

Generalized Linear Mixed Models

An Introduction for Tree Breeders and Pathologists

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Introduction

Generalized Linear Mixed Models (GLMM) have attracted considerable attention over the last years. The word “Generalized” refers to non-normal distributions for the response variable, and the word “Mixed” refers to random effects in addition to the usual fixed effects of regression analysis. With the development of modern statistical packages such as SAS, R, and ASReml, a large variety of statistical analyses are available to a larger audience. However, along with being able to handle more sophisticated models comes a responsibility on the part of the user to be informed on how these advanced tools work.

The objective of this workshop is to provide an introduction to generalized linear mixed models by first discussing some of the assumptions and deficiencies of statistical linear models in general, then giving examples of uses in common situations in the natural sciences.

The first section reviews linear models and regression analysis for simple and multiple variables. Two numerical examples are solved using the SAS REG software.

The second section presents linear mixed models by adding the random effects to the linear model. A simple numerical example is presented using the SAS MIXED Procedure.

The third (last) section introduces generalized linear models. Two illustrative examples of binary and count data are presented using the SAS GLIMMIX procedure and ASReml software.

Linear Models

Linear models (regression) are often used for modeling the relationship between a single variable y , called the *response* or *dependent* variable, and one or more *predictor*, *independent* or *explanatory* variables, X_1, \dots, X_p . When $p=1$, it is called simple regression but when $p > 1$ it is called multiple regression.

Regression analysis can be used to assess the relationship between explanatory variables on the response variable. It is also a useful tool to predict future observations or merely describe the structure of the data.

To start with a simple example, suppose that y is the weight of trees, the predictors are the height (X_1), and the age of the trees (X_2). Typically the data will be available in the form of an array like the following

$$\begin{array}{ccc} y_1 & x_{11} & x_{12} \\ y_2 & x_{21} & x_{22} \\ \vdots & \vdots & \vdots \\ y_n & x_{n1} & x_{n2} \end{array}$$

where y_i is the observation of the i -th tree and n is the number of observations.

There is an infinite number of ways to model the relationship between the response and the explanatory variables. However, to keep it simple, the relationship can be modeled through a linear function in the parameters as follows

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$$

where β_i for $i = 0, 1, 2$ are unknown parameters and ε is the error term. Thus, the problem is reduced to the estimation of three parameters.

Notice that in a linear model the parameters enter linearly, but the predictors do not necessarily have to be linear. For instance, consider the following two functions

$$y = \beta_0 + \beta_1 \exp(X_1) + \beta_2 \log(X_2) + \varepsilon$$

$$y = \beta_0 + X_1^{\beta_1} + X_2 \exp(\beta_2) + \varepsilon$$

The first one is linear in the parameters, but the second one is not.

Using matrix representation, the regression equation for the above example can be written as:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\varepsilon}$$

where $\mathbf{y} = (y_1, \dots, y_n)^T$, $\boldsymbol{\beta} = (\beta_0, \beta_1, \beta_2)^T$, $\boldsymbol{\varepsilon} = (\varepsilon_0, \dots, \varepsilon_n)^T$, and the design matrix \mathbf{X} is

$$\mathbf{X} = \begin{bmatrix} 1 & x_{11} & x_{12} \\ 1 & x_{21} & x_{22} \\ \vdots & \vdots & \vdots \\ 1 & x_{n1} & x_{n2} \end{bmatrix}$$

The estimation of $\boldsymbol{\beta}$ can be carried out using the least square approach. That is, we define $\hat{\boldsymbol{\beta}}$ as the best estimate of $\boldsymbol{\beta}$ in the sense that minimizes the sum of the squared errors.

$$\sum_{i=1}^n \varepsilon_i^2 = \boldsymbol{\varepsilon}^T \boldsymbol{\varepsilon} = (\mathbf{y} - \mathbf{X}\boldsymbol{\beta})^T (\mathbf{y} - \mathbf{X}\boldsymbol{\beta})$$

Differentiating with respect to $\boldsymbol{\beta}$ and setting equal to zero, it can be shown that $\hat{\boldsymbol{\beta}}$ satisfies the normal equations

$$\mathbf{X}^T \mathbf{X} \hat{\boldsymbol{\beta}} = \mathbf{X}^T \mathbf{y}$$

Thus, provided that $\mathbf{X}^T \mathbf{X}$ is invertible

$$\hat{\boldsymbol{\beta}} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{y}$$

So far, we have not assumed any distributional form for the errors $\boldsymbol{\varepsilon}$. The usual assumption is that the errors are normally distributed and in practice this is often, although not always, a reasonable assumption.

If we assume that the errors are independent and identically normally distributed with mean 0 and variance σ^2 , that is to say $\boldsymbol{\varepsilon} \sim N(0, \sigma^2 \mathbf{I})$, then the expectations of observations are

$$\mathbf{y} \sim N(\mathbf{X}\boldsymbol{\beta}, \sigma^2 \mathbf{I})$$

and expectations of parameters are

$$\hat{\boldsymbol{\beta}} \sim N(\boldsymbol{\beta}, (\mathbf{X}^T \mathbf{X})^{-1} \sigma^2)$$

Linear regression example

We would like explore linear dependency between height and weight. The linear model can be run using SAS GLM or REG procedures.

```

title 'Simple Linear Regression';
data A;
  input Height Weight Age @@;
  datalines;
  69.0 112.5 14 56.5 84.0 13 65.3 98.0 13
  62.8 102.5 14 63.5 102.5 14 57.3 83.0 12
  59.8 84.5 12 62.5 112.5 15 62.5 84.0 13
  59.0 99.5 12 51.3 50.5 11 64.3 90.0 14
  56.3 77.0 12 66.5 112.0 15 72.0 150.0 16
  64.8 128.0 12 67.0 133.0 15 57.5 85.0 11
  66.5 112.0 15
  ;

/* Create a unique age for each */

data B (drop =i ); set A;
  do i = 1 to 1 ;
    b = ranuni (i) ;
    output ;
  end;
run;

data B; set B ;
  Age=round (sum (year,b) , .001) ;
run;

/* Simple linear regression */

```

```
ods graphics on;
ods listing style=statistical sge=on;
proc reg data=A ;
    model weight = height / clm cli;
run; quit ;
```

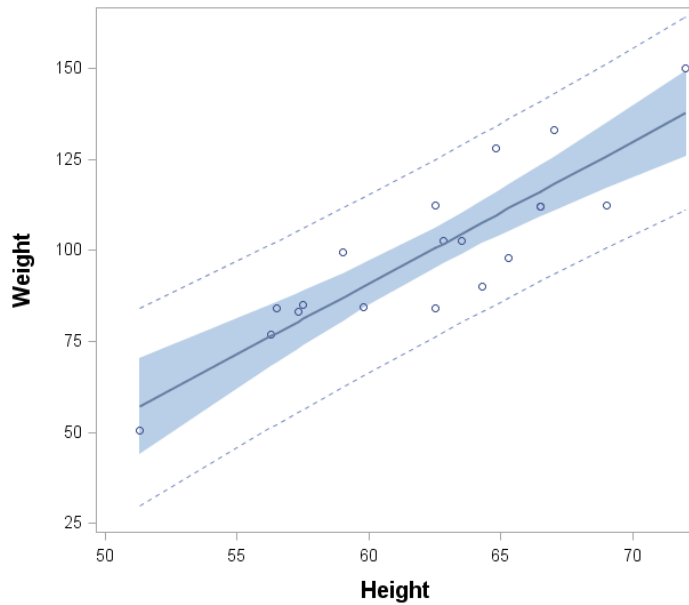


Figure1. Scatter plot and regression line of Height on Weight

The SAS output:

The REG Procedure
Dependent Variable: Weight

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7193.24912	7193.24912	57.08	<.0001
Error	17	2142.48772	126.02869		
Corrected Total	18	9335.73684			

Root MSE	11.22625	R-Square	0.7705
Dependent Mean	100.02632	Adj R-Sq	0.7570
Coeff Var	11.22330		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	-143.02692	32.27459	-4.43	0.0004
Height	1	3.89903	0.51609	7.55	<.0001

The intercept is $\hat{\beta}_0 = -143.027$ and the slope is $\hat{\beta}_1 = 3.899$

Multiple linear regression

Now consider that we also want to include tree *age* as another predictor. That is, we want to assess the relationship between *Age* and *Weight* while keeping the effect of *Height* constant. In simple regressions, a line is fit to the data, whereas in multiple regressions, a p -th dimensional plane is fit, where p is the number of explanatory variables. To visualize the data and the fitted values, a 3D plot is necessary.

```
/* Create 3d plot */  
  
proc g3grid data=ws.a out=a;  
    grid age*height=weight / spline ;  
run;  
  
options device=png xpixels=720 ypixels=600 noborder  
gunit=pct htitle=3 htext=2 reset=all ;  
  
ODS LISTING CLOSE;  
proc g3d data=A;  
    plot age*height=weight /grid ctop=red  
    cbottom=blue caxis=black ;  
run; quit;
```

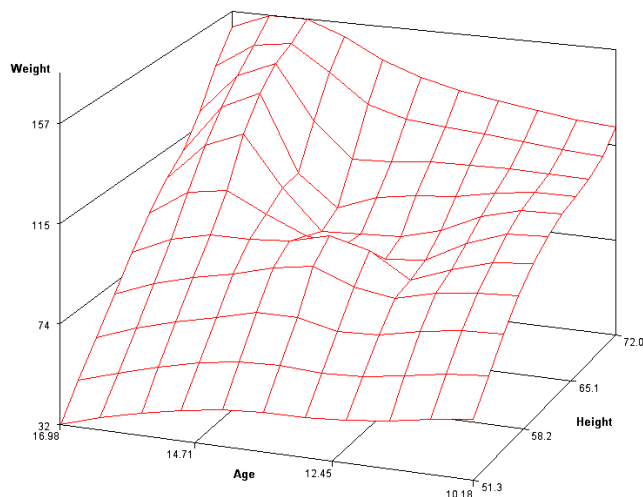


Figure2. Regression plane of weight, age, and height

In this case, we can use the multiple linear regression approach. So, we will need to estimate three parameters from the data. In SAS, this can be easily done by adding the following lines to the code shown above.

```

/* Multiple linear regression */

ods html image_dpi=300; * Image resolution ;
ods graphics on;
ods listing style=statistical sge=on; * Graphic type;

proc reg data=ws.A ;
    model weight = height age;
run; quit;

```

The SAS output is

The REG Procedure
Dependent Variable: Weight

Analysis of Variance

Source	DF	Squares	Sum of Square	Mean F Value	Pr > F
Model	2	7574.69595	3787.34798	34.41	<.0001
Error	16	1761.04089	110.06506		
Corrected Total	18	9335.73684			

Root MSE	10.49119	R-Square	0.8114
Dependent Mean	100.02632	Adj R-Sq	0.7878
Coeff Var	10.48843		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > t
Intercept	1	-155.29024	30.87234	-5.03	0.0001
Height	1	3.49864	0.52808	6.63	<.0001
Age	1	2.68549	1.44255	1.86	0.0811

Following with the notation, the estimated parameters of the multiple linear regression are $\hat{\beta}_0 = -155.29$, $\hat{\beta}_1 = 3.498$ and $\hat{\beta}_2 = 2.685$. Thus, the relationship among variables can be expressed as: $Weight = -155.29 + 3.498 Height + 2.685 Age$

Linear Mixed Model

A linear mixed model is a statistical model containing both fixed effects and random effects. These models are widely used in the biological and social sciences. In matrix notation, linear mixed models can be represented as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\boldsymbol{\gamma} + \boldsymbol{\varepsilon}$$

where:

\mathbf{y} is the $n \times 1$ vector of observations,

$\boldsymbol{\beta}$ is a $p \times 1$ vector of fixed effects,

$\boldsymbol{\gamma}$ is a $q \times 1$ vector of random effects,

$\boldsymbol{\varepsilon}$ is a $n \times 1$ vector of random error terms,

\mathbf{X} is the $n \times p$ design matrix for the fixed effects relating observations \mathbf{y} to $\boldsymbol{\beta}$,

\mathbf{Z} is the $n \times q$ design matrix for the random effects relating observations \mathbf{y} to $\boldsymbol{\gamma}$.

We assume that $\boldsymbol{\gamma}$ and $\boldsymbol{\varepsilon}$ are uncorrelated random variables with zero means and covariance matrices \mathbf{G} and \mathbf{R} , respectively.

$$E[\boldsymbol{\gamma}] = \mathbf{0}, \quad Var[\boldsymbol{\gamma}] = \mathbf{G}$$

$$E[\boldsymbol{\varepsilon}] = \mathbf{0}, \quad Var[\boldsymbol{\varepsilon}] = \mathbf{R}$$

$$cov(\boldsymbol{\varepsilon}, \boldsymbol{\gamma}) = \mathbf{0}$$

Thus, the expectation and variance (\mathbf{V}) of the observation vector \mathbf{y} are given by:

$$E[\mathbf{y}] = \mathbf{X}\boldsymbol{\beta}$$

$$Var[\mathbf{y}] = \mathbf{V} = \mathbf{Z}\mathbf{G}\mathbf{Z}^T + \mathbf{R}$$

Understanding the \mathbf{V} matrix is a very important component of working with mixed models since it contains both sources of random variation and defines how these models differ from computations with Ordinary Least Squares (OLS).

If you only have random effects models (such as a randomized block design) the \mathbf{G} matrix is the primary focus. On the other hand, for repeated measures or for spatial analysis, the \mathbf{R} matrix is relevant.

If we also assume the random terms are normally distributed as:

$$\boldsymbol{\gamma} \sim N(\mathbf{0}, \mathbf{G}), \quad \boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{R})$$

Then, the observation vector will be normally distributed $\mathbf{y} \sim N(\mathbf{X}\boldsymbol{\beta}, \mathbf{V})$.

For the general linear mixed model described above, the Henderson's mixed model equations (MME) can be used to find $\hat{\beta}$ and $\hat{\gamma}$, the best linear unbiased estimator (BLUE) of β , and the best linear unbiased predictor (BLUP) of γ , respectively.

$$\begin{pmatrix} \mathbf{X}^T \mathbf{R}^{-1} \mathbf{X} & \mathbf{X}^T \mathbf{R}^{-1} \mathbf{Z} \\ \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{X} & \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{Z} + \mathbf{G}^{-1} \end{pmatrix} \begin{pmatrix} \hat{\beta} \\ \hat{\gamma} \end{pmatrix} = \begin{pmatrix} \mathbf{X}^T \mathbf{R}^{-1} \mathbf{y} \\ \mathbf{Z}^T \mathbf{R}^{-1} \mathbf{y} \end{pmatrix}$$

The solutions can also be written as:

$$\hat{\beta} = (\mathbf{X}^T \mathbf{V}^{-1} \mathbf{X})^{-1} \mathbf{X}^T \mathbf{V}^{-1} \mathbf{y}$$

$$\hat{\gamma} = \mathbf{G} \mathbf{Z}^T \mathbf{V}^{-1} (\mathbf{y} - \mathbf{X} \hat{\beta})$$

If the \mathbf{G} and \mathbf{R} matrices are known, generalized least squares can estimate any linear combination of the fixed effects β . However, as usually is the case these matrices are not known, so a complex iterative algorithm for fitting linear models must be used to estimate them.

Consider the following example. Suppose that we have collected data on the growth of different trees measured in two different locations. We can assume that the trees come from a large population, which is a reasonable assumption, and therefore, we will treat them as random.

Tree (t)	Location (l)	Height (y)
1	1	87
2	2	84
3	2	75
4	1	90
5	2	79

The linear mixed model can be expressed in matrix notation as follows

$$\begin{bmatrix} 87 \\ 84 \\ 75 \\ 90 \\ 79 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & 1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} l_1 \\ l_2 \end{bmatrix} + \begin{bmatrix} 1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} t_1 \\ t_2 \\ t_3 \\ t_4 \\ t_5 \end{bmatrix} + \begin{bmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \\ \varepsilon_4 \\ \varepsilon_5 \end{bmatrix}$$

which has the matrix form $\mathbf{Y} = \mathbf{X}\beta + \mathbf{Z}\gamma + \boldsymbol{\varepsilon}$. Notice that we are treating location as fixed effects. Matrices \mathbf{X} and \mathbf{Z} relate phenotypic observations to location and random tree effects.

We need to make some assumptions for the variance components of the model. In this case, we will assume that trees are independent of each other, and the errors are independent. So, the structure of the variance matrices can be expressed as:

$$\mathbf{R} = \mathbf{I}_n \sigma_\varepsilon^2 \quad \mathbf{G} = \mathbf{I}_n \sigma_\gamma^2 \quad \mathbf{V} = \mathbf{Z} \mathbf{G} \mathbf{Z}^T + \mathbf{R}$$

where \mathbf{I}_n represents the $n \times n$ identity matrix. Also, we assume that:

$$\boldsymbol{\gamma} \sim N(\mathbf{0}, \mathbf{G}) \quad \boldsymbol{\varepsilon} \sim N(\mathbf{0}, \mathbf{R}) \quad \mathbf{Y} \sim N(\mathbf{X}\boldsymbol{\beta}, \mathbf{V})$$

We can compute the solutions using R software or any other software that uses matrix algebra.

$$\hat{\boldsymbol{\beta}} = \begin{bmatrix} l_1 \\ l_2 \end{bmatrix} = \begin{bmatrix} 88.50 \\ 79.33 \end{bmatrix}$$

$$\hat{\boldsymbol{\gamma}} = \begin{bmatrix} t_1 \\ t_2 \\ t_3 \\ t_4 \\ t_5 \end{bmatrix} = \begin{bmatrix} -1.0067 \\ 3.1320 \\ -2.9083 \\ 1.0067 \\ -0.2237 \end{bmatrix}$$

Below is a sample R code to compute the above solution.

```
# Linear Mixed Model Example y = X*B + Z*U + e
# Y, observations
Y=c(87, 84, 75, 90, 79)

# X, design matrix for the fixed effects
X=matrix(c(1,0,0,1,0,0,1,1,0,1),5,2)

# Z, design matrix for the random effects
Z=diag(5); #identity matrix of size 5x5

# G = var(U)
Su2=100; # random effect variance
G=diag(5)*Su2;

# R = var(e)
Se2=49; # error variance
R=diag(5)*Se2;

# V=var(Y)
V=Z%*%G%*%t(Z) + R

# Solutions
Vi= solve(V)
B = solve( t(X)%*%Vi%*%X )%*%t(X)%*%Vi%*%Y;
U = G%*%t(Z)%*%Vi%*%(Y - X%*%B);

# Print out solutions
print (U, quote=T, row.names=F)

# Or produce histogram of BLUP values
hist(U, col="lightblue")
```

Generalized Linear Models

Recall that linear models, as its name states, assumes that

- the *relationship between the dependent variable and the fixed effects can be modeled through a linear function,*
- *The variance is not a function of the mean,* and
- the random terms follow a normal distribution

In some situations, any or even all these assumptions may be violated.

They are an extension of ordinary least squares regression.

The GLM generalizes linear regression by

- Allowing the linear model to be related to the response variable *via a link function* and
- Allowing the magnitude of the variance of each measurement to be a function of its predicted value.

In a GLM, each outcome of the dependent variables, \mathbf{y} , is assumed to be generated from a particular distribution in the exponential family. The most common distributions from this family are Binomial, Poisson, and Normal.

The mean, $\boldsymbol{\mu}$, of the distribution depends on the independent variables, \mathbf{X} , through the inverse link function (g^{-1}).

$$E(\mathbf{Y}) = \boldsymbol{\mu} = g^{-1}(\mathbf{X}\boldsymbol{\beta}) = g^{-1}(\boldsymbol{\eta})$$

where $E(\mathbf{y})$ is the expected value of \mathbf{y} ; $\boldsymbol{\eta} = \mathbf{X}\boldsymbol{\beta}$ is called the *linear predictor*, a linear combination of unknown parameters, $\boldsymbol{\beta}$, and g is the link function.

In this framework, the variance \mathbf{V} is typically a function of the mean:

$$Var(\mathbf{Y}) = V(\boldsymbol{\mu}) = V(g^{-1}(\mathbf{X}\boldsymbol{\beta})) = V(g^{-1}(\boldsymbol{\eta}))$$

(Inverse) Link Function converts a linear predictor into a mean

$$E(y_i) = \mu_i$$

<i>Distribution</i>	<i>Link</i>	<i>Inverse Link</i>
Normal	Identity	η
Binomial/n	Logit = $\ln(\mu_i(1 - \mu_i))$	$e^\eta = 1/(1 + e^{-\eta})$
Poisson	Log	e^η

Selection of inverse link functions is typically based on the error distribution. *The logit link function, unlike the identity link function, will always yield estimated means in the range of zero to one.* For most univariate link functions, link and inverse link functions are increasing

monotonic functions. In other words, an increase in the linear predictor results in an increase in the conditional mean, but not at a constant rate.

Variance Function

The variance function is used to model non-systematic variability. Typically with a generalized linear model, residual variability arises from two sources. First, variability arises from the sampling distribution. For example, a Poisson random variable with mean μ has a variance of μ . Second, additional variability, or over-dispersion, is often observed.

Variance function models the relationship between the variance of y and μ .

<i>Distribution</i>	<i>Variance - $v(\mu)$</i>
Normal	1
Binomial	$\mu (1 - \mu)$
Poisson	μ

Consider the situation where individual seeds are laid on damp soil in different pots. In this experiment, the pots are kept at different temperatures T for a number of days D . After an arbitrary number of days, the seeds are inspected and the outcome $y=1$ is recorded if it has germinated and $y=0$ otherwise. The probability of germination p can be modeled through a linear function of the form:

$$\eta = \beta_0 + \beta_1 D + \beta_2 T$$

Where η is the linear predictor and β_0 , β_1 and β_2 are parameters to be estimated. Note that there is nothing holding the linear predictor to be between 0 and 1.

In GLM, however, the probability is restricted to the interval (0,1) through the inverse link function:

$$p = \frac{1}{1 + e^{-\eta}} = g^{-1}(\eta)$$

It is worth noting that p is the expected value of y for a binomial distribution. Also notice the non-linear relationship between the outcome p and the linear predictor η is modeled by the inverse link function. In this particular case, the link function is the logistic link function or logit:

$$\eta = \log\left(\frac{p}{1-p}\right) = g(p)$$

Binary Data Example – Disease incidence probability

Here is a SAS code to reproduce data.

```

goptions reset=all cback=white htitle=15pt htext=15pt;
data pgerm;
  do T=0 to 40 by 0.5;
    do D=0 to 15 by 0.5;
      p=1/(1+exp(8 - 0.19*T - 0.37*D));
      output;
    end;
  end;
run;

```

SAS code to produce 3D plot of Figure 4

```

/* Designate a GIF file for the G3D output. */
filename anim 'c:\users\fisik\pond.gif';

/** animation. **/
goption reset dev=gifanim gsfmode=replace noborder htext=1.4
gsfname=anim xpixels=640 ypixels=480
iteration=0 delay=5
gepilog='3B'x /* add a termination char to the end of the GIF file */
disposal=background;

proc g3d data=pgerm;
  plot D*T=p / tilt=60 grid rotate=0 to 350 by 10
          xticknum=4 yticknum=5 zticknum=5
          zmin=0 zmax=1
          caxis = black ctop=red cbottom=blue;
          label T='Temperature' D='Days' p='p' ;
run; quit;

```

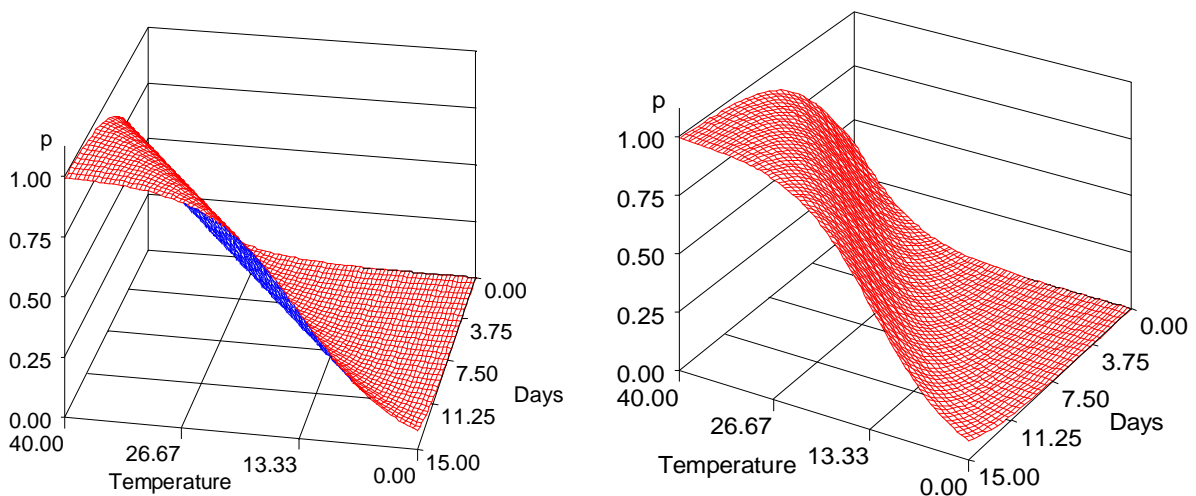


Figure 4: Disease incidence probability as a function of Temperature and Day. The values of β_i for $i = 0, 1, 2$ have been chosen for illustrative purposes

The inverse link function is, $p = [1 + \exp(-\eta)]^{-1}$. Then, the linear predictor takes the form: $\eta = -8 + 0.19T + 0.37D$. Using the model we can estimate how many days are needed for the probability of disease incidence to exceed 80% at a given temperature. After some simple algebra it can be shown that at temperature 10 at least 20 days are needed to reach a probability of 0.80 germination.

The above example has no random effects so it is a generalized linear model (GLM), not a generalized mixed model (GLMM).

Count Data Example – Number of trees infected

We have previously considered an example where the outcome variable is numeric and binary. Often the outcome variable is numeric but in the form of counts. Sometimes it is a count of rare events such as the number of new cases of fusiform rust of loblolly pine occurring in a population over a certain period of time.

The probability distribution of a Poisson random variable X representing the number of successes occurring in a given time interval or a specified region of space is given by the formula:

The Poisson probability function is appropriate for count data with no upper bound to the range, that is, y could be any non-negative integer.

$$\Pr(y = k) = \frac{\lambda^k e^{-\lambda}}{k!}, \quad k = 0, 1, 2, \dots$$

Where, λ is the mean number of success in a given time or space interval. This λ is often referred to as the Poisson “intensity” as it is the average event count. k is the random variable representing the number of success occurring in given a time interval or specified region of space ($k = 0, 1, 2, 3, \dots$). e is 2.71828.

As a hypothetical example, we can use data that relate the number of newly infected trees over a three-month period with its age, A , and height, H , within a population.

Our interest lies in modeling λ as a function of *age* (A) and *height* (H) for a given family and location. Using a linear model for λ could result in negative intensities, $\lambda < 0$, which would make no sense. The natural link function $g(\lambda)$ for a Poisson is the logarithm, so the model can be the following:

$$\eta = \beta_0 + \beta_1 A + \beta_2 H$$

Where η is the linear predictor and β_0 , β_1 , and β_2 are parameters to be estimated.

The link function and its inverse are given by:

$$\eta = \ln(\lambda) = g(\lambda)$$

$$\lambda = e^\eta = g^{-1}(\eta)$$

In Figure 5 the intensity λ as a function of *age* and *height* is plotted. Again, the values of β_i for $i = 0, 1, 2$ have been chosen for illustrative purposes only. Then, the linear predictor takes the form: $\eta = -2 - 0.03A - 0.01H$.

Here is a SAS code to reproduce Figure 5.

```

goptions reset=all cback=white htitle=15pt htext=15pt;
data intensity;
  do A=0 to 50 by 0.5;
    do H=0 to 30 by 0.5;
      I=exp(-2 - 0.03*A - 0.01*H);
      output;
    end;
  end;
run;

proc g3d data=intensity;
title 'Intensity';
  plot A*H=I / rotate=160 tilt=80 grid
          xticknum=4 yticknum=3 zticknum=5
          zmin=0 zmax=0.15
          caxis = black ctop=blue cbottom=red;
          label A='Age (years)' H='Height (meters)' I='lambda';
run; quit;

```

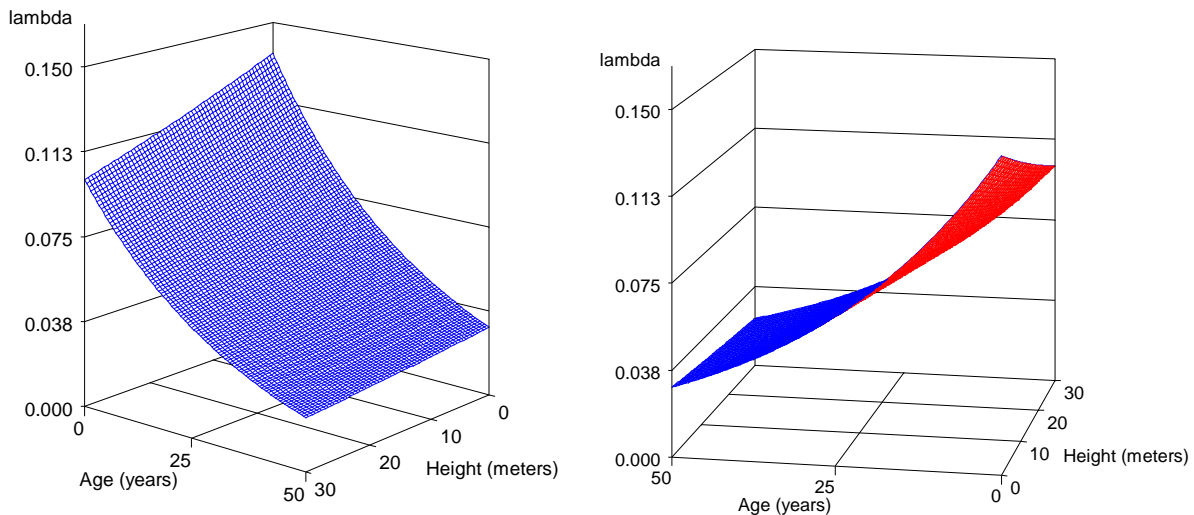


Figure 5: Intensity as a function of Age and Height. The inverse link function is defined as $\lambda = \exp(\eta)$, where $\eta = -2 - 0.03A - 0.01H$.

Notice that the above count data example does not include random effects, therefore, it is a generalized linear model, not a generalized linear mixed model. In the next section the generalized linear mixed model is presented.

Generalized Linear Mixed Model

A Generalized linear mixed models (GLMM) is an extension to the generalized linear model (GLM) in which the linear predictor contains random effects in addition to the fixed effects.

The expectations of the GLMM are:

$$E[\mathbf{y}|\mathbf{u}] = g^{-1}(\mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u}) = g^{-1}(\boldsymbol{\eta})$$

Where

\mathbf{y} represents the $(n \times 1)$ response vector,

\mathbf{X} the $(n \times p)$ design matrix of rank k for the $(p \times 1)$ fixed effects $\boldsymbol{\beta}$ and

\mathbf{Z} the $(n \times q)$ design matrix for the $(q \times 1)$ random effects \mathbf{u} .

The random effects \mathbf{u} are assumed to be normally distributed with mean $\mathbf{0}$ and variance matrix \mathbf{G} , that is to say $\mathbf{u} \sim N(\mathbf{0}, \mathbf{G})$.

$$E[\mathbf{u}] = \mathbf{0}, \quad \text{Var}[\mathbf{u}] = \mathbf{G}$$

The fixed and random effects are combined to form a linear predictor

$$\boldsymbol{\eta} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u}$$

The model for the vector of observations \mathbf{y} is obtained by adding a vector of residuals, $\boldsymbol{\varepsilon}$, as follows:

$$\mathbf{Y} = \boldsymbol{\eta} + \boldsymbol{\varepsilon} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$$

The relationship between the linear predictor and the vector of observations is modeled as

$$\mathbf{y}|\mathbf{u} \sim (g^{-1}(\boldsymbol{\eta}), \mathbf{R})$$

The above notation denotes that the conditional distribution of \mathbf{y} given \mathbf{u} has mean $g^{-1}(\boldsymbol{\eta})$ and variance \mathbf{R} . The conditional distribution of $\mathbf{y}|\mathbf{u}$ is usually referred as the error distribution. Note that Instead of specifying a distribution for \mathbf{y} , as in the case of a GLM, we now specify a distribution for the conditional response, $\mathbf{y}|\mathbf{u}$. This formulation is also known as the conditional model specification.

Last, the variance matrix of the observations is given by:

$$V(\mathbf{y}) = E[V(\mathbf{y}|\mathbf{u})] + V[E(\mathbf{y}|\mathbf{u})] = \mathbf{A}^{1/2}\mathbf{R}\mathbf{A}^{1/2} + \mathbf{ZGZ}^T$$

Where matrix \mathbf{A} is a diagonal matrix that contains the variance functions of the model.

The class of generalized linear mixed models contains several important types of statistical models. For example,

- Linear models: no random effects, identity link function, and normal distribution
- Generalized linear models: no random effects present
- Linear mixed models: random effects, identity link function, and normal distribution

The generalized linear mixed models have been developed to address the deficiencies of linear mixed models.

There are many cases when the implied assumptions are not appropriate.

For instance, the linear mixed model assumes that the relationship between the mean of the dependent variable \mathbf{y} and the fixed and random effects can be modeled through a linear function. This assumption is questionable, for example, in modeling disease incidence.

Another assumption of the linear mixed model is that the variance is not a function of the mean, and the random effects follow a normal distribution. The assumption of constant variance is violated when analyzing a zero/one trait, such as diseased (1) or not diseased (0). In this case, the response variable is Binomial. So, for a predicted disease incidence, the variance is $\mu(1 - \mu)$, which is a function of the mean.

The assumption of normality is not valid for a binary trait. The outcome is a random variable that can only take two values, zero or one. In contrast, the normal distribution is a bell shaped curve that can take any real number.

Finally, the predictions from linear mixed models can take any value. Whereas predictions for a binary variable is bounded (0,1) or for a count variable, it cannot take negative values.

Probability, Odds and Odds Ratio

In order to understand the output and correctly interpret results, we need to be familiar with the probability, odds and odds ratio. Here is an example data and the P, ODDS and OR.

	Outcome		Sum
	No	Yes	
Sample1	30	70	100
Sample2	20	180	200
Sum	50	250	300

Sample1

Probability of a **Yes outcome** = 70/100 (**0.70**)

Probability of a **No outcome** = 30/100 (**0.30**)

The **odds** of Outcome in Sample1 Odds = $p / (1-p) = 0.70 / 0.30 = 2.3$

We expect 2.3 times as many occurrences as non-occurrences in Sample1.

Sample2

Probability of a **Yes outcome** = 180/200 (**0.90**)

Probability of a **No outcome** = 20/200 (**0.10**)

The **odds** of Outcome in Sample2 Odds = $p / (1-p) = 0.90 / 0.10 = 9$

We expect 9 times as many occurrences as non-occurrences in Sample2.

The odds ratio of Sample2 to Sample1 is $OR = 9/2.3 = 3.86$

The odds of having outcome (Yes) in Sample2 is almost 4 times those in Sample1.

Over-dispersion in Binomial and Poisson Regression Models

(Modified from http://rfd.uoregon.edu/files/rfd/StatisticalResources/gnmd08_overdisp.txt)

Over-dispersion results when the data appear more dispersed than is expected under some reference model. It may occur with count data analyzed with binomial or Poisson regression models, since the variance of both distributions is a function of the mean. That is,

$$\text{Var}[Y] = f(E[Y]) * \phi$$

With both distributions the scale parameter phi is assigned a value of 1.

To understand what over-dispersion implies, first review the linear regression model (computed with ordinary least squares). Under the normal distribution data are never over-dispersed because the mean and variance are not related. The expectations of a linear model ($\mathbf{y} = \mathbf{X}'\boldsymbol{\beta} + \mathbf{e}$) are

$$\mathbf{e} \sim \text{NID}(0, \sigma_e^2)$$

The variance of the residuals (σ^2) is assumed constant for all linear combinations of the covariates. This variance is estimated from the data and can assume any value greater than zero no matter what the mean value is. Thus, the response values are assumed to have constant variance:

$$\text{Var}(\mathbf{y}) = \sigma_e^2 * \mathbf{1}$$

The normal errors and identity link function (linear regression) have the variance function $\text{Var}(\mu) = 1$. This variance is constant for all y_i .

For a generalized linear model:

$$g(\mu) = \mathbf{X}'\boldsymbol{\beta}$$

where $\mu = E(y)$ and g is the link function. The variance of y is:

$$\text{Var}(y) = \phi * V(\mu)$$

That is, the variance of an observation equals some constant ϕ (**the scale parameter**) times a function of the mean of y .

- For a binary variable y , the variance is multiplicative function of its mean: $\text{Var}(y) = \mu (1-\mu)$
- Under the Poisson distribution the variance of is the mean itself: $\text{Var}(y) = E(y) = \mu$.

In either case, the observed counts have variances that are functions which depend on the value of the mean. That is, the variance of y depends on the expectation of y , which is estimated from the data.

When either model is fit under the assumption that the data were generated from a binomial distribution or by a Poisson process, the scale parameter, ϕ , is automatically set equal 1. That is why we see 1.00 for error variance in the ASREML output or in SAS GENMOD procedure output when we fit Poisson or Binomial distributions. The value 1 is not error variance, but it is a scale parameter and should not be used as variance component to calculate heritability for binomial distribution.

For binomial and Poisson regression models, the covariance matrix (and hence the standard errors of the parameter estimates) is estimated under the assumption that the chosen model is appropriate. More variation in the data may be present than is expected by the distributional assumption. This is called **over-dispersion** (also known as heterogeneity) which typically occurs when the observations are correlated or are collected from "clusters".

To identify possible over-dispersion in the data for a given model, divide the deviance by its degrees of freedom: this is called the **dispersion parameter**. If the deviance is reasonably "close" to the degrees of freedom (i.e., the scale parameter=1) then evidence of over-dispersion is lacking.

$$\text{Dispersion parameter (or scaled deviance)} = \text{Deviance/DF}$$

A scale parameter that is greater than 1 does not necessarily imply over-dispersion is present. This can also indicate other problems, such as an incorrectly specified model (omitted variables, interactions, or non-linear terms), an incorrectly specified functional form (an additive rather than a multiplicative model may be appropriate), as well as influential or outlying observations.

If you believe you have correctly specified the model and the scale estimate is greater than 1, then conclude your data are over-dispersed. You should be able to identify possible reasons why your data are over-dispersed. If you do not correct for over-dispersion, the estimates of the standard

errors are too small which leads to biased inferences (i.e. you will observe smaller p-values than you should and thus make more Type I errors). As a result, confidence intervals will also be incorrect.

When you have the "correct" model, outliers are not a problem, and the scaled deviance is large, there are various choices for SAS procedures (GENMOD, GLIMMIX, NLMIXED) or for ASReml to correct for over-dispersion.

Example1: Binomial Counts in Randomized Blocks

(Modified from SAS GLIMMIX Help system)

In the context of spatial prediction in generalized linear models, Gotway and Stroup (1997) analyze data from an agronomic field trial. Researchers studied sixteen varieties (entries) of wheat for their resistance to infestation with the Hessian fly. They arranged the varieties in a randomized complete block design on an 8×8 grid. Each 4×4 quadrant of that arrangement constitutes a block. The outcome of interest was the number of damaged plants (Y_{ij}) out of the total number of plants growing on the unit (n_{ij}). In other words, determine if there are significant differences between entries (Plant varieties). The two subscripts identify the block ($i = 1, \dots, 4$) and the entry ($j = 1, \dots, 16$).

The following SAS statements create the data set. The variables *lat* and *lng* denote the coordinate of an experimental unit on the 8×8 grid.

```
data HessianFly;
label Y = 'No. of damaged plants'
      n = 'No. of plants';
input block entry lat lng n Y @@;
datalines;
1 14 1 1 8 2 1 16 1 2 9 1
1 7 1 3 13 9 1 6 1 4 9 9
1 13 2 1 9 2 1 15 2 2 14 7
1 8 2 3 8 6 1 5 2 4 11 8
1 11 3 1 12 7 1 12 3 2 11 8
1 2 3 3 10 8 1 3 3 4 12 5
1 10 4 1 9 7 1 9 4 2 15 8
1 4 4 3 19 6 1 1 4 4 8 7
2 15 5 1 15 6 2 3 5 2 11 9
2 10 5 3 12 5 2 2 5 4 9 9
2 11 6 1 20 10 2 7 6 2 10 8
2 14 6 3 12 4 2 6 6 4 10 7
2 5 7 1 8 8 2 13 7 2 6 0
2 12 7 3 9 2 2 16 7 4 9 0
2 9 8 1 14 9 2 1 8 2 13 12
2 8 8 3 12 3 2 4 8 4 14 7
3 7 1 5 7 7 3 13 1 6 7 0
3 8 1 7 13 3 3 14 1 8 9 0
```

```

3 4 2 5 15 11 3 10 2 6 9 7
3 3 2 7 15 11 3 9 2 8 13 5
3 6 3 5 16 9 3 1 3 6 8 8
3 15 3 7 7 0 3 12 3 8 12 8
3 11 4 5 8 1 3 16 4 6 15 1
3 5 4 7 12 7 3 2 4 8 16 12
4 9 5 5 15 8 4 4 5 6 10 6
4 12 5 7 13 5 4 1 5 8 15 9
4 15 6 5 17 6 4 6 6 6 8 2
4 14 6 7 12 5 4 7 6 8 15 8
4 13 7 5 13 2 4 8 7 6 13 9
4 3 7 7 9 9 4 10 7 8 6 6
4 2 8 5 12 8 4 11 8 6 9 7
4 5 8 7 11 10 4 16 8 8 15 7
;

```

If infestations are independent among experimental units, and all plants within a unit have the same propensity of infestation, then the Y_{ij} are binomial random variables.

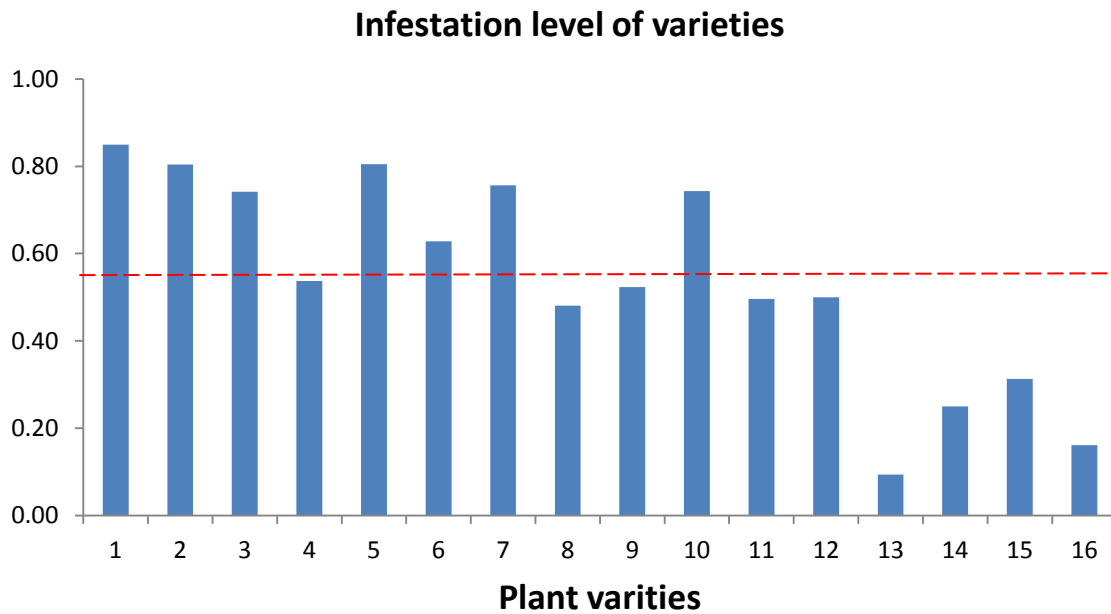


Figure 1. Data visualization and summary is an important step before any statistical analysis. The chart shows large differences between varieties for infestation. The horizontal dashed line shows the overall mean (0.54) incidence.

Analysis as a GLM

Let's consider first a standard generalized linear model for independent binomial counts. The SAS statements would be as follows:

```

proc glimmix data=HessianFly;
class block entry;

```

```

model y/n = block entry / solution;
run;

```

It may worth noting that the GLIMMIX procedure supports two kinds of syntax for the response variable. This example uses the *events/trials* syntax. The variable *y* represents the number of successes (events) out of *n* Bernoulli trials.

When the *events/trials* syntax is used, the GLIMMIX procedure automatically selects the binomial distribution as the response distribution. Once the distribution is determined, the procedure selects the *link function* for the model. The default link for binomial data is the *logit link*. The above statement is equivalent of the following statements.

```

proc glimmix data=HessianFly ;
class block entry;
model y/n = block entry/solution dist=binomial link=logit ;
run;

```

Part of the output is given below:

The “Model Information” table describes the model and methods used in fitting the statistical model.

Model Information

Data Set	WORK.HESSIANFLY
Response Variable (Events)	Y
Response Variable (Trials)	n
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Diagonal
Estimation Technique	Maximum Likelihood
Degrees of Freedom Method	Residual

The GLIMMIX procedure recognizes that this is a model for uncorrelated data (variance matrix is diagonal) and that parameters can be estimated by maximum likelihood.

The “Class Level Information” table lists the levels of the variables specified in the **CLASS** statement and the ordering of the levels.

Class Level Information

Class	Levels	Values
block	4	1 2 3 4
entry	16	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Number of Observations Read 64

Number of Observations Used	64
Number of Events	396
Number of Trials	736

The “Dimensions” table lists the size of relevant matrices.

Dimensions

Columns in X	21
Columns in Z	0
Subjects (Blocks in V)	1
Max Obs per Subject	64

Because of the absence of random effects in this model, there are no columns in the Z matrix. The 21 columns in the X matrix comprise the intercept, 4 columns for the block effect and 16 columns for the entry effect.

The “Fit Statistics” table lists information about the fitted model.

Fit Statistics

-2 Log Likelihood	265.69
AIC (smaller is better)	303.69
AICC (smaller is better)	320.97
BIC (smaller is better)	344.71
CAIC (smaller is better)	363.71
HQIC (smaller is better)	319.85
Pearson Chi-Square	106.74
Pearson Chi-Square / DF	2.37

The -2 Log Likelihood values are useful for comparing nested models, and the information criteria AIC, AICC, BIC, CAIC, and HQIC are useful for comparing non-nested models. On average, the ratio between the Pearson Chi-square statistic and its degrees of freedom should equal one in GLMs. Values larger than one are indicative of *over-dispersion*. With a ratio of 2.37, these data appear to exhibit more dispersion than expected under a binomial model with block and varietal effects.

The “Type III Tests of Fixed Effect” table displays significance tests for the two fixed effects in the model.

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
block	3	45	1.42	0.2503
entry	15	45	6.96	<.0001

These tests are Wald-type tests, not likelihood ratio tests. The entry effect is clearly significant in this model with a p-value of < 0.0001 , indicating that the 16 wheat varieties are not equally susceptible to damage by the Hessian fly.

Analysis as GLMM – Random block effects

There are several possible reasons for the *over-dispersion* noted in the “Fit Statistics” table (Pearson ratio = 2.37). The data may not follow a binomial distribution, one or more important effects may have not been accounted for in the model, or the data are positively correlated.

If important fixed effects have been omitted, then you might need to consider adding them to the model. Since this is a designed experiment, it is reasonable not to expect further effects apart from the block and entry effects that represent the treatment and error control design structure. The reasons for the over-dispersion must lie elsewhere. If over-dispersion stems from correlations among the observations, then the model should be appropriately adjusted. The correlation can have multiple sources. First, it may not be the case that the plants within an experimental unit responded independently.

If the probability of infestation of a particular plant is altered by the infestation of a neighboring plant within the same unit, the infestation counts are not binomial, and a different probability model should be used. A second possible source of correlations is the lack of independence of experimental units. Even if treatments were assigned to units at random, they may not respond independently. Shared spatial soil effects, for example, may be the underlying factor. The following analyses take these spatial effects into account.

First, assume that the environmental effects operate at the scale of the blocks. By making the block effects random, the marginal responses will be correlated due to the fact that observations within a block share the same random effects. Observations from different blocks will remain uncorrelated, in the spirit of separate randomizations among the blocks.

The next set of SAS statements fits a **generalized linear mixed model (GLMM)** with random block effects:

```
proc glimmix data=HessianFly;
class block entry;
model y/n = entry / solution;
random block;
run;
```

Treating the block effects as random changes the estimates compared to a model with fixed block effects.

Selected tables of output are given below.

[Model Information](#)

Data Set	WORK.HESSIANFLY
Response Variable (Events)	Y
Response Variable (Trials)	n
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix	Not blocked
Estimation Technique	Residual PL
Degrees of Freedom Method	Containment

In the presence of random effects and a conditional binomial distribution, PROC GLIMMIX does not use maximum likelihood for estimation. Instead, the GLIMMIX procedure applies a restricted (residual) pseudo-likelihood algorithm.

The “Dimensions” table has changed from the previous model. The “Dimensions” table indicates that there is a single **G**-side parameter, the variance of the random block effect.

Dimensions

G-side Cov. Parameters	1
Columns in X	17
Columns in Z	4
Subjects (Blocks in V)	1
Max Obs per Subject	64

Note that although the block effect has four levels, only a single variance component is estimated. The Z matrix has four columns, however, corresponding to the four levels of the block effect. Because no SUBJECT= option is used in the **RANDOM** statement, the GLIMMIX procedure treats these data as having arisen from a single subject with 64 observations.

The “Optimization Information” table indicates that a Quasi-Newton method is used to solve the optimization problem. This is the default method for GLMM models.

Optimization Information

Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	1
Lower Boundaries	1
Upper Boundaries	0
Fixed Effects	Profiled
Starting From	Data

The “Fit Statistics” table shows information about the fit of the GLMM.

Fit Statistics

-2 Res Log Pseudo-Likelihood	182.21
Generalized Chi-Square	107.96

The generalized chi-square statistic measures the residual sum of squares in the final model and the ratio with its degrees of freedom is a measure of variability of the observation about the mean model. The over-dispersion parameter (2.25) is still larger than 1.

The variance of the random block effects in the following table is rather small. The random block model does not provide a suitable adjustment for dispersion.

Covariance Parameter Estimates		
Cov Parm	Estimate	Standard Error
block	0.01116	0.03116

Because the block variance component is small, the Type III test for the variety effect in is affected only very little compared to the standard GLM.

Type III Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Entry	15	45	6.90	<.0001

In the experimental designs, researchers had row-column (longitude and latitude) values for each data to account for spatial scale micro-site variation.

Analysis with Smooth Spatial Trends

If the environmental effects operate on *a spatial scale smaller than the block size*, the random block model does not provide a suitable adjustment. From the coarse layout of the experimental area, it is not surprising that random block effects alone do not account for the over-dispersion in the data. We need to add a multiplicative over-dispersion component in PROC GLIMMIX.

```
random _residual_;
```

Such over-dispersion components do not affect the parameter estimates, only their standard errors. A genuine random effect, on the other hand, affects both the parameter estimates and their standard errors.

```

Ods graphics on/noborder ;
proc glimmix data=HessianFly PLOTS=All;
  class entry ;
  model y/n = entry / solution ddfm=contain;
  random _residual_ / subject=intercept type=sp(exp)(lng lat);
  lsmeans entry / plots=mean(sliceby=entry join);
run;

```

The keyword `_RESIDUAL_` in the `RANDOM` statement instructs the `GLIMMIX` procedure to model the **R** matrix. Here, **R** is to be modeled as an *exponential covariance structure matrix*. The `SUBJECT=INTERCEPT` option means that all observations are considered correlated. The block effects can be kept in the model. But correlated **R** structure sucks out all the variation and does not leave anything (zero variance) for Block effect to explain. We dropped the Block effects from the final model. `PLOTS=All` option produces model diagnostic plots.

Model Information

Data Set	WORK.HESSIANFLY
Response Variable (Events)	Y
Response Variable (Trials)	n
Response Distribution	Binomial
Link Function	Logit
Variance Function	Default
Variance Matrix Blocked By	Intercept
Estimation Technique	Residual PL
Degrees of Freedom Method	Containment

Dimensions

R-side Cov. Parameters	2
Columns in X	17
Columns in Z per Subject	0
Subjects (Blocks in V)	1
Max Obs per Subject	64

Fit Statistics

-2 Res Log Pseudo-Likelihood	158.85
Generalized Chi-Square	121.51
Gener. Chi-Square / DF	2.53

Covariance Parameter Estimates

Cov Parm	Subject	Estimate	Standard Error
----------	---------	----------	----------------

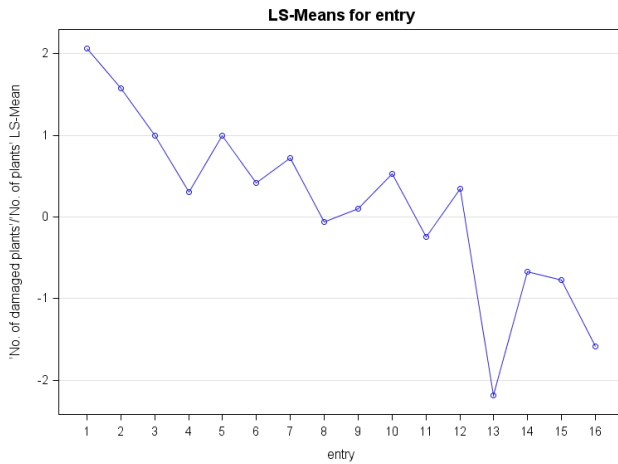
SP (EXP)	Intercept	0.9052	0.4404
Residual		2.5315	0.6974

The sill of the spatial process, the variance of the underlying residual effect, is estimated as 2.5315. The one third of practical range of a spatial process is 0.9052.

Type III Tests of Fixed Effects

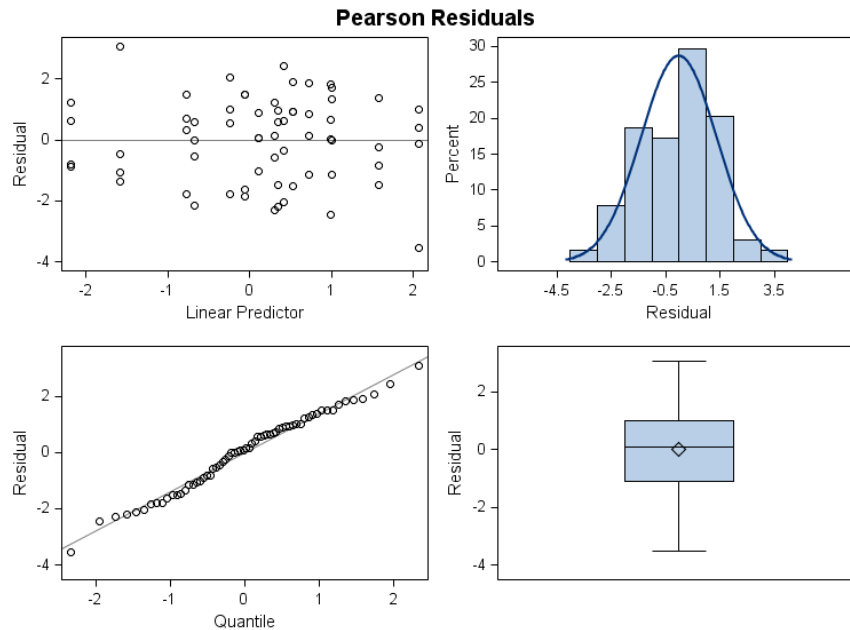
Effect	Num DF	Den DF	F Value	Pr > F
entry	15	48	3.60	0.0004

The F value (3.6) for the entry effect has been sharply reduced compared to the previous analyses. The smooth spatial variation accounts for some of the variation among the varieties



The following plot compares the LS-means of varieties. Varieties with negative LS-means have less infestation with Hessian fly.

The `PLOTS=All` statement produces diagnostic plots



Conclusions

In this example three models were considered for the analysis of a randomized block design with binomial outcomes.

If data are correlated, a standard generalized linear model often will indicate over-dispersion relative to the binomial distribution. Two courses of action are considered in this example to address this over-dispersion.

First, the inclusion of *G-side random effects* models the correlation indirectly; it is induced through the sharing of random effects among responses from the same block.

Second, *the R-side spatial covariance structure* models covariation directly.

In generalized linear (mixed) models, these two modeling approaches can lead to different inferences, because the models have different interpretation. The random block effects are modeled on the linked (logit) scale, and the spatial effects were modeled on the mean scale.

Only in a linear mixed model are the two scales identical.

GLMM with ASReml

ASReml is widely used in genetic data analysis designed experiments. We analyzed Hessian Fly data using the following ASReml code. The block effect is fitted as random again.

```

Title: Hessianfly.
#Damaged,Plants,block,Entry,lat,lng
#2,8,1,14,1,1
#1,9,1,16,1,2
Y
N
block *
entry *
lat *
lng *
yRatio !=y !/N

Hessianfly.csv !SKIP 1

y !bin !TOTAL=N ~ mu entry !r block

```

Partial output from the primary output file (HessianFly.ASR) file is given below.

```

Distribution and link: Binomial; Logit   Mu=P=1/(1+exp(-XB))
                                     V=Mu(1-Mu)/N
Warning: The LogL value is unsuitable for comparing GLM models

Notice:      1 singularities detected in design matrix.
 1 LogL=-46.8426      S2=  1.0000      48 df      Dev/DF=   2.671
 2 LogL=-46.8446      S2=  1.0000      48 df      Dev/DF=   2.671
 3 LogL=-46.8446      S2=  1.0000      48 df      Dev/DF=   2.671
 4 LogL=-46.8446      S2=  1.0000      48 df      Dev/DF=   2.671
 5 LogL=-46.8446      S2=  1.0000      48 df      Dev/DF=   2.671

Final parameter values                                1.0000
Deviance from GLM fit                                48      128.23
Variance heterogeneity factor [Deviance/DF]          2.67

```

The heterogeneity factor [Deviance / DF] gives some indication as how well the discrete distribution fits the data. A value greater than 1 suggests the data are over-dispersed, that is the data values are more variable than expected under the chosen distribution.

- - - Results from analysis of y - - -

Source	Model terms	Gamma	Component	Comp/SE	% C
Variance	64 48	1.00000	1.00000	0.00	0 F

Wald F statistics					
Source of Variation	NumDF	DenDF	F-inc	P-inc	
7 mu	1	48.0	4.64	0.036	
4 entry	15	48.0	6.87	<.001	

Finished: 26 Jul 2011 13:38:06.968 LogL Converged

The F-value for the Entry (plant varieties) is large and significant.

We can adjust for heterogeneity (over-dispersion) by using !DISPERSION qualifier in ASReml. The dispersion parameter is estimated from the residuals if not supplied by the analyst. Here is the model to account for over-dispersion.

```
y !bin !TOTAL=N !dispersion ~ mu entry
```

Output

Source of Variation	Wald F statistics			
	NumDF	DenDF	F-inc	P-inc
8 mu	1	48.0	2.05	0.159
4 entry	15	48.0	3.03	0.002

After adjusting for heterogeneity in the variance we see a much smaller F test for Entry. It is still significant. The predictions for plant varieties do not change but their standard errors change.

Spatial R structure with ASReml

ASReml is powerful to model the **R** side of the GLMM models, especially fitting spatial and power models. In the following example, we used two dimensional autoregressive order 1 (AR1 x Ar1) correlation structure for **R** to account for heterogeneity in data as a demonstration.

```
Title: Hessianfly.
#Damaged,Plants,block,Entry,lat,lng
#2,8,1,14,1,1
#1,9,1,16,1,2
y
N
block *
entry *
lat *
lng *
yRatio !=y !/N
Hessianfly.csv !SKIP 1 !DOPART 2

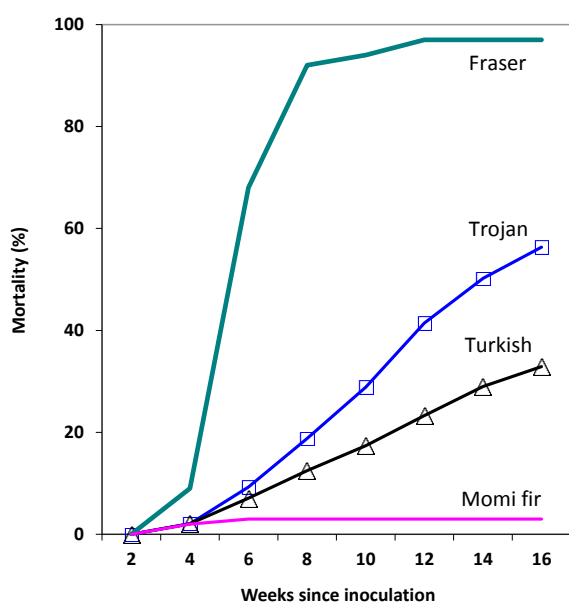
!PART 2
! Generalized Linear Mixed Model with spatial R
y !bin !TOTAL=N ~ mu entry mv
1 2 0
```


Seedlings were grown in a greenhouse and inoculated with *Phytophthora cinnamomi*, a soil borne pathogen. Subsequently, survival or mortality of each seedling was assessed biweekly. Fraser fir was used as control. The objective of the research was to examine the genetic variation between and within seed sources of Turkish firs and estimate heritability values for disease susceptibility.

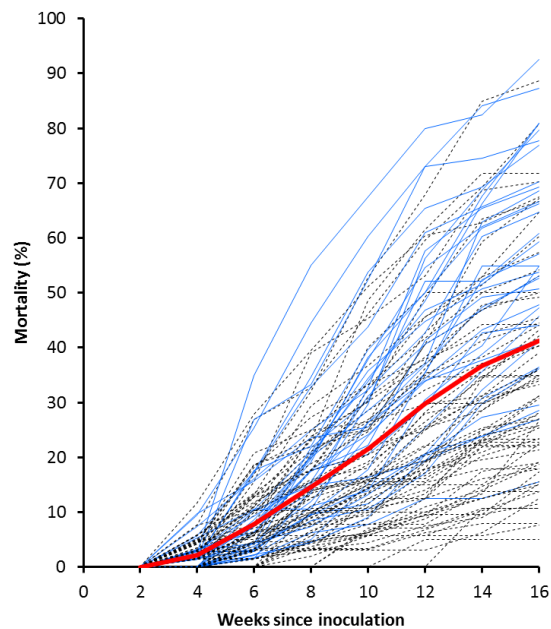
The first 10 lines of data are presented here

```
Sort,Rep,Tray,Species,Prov,Family,Tree,Wk2,Wk4,Wk6,Wk8,Wk10,Wk12,Wk14,Wk16
1,1,1,Turkish,SAF,120, 1, 0,0,0,0,0,0,0,0,0
2,1,1,Turkish,SAF,120, 2, 0,0,0,0,0,0,0,0,0
3,1,1,Turkish,SAF,120, 3, 0,0,0,0,0,0,0,0,0
4,1,1,Turkish,SAF,120, 4, 0,0,0,0,0,0,0,0,0
5,1,1,Turkish,SAF,120, 1, 0,0,0,0,0,0,0,0,0
6,1,1,Turkish,SAF,120, 6, 0,0,0,0,0,0,0,0,0
7,1,1,Turkish,SAF,120, 7, 0,0,0,0,0,0,0,0,0
8,1,1,Turkish,SAF,120, 8, 0,0,0,0,0,0,0,0,0
9,1,1,Turkish,SAF,120, 9, 0,0,0,0,0,0,0,0,0
10,1,1,Turkish,SAF,120,10, 0,0,0,0,0,0,0,0,0
```

The means were plotted against time (weeks) to examine the trends (linear, quadratic etc.) in disease incidence but also visually depict the interactions of species and provenances with time.



Mortality means (%) of fir species over time. The mean mortality of Fraser fir was 97%,



Plot of 105 family profiles and the average trend of mortality (thick red line) over time.

whereas the mean mortality of Trojan (56.5%) and Turkish (35%) were considerably smaller. The blue lines are Trojan fir families. Black lines are families from Turkish fir.

The probability of mortality ($\hat{\pi}$) of a seedling was modeled with the generalized linear mixed model using a logit (canonical) link function to partition phenotypic variance into genetic and environmental components.

$$\eta_{ijkl} = \log [\pi/(1-\pi)] = \mu + R_i + P_j + RP_{ij} + F(P)_{k(j)} + RF(P)_{ik(j)} + e_{ijkl}$$

Where;

η_{ijkl} is the link function $[g(\boldsymbol{\mu})]$,

$\boldsymbol{\mu}$ is the conditional mean,

π is the proportion of seedlings,

R_i is the fixed effect of the i th replication,

P_j is the fixed effect of the j th provenance,

RP_{ij} is the fixed interaction effect between i th replication and j th provenance,

$F(P)_{j(k)}$ is the random effect of k th family nested within provenance with $N(0, \mathbf{I}\sigma_{f(p)}^2)$,

$RF(P)_{ij(k)}$ is the random interaction of the k th family and i th replication with $N(0, \sigma_{rf(p)}^2)$, and

e_{ijkl} is the random residual with $N(0, \mathbf{I}\sigma_e^2)$.

The model was run using the ASReml software (Gilmour et al. 2009).

```
Title: Pc inoculation
Sort *
Rep *          # Rep is numeric
Tray *         # Tray is numeric
Species !A    # Specify is alpha numeric, 1 level
Prov !A       # Provenance is alpha numeric, 3 levels
Family !I     # Family Id is non-integer numeric
Tree *
Wk_2          Wk_4          Wk_6          Wk_8
Wk_10         Wk_12         Wk_14         Wk_16

Tfir_dat.csv !SKIP 1 !DOPART 1 # Data file

!PART 1
Wk_16 !bin !logit ~ mu Rep*Prov ,          # Specify fixed model
      !r Prov.Family Rep.Prov.Family # Random effects
```

WK_16 is the response variable.

Here is a simple ASReml command file written using a text editor (ConTEXT) with more description.

ConTEXT - [C:\Users\fisik\Documents\2011 May Genetic Data Analysis Workshop\Examples\Example1.asr]

File Edit View Format Project Tools Options Window Help

ASReml

Example1.asr * Example1.asr

File Explorer Favorites

C:\Examples

Name

ainverse.bin
Example1.aov
Example1.es
Example1.esk
Example1.asr

Example1_data.txt
Example1_pedigree.txt
Example1_FvE_1.ps

Title. Example1-Provenance progeny Data

#treeid, fam, male, PROV, REP, PLOT, TREE, HT, DBH, VOL

#191_1_1_1, 191, 0, 10, 1, 1, 1, 10.7, 15, 0.0722

#191_1_1_2, 191, 0, 10, 1, 1, 2, 11.5, 22, 0.167

#191_1_1_3, 191, 0, 10, 1, 1, 3, 12.1, 23.8, 0.2056

treeid !A 914

family !I

male !I

prov !I

rep *

plot *

tree *

ht dbh vol

#example1_pedigree.txt

Example1_data.txt !S

!PART 1

TABULATE ht ~ rep plot !STATS

ht ~ mu

You MUST have a TITLE

Field definitions in the

There MUST be a blank field before names

!A Alphanumeric fields are taken as factors. There are 914 subjects

!I Count integer level

* Sequential integer fields are taken as simple factors

Missing fields and those with decimal points are taken as covariates

Linear Model - Factor names are case sensitive. plot ≠Plot

Notice that we define the distribution using the **!BIN** qualifier (binomial) and underlying link function using **!LOGIT** qualifier in ASReml. The logit is the default link function. The variance on the underlying scale is $\pi^2/3 = 3.28987 \approx 3.298$ (underlying logistic distribution) for the logit link (Gilmour et al. 2006).

The results are

```
ASReml 3.0 [01 Jan 2009] Title: Pc inoculation master thru wk16 filtered.
Build fl [ 2 Sep 2009] 64 bit
31 Jul 2011 11:52:06.419 32 Mbyte Windows x64 Tfir21_Wk_16
Licensed to: North Carolina State University 30-sep-2011
*****
* Contact support@asreml.co.uk for licensing and support *
***** ARG *
Folder: C:\Users\fisik\Documents\_Research\PROJECTS\Christmas
Tree\Phytophthora Inoculations\ASREML
Rep * !SKIP 1
Species !A
Prov !A
Family !I
QUALIFIERS: !SKIP 1 !DDF 2
! Turkish fir
QUALIFIER: !DOPART 1 is active
Reading Tfir_dat.csv FREE FORMAT skipping 1 lines

Univariate analysis of Wk_16
Summary of 3662 records retained of 3675 read
```

Model term	Size	#miss	#zero	MinNon0	Mean	MaxNon0	StndDevn
1 Rep	4	0	0	1	2.4913	4	
2 Tray	9	0	0	1	4.6182	9	
3 Species	2	0	0	1	1.0000	1	
4 Prov	4	0	0	1	2.5800	4	
5 Family	66	0	0	1	32.8610	66	

```

6 Tree          18      0      0      1      7.0866      18
7 Wk_2          0 3662 0.000      0.000      0.000      0.000
8 Wk_4          0 3578 1.000      0.2294E-01 1.000      0.1497
9 Wk_6          0 3394 1.000      0.7318E-01 1.000      0.2605
10 Wk_8         0 3176 1.000      0.1327      1.000      0.3393
11 Wk_10        0 2988 1.000      0.1841      1.000      0.3876
12 Wk_12        0 2751 1.000      0.2488      1.000      0.4324
13 Wk_14        0 2531 1.000      0.3088      1.000      0.4621
14 Wk_16        0 2379 1.000      0.3504      1.000      0.4771
15 mu          1
16 Rep.Prov     16 1 Rep      : 4 4 Prov      : 4
17 Prov.Family 264 4 Prov     : 4 5 Family    : 66
18 Rep.Prov.Family 1056 1 Rep      : 4 17 Prov.Family : 264

```

Forming 1345 equations: 25 dense.

Initial updates will be shrunk by factor 0.010

Notice: Algebraic Denominator DF calculation is not available
Numerical derivatives will be used.

Distribution and link: Binomial; Logit $\mu = P / (1 + \exp(-XB))$
 $V = \mu(1 - \mu) / N$

Warning: The LogL value is unsuitable for comparing GLM models

Notice: 9 singularities detected in design matrix.

```

1 LogL=-4751.08 S2= 1.0000 3646 df Dev/DF= 1.126
2 LogL=-4751.23 S2= 1.0000 3646 df Dev/DF= 1.126
3 LogL=-4752.53 S2= 1.0000 3646 df Dev/DF= 1.124
4 LogL=-4757.13 S2= 1.0000 3646 df Dev/DF= 1.119
5 LogL=-4765.90 S2= 1.0000 3646 df Dev/DF= 1.114
6 LogL=-4778.73 S2= 1.0000 3646 df Dev/DF= 1.109
7 LogL=-4785.19 S2= 1.0000 3646 df Dev/DF= 1.106
8 LogL=-4786.54 S2= 1.0000 3646 df Dev/DF= 1.106
9 LogL=-4786.64 S2= 1.0000 3646 df Dev/DF= 1.106
10 LogL=-4786.64 S2= 1.0000 3646 df Dev/DF= 1.106
11 LogL=-4786.64 S2= 1.0000 3646 df Dev/DF= 1.106

```

Final parameter values 0.45778 0.12291 1.0000

Deviance from GLM fit 3646 4031.00

Variance heterogeneity factor [Deviance/DF] 1.11

- - - Results from analysis of Wk_16 - - -

Notice: While convergence of the LogL value indicates that the model has stabilized, its value CANNOT be used to formally test differences between Generalized Linear (Mixed) Models.

Approximate stratum variance decomposition

Stratum	Degrees-Freedom	Variance	Component	Coefficients
Prov.Family	39.25	2.06632	4.2	1.0
Rep.Prov.Family	11.64	0.122911	0.0	1.0

Source	Model	terms	Gamma	Component	Comp/SE	% C
Prov.Family	264	264	0.457785	0.457785	4.14	0 P
Rep.Prov.Family	1056	1056	0.122911	0.122911	2.41	0 P
Variance	3662	3646	1.00000	1.00000	0.00	0 F

Wald F statistics

Source of Variation	NumDF	DenDF	F-inc	P-inc
15 mu	1	58.7	60.66	<.001
1 Rep	3	158.6	6.09	<.001
4 Prov	3	59.1	9.79	<.001

```

16 Rep.Prov          9      167.8      0.85          0.567
Notice: The DenDF values are calculated ignoring fixed/boundary/singular
variance parameters using numerical derivatives.

```

Warning: These Wald F statistics are based on the working variable and are not equivalent to an Analysis of Deviance. Standard errors are scaled by the variance of the working variable, not the residual deviance.

```

17 Prov.Family      264 effects fitted (    198 are zero)
18 Rep.Prov.Family 1056 effects fitted (    792 are zero)
Finished: 31 Jul 2011 11:52:09.270   LogL Converged

```

We are interested in the variance components (Component) in this study to understand the effect of genetics and environment on the disease incidence. Heritability will tell us about the effects of genetics (family differences) on the incidence compared to phenotypic variance.

The family effect and other random effects are on logistic scale with a variance of $3.28987 = \pi^2/3$ (Gilmour et al. 1985). Because we have wind-pollinated families assumed to be half-siblings, variance explained by family effect is 1/4 of additive genetic variance (Falconer and Mackay 1996). The total additive genetic variance would be $4 * \text{Var}(\text{Prov.Family})$.

We are mostly interested in selection of families and thus the heritability of interest would be family mean heritability.

$$h_f^2 = \frac{\sigma_{f(p)}^2}{\left(\sigma_{f(p)}^2 + \frac{\sigma_{rf(p)}^2}{r} + \frac{\sigma_e^2}{n}\right)}$$

Where $\sigma_{f(p)}^2$ is the aggregate family variance component across provenances, $\sigma_{rf(p)}^2$ is the replication by family interaction variance, σ_e^2 is the fixed error variance, r is the number of replications and n is the number of seedlings per family. The error variance was set to 3.29 in calculation of phenotypic variances as suggested by Gilmour *et al.* (1985). Standard errors of heritabilities were estimated using Delta method (Lynch and Walsh 1998).

The denominator in the above formula is the phenotypic variance of family means, r is the number of replications (4), and n is the number of seedlings per family (on average it is 52 seedlings). Using the numbers from the output file given above column named 'Component', the heritability would be $= 0.457785 / (0.457785 + 0.1229/4 + 3.29/52) = 0.83$

We can use ASReml to calculate all sorts of functions of variance components.

```

Title: Pc inoculation
Sort  *
Rep   *          # Rep is numeric
Tray  *          # Tray is numeric
Species !A      # Species is alpha numeric, 1 level
Prov  !A        # Provenance is alpha numeric, 3 levels
Family !I       # Family Id is non-integer numeric
Tree  *

```

```

Wk_2      Wk_4      Wk_6      Wk_8
Wk_10     Wk_12     Wk_14     Wk_16

Tfir_ped.csv !SKIP 1 !MAKE !ALPHA !GROUPS 4 # Pedigree file
Tfir_dat.csv !SKIP 1 !DOPART 1 !CONTINUE # Data file

!PART 1
Wk_16 !bin !logit ~ mu Rep*Prov , # Specify fixed model
      !r Prov.Family Rep.Prov.Family # Random effects

!PIN !DEFINE
P Total 1+2+3*3.29 # 4 Total Variance
P Pheno 1+2+3*3.29 # 5 Phenotypic Variance
P Pheno_FamMean 1+2*0.25+3*0.0598 # 6 Family Mean Phen Variance
P ErrorVar 3*3.29 # 7 Error Variance
P AddVar 1*4 # 8 Additive Genetic Variance
H Percent_Fam 1 4 # % Variance of Family(Prov)
H Percent_RepFam 2 4 # % Variance of Rep*Family
H Percent_Error 7 4 # % Variance of Error
H H2I 8 5 # Individual Tree herit
H H2F 1 6 # Family Mean Heritability

```

Output

```

4 Total 1 3.871 0.1166
5 Pheno 1 3.871 0.1166
6 Pheno_Fam 1 0.5483 0.1099
7 ErrorVar 3 3.290 0.000
8 AddVar 1 1.831 0.4421
P_Fam = Family 1/Total 1 4= 0.1183 0.0254
P_RepFam = Rep.Fami 2/Total 1 4= 0.0318 0.0129
P_Error = ErrorVar 7/Total 1 4= 0.8500 0.0256
H2I = AddVar 8/Pheno 1 5= 0.4731 0.1016
H2F = Family 1/Pheno_Fa 6= 0.8349 0.0403
Notice: The parameter estimates are followed by their approximate
standard errors.

```

Additive genetic variance is 1.83 ± 0.442 . Family effect explained about 12% of total variance (0.118) observed in the study. Family mean heritability is 0.83 which is high, suggesting that if we select families with low disease incidence and use them for plantations, we will be able to control the disease successfully.

Accounting for Genetic Groups Effect

Provenances can be considered genetic groups or founders in the data because the F-test showed significant differences between provenances for mortality. We fit Provenance as a fixed effect in the GLMM model above to account for their effects on the variances components, or adjust for Provenance effect while estimating family variance (*Prov.Family* term in the model above).

The following are a few lines from the prediction file of ASReml (.sln). The predictions from the model are on the logit scale and they do not include the Provenance effect in which they were sampled.

EFFECT	LEVEL	BLUP	Stderr
Family	ULU	0.3549	0.2242
Family	AKY	0.000	0.000
Family	BOL	-1.086	0.2838
Family	SAF	-1.150	0.2553
Family	1	1.376	0.2915
Family	2	0.6037	0.3092
Family	3	-0.9268	0.4280
Family	4	0.4487	0.3716

In order to rank families across the provenances we need to add the predicted values of Provenances to the families. Let's assume the families 1, 2 and 3 are from ULU provenance and family 4 is from BOL provenance. The predictions of those families would be

It is also more straightforward to interpret probabilities (which range between 0 and 1) than predictions on the logit scale. In order to obtain the probabilities, we need to apply the inverse of link function.

$$\hat{p} = \exp(\hat{\mathbf{u}}) / [1 + \exp(\hat{\mathbf{u}})]$$

Where, $\hat{\mathbf{u}}$ is the vector of solutions for families (Best Linear Unbiased Prediction (BLUP)). Predicted probability values (\hat{p}) range between 0.0 and 1.0. A high probability value indicates a high probability of mortality.

Family ID	GCA	Breeding value (2*GCA)	Provenance	Sum	Predicted probability
1	1.376	2.7520	ULU = 0.3545	3.1065	0.96
2	0.6073	1.2146	ULU = 0.3545	1.5691	0.83
3	-0.9268	-1.8536	ULU = 0.3545	-1.4991	0.18
4	0.4487	0.8974	BOL= -0.1086	1.2519	0.78

Family 1 has the predicted probability of 0.96 for mortality, whereas family 3 had only 0.18 probability of mortality.

Example for rust disease in pines

Fusiform rust disease caused by a fungus is a serious threat to pine plantations in the southern United States. Offspring of four crosses (full-sib families) were cloned to select disease resistant clones for deployment. The total number of clones used in the experiment was 282. A

randomized complete block design was used with 9 replications. Each clone had one copy in a block (single-tree plot design). Block effect was considered fixed and the family and clone effects were random. When the trees were 3 years old in the field, the presence=1 and absence=0 of disease (galls) were recorded. We are interested in partitioning phenotypic variance into genetics and environmental components and to predict genetic values of clones. (Isik et. al. 2005. Predicted genetic gains and testing efficiency from two loblolly pine clonal trials. Canadian J. Forest Research 35: 1754-1766).

The probability of infection (p) of a single tree was modeled with the generalized linear mixed model using a logit (canonical) link function.

$$\eta_{ijk} = \log [p/(1-p)] = \mu + r_i + f_j + c(f)_{kj} + e_{ijk}$$

in a matrix form the model is

$$\boldsymbol{\eta} = \boldsymbol{\mu} + \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

where η_{ijk} is the link function $g(\boldsymbol{\mu})$, and $\boldsymbol{\mu}$ is the conditional mean, p is the proportion of infected trees, r_i is the fixed effect of the i th block, f_j is the random effect of the j th family with $N(0, \mathbf{I}\sigma_f^2)$, $c(f)_{kj}$ is the random effect of the k th clone within the j th family with $N(0, \mathbf{I}\sigma_{c(f)}^2)$, and e_{ijk} is the random residual with $N(0, \mathbf{I}\sigma_e^2)$.

The variance of observations is

$$\begin{aligned} \text{Var}(\mathbf{y}) &= E[\text{Var}(\mathbf{y} | \mathbf{u})] + \text{Var}[E(\mathbf{Y} | \mathbf{u})] \\ &= \mathbf{A}^{1/2} \mathbf{R} \mathbf{A}^{1/2} + \mathbf{Z} \mathbf{G} \mathbf{Z}^T \end{aligned}$$

Where the \mathbf{A} is diagonal matrix and contains the variance function of the model. That is $\mathbf{A} = \text{diag}\{p(1-p)\}$ and $p = \Pr(y_i = 1)$. The \mathbf{R} is the variance matrix for residuals random effects. The vector of random effects \mathbf{u} , was assumed to be multivariate normal with a variance-covariance matrix of $\mathbf{G} = \text{Var}(\mathbf{u})$ (SAS Institute Inc. 1996). The \mathbf{Z} and \mathbf{Z}^T are design matrix and transposed design matrix, respectively, for random effects. The validity of the model fitted to the rust data and the predicted values of clones are closely related to the average rust infection. The average infection in the experiment was 0.38. We assumed that an average rust infection 0.38 is within acceptable boundaries. An infection average smaller than 0.2 or greater than 0.8 would be associated with high environmental variance (error) not suitable for analysis.

Because linear predictors for rust infection were computed on a logit scale, the solutions from the generalized linear mixed model are difficult to interpret. Therefore, predicted probabilities (\hat{p}) of the clones were calculated by applying the inverse of the link function and using the BLUP of the random solution vector ($\hat{\mathbf{u}}$).

$$\hat{p} = [\exp(\mathbf{X}\boldsymbol{\beta} + \hat{\mathbf{u}})] / [1 + \exp(\mathbf{X}\boldsymbol{\beta} + \hat{\mathbf{u}})]$$

Where, \mathbf{X} is the design matrix for fixed effects, $\boldsymbol{\beta}$ is the solutions for fixed effects (i.e., the intercept), and $\hat{\mathbf{u}}$ is the solution for random effects or the Best Linear Unbiased Prediction (BLUP) of clones. Clone rust infection predicted probability values (\hat{p}) range between 0.0 and 1.0. A high probability value for a clone indicates a high probability of disease infection. These values are best linear unbiased predictors for clones.

Using variance components, we can easily calculate repeatability of clone means or heritability of clone means.

$$H^2 = \sigma_c^2 / [\sigma_c^2 + \sigma_e^2 / n]$$

Where H^2 is the repeatability of clone means, σ_c^2 is the variance explained by the clone effects, σ_e^2 is the variance of residuals which is fixed to 3.29, and n is the number of trees per clone in the experiment.

Fitting the model with ASReml

ASReml command file (.AS)

```
Loblolly Clonal data, MWV SC
block      9  !I  # There are 9 reps
family     4  !A  # there are 4 parents
clone     282 !A  # clone
height1
height2
height3
rust3      # 1=infected, 0=no infection
c:\research\handbook\data\MW_rust_data.csv !SKIP 1
# file has 1 header line

#univariate for rust age 3
rust3 !BIN !LOGIT ~ mu block !r family clone
```

Variance components from ASReml .ASR output file

Source	Model	Gamma	Component	Comp/SE	% C
fam	4	0.579831	0.579831	1.10	0 P
clone	1128	2.70983	2.70983	7.94	0 P
Variance	2369	1.00000	1.00000	0.00	0 F

The variance due to clone differences explained a large proportion of disease incidence (2.7098). Family component was 0.5798. The repeatability of clones is calculated as follows.

$$\begin{aligned}
 H^2 &= \sigma_c^2 / [\sigma_c^2 + \sigma_e^2 / n] \\
 &= 2.7 / [2.7 + (3.3 / 4.3)]
 \end{aligned}$$

Partial output from .SLN prediction output file

Effect	Level	Estimate	Std error
mu	1	-1.046	0.4251
fam	F	0.2428	0.4295
fam	H	0.0354	0.4307
fam	I	-0.9985	0.4398
fam	K	0.7203	0.4242
fam.clone	F.F0101	-1.213	1.137
fam.clone	F.F0103	0.7804	0.7163
fam.clone	F.F0104	-1.364	1.099
fam.clone	F.F0107	-1.306	1.114

Best linear unbiased estimates of some clones on measured scale (inverse link) are given below. The last column (inverse link) is back-transformed predicted probability (BLUP) of the clones after adding the MU to the ESTIMATE as follows.

$$p = \exp[\mu + \text{BLUP}(\text{clone})] / [1 + \exp(\mu + \text{BLUP}(\text{clone}))]$$

Clone	Estimate	Std Err	Inverse link
F.F0101	-1.213	1.137	0.09
F.F0103	0.7804	0.7163	0.43
F.F0104	-1.364	1.099	0.08
F.F0107	-1.306	1.114	0.09
F.F0108	-1.303	1.115	0.09
F.F0110	-1.292	1.118	0.09
F.F0111	0.8226	0.7198	0.44
F.F0112	1.118	0.6705	0.52
F.F0113	-0.4338	0.8745	0.19
F.F0116	0.7804	0.7163	0.43

Clone F0104 had the lowest probability of disease infection ($\hat{p} = 0.08$), whereas clone F0112 had the highest probability of infection. Assuming a probability of infection of 0.50 for a Checklot family, how much genetic gain can be realized if the top 3 clones are selected over the Checklot tree?

Fitting the model with the SAS GLIMMIX

The following code imports comma separated value data into SAS environment.

```
Proc import out= WORK.rust
  datafile= "C:\research\handbook\data\MW_rust_data.csv"
```

```

    dbms=CSV replace;
    getnames=YES;
    datarow=2;
run;

```

Fitting the model using SAS GLIMMIX procedure.

```

proc glimmix data=rust asycov;
  class rep fam clone ;
  model rust3 (event='1')= rep /s dist=binary link=logit;
  random fam clone /s ;
  output out=p pred(blup ilink)=predicted lcl=lower ;
  ods output solutionr=s_r solutionf=s_f;
  ods exclude solutionr solutionf;
run;

```

1. In the MODEL statement, the probability of the event=1 (infection) is modeled. If you do not specify the event, the code may choose 0, depending on the order. After the slash in MODEL, Best Linear Unbiased Estimates of fixed effects (BLUEs) are requested. The distribution of data is defined as binary (DIST=BINARY) and LOGIT link function is used for transformation.
2. FAM and CLONE effects are random. Best linear unbiased predictors (BLUP) of random effects are requested using /S option.
3. In the OUTPUT OUT statement inverse link of BLUP estimates were requested with the lower confidence level (LCL).
4. The ODS OUTPUT statement creates two data sets; one for random effects predictions and one for fixed effects estimates.

OUTPUT

The GLIMMIX Procedure

Model Information

Data Set	WORK.RUST
Response Variable	rust3
Response Distribution	Binary
Link Function	Logit
Variance Function	Default
Variance Matrix	Not blocked
Estimation Technique	Residual PL
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
rep	9	1 2 3 4 5 6 7 8 9
fam	4	F H I K
clone	282	F0100 F0101 F0103 F0104 F0107 F0108 F0110

Number of Observations Read	2528
Number of Observations Used	2369

Response Profile

Ordered Value	rust3	Total Frequency
1	0	1831
2	1	538

The GLIMMIX procedure is modeling the probability that rust3='1'.

The order of the outcome (observations) whether it is 0 or 1 is important. Check above table to make sure modeling the 'event=1'.

Dimensions

G-side Cov. Parameters	2
Columns in X	10
Columns in Z	286
Subjects (Blocks in V)	1
Max Obs per Subject	2369

Optimization Information

Optimization Technique	Dual Quasi-Newton
Parameters in Optimization	2
Lower Boundaries	2
Upper Boundaries	0
Fixed Effects	Profiled
Starting From	Data

Iteration History

Convergence criterion (PCONV=1.11022E-8) satisfied.

Fit Statistics

```

-2 Res Log Pseudo-Likelihood      11916.75
Generalized Chi-Square             1469.74
Gener. Chi-Square / DF             0.62

```

Covariance Parameter
Estimates

Cov Parm	Estimate	Standard Error
fam	0.5798	0.5285
clone	2.7098	0.3439

Type III Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
rep	8	2079	6.39	<.0001

The output includes model information, variance components as well as solutions for fixed effects (BLUE) and solutions for random effects (BLUPs). The following is a partial output from the S_R (prediction file for random effects) file.

Solution for Random Effects

Effect	fam	clone	Estimate	Std Err
fam	F		-0.2428	0.4295
fam	H		-0.03543	0.4307
fam	I		0.9985	0.4398
fam	K		-0.7203	0.4242
clone		F0101	1.2134	1.1374
clone		F0103	-0.7804	0.7163
clone		F0104	1.3638	1.0992
clone		F0107	1.3064	1.1138
clone		F0108	1.3030	1.1146

The “Estimate” column displays the BLUP estimates on the logit scale. Since linear predictors for rust incidence were computed on logit scale, predicted probability (p) of random effects can be calculated by applying the inverse link function. For example, probability of a clone being infected by the disease can be calculated as follows:

$$p = \exp[\mu + \text{BLUP}(\text{clone})] / [1 + \exp(\mu + \text{BLUP}(\text{clone}))]$$

clone	Estimate	StdErr	inverse link
-------	----------	--------	--------------

F0101	-1.213	1.137	0.08
F0103	0.780	0.716	0.37
F0104	-1.364	1.099	0.07
F0107	-1.306	1.114	0.07
F0108	-1.303	1.115	0.07
F0110	-1.292	1.118	0.07
F0111	0.823	0.720	0.38
F0112	1.118	0.671	0.46
F0113	-0.434	0.874	0.15
F0116	0.780	0.716	0.37

The predicted probabilities of clones are similar to what we calculated from ASReml.

Acknowledgement

Alfredo Farjat, PhD student with the Cooperative Tree Improvement Program in the Department of Forestry and Environmental Resources at NC State University contributed the document, especially the theory parts.

References

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