# Statistical analyses for nested eperimental design

Jixiang Wu, South Dakota State University

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```
## Warning: package 'knitr' was built under R version 3.3.2
```

## Introduction

When two or more factors are considered in an experimental design, these treatment factors can be in a factorial way, which has been introduced in the previous chapter. However, other ways related to these treatment factors are possible too. Another common one is nested treatment designs. In this case, one factor is nested within another factor. The data analysis for such treatment designs can be conducted by either ANOVA methods or linear mixed model approaches, depending on the data structures. We will use several data sets to demonstrate the use of different methods.

## Two-factor nested experimental designs

There are several models to analyze two-factor nested experimental designs based on if blocks are included. We assume that block effects are included.

Assume the above used data come from a nested genetic mating design, we can work on the nested experimental designs. When demonstrating data analysis, we frequentyly use the term "assume" or "assumption" or related word to use an appropirate method. In reality, you need to choose particular linear models and/or particular methods based on the objectives of a research project. It is very important to clarify in your own research work.

In this demonstration, we will use a previously used data set, which is available in the minque package. It is easy to load this data set following these R codes provided below.

```
require(minque)

## Loading required package: minque

## Loading required package: klaR

## Loading required package: MASS

## Loading required package: Matrix

data(ncii)
```

# ANOVA based analysis

We may try to use the built-in function to run Unlike factorial factor design, the model system for nested factor design used in the aov will be a little different. Before we run an ANOVA analysis, we need to convent several variables in this data set.

```
dat=ncii
dat=transform(dat,Female=factor(Female),Male=factor(Male),Rep=factor(Rep))
```

I found three model expressions showed the same results. Knowing these expression sometimes is very helpful.

```
# Model expression 1
mod=aov(Yld~Male+Male/Female,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.764 | 859.38216 | 9.587483 | 0.0003401 |
| Male:Female | 12 | 24777.782 | 2064.81514 | 23.035596 | 0.0000000 |
| Residuals | 45 | 4033.613 | 89.63585 | NA | NA |

```
# Model expression 2
mod=aov(Yld~Male+Female:Male,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.764 | 859.38216 | 9.587483 | 0.0003401 |
| Male:Female | 12 | 24777.782 | 2064.81514 | 23.035596 | 0.0000000 |
| Residuals | 45 | 4033.613 | 89.63585 | NA | NA |

```
# Model expression 3
mod=aov(Yld~Male+Male:Female,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.764 | 859.38216 | 9.587483 | 0.0003401 |
| Male:Female | 12 | 24777.782 | 2064.81514 | 23.035596 | 0.0000000 |
| Residuals | 45 | 4033.613 | 89.63585 | NA | NA |

Rember that the above data analysis is only suitable when both female and male are fixed effects and no block effect is assumed.

If you want to include a block effect in the model, it is very easy to add that effect in the model as showed below.

```
# Model expression 1
mod=aov(Yld~Male+Male/Female+Rep,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.7643 | 859.38216 | 9.2088698 | 0.0004828 |
| Rep | 3 | 114.1255 | 38.04184 | 0.4076444 | 0.7482971 |
| Male:Female | 12 | 24777.7816 | 2064.81514 | 22.1259116 | 0.0000000 |
| Residuals | 42 | 3919.4876 | 93.32113 | NA | NA |

```
# Model expression 2
mod=aov(Yld~Male+Female:Male+Rep,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.7643 | 859.38216 | 9.2088698 | 0.0004828 |
| Rep | 3 | 114.1255 | 38.04184 | 0.4076444 | 0.7482971 |
| Male:Female | 12 | 24777.7816 | 2064.81514 | 22.1259116 | 0.0000000 |
| Residuals | 42 | 3919.4876 | 93.32113 | NA | NA |

```
# Model expression 3
mod=aov(Yld~Male+Male:Female+Rep,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|  | Df | Sum.Sq | Mean.Sq | F.value | Pr..F. |
|---|---|---|---|---|---|
| Male | 2 | 1718.7643 | 859.38216 | 9.2088698 | 0.0004828 |
| Rep | 3 | 114.1255 | 38.04184 | 0.4076444 | 0.7482971 |
| Male:Female | 12 | 24777.7816 | 2064.81514 | 22.1259116 | 0.0000000 |
| Residuals | 42 | 3919.4876 | 93.32113 | NA | NA |

When female is random, based on EMS provided in this class, the F-test for factor Male is not right, by doing so, we may slightly change the model expression to obtain a right F-test for factor Male.

```
mod=aov(Yld~Male+Error(Male:Female)+Rep,data=dat)

## Warning in aov(Yld ~ Male + Error(Male:Female) + Rep, data = dat): Error()
## model is singular

summary(mod)

##
## Error: Male:Female
```

```
##            Df Sum Sq Mean Sq F value Pr(>F)
## Male        2   1719   859.4   0.416  0.669
## Residuals  12  24778  2064.8
##
## Error: Within
##            Df Sum Sq Mean Sq F value Pr(>F)
## Rep         3    114   38.04   0.408  0.748
## Residuals  42   3919   93.32
```

Now you have a right F-test for factor male. All other F-tests please refer to the regular ANOVA results.Accordingly, if we want to compare the differences between any two males, we will need to use the mean square for female instead of the MSE in the original ANOVA analysis. The degrees of freedom will be that for female when a test or any pair-wise mean comparisons are performed.

## Linear mixed model analysis

We may use linear mixed model analysis for this nested factor experimental design. By doing so, we will need to determine which effects are fixed or random. Again, the vertical bar "|" will be used to separate fixed effect(s) and random effects.The first case is for that both male and female are fixed.

### Case 1: Both male and female are fixed
```
res=lmm.jack(Yld~Male+Male:Female|Rep,data=dat)[[1]]
data.frame(res$Var[[1]])
```

|        | Estimate   | SE        | PValue    | X2.5.LL    | X97.5.UL    |
|--------|------------|-----------|-----------|------------|-------------|
| V(Rep) | 0.0194057  | 0.0613661 | 0.9780305 | -0.1973558 | 0.2361671   |
| V(e)   | 93.9641254 | 5.6661721 | 0.0000002 | 73.9496834 | 113.9785673 |

```
data.frame(res$PVar[[1]])
```

|           | Estimate  | SE        | PValue    | X2.5.LL    | X97.5.UL  |
|-----------|-----------|-----------|-----------|------------|-----------|
| V(Rep)/VP | 0.0002351 | 0.0007435 | 0.9780305 | -0.0023910 | 0.0028612 |
| V(e)/VP   | 0.9997649 | 0.0007435 | 0.0000000 | 0.9971388  | 1.0023910 |

```
data.frame(res$FixedEffect[[1]])
```

|                  | Estimate    | SE        | PValue    | X2.5.LL     | X97.5.UL    |
|------------------|-------------|-----------|-----------|-------------|-------------|
| mu               | 86.0898489  | 0.4210378 | 0.0000000 | 84.6026302  | 87.577067   |
| Male(6)          | -0.0405724  | 0.5213790 | 0.9937588 | -1.8822230  | 1.801078    |
| Male(7)          | 5.5177798   | 0.3578130 | 0.0000004 | 4.2538883   | 6.781671    |
| Male(8)          | -5.4772074  | 0.3373617 | 0.0000002 | -6.6688594  | -4.285555   |
| Male:Female(6:1) | -17.4100053 | 0.7450692 | 0.0000000 | -20.0417900 | -14.778221  |
| Male:Female(7:1) | 16.1111281  | 1.7422977 | 0.0000274 | 9.9568646   | 22.265391   |

| | | | | | |
|---|---|---|---|---|---|
| Male:Female(8:1) | -27.9503081 | 0.8455030 | 0.0000000 | -30.9368520 | -24.963764 |
| Male:Female(6:2) | -15.2984431 | 1.7201770 | 0.0000376 | -21.3745705 | -9.222316 |
| Male:Female(7:2) | 6.0323713 | 1.8531502 | 0.0389770 | -0.5134531 | 12.578196 |
| Male:Female(8:2) | 4.9460252 | 1.4527019 | 0.0308352 | -0.1853083 | 10.077359 |
| Male:Female(6:3) | 14.8386596 | 0.9707360 | 0.0000004 | 11.4097593 | 18.267560 |
| Male:Female(7:3) | 20.0430379 | 1.1039191 | 0.0000001 | 16.1436993 | 23.942377 |
| Male:Female(8:3) | 37.1223585 | 1.0908651 | 0.0000000 | 33.2691299 | 40.975587 |
| Male:Female(6:4) | 19.7284929 | 1.8524965 | 0.0000085 | 13.1849775 | 26.272008 |
| Male:Female(7:4) | -27.1576287 | 1.9519811 | 0.0000009 | -34.0525503 | -20.262707 |
| Male:Female(8:4) | -33.8026415 | 1.3939913 | 0.0000000 | -38.7265932 | -28.878690 |
| Male:Female(6:5) | -1.8992764 | 2.1877705 | 0.8387200 | -9.6270697 | 5.828517 |
| Male:Female(7:5) | -9.5111287 | 1.8232955 | 0.0022051 | -15.9514982 | -3.070759 |
| Male:Female(8:5) | 14.2073585 | 1.5388521 | 0.0000277 | 8.7717195 | 19.642998 |

## Case 2: Both male and female are random

The second case is that both male and female are random.

```
res=lmm.jack(Yld~1|Male+Male:Female+Rep,data=dat)[[1]]
data.frame(res$Var[[1]])
```

| | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Male) | 0.0000000 | 0.0000000 | 1.0000000 | 0.000000 | 0.000000 |
| V(Rep) | 0.1965852 | 0.4555052 | 0.9631591 | -1.412381 | 1.805552 |
| V(Male:Female) | 492.0468867 | 19.7713325 | 0.0000000 | 422.209229 | 561.884545 |
| V(e) | 93.0763519 | 7.9319043 | 0.0000037 | 65.058735 | 121.093969 |

```
data.frame(res$PVar[[1]])
```

| | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Male)/VP | 0.0000000 | 0.0000000 | 1.0000000 | 0.0000000 | 0.0000000 |
| V(Rep)/VP | 0.0003424 | 0.0007928 | 0.9630983 | -0.0024578 | 0.0031427 |
| V(Male:Female)/VP | 0.8404374 | 0.0151787 | 0.0000000 | 0.7868223 | 0.8940525 |
| V(e)/VP | 0.1592202 | 0.0154661 | 0.0000112 | 0.1045898 | 0.2138506 |

```
data.frame(res$FixedEffect[[1]])
```

| | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| mu | 86.12719 | 0.5796184 | 0 | 84.07982 | 88.17456 |

```
data.frame(res$RandomEffect[[1]])
```

| | Pre | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| Male(6) | 0.0000000 | 0.0000000 | 1.0000000 | 0.0000000 | 0.0000000 |
| Male(7) | 0.0000000 | 0.0000000 | 1.0000000 | 0.0000000 | 0.0000000 |
| Male(8) | 0.0000000 | 0.0000000 | 1.0000000 | 0.0000000 | 0.0000000 |
| Rep(1) | -0.1482982 | 0.3135516 | 0.9562620 | -1.2558469 | 0.9592504 |
| Rep(2) | -0.1648107 | 0.3809187 | 0.9629888 | -1.5103177 | 1.1806964 |
| Rep(3) | 0.0843874 | 0.1803467 | 0.9571511 | -0.5526457 | 0.7214205 |
| Rep(4) | 0.2287215 | 0.5086158 | 0.9602497 | -1.5678463 | 2.0252893 |
| Male:Female(6:1) | -18.4872867 | 1.4838209 | 0.0000022 | -23.7285408 | -13.2460325 |
| Male:Female(7:1) | 17.2282551 | 1.9636810 | 0.0000421 | 10.2920062 | 24.1645040 |
| Male:Female(8:1) | -29.2982991 | 2.9900500 | 0.0000169 | -39.8599589 | -18.7366392 |
| Male:Female(6:2) | -15.9907095 | 1.9515285 | 0.0000731 | -22.8840324 | -9.0973866 |
| Male:Female(7:2) | 6.7067474 | 1.6651579 | 0.0118711 | 0.8249623 | 12.5885324 |
| Male:Female(8:2) | 5.2397503 | 2.3881439 | 0.2028366 | -3.1958155 | 13.6753161 |
| Male:Female(6:3) | 15.5824360 | 1.4869107 | 0.0000097 | 10.3302681 | 20.8346039 |
| Male:Female(7:3) | 21.2069782 | 1.7776041 | 0.0000032 | 14.9280031 | 27.4859533 |
| Male:Female(8:3) | 38.4966882 | 1.8263558 | 0.0000000 | 32.0455089 | 44.9478676 |
| Male:Female(6:4) | 20.8556309 | 2.6123627 | 0.0000900 | 11.6280639 | 30.0831979 |
| Male:Female(7:4) | -28.2849839 | 1.9834755 | 0.0000007 | -35.2911522 | -21.2788155 |
| Male:Female(8:4) | -35.8561715 | 2.4805515 | 0.0000006 | -44.6181459 | -27.0941970 |
| Male:Female(6:5) | -1.9990954 | 2.2839311 | 0.8356955 | -10.0665536 | 6.0683628 |
| Male:Female(7:5) | -9.8162580 | 1.9259665 | 0.0025889 | -16.6192890 | -3.0132271 |
| Male:Female(8:5) | 14.4163180 | 2.2360906 | 0.0004741 | 6.5178451 | 22.3147908 |

## Case 3: Male is fixed while female is random

The second case is that male is fixed while female is random.

```
res=lmm.jack(Yld~Male|Male:Female+Rep,data=dat)[[1]]
data.frame(res$Var[[1]])
```

| | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Rep) | 0.0090026 | 0.0284687 | 0.9780305 | -0.0915565 | 0.1095617 |
| V(Male:Female) | 494.5684316 | 15.6889615 | 0.0000000 | 439.1508046 | 549.9860585 |
| V(e) | 93.1527521 | 5.7332742 | 0.0000002 | 72.9012875 | 113.4042166 |

```
data.frame(res$PVar[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Rep)/VP | 0.0000159 | 0.0000501 | 0.9780305 | -0.0001612 | 0.0001930 |
| V(Male:Female)/VP | 0.8414660 | 0.0091246 | 0.0000000 | 0.8092356 | 0.8736965 |
| V(e)/VP | 0.1585181 | 0.0091327 | 0.0000001 | 0.1262590 | 0.1907772 |

```
data.frame(res$FixedEffect[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| mu | 86.1566203 | 0.4437518 | 0.0000000 | 84.589170 | 87.724071 |
| Male(6) | -0.0875655 | 0.5473883 | 0.9900794 | -2.021088 | 1.845957 |
| Male(7) | 6.6900818 | 0.7274284 | 0.0000286 | 4.120609 | 9.259554 |
| Male(8) | -6.6025162 | 0.6648016 | 0.0000152 | -8.950774 | -4.254258 |

```
data.frame(res$RandomEffect[[1]])
```

|  | Pre | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| Rep(1) | -0.0135217 | 0.0427593 | 0.9780305 | -0.1645591 | 0.1375157 |
| Rep(2) | -0.0353696 | 0.1118484 | 0.9780305 | -0.4304483 | 0.3597092 |
| Rep(3) | 0.0184557 | 0.0583621 | 0.9780305 | -0.1876949 | 0.2246064 |
| Rep(4) | 0.0304355 | 0.0962456 | 0.9780305 | -0.3095299 | 0.3704010 |
| Male:Female(6:1) | -18.3996020 | 1.8451663 | 0.0000147 | -24.9172250 | -11.8819789 |
| Male:Female(7:1) | 15.6203008 | 2.8033656 | 0.0013852 | 5.7180603 | 25.5225412 |
| Male:Female(8:1) | -28.1483675 | 2.7057003 | 0.0000103 | -37.7056278 | -18.5911073 |
| Male:Female(6:2) | -16.0852564 | 1.7666733 | 0.0000311 | -22.3256211 | -9.8448918 |
| Male:Female(7:2) | 5.0711576 | 2.0849847 | 0.1417215 | -2.2935683 | 12.4358836 |
| Male:Female(8:2) | 6.3273876 | 2.2475897 | 0.0780198 | -1.6117029 | 14.2664781 |
| Male:Female(6:3) | 15.5572582 | 1.2702584 | 0.0000026 | 11.0703642 | 20.0441523 |
| Male:Female(7:3) | 19.7213502 | 2.0402493 | 0.0000190 | 12.5146414 | 26.9280589 |
| Male:Female(8:3) | 40.2594507 | 1.4437948 | 0.0000000 | 35.1595794 | 45.3593219 |
| Male:Female(6:4) | 20.9083422 | 2.6889826 | 0.0001111 | 11.4101331 | 30.4065513 |
| Male:Female(7:4) | -29.9442983 | 1.9964438 | 0.0000005 | -36.9962742 | -22.8923224 |
| Male:Female(8:4) | -34.5788162 | 2.1852240 | 0.0000003 | -42.2976144 | -26.8600180 |
| Male:Female(6:5) | -1.9807420 | 2.2712069 | 0.8370424 | -10.0032549 | 6.0417709 |
| Male:Female(7:5) | -10.4685103 | 3.7694202 | 0.0828263 | -23.7831152 | 2.8460946 |
| Male:Female(8:5) | 16.1403454 | 1.6288323 | 0.0000154 | 10.3868721 | 21.8938187 |

# Two-factor experimental designs with nested block effects

It is very often that some experiments are conducted in different environments. For such experiments, randomized complete blocks in each environment are independent of those in another one. That means block effects are nested within each environment.

```
library(agricolae)

data(plrv)
names(plrv)

## [1] "Genotype"     "Locality"     "Rep"          "WeightPlant" "WeightPlot"
## [6] "Yield"

dat=plrv
dat=transform(dat,Geno=Genotype,Loc=Locality, Rep=factor(Rep))
names(dat)

## [1] "Genotype"     "Locality"     "Rep"          "WeightPlant" "WeightPlot"
## [6] "Yield"        "Geno"         "Loc"
```

## ANOVA methods

```
require(minque)
mod=aov(Yield~Geno*Loc+Loc/Rep,data=dat)
a=summary(mod)
data.frame(a[[1]])
```

|          | Df  | Sum.Sq     | Mean.Sq     | F.value    | Pr..F.    |
|----------|-----|------------|-------------|------------|-----------|
| Geno     | 27  | 17533.281  | 649.38078   | 17.535906  | 0.0000000 |
| Loc      | 5   | 122284.346 | 24456.86916 | 660.434332 | 0.0000000 |
| Geno:Loc | 135 | 23761.795  | 176.01330   | 4.753070   | 0.0000000 |
| Loc:Rep  | 12  | 1141.785   | 95.14878    | 2.569402   | 0.0028887 |
| Residuals| 324 | 11998.204  | 37.03149    | NA         | NA        |

If we assume all effects are fixed, then the results in the above ANOVA table look ok. However, if block effect is random or any other factor is random, based on the derived EMS, some F-tests may not be right. Thus, with such a model, ANOVA analysis for F-tests may be problematic and linear mixed model approached are more preferred.

## Linear mixed model approaches

### Case 1: Both genotype and environment are fixed

```
require(minque)

res=lmm.jack(Yield~Loc*Geno|Loc:Rep,data=dat)[[1]]
data.frame(res$Var[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Loc:Rep) | 2.202455 | 0.6632316 | 0.035163 | -0.1402571 | 4.545167 |
| V(e) | 36.972805 | 1.3270367 | 0.000000 | 32.2853549 | 41.660255 |

```
data.frame(res$PVar[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Loc:Rep)/VP | 0.0563057 | 0.0172517 | 0.0384545 | -0.0046318 | 0.1172432 |
| V(e)/VP | 0.9436943 | 0.0172517 | 0.0000000 | 0.8827568 | 1.0046318 |

```
head(data.frame(res$FixedEffect[[1]]))
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| mu | 29.7436691 | 2.698851 | 0.0000063 | 20.210601 | 39.276738 |
| Loc(Ayac) | -3.6389076 | 3.287643 | 0.7237249 | -15.251745 | 7.973930 |
| Loc(LM-02) | -3.6389068 | 3.287642 | 0.7237249 | -15.251743 | 7.973929 |
| Loc(SR-02) | -0.3181576 | 1.658233 | 0.9882247 | -6.175483 | 5.539168 |
| Loc(Hyo-02) | 1.5261900 | 3.107071 | 0.9529330 | -9.448818 | 12.501199 |
| Loc(LM-03) | 3.7239352 | 5.095013 | 0.8906925 | -14.273021 | 21.720891 |

```
head(data.frame(res$RandomEffect[[1]]))
```

|  | Pre | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| Loc:Rep(Ayac:1) | 0.1693823 | 0.4632113 | 0.9723861 | -1.4668046 | 1.8055692 |
| Loc:Rep(Ayac:2) | 0.6057069 | 0.3347713 | 0.3472509 | -0.5767954 | 1.7882093 |
| Loc:Rep(Ayac:3) | -0.7750893 | 0.3992675 | 0.2909507 | -2.1854092 | 0.6352307 |
| Loc:Rep(LM-02:1) | 2.2544395 | 0.4114606 | 0.0015614 | 0.8010501 | 3.7078289 |
| Loc:Rep(LM-02:2) | 0.6566264 | 0.3493362 | 0.3163697 | -0.5773230 | 1.8905758 |
| Loc:Rep(LM-02:3) | -2.9110659 | 0.5721572 | 0.0026202 | -4.9320788 | -0.8900530 |

## Case 2: Both genotye and environment are random

```
res=lmm.jack(Yield~1|Geno*Loc+Rep:Loc,data=dat)[[1]]
data.frame(res$Var[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Geno) | 26.264434 | 1.9588603 | 0.0000012 | 19.3452131 | 33.183655 |
| V(Loc) | 288.643219 | 5.0426925 | 0.0000000 | 270.8310744 | 306.455364 |
| V(Geno:Loc) | 45.993977 | 1.2512883 | 0.0000000 | 41.5740909 | 50.413864 |
| V(Loc:Rep) | 1.964619 | 0.4560123 | 0.0078398 | 0.3538612 | 3.575377 |
| V(e) | 37.362891 | 1.6082036 | 0.0000000 | 31.6822835 | 43.043498 |

```
data.frame(res$PVar[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| V(Geno)/VP | 0.0656085 | 0.0045344 | 0.0000006 | 0.0495919 | 0.0816251 |
| V(Loc)/VP | 0.7211740 | 0.0059276 | 0.0000000 | 0.7002361 | 0.7421119 |
| V(Geno:Loc)/VP | 0.1149207 | 0.0028200 | 0.0000000 | 0.1049596 | 0.1248817 |
| V(Loc:Rep)/VP | 0.0049131 | 0.0011560 | 0.0085357 | 0.0008299 | 0.0089964 |
| V(e)/VP | 0.0933837 | 0.0045735 | 0.0000000 | 0.0772287 | 0.1095386 |

```
data.frame(res$FixedEffect[[1]])
```

|  | Estimate | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| mu | 30.67555 | 0.1212494 | 0 | 30.24727 | 31.10384 |

```
head(data.frame(res$RandomEffect[[1]]))
```

|  | Pre | SE | PValue | X2.5.LL | X97.5.UL |
|---|---|---|---|---|---|
| Geno(102.18) | -3.6511460 | 0.5944079 | 0.0006808 | -5.7507544 | -1.5515377 |
| Geno(104.22) | 0.5418619 | 0.2408313 | 0.1867132 | -0.3088189 | 1.3925427 |
| Geno(121.31) | -0.4919683 | 0.4131696 | 0.6781046 | -1.9513942 | 0.9674576 |
| Geno(141.28) | 7.7195038 | 0.6655875 | 0.0000041 | 5.3684701 | 10.0705375 |
| Geno(157.26) | 5.3266253 | 0.4751735 | 0.0000055 | 3.6481848 | 7.0050658 |
| Geno(163.9) | -7.7677790 | 0.6176531 | 0.0000021 | -9.9494958 | -5.5860621 |

## Conclusions

When two factors or more factors involve, experimental designs can be very complicated. In the same manner, the corresponding data analyses can be complicated too. The key for these experimental analyses is to write an appropriate linear model. ANOVA based approaches can be used for data analyses once you can provide an appropriate linear model. The ANOVA results, especially F-testing results, generated by the function of aov are based on that all effects are fixed. When at least one effect is random, some F-tests can be very complicated. Thus, linear mixed model approaches are prefered.