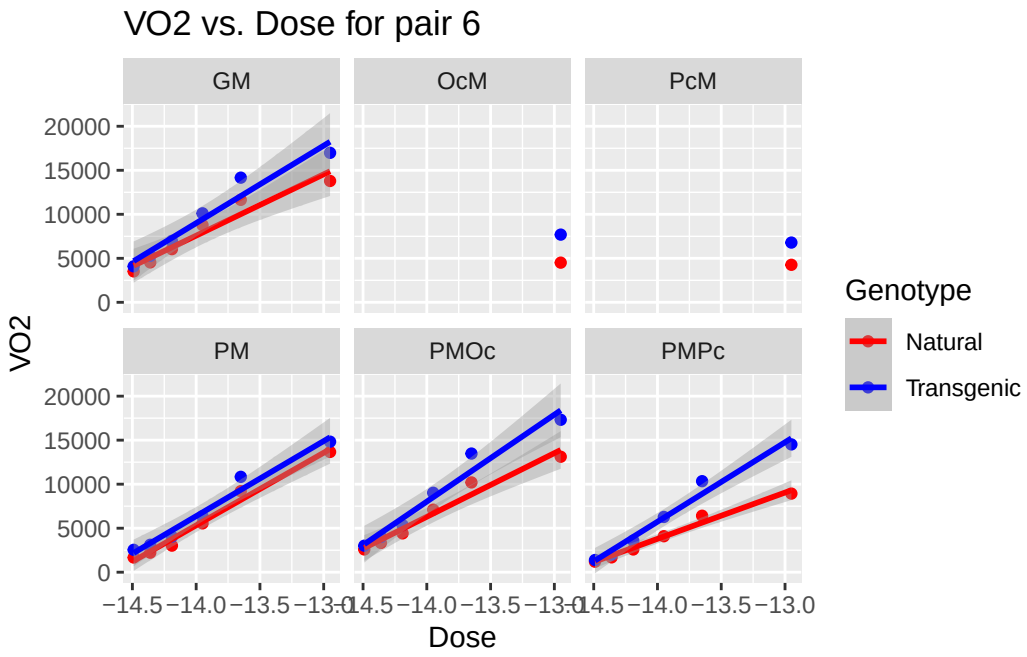



Table 1: Model comparison using AIC and Adjusted $R^2$

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

**Modeling**

```
Call:
lm(formula = 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 ***
```

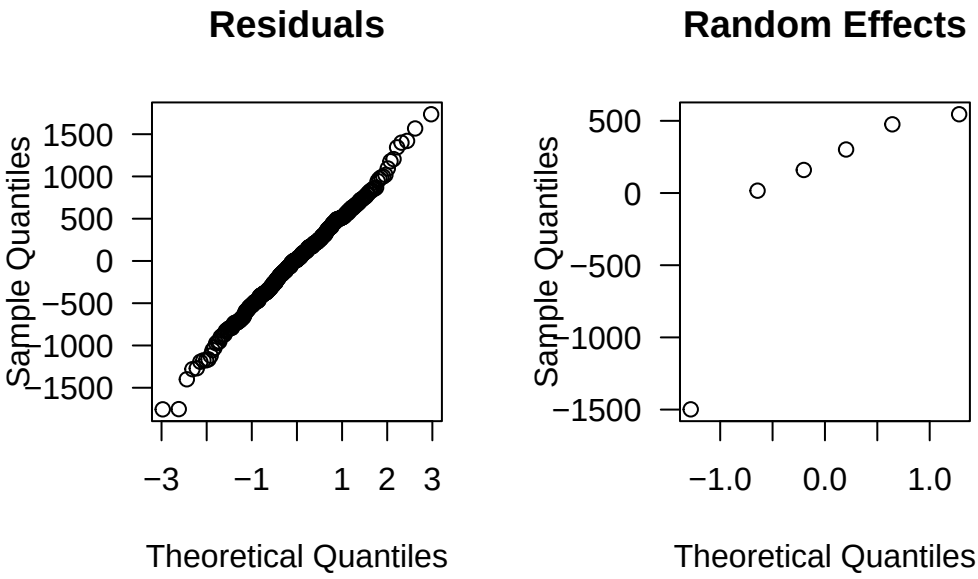
```
SubstratePcM    4926.5      379.4   12.98   <2e-16 ***
SubstratePM     6215.9      346.4   17.95   <2e-16 ***
SubstratePMOc   7716.4      346.4   22.28   <2e-16 ***
SubstratePMPc   6563.2      346.4   18.95   <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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) 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
```

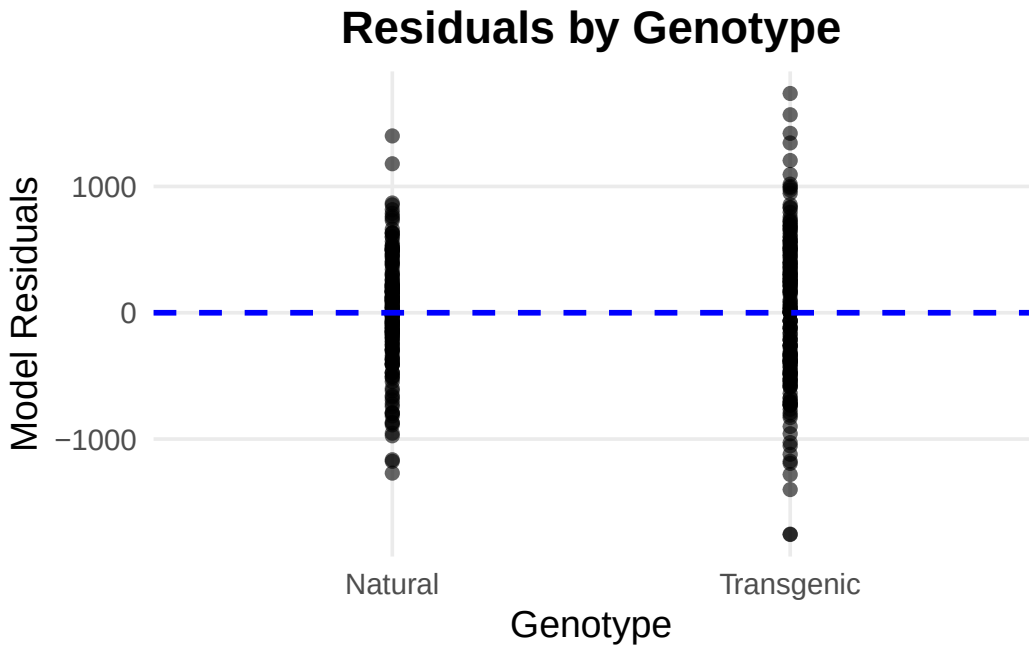
```
naturalTransgenic:SubstratePM         -11623.31    9782.99     310.97  -1.188
naturalTransgenic:SubstratePMOc         7909.05    9782.99     310.97   0.808
naturalTransgenic:SubstratePMPc        26976.20    9782.99     310.97   2.757
Dose:SubstrateOcM                      -7485.71     513.42     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: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
```

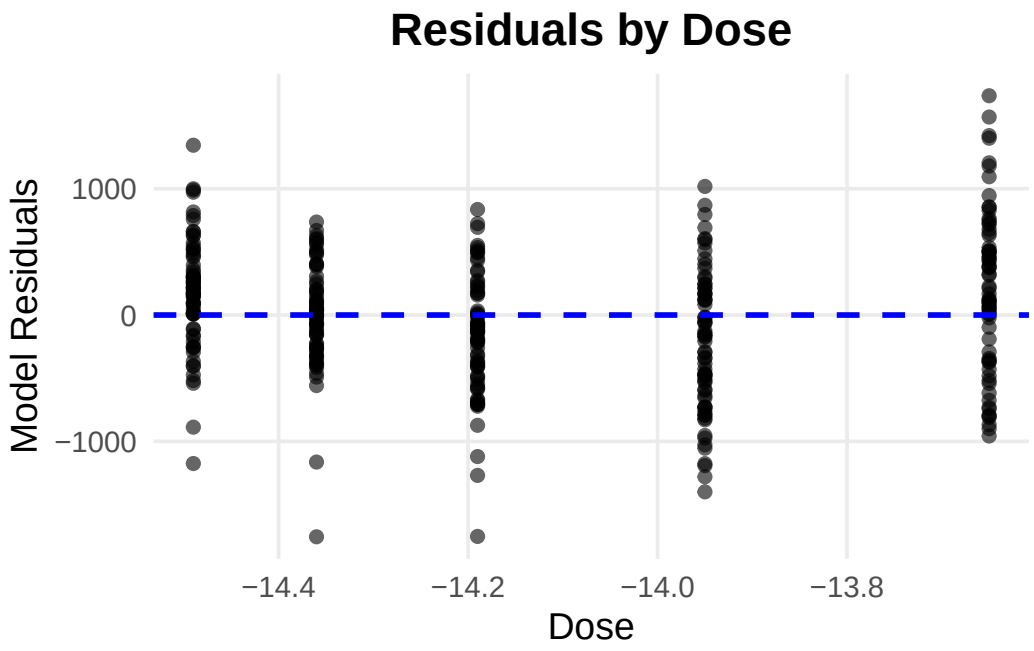
```
Correlation matrix not shown by default, as p = 24 > 12.
Use print(x, correlation=TRUE)  or
    vcov(x)        if you need it

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 4701.9667 < 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: 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)
```

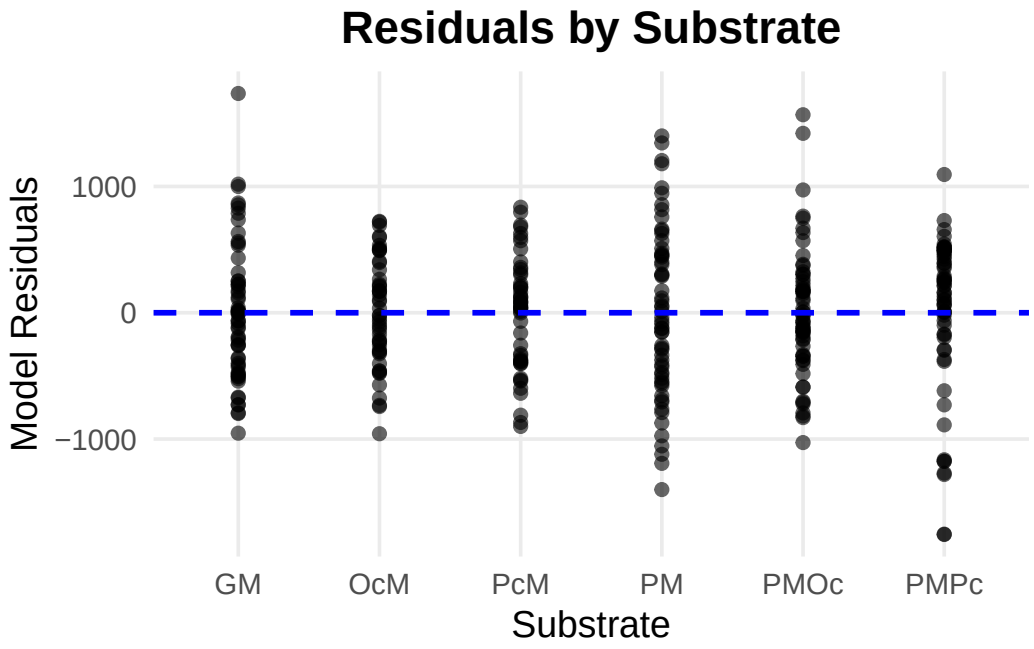
```
1  26 -2489.7
2  14 -2724.9 -12 470.45  < 2.2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# 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 *
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
```

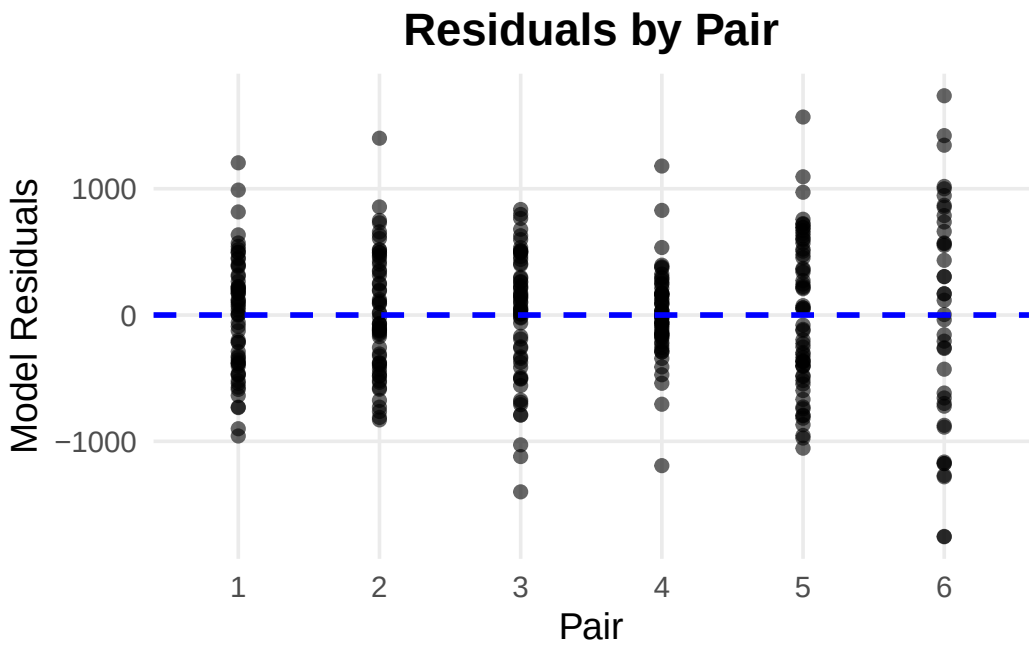
```
Dose:SubstratePMOc                       -532.46     723.63  -0.736   0.4624
Dose:SubstratePMPc                      -3706.61     723.63  -5.122 5.26e-07 ***
naturalTransgenic:Dose:SubstrateOcM     -1278.14    1073.31  -1.191   0.2346
naturalTransgenic:Dose:SubstratePcM      -784.42    1073.31  -0.731   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: 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
```

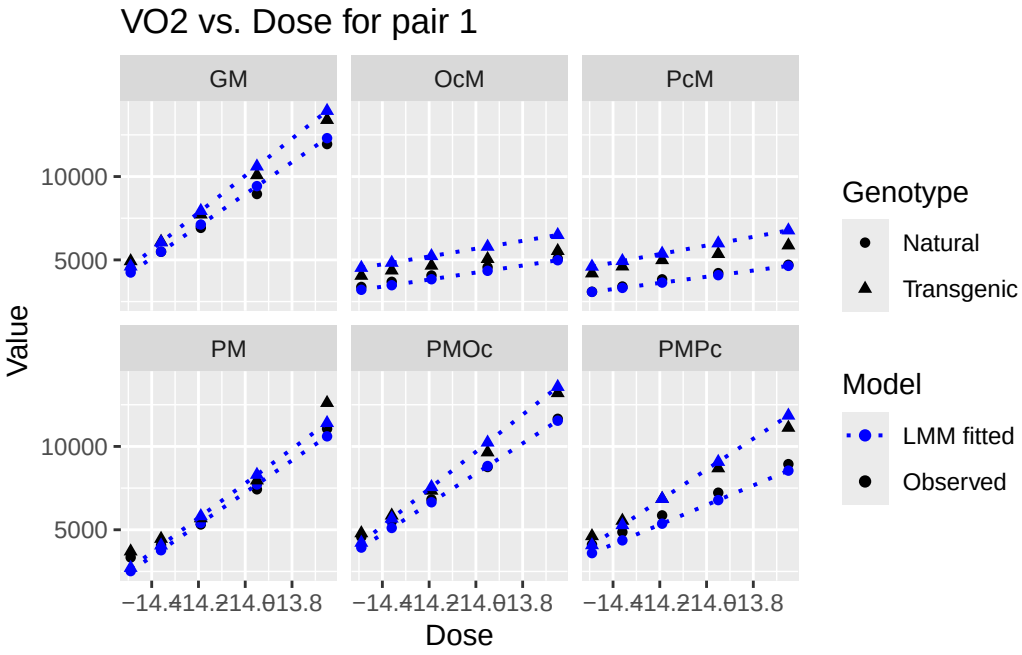

Residuals
Sample Quantiles
1500
1000
500
0
−500
−1000
−1500
−3
−1
1
2
3
Theoretical Quantiles
Random Effects
Sample Quantiles
500
0
−500
−1000
−1500
−1.0
0.0
1.0
Theoretical Quantiles

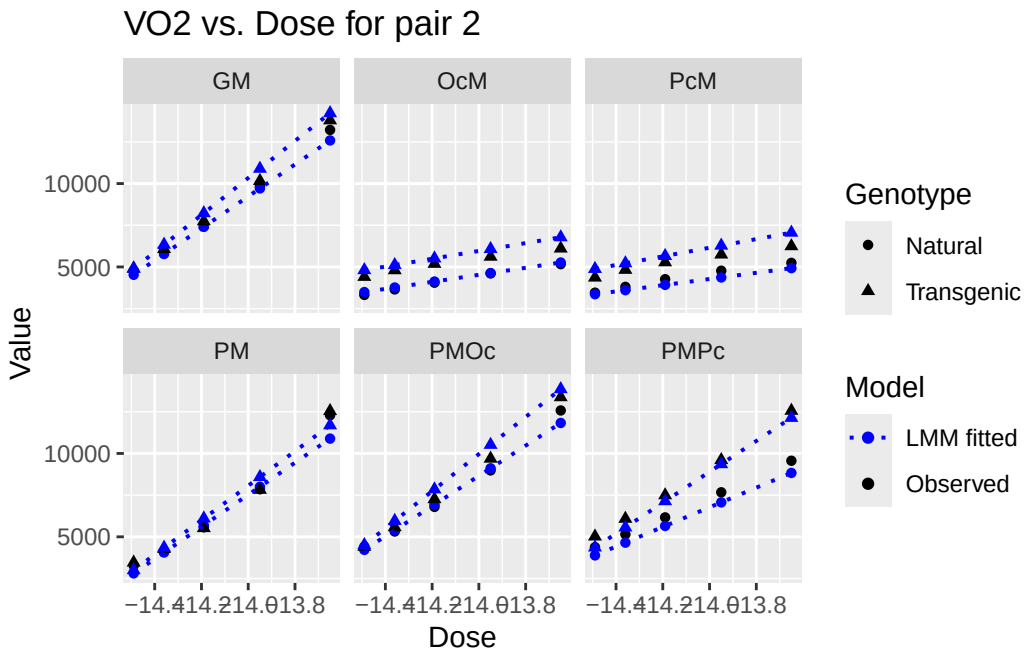

Residuals by Genotype
Model Residuals
1000
0
−1000
Natural
Transgenic
Genotype

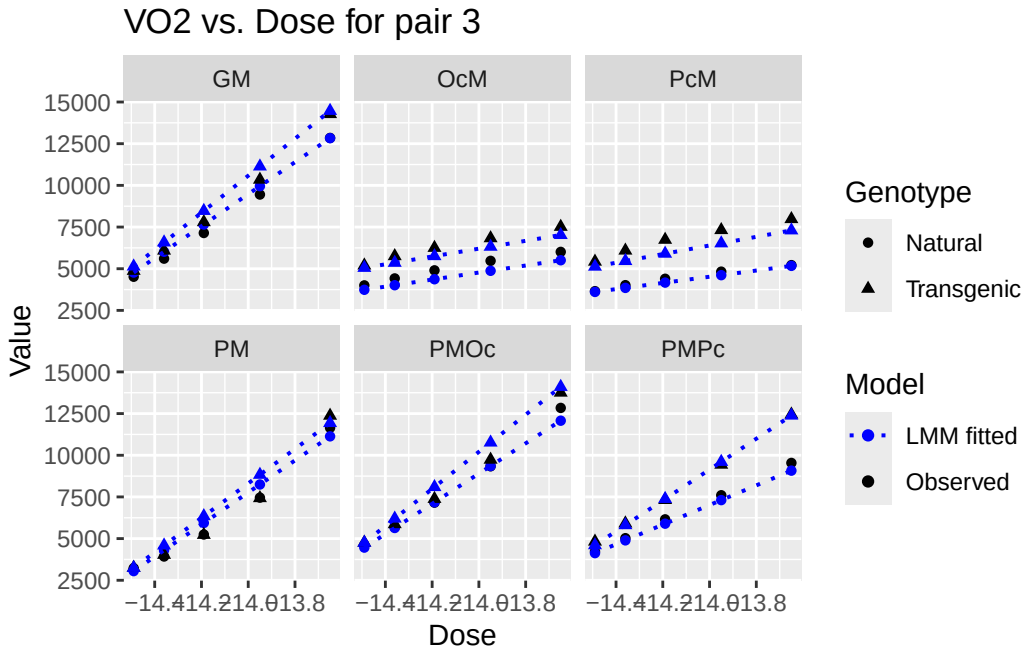

Residuals by Dose
Model Residuals
1000
0
−1000
−14.4
−14.2
−14.0
−13.8
Dose

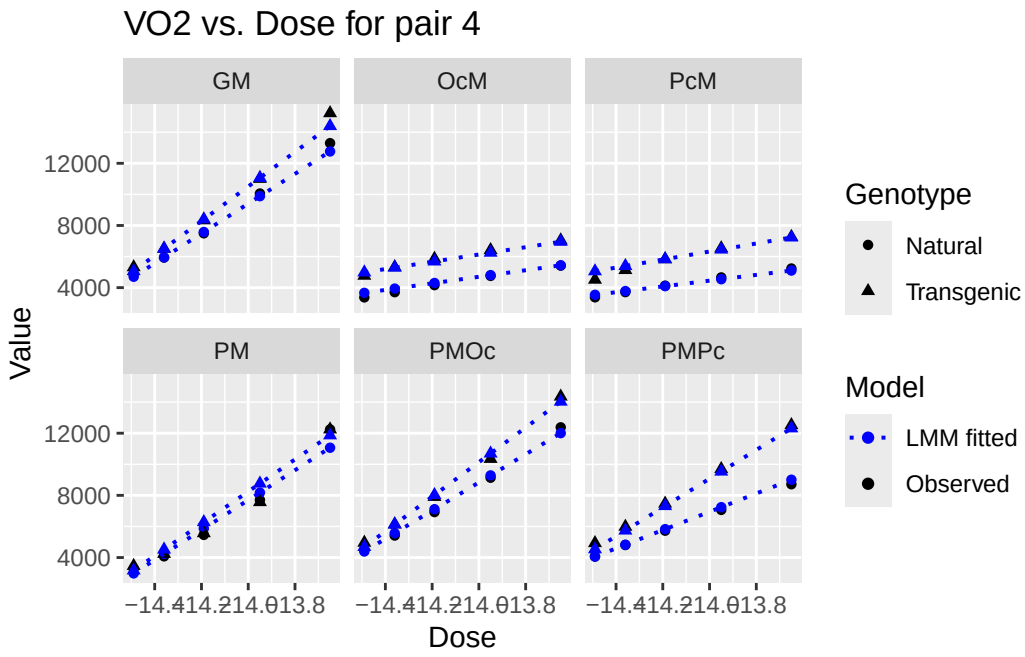

Residuals by Substrate
Model Residuals
1000
0
−1000
GM
OcM
PcM
PM
PMOc
PMPc
Substrate

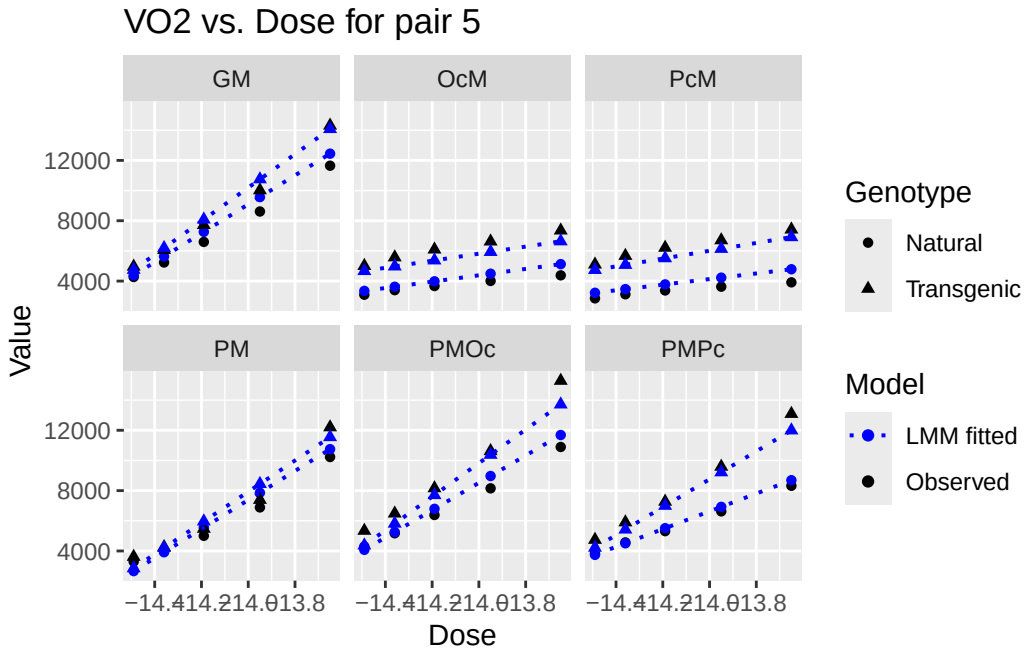

Residuals by Pair
Model Residuals
1000
0
−1000
1
2
3
4
5
6
Pair


VO2 vs. Dose for pair 1
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

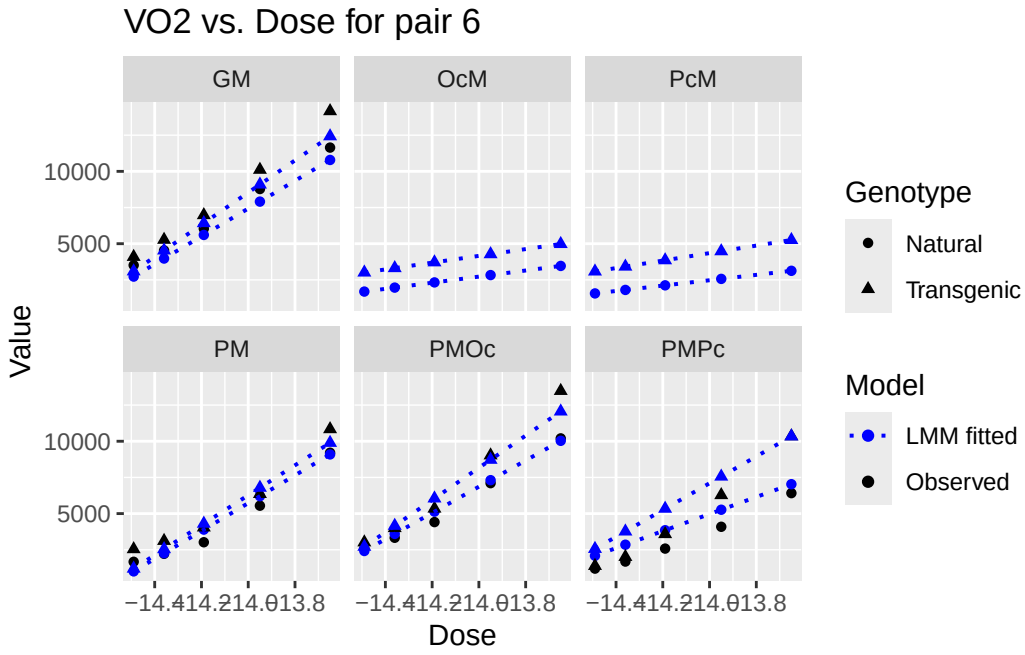

VO2 vs. Dose for pair 2
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

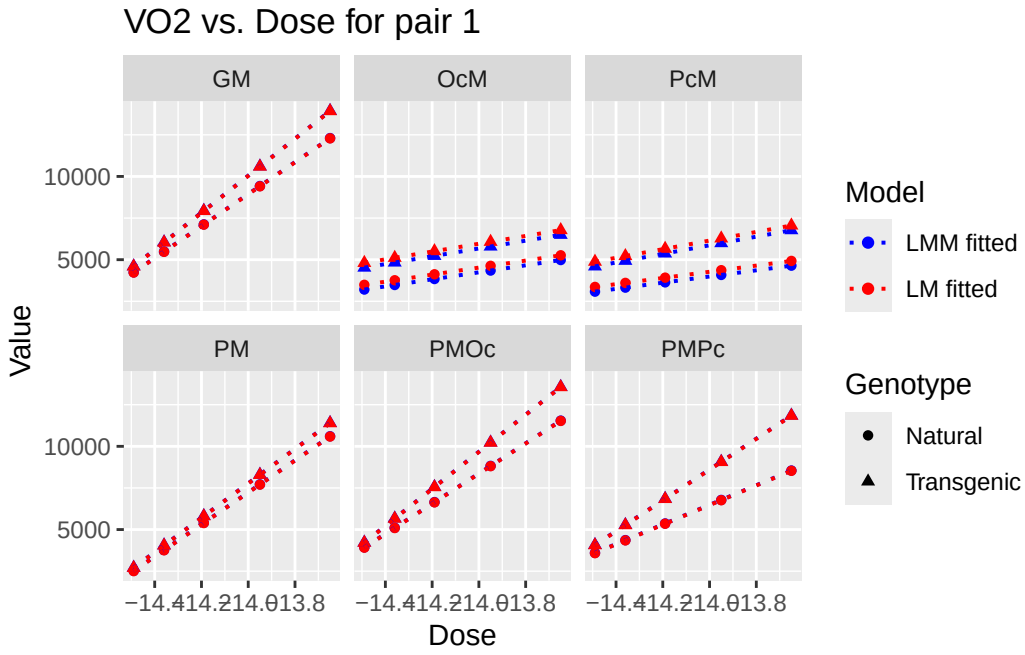

VO2 vs. Dose for pair 3
GM
OcM
PcM
PM
PMOc
PMPc
Value
15000
12500
10000
7500
5000
2500
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

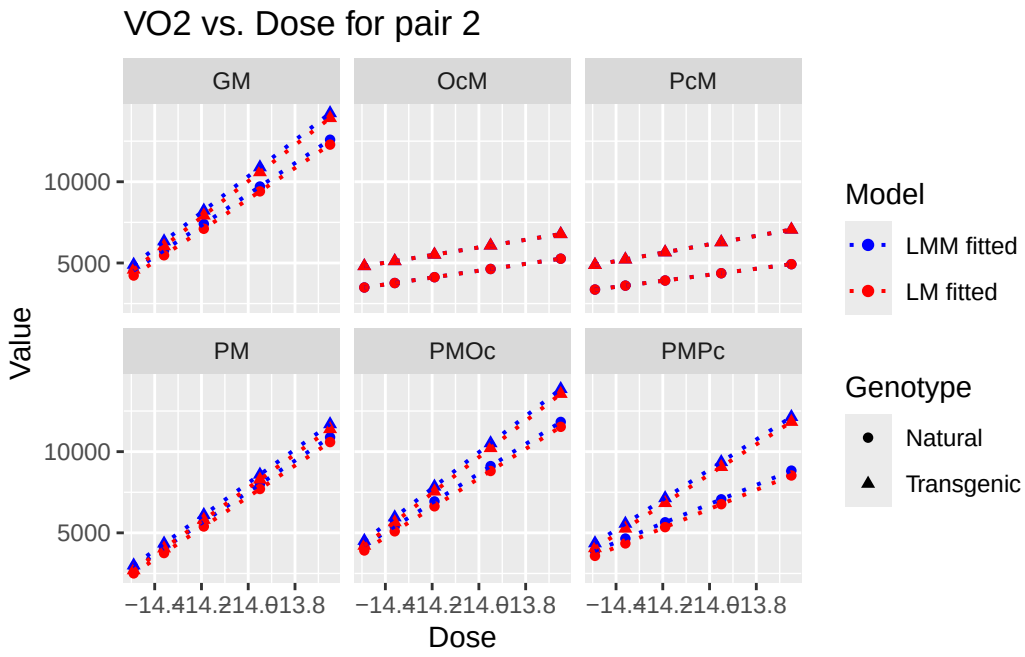


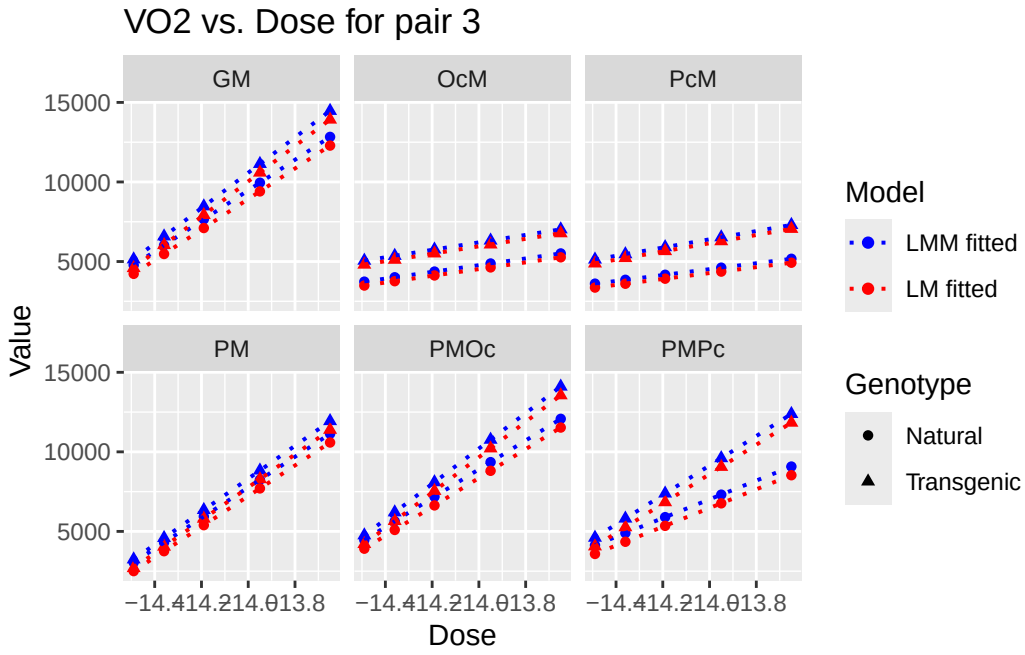



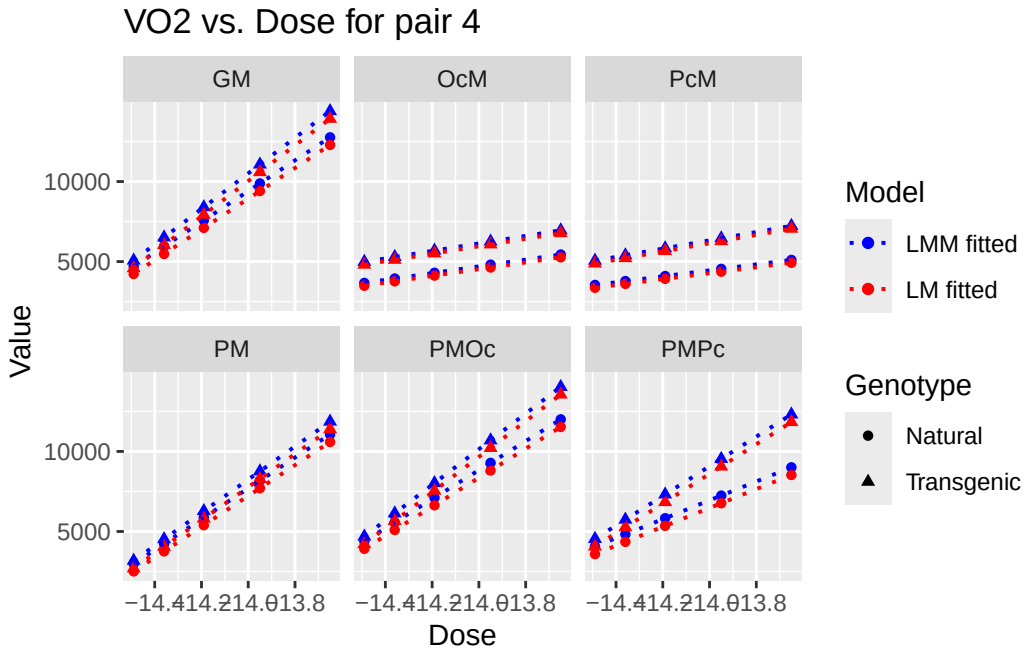

```
Warning: Removed 20 rows containing missing values or values outside the scale range
(`geom_point()`).
```

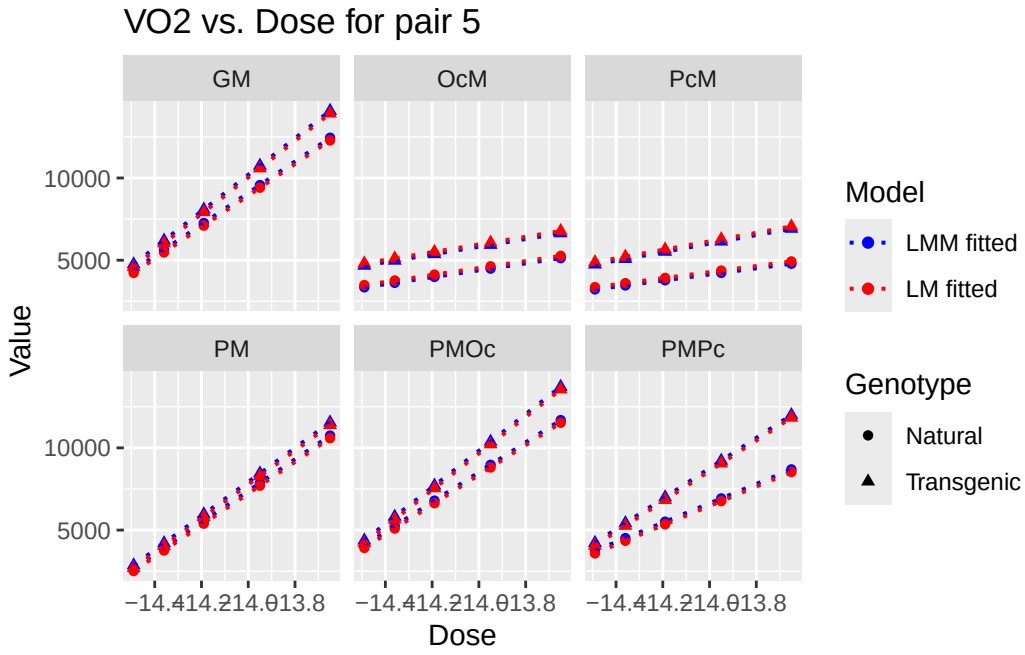

VO2 vs. Dose for pair 6
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Genotype
Natural
Transgenic
Model
LMM fitted
Observed

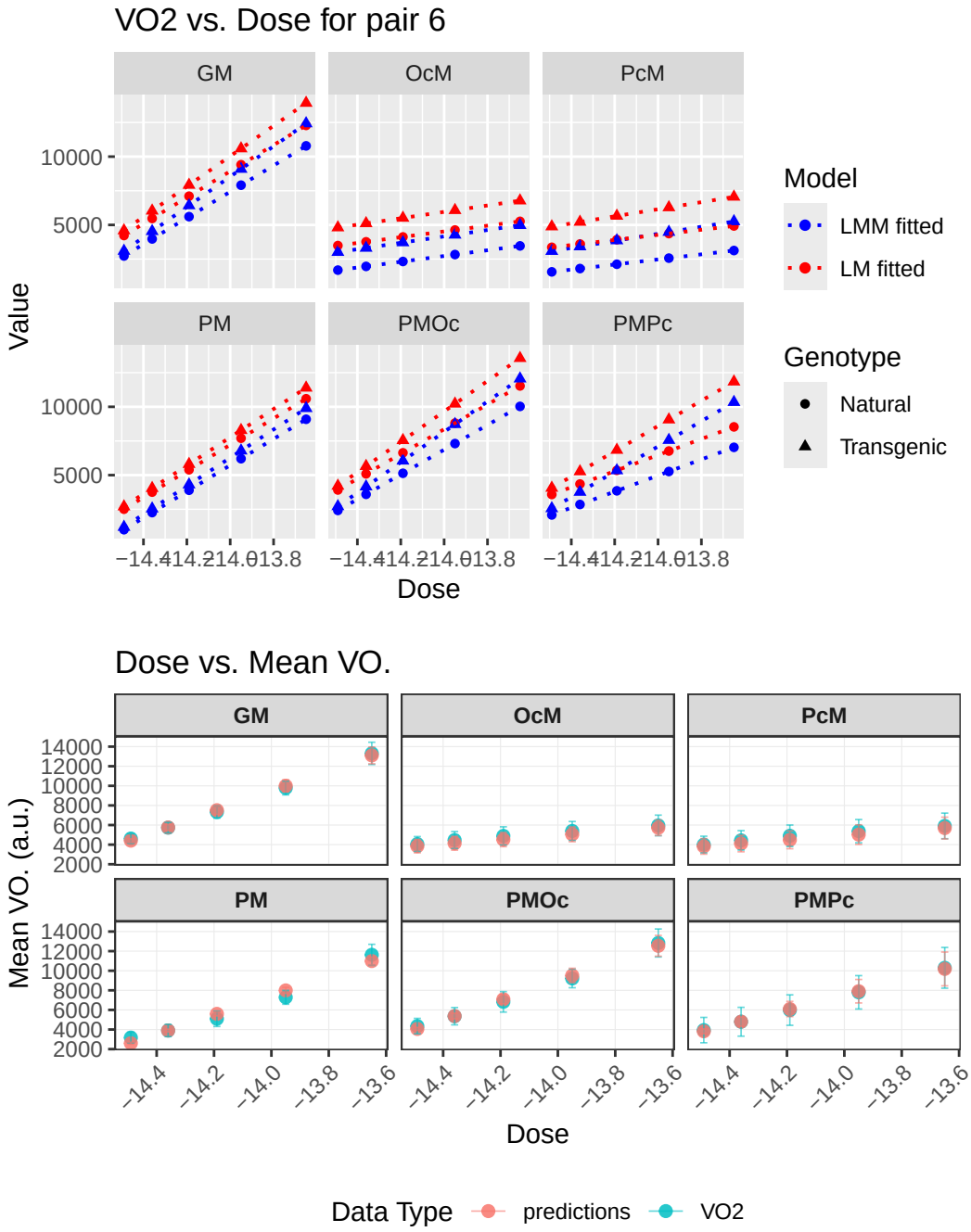

VO2 vs. Dose for pair 1
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 2
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic

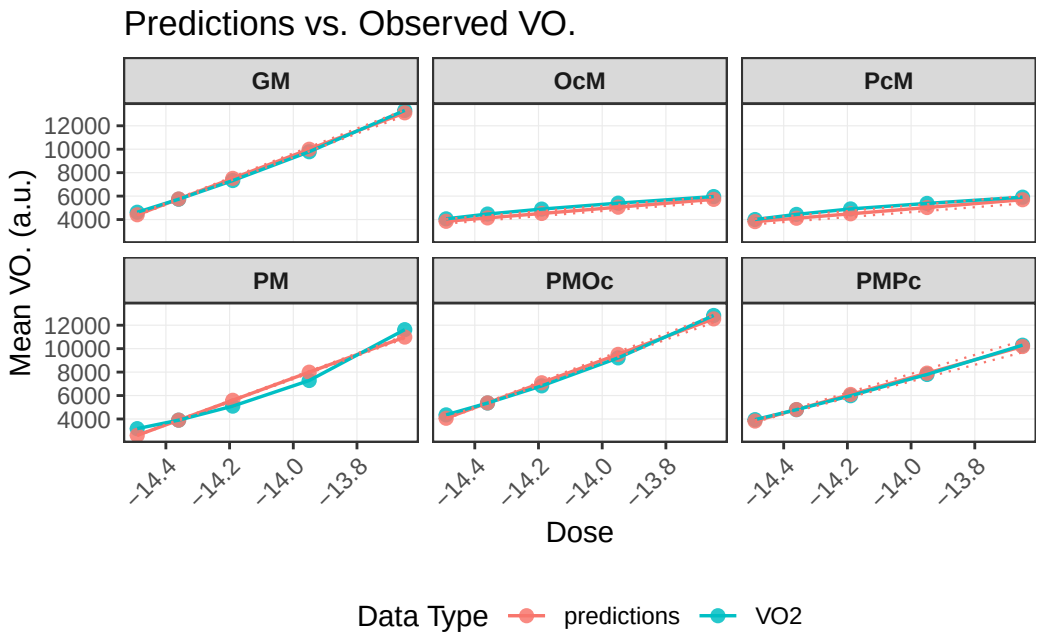

VO2 vs. Dose for pair 3
GM
OcM
PcM
PM
PMOc
PMPc
Value
15000
10000
5000
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 4
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 5
GM
OcM
PcM
PM
PMOc
PMPc
10000
5000
Value
−14.4
14.2
14.0
13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


VO2 vs. Dose for pair 6
GM
OcM
PcM
PM
PMOc
PMPc
Value
10000
5000
−14.4
−14.2
−14.0
−13.8
Dose
Model
LMM fitted
LM fitted
Genotype
Natural
Transgenic


Dose vs. Mean VO.
GM
OcM
PcM
PM
PMOc
PMPc
Mean VO. (a.u.)
14000
12000
10000
8000
6000
4000
2000
−14.4
−14.2
−14.0
−13.8
−13.6
Dose
Data Type
predictions
VO2


Predictions vs. Observed VO.
GM
OcM
PcM
PM
PMOc
PMPc
Mean VO. (a.u.)
12000
10000
8000
6000
4000
−14.4
−14.2
−14.0
−13.8
Dose
Data Type
predictions
VO2